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EFFECTS OF 2,4,5-T ON MAN AND THE ENVIRONMENT

HEARINGS

BEFORE THE

SUBCOMMITTEE ON ENERGY, NATURAL RESOURCES, AND THE ENVIRONMENT

OF THE

COMMITTEE ON COMMERCE
UNITED STATES SENATE

NINETY-FIRST CONGRESS

SECOND SESSION

ON

EFFECTS OF 2,4,5-T ON MAN AND THE ENVIRONMENT

APRIL 7 AND 15, 1970

Serial 91-60

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EFFECTS OF 2,4,5-T ON MAN AND THE ENVIRONMENT

TUESDAY, APRIL 7, 1970

U.S. SENATE,
COMMITTEE ON COMMERCE,
SUBCOMMITTEE ON ENERGY, NATURAL RESOURCES,
AND THE ENVIRONMENT,
Washington, D.C.

The subcommittee met, pursuant to notice, at 10 a.m., in room 1318, New Senate Office Building, Hon. Philip A. Hart, presiding.

Present: Senators Hart, Inouye, and Baker.

OPENING STATEMENT BY THE CHAIRMAN

Senator HART. The committee will be in order.

Permit me a brief opening statement. For the Subcommittee on Energy, Natural Resources, and the Environment, I welcome those present for the first of these 2 days of hearings we are holding to examine the effect of the herbicide known as 2,4,5-T on man and the environment. I suggest that what is at stake at these hearings is virtually impossible to evaluate at this moment, in light of the uncertainty about this frequently used pesticide.

The questions which have been raised recently concerning the hazards of 2,4,5,-T and related chemicals may in the end appear to be much ado about very little indeed.

On the other hand, they may ultimately be regarded as portending the most horrible tragedy ever known to mankind.

What does emerge clearly from this uncertainty is that we must take steps to eliminate it. In view of the potential disaster that could befall us—or conceivably has insidiously already befallen us—absolutely no delay is tolerable in the search for answers to the questions posed.

It is with the hope that Congress will be able to play an active role in that search that these hearings have been scheduled.

Although the title of the hearing refers to the effects of 2,4,5,-T alone, it should be made clear early in the game that other similar chemicals may give rise to similar problems for the environment.

Witnesses should feel free, therefore, to address themselves to any such chemical to the extent that it bears upon the central problems at issue.

Our first witness was to have been Congressman Richard McCarthy, of New York, but unfortunately he will not be able to be

Staff member assigned to this hearing: Leonard Bickwit, Jr.

with today. We will accordingly, if there is no proxy, submit his testimony for the record.

(The statement follows:)

STATEMENT OF HON. RICHARD D. MCCARTHY,
U.S. REPRESENTATIVE FROM NEW YORK

Mr. CHAIRMAN: I appreciate the opportunity to appear before this distinguished committee in order to comment on the policies controlling the regulation and use of the herbicide commonly known as 2,4,5-t. Your examination is most timely, for the effects of this defoliant on plant and animal life suggest that more stringent regulations regarding its application are called for.

As you may know, I have spent much of the past year investigating the government's chemical and biological warfare program. I am happy to report that several significant changes have taken place in U.S. policies; changes which I have advocated for many months. These include a promise to resubmit the 1925 Geneva Protocol outlawing chemical and biological agents in warfare, and a ban on any further biological warfare development, including toxins.

These are small steps forward, but the chemical warfare program still contains many features which are of questionable value and safety. One of these is the defoliation program in Southeast Asia. There is evidence that one of the compounds sprayed on a widespread basis is teratogenic, or birth-deforming. Without objection, I wish to insert an article from the *New York Times* of March 15 which examines possible birth defects in babies born in areas where compounds containing the chemical 2,4,5-trichlorophenoxyacetic acid are used.

This spray, containing an agent known as 2,4,5-trichoirophenoxyacetic acid, has also been in common use as a brush killer in the United States. The commercial compound is known as Silvex, and is produced commercially by Dow Chemical Company under the trade name, Kuron. It is used by the U.S. Forest Service to kill brush as part of watershed projects in the Southwest United States.

Last year, I learned some startling facts about 2,4,5-t. As I have noted before, laboratory tests conducted by the Bionetics Laboratories for the National Cancer Institute indicated that the compound is teratogenic. A recent discussion of these laboratory tests appears in the *Medical World News* of February 25, 1970. Without objection, I wish to insert the article, "Defoliants, Deformaties: What Risk?" in the *Record* at this point.

I was therefore relieved when the White House, on October 29, 1969, announced that the Agriculture Department was to terminate its use around food crops after the first of the year. The new year came, but instead of receiving word of compliance, I was informed that the Department of Agriculture had no intention of restricting its use. In their words:

"We are awaiting advice from DHEW as to whether or not they intend to establish tolerances for 2,4,5-t before we decide whether to cancel or extend uses of 2,4,5-t on food crops. Our January 1, 1970, date was based on DHEW's expectation that they would have reached a decision by that time. That agency believes that the public interest would best be served by waiting for additional research data which will be available shortly. We concur in their judgment."

I tried in vain to find out why the White House directive had not been put into force. The explanations from all agencies involved, the White House, the Department of Agriculture, and the Food and Drug Administration, simply ignored the original directive, and I was informed that the spray would be authorized until further laboratory tests were completed sometime this Spring. The best analysis of this confusing situation appeared in the *New Yorker Magazine* of February 4, 1970. Without objection, I wish to insert the article entitled "A Reporter at Large: Defoliation", by Thomas Whiteside in the *Record* at this point.

I have not had the opportunity to examine the effects of defoliation in Vietnam firsthand, but I am happy to know that Dr. Matthew Messelson, a distinguished biologist and expert on chemical and biological warfare, is in the process of initiating a thorough investigation on this matter under the auspices of the American Association for the Advancement of Science.

I can only say that I am shocked by the unregulated manner in which the Department of Defense permits its widespread application, and I am appalled that no field investigation regarding its long-range effects has been conducted by the military. I have, however, had the opportunity to review the policies and examined the effects of 2,4,5-t spraying operations in this country. I hope my observations on this matter will be of interest to the members of this committee.

Last year, I learned about a controversy over one spray program carried out by the Forest Service in Eastern Arizona. Reports from Globe, Arizona indicated that the Chapparel Management Program, a part of the Salt River Watershed Project had been suspended because residents of the area had complained of irregularities in the program.

Because of the difficulties I had in obtaining adequate information from Administration officials in Washington, and because of the nature of the controversy in Globe, Arizona, I made arrangements to conduct two days of public hearings in that town to learn first-hand about the spray program and its problems.

The Department of Agriculture plays a unique role in a State like Arizona. It has jurisdiction over approximately 80 percent of the land area, most of which is administered by the U.S. Forest Service.

Originally established to preserve forest regions in their natural state of wilderness, the Forest Service now participates in a series of commercial projects which attempt to increase the water supply for human consumption. One step is to kill unwanted flora—in this case chapparel trees, which absorb what officials believe is too much water. Traditionally, burning accomplished this mission. In the past few years, however, chemical sprays, including Silvex Kuron, have been used extensively.

According to Forest Service officials, Kuron has been sprayed on four occasions since 1965. Following the latest spraying operation in June 1969, residents complained of illnesses to themselves and to their livestock, including respiratory problems and deformed offspring to their animals.

My purposes in conducting hearings on February 12 and 13, 1970 were the following:

(1) To learn the regulations under which the Department of Agriculture authorizes the use of the spray, and how they are enforced. In addition, I wanted to learn how new policies from Washington were transmitted and implemented, and how each regional office of the U.S. Forest Service was advised of the latest scientific information on chemicals which were in use.

(2) To ascertain the degree of scientific understanding of the ecological effects of the herbicide 2,4,5-t, and

(3) To learn the scope and nature of citizen complaints, and what if any, relation they have with the chapparel spraying operations.

Accordingly, I requested six witnesses to appear at the public hearing. They included Dr. Arthur Galston, Department of Biology, Yale University; Dr. John Pierovich, Assistant Regional Commissioner, National Forest Service, Albuquerque, New Mexico; Dr. F. I. Skinner, Veterinarian, Globe, Arizona; and Dr. Paul Martin, Department of Geochronology, University of Arizona, Tuscon, Arizona. In addition, two residents of Globe, Mrs. Billie Shoecraft and Mr. Robert McKusiak gave accounts of their experiences following the June 1969 spraying.

Mr. Pierovich gave me a full explanation of the chapparel management program, including the regulations to be followed while the compound is being sprayed.

These include restrictions around crops, over water, and above certain wind speeds.

Mr. Pierovich admitted that violations had occurred; the spray has found its way into bodies of water, and the compound had drifted onto private property as a result of wind speeds above the prescribed ten miles per hour. In addition, he informed me that new policies regarding the use of sprays containing 2,4,5-t were transmitted to the field office long after the fact. For example, Forest Service officials in Albuquerque were furnished with a policy statement from the Department of Agriculture in December which referred to the DuBridge statement. Mr. Pierovich also admitted that he has learned of the 1966 laboratory tests on 2,4,5-t from the press. Evidently no effort was made by the Department of Agriculture to inform the field offices of the latest scientific evidence regarding the possible dangers of 2,4,6-t.

I therefore concluded that the U.S. Forest Service is negligent in enforcing current regulations regarding herbicides; that it fails to transmit new policies

quickly, and that no adequate system exists to transmit new scientific information. Mr. Pierovich himself stated:

"The most healthy thing that could happen in this area would be a definite summary of literature that our technicians could refer to. There are abstracts available now, but the combination of inputs from the universities and from the various departments of government in one abstract bulletin would be helpful to us."

I intend to write the Secretary of Agriculture urging him to arrange such an information system.

A second purpose of my visit was to duscuss with a competent scientist, the ecological effects of herbicides on a semi-arid region.

Dr. Arthur Galston assisted me in this effort. An experienced biologist who has studied at length the impact of defoliants at home and abroad, he outlined in his testimony the chemical reactions of these compounds and discussed certain government investigations which caution against untested use of herbicides and pesticides. He refers, for example, to the Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health, prepared by the distinguished panel shared by Doctor Emil Mrak, the Chancellor Emeritus of the University of California at Davis. He goes on to say:

"This report to which I have alluded includes as its final statement, a chapter on Teratology. I would like to read you the summary which emphasizes my concern: 'All currently used pesticides should be tested for teratogenicity in the near future in two or more mammalian species chosen on the basis of the closest metabolic and pharmacologic similarity to human beings possible. Pesticides should be tested at various concentrations including levels substantially higher than those to which the human population is likely to be exposed. Test procedures should also reflect routes related to human exposures. Apart from the obvious route of ingestion, attention should be directed to other routes of exposure, including inhalation exposures from pesticide aerosols and vaporizing pesticide strips used domestically and exposures from skin absorption. Parenteral administration is an appropriate test route for pesticides to which humans are exposed by inhalation, or for pesticides which are systematically absorbed following ingestion.

"'The use of currently registered pesticides to which humans are exposed and which are found to be teratogenic by suitable test procedures in one or more mammalian species should be immediately restricted to prevent risk of human exposure.' . . .

Dr. Galston continues:

"Here, then, is the Government's most distinguished panel saying that there is evidence that 2,4,5-t has produced teratogenic effects in one or more mammalian species. To me, this means should be restricted immediately. The committee also said no new pesticide found to be teratogenic, should be used only in circumstances where risk of human exposure is minimal."

In spite of this recent information, the Forest Service—in Arizona, at least—was unaware of its contents. Dr. Galston's remarks about the overall purpose of the project are worth repeating. He states:

"My overall view after one day of looking around is one of puzzlement. I wonder why anyone desired to initiate this kind of an operation in this kind of an environment. The stated objective is to improve water runoff, and water runoff will benefit, I presume, the citizens of a nearby urban area, Phoenix, which is growing rapidly, and which has a lot of water requirements, and their water requirements will grow as the years go by, and we know this is an arid area.

"Truly, water is going to be wilting in this area for all activities. So far as I can see unless nuclear technology makes it available on a massive scale, taking water from this area to give to another area, is, in fact, robbing Peter to pay Paul. If you are removing water from this area you are going to partially change the vegetation. Perhaps you are going to denude some of the areas in order to increase the runoff. This involves a comparative set of valves. Whose ox is going to be gored here? Whose interests are paramount? Clearly, cities are not going to be able to grow indefinitely; we are going to have to put some limit on them. We know, for example, that the city of Los Angeles got into a lot of trouble with smog because there are just too many people there. In the same way, cities in the Southwest may have to limit their size ultimately based on the number of people they can support on the basis of the amount of water resources available.

"Now that President Nixon among others is calling for a campaign to restore the environment, it might be that we would want to look at this project in the context of what we are doing to the entire Stae and to the entire countryside."

I cannot help but conclude that such land management programs involve much more than the immediate water needs of urban sprawl. The natural regions of the Southwest are a great natural resource. They must not be sacrificed so recklessly.

My third interest was to determine whether any relationship existed between the maladies of certain residents and their livestock and the spraying of Silvex.

The Agriculture Department readily admitted that the spray drifted out to private property. The owners have a rightful complaint regarding this fact which has not yet been resolved. The local veterinarian, Dr. F. I. Skinner informed me that he would not have recommended the use of 2,4,5-t compounds had he been familiar with the results of the Bioneties Laboratory's tests.

It is also true that illnesses developed whose symptoms are similar to those which are known to be associated with herbicides. In addition, I saw malformed animals who were born after the incidents of last June. Statements were made regarding this fact by persons testifying in good faith, and should not be dismissed outright. They obviously have some bearing because the Forest Service has suspended further sprayings in the area. This decision followed the complaints, and while Federal investigation found no direct relationship between the spray operations and the illnesses, Forest Service officials await further developments before resuming.

No one is being helped by the procrastination of officials in Washington.

Three agencies are now involved in the 2,4,5-t controversy, yet none have assumed responsibility for regulating this herbicide. The Food and Drug Administration, which under the 1954 amendments to the Cosmetic Act of 1938, has the obligation to establish safe tolerance levels before a chemical of this kind is put on the market, has failed to enforce the Law. The Agriculture Department continues to ignore other agencies in administering the Federal Insecticide, Fungicide and Rodenticide Act. The Pesticide Regulation Division established by this act, was sharply condemned by the House Government Operations Committee report of November 13, 1969 for not carrying out its responsibility to police the licensing of herbicides. In addition to the charge that no legal steps have ever been taken against firms which violate licensing regulations, the Committee report brought to light repeated instances of conflict of interest among various officials of the Pesticide Regulation Division and agro-chemical companies.

Finally, the White House has backed down from its assertive position of last October. After reversing an earlier ban, I am now told boldly by Dr. Lee A. DuBridge in a letter of March 2, 1970, that "we anticipate, indeed we will insist upon final action of 2,4,5-t before its period of principal usage in late spring."

I will not hold my breath.

Mr. Chairman, there are obvious irregularities in the regulation and management of herbicide compounds containing 2,4,5-t. It is clear that the National Forest Service no longer regards preservation of lands in their natural state as a primary responsibility. There is insufficient information regarding its risks and inadequate statistics on its effects to animal and plant life. Its use must not be continued until its safety is assured.

Accordingly, I recommend an immediate five year ban on the use of herbicides containing 2,4,5-t. During this period of suspension, the Food and Drug Administration should establish, once and for all, whether the chemical has a safe tolerance level. The latest word from FDA is that the officials "are in no position to say that the chemical 2,4,5-t or the dioxin is without hazardous effects". A letter to me of March 12, 1970, reflects the inconclusive evidence regarding its safety. It is worth repeating in full. Without objection, I wish to insert the letter from M. J. Ryan of the Food and Drug Administration, along with an attached fact sheet in the *Record* at this point.

In addition, steps should be taken immediately to collect information on the number of children born with birth defects, including those which might be caused by herbicides.

Accordingly, I will be writing to the Secretary of Health, Education and Welfare urging him to instruct the National Institute of Neurological Diseases and Strokes of the National Institutes of Health to begin gathering information on these phenomena.

Mr. Chairman, I am confident that these hearings will help resolve a most important issue. There can be no more delay.

Mr. Chairman, without objection, I wish to insert various documents pertaining to my hearings in Globe, Arizona for inclusion as an appendix to these hearings.[1]

Senator HART. We will move to the next scheduled witnesses, James Turner and Harrison Wellford of the Center for Responsive Law.

STATEMENT OF HARRISON WELLFORD, CENTER FOR STUDY OF RESPONSIVE LAW, WASHINGTON, D.C.

Mr. WELLFORD. Thank you, Mr. Chairman.

We appreciate very much the invitation to appear at these hearings this morning. I would like, if I may, to read excerpts from my testimony rather than reading the entire testimony.

In this testimony we wish to bring to your attention that herbicides containing 2,4,5,-T are widely marketed for casual use by the individual consumer in residential and other populated areas. The herbicide 2,4,5-T even in extremely small doses, causes massive and severe birth defects in test animals, including mice, rats, and hamsters. The herbicide 2,4-D, which is often mixed with 2,4,5-T in the popular herbicides such as Ortho Weed-Be-Gone, is also teratogenic, but at higher dose levels. We feel that these herbicides, as currently used, may pose a grave and unnecessary danger to public health.

In a recent article, the biologists Arthur W. Galston, William Cooke, and William Haseltine stated that 2,4,5-T "may represent the ecological equivalent of thalidomide." (Congressional Record, Feb. 19, 1970, S. 1984). Professor John T. Edsall of Harvard University, stated before the American Association for the Advancement of Science's Committee on Chemical and Biological Warfare that "the use of these compounds is much more seriously questionable than the use of cyclamates. If one applies the same criteria, one would consider the risks quite unacceptable." (Quoted in the New York Times, Dec. 29, 1969). The tests performed by the Bionetics Laboratory, by the Food and Drug Administration, National Institute of Dental Research, and the National Institute of Environmental Health Sciences clearly show that these chemicals are potentially harmful. Whether or not human beings are more or less susceptible than test animals to these chemicals, we do not yet know. But clearly on the evidence now available, the burden of proof should be on the industry to demonstrate that they are not harmful. In the meantime, all uses of these herbicides around the home and in populated areas should be immediately suspended.

We wish to discuss this morning the nature of the danger as we see it and the serious failures of both the Federal Government and private industry to reduce the unnecessary risks to public health of exposure to 2,4,5-T and other suspicious weedkillers. Specifically I am concerned about:

(1) The attempts by Government and industry to conceal the facts about 2,4,5-T and 2,4-D from the public and from other scientists.

[1] See p. 102.

(2) The hazards to public health which may result from widespread use of 2,4-D and 2,4,5-T in populated areas.

My colleague, Mr. James Turner, will discuss the profound implications which the 2,4,5-T–2,4-D case has for the effectiveness of Government efforts to protect the public from pesticide hazards.

2,4,5-T was developed from research done at the Chemical and Biological Warfare Center at Fort Detrick during the 1940's. It has been massively applied to the human environment for 20 years, but until very recently no studies have been conducted by any Government agency on the possible carcinogenic, mutagenic, or teratogenic properties of this herbicide, or on the ecological consequences of its use. In 1966 the Bionetics Laboratory was commissioned by the National Cancer Institute to begin research into the birth defect properties of a variety of pesticides and herbicides, including 2,4-D and 2,4,5-T. By 1968 these tests had revealed substantial evidence that 2,4,5-T caused birth defects in test animals. In February of 1969, the preliminary results from the Bionetics testing were to be presented to the annual meeting of the American Society of Toxicology. The report included tests results which showed that 2,4-D and 2,4,5-T cause gross abnormalities and birth defects in mice. 2,4-D was termed "potentially dangerous, but needing further study," while 2,4,5-T was labeled "probably dangerous." This report would have provided an early warning on potential hazards from these herbicides, but for reasons still unknown, the Bionetics presentation was canceled at the last minute, although its paper was listed on the printed agenda of the meeting. The Bionetics report thereafter returned to obscurity. Only a few scientists in the Government knew of its existence. Even members of the Mrak Commission. Panel on Teratogenicity were initially rebuffed when they asked to see the report. The report, however, had been presented to selected officials in the Food and Drug Administration, the U.S. Department of Agriculture, and the Defense Department. These delays contradict the recommendation of the Mrak report that "any teratogenic pesticide to which the population is exposed should be promptly identified so that appropriate precautions can be taken to prevent risk of human exposure" (p. 657).

On October 29, after the existence of the report finally became public (it has still not been officially released), Dr. Lee Dubridge, the Science Adviser to the President, announced that "a coordinated series of actions" was being undertaken by several Federal agencies to ban the use of 2,4,5-T on food crops and Government use of the herbicide in populated areas. Dr. Dubridge stated that these actions were being taken to "assure the safety of the public while further evidence is sought." The ban on use of 2,4,5-T on crops was to go into effect on January 1, 1970 unless by that time the FDA had found a means to establish safe residue tolerances. Apparently because Dr. Dubridge estimated that "almost none (of 2,4,5-T) is used by home gardeners, or in residential areas," nothing was said about stopping individual consumers from using 2,4,5-T in their own backyards.

The Department of Interior did follow Dr. Dubridge's recommendation and ceased the use of 2,4,5-T in its operations. The deadline

of January 1, 1970, passed without FDA setting a tolerance. By late January, it was clear that the Department of Agriculture and other Federal agencies (with the exception of Interior) had no intention of restricting the use of 2,4,5-T. On February 6, the Department of Agriculture announced that the original 2,4,5-T used in the Bionetics test had been contaminated with a tetradioxin and that further testing with a purer batch of 2,4,5-T had shown no adverse effect. The Department relied on tests conducted by the Dow Chemical Co., a major manufacturer of 2,4,5-T. The Dow evidence was immediately contradicted by tests conducted at several other Federal agencies which clearly showed that even the purest 2,4,5-T, where the dioxin contaminant was present at less than one part per million, still produced birth defects in test animals at significant levels. Recent tests on 2,4-D conducted by the FDA have confirmed that this herbicide, which is very often mixed with 2,4,5-T, is also teratogenic. Many other questions with significance for public health remain to be answered: How persistent are 2,4,5-T and 2,4-D once applied? How persistent is the dioxin contaminant? Is it cumulative in human tissue? How much dioxin is present in herbicides already on the shelves of local hardware stores?

Therefore, Mr. Chairman, 5 months after Dr. Dubridge urged that most uses of 2,4,5-T be banned to "assure the safety of the public while further evidence is sought," these herbicides continue to be teratogenic and are as widely used as ever. The most recent tests provide the "further evidence" Dr. Dubridge asked for. The burden of proof on those who wish to demonstrate the safety of these herbicides has greatly expanded since the first Bionetics test.

When tests indicate that a pesticide is teratogenic in animals, the burden of proving that it is safe should be placed on the manufacturers of the pesticide, not on its possible victims in the general population. If the manufacturers do not cooperate, the Federal Government has a statutory responsibility to minimize human exposure to teratogenic pesticides by appropriate regulatory preventive action. The Government must not fail in its trust, for nationwide statistics on birth defects are so inadequate that even an increase of several thousand deformities could probably go undetected. Therefore, if Government and industry act irresponsibly, there will probably be few complaints from the medical profession or the general public to call them to task.

Present population monitoring techniques do not provide adequate gages of the incidence of birth defects in the population. Federal regulators charged with protecting the public from pesticide hazards are being very irresponsible if they assume, as did Dr. Lindsey of the Food and Drug Administration in a recent interview that, "the National Institute of Neurological Disease and Stroke has recorded birth defects for some 15 years and would be telling us if they were on the rise." [1] Dr. Hines Berendes, Chief of NINDS' Perinatal Research Branch, has unhappily conceded that, "no nationwide data are available on the frequency or incidence of malformation." [1] Even in States where birth certificates request that doctors record birth defects, the completeness and accuracy of the reporting depends on

[1] Medical World News, Feb. 27, 1970.

the interest and diligence of the physician and on the conspicuousness of the abnormality. Nationally, no attempt has been made to collect and evaluate all the data on birth defects that are presently available on birth certificates. After careful study of this problem, the writers of the Mrak report concluded that "epidemiologic data on possible effects of pesticides on human reproduction and teratologf are grossly inadequate." [2]

The Mrak Commission report states that when animal experiments indicate that a pesticide is teratogenic, the effect should be retrospectively evaluated when possible by a study of pregnancies during which the mothers were inadvertently exposed to the pesticide, such as in farmwork and industrial exposure and through accidental ingestion. As far as we know, this has not been done for 2,4,5-T and other weedkillers. Because of the need to minimize human exposure, it is not possible to test on human populations pesticides previously shown to be teratogenic by experimental animal studies.

The Mrak report states unequivocally that there is little comfort to be gained from the expectation that present epidemiological surveys of pesticides in current use will discover in time chemical compounds causing birth defects. It states that no major teratogen (term for substance causing birth defects) has been discovered in this way. The malformations induced by X-rays, german measles, thalidomide, and mercury were each recognized by "an alert medical practitioner who observed a cluster of cases and then traced the cause to its source." [3] Tracing observed defects to a specified cause is much more difficult when the defect commonly occurs. In the case of 2,4,5-T, the most common defects produced in test animals are kidney abnormalities and cleft palates, neither of which is unusual in humans. Had thalidomide produced such ordinary malformations instead of bizarre and unusual ones, it probably would never have been discovered. Thus any birth defects produced by human exposure to 2,4,5-T are unlikely to be traced to the weedkiller because they are already common in the population.

If 2,4,5-T and 2,4-D, as commonly used in populated areas, do produce birth defects in humans, the birth defects will remain a very private family tragedy. Because 2,4,5-T leaves no unique fingerprints on the fetus to indicate its specific role as the teratogen, the parents of the deformed child would probably remain silent, with no knowledge of the cause of their distress, They would probably never know that they belong to a class of victims of a preventable tragedy.

There is another reason why Government has a special responsibility here to protect the public. Because a 2,4,5-T or 2,4-D induced birth defect is not unique, the parents of deformed children will have great difficulty in using the courts to discipline the manufacturers of dangerous herbicides. The parents will gain no compensation for their loss. Moreover, there will be no lawsuits to force the chemical companies to test more thoroughly their products for teratogenic effects before they are released on the market or to maintain strict quality control standards which will keep the level of contami-

[2] Report of the Secretary's Commission on Pesticides (the Mrak report, U.S. Department of Health, Education, and Welfare. December 1969, p. 674.
[3] Mrak report, p. 661.

nation of dangerous dioxins as low as possible. In the absence of legal remedies for private citizens, protection must come from the Federal Government.

As a first step, we suggest that the committee consider the recommendation of the Mrak Commission that efforts must be made to improve and use information on congenital malformations recorded on birth certificates and that new systems of collecting birth defect data be established.

POTENTIAL HAZARDS FROM CONSUMER USE OF TERATOGENIC HERBICIDES IN RESIDENTIAL AREAS

Weed-killers containing 2,4,5-T are readily available to the home gardener. These products come both as premixed, ready-to-use liquid, spray or dust, and as liquid preparations which the user dilutes at home, or uses with the garden hose atomizer. The concentrated liquids are obviously the most dangerous.

Last week two of my assistants made a survey of herbicide products in 10 Washington area stores: Eight hardware stores, including Meenehans, Sears, Hechingers, McIntyre, Community Paint and Hardware, Kresge's at 7th and Pennsylvania, Chevy Chase Hardware; one grocery store, the Giant at Western Ave. and Wisconsin; and one gardening store, Sheridan Garden Store on Old Georgetown Road.

My assistants found the following:

(1) Eight of the stores carried lawn and garden weedkillers containing 2,4,5-T, nine of the stores carried product lines which included 2,4,5-T products. All of the stores carried weed-killers containing 2,4-D. They found nine product lines containing 2,4-D; six with 2,4,5-T.

(2) In addition to 2,4-D and 2,4,5-T, at least seven of these stores carried all but one of the other products cited in the Bionetics Report as at least potentially causes of birth defects: Captan, Folpet, Sevin; as well as organo-mercurial products which have been known to produce birth defects in humans since an epidemic in Japan led to their being banned in that country.[4]

(3) Some of the products, the Scotts line in particular, were very badly packaged. Most Scotts products and some others are packaged in flimsy paper bags; a number of the bags which my assistants examined were in such bad repair that they could not be handled without spilling dust on the handler. As a result, there was frequently a coating of chemical dust on products nearby. In an unintended irony, most of the packages bore warnings to the user to "avoid contact with skin, eyes or clothing."

(4) A number of products and again the Scotts line in particular, were fertilizers with various herbicides added, including 2,4-D, and 2,4,5-T (in Greenfield products.) Since the contents were not very conspicuously displayed, many users might well assume that these were only "super fertilizers," and therefore not handle them with the care that their contents warranted. These products, containing a mixture of fertilizer and herbicide are heavily promoted on radio and television, with no warning of potential danger. Their names,

[4] Mrak report, p. 601.

for example, Scotts Turf Builder Plus-1, which contains a mercurial compound, often disguise or play down the herbicide content. Mixing herbicides with fertilizer tends to identify potentially dangerous chemicals with innocuous fertilizers and promotes unnecessary, as well as careless use.

(5) The manner in which these herbicides are used magnifies their potential risk. Their labels all read: "Avoid contact with skin, eyes or clothes. Avoid inhaling dust." Yet these products are dispensed in a manner which makes contamination of the user with either dust or spray inevitable. Herbicides in dust or powder form are often applied by dumping them into a wheelbarrow with a hole in the bottom and a propeller underneath which the user pushes across his lawn raising a cloud of dust. Liquid herbicides are applied through attachments to a garden hose or through hand applicators. In both cases, contact with the spray is unavoidable. Many of these products on their packages depict homeowners spraying with bare hands and bare arms. On no products, even the most poisonous, was there any suggestion that the user wear rubber gloves. Several of the herbicides did contain the difficult instruction that children and pets be kept off the treated areas, sometimes for an unspecified amount of time, until after the area had been watered and dried or it had rained.

Whether applied in spray or dust form, the application of herbicides containing 2,4,5-T presents serious problems of drift. The report of the Subcommittee on Weeds of the National Research Council stated in 1968 that spray with "droplets of 10 microns in diameter can drift up to 1 mile when released at a height of 10 feet with a 3-mile per-hour wind." (p. 248). Even when kept in perfect condition, few nozzles used for spray application would produce uniform droplets large enough to minimize drift and yet small enough to provide even coverage. The hazards of drift, even when the herbicide is applied in dust or powder form is also great. The Department of Agriculture, in its caution suggested for use on weed-killers containing 2,4,5-T and 2,4-D, warns that "this dust may drift for miles even on quiet days." (Federal Register, May 21, 1969).

It is a conservative estimate that even on a relatively calm day children playing within 100 yards of an area where a yard is being sprayed or dusted with 2,4,5-T are probably going to be exposed to the chemical. Droplets and dust particles of 2,4,5-T can be carried by the wind into open windows and onto screen porches. In heavily populated residential areas, one simply cannot defoliate his backyard of chickweed and dandelions without running the risk of contaminating his neighbors or their children. The economic and horticultural benefits of these herbicides in residential areas do not outweigh their risks to those who wish to enjoy the outdoors without being contaminated by teratogenic spray.

(6) A few products did not bear even the minimum federally required warning, "Caution, Keep out of reach of children," which must be displayed prominently on the front. For example, Scotts Kansel Weed Killer, in a salt-shaker like container, had no warnings at all on the front, and only "Avoid contact with skin, eyes, clothing, etc." written very inconspicuously on the back. The same was true of an identically packaged Amchem Garden Weeder.

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(7) The potential hazards of 2,4,5-T herbicides are increased by the fact that consumers frequently do not read the label on the pesticide package, or if they do, do not usually understand it. The Mrak report cites the 1969 study of home pesticide use in Charleston, S.C. which found that of the 83 percent white and 97 percent nonwhite families using pesticides:

> Both white and nonwhite families commonly ignored safety precautions in the use of household chemicals. Locked storage was not employed by 88 percent of all families; 66 percent stored the pesticides within easy reach of small children; 54 percent stored the chemicals near food or medicine; and 66 percent never wore protective gloves during use or washed their hands after application.[5]

It is unlikely that the labels of the 2,4,5-T herbicides we examined in local stores could be changed sufficiently to prevent users from contaminating vulnerable individuals.

I might add while it is not included in my prepared testimony that we did come across yesterday herbicides on local hardware shelves which have disclaimers of liabilities which are clearly in violation of the notice to manufacturers, formulators, and distributors issued by the Pesticide Regulation Division of the Department of Agriculture on November 16, 1966.

(The notice follows:)

> UNITED STATES DEPARTMENT OF AGRICULTURE
> AGRICULTURAL RESEARCH SERVICE
> PESTICIDES REGULATION DIVISION,
> *Washington, D.C., November 16, 1966.*
>
> NOTICE TO MANUFACTURERS, FORMULATORS, DISTRIBUTORS, AND REGISTRANTS OF ECONOMIC POISONS
>
> STATUS OF DISCLAIMER STATEMENTS ON PESTICIDE LABELING
>
> Attention: Person responsible for Federal Registration of Economic Poisons.
>
> The Federal Insecticide, Fungicide, and Rodenticide Act, and the regulations promulgated thereunder, provide that an economic poison is misbranded if its labeling bears any statement, graphic representation, or design which is false or misleading in any particular.
>
> Labeling disclaimers which negate or detract from labeling information required under the Act and regulations are not acceptable on products proposed for registration.
>
> An example of a disclaimer which would render a product misbranded is as follows:
>
> "The information furnished hereon is provided gratuitously by the manufacturer who assumes no responsibility whatsoever for the effectiveness or safety of this product regardless of whether or not it is used as directed."
>
> Such disclaimer is both false and misleading, since adequate directions for use, necessary warnings and cautions, and other essential information on the safe handling and use of a product are required under the Federal Act.
>
> Labeling for registered products bearing disclaimer statements which are either false or misleading must be revised or deleted. Such revisions or deletions will not require reregistration by this Division. However, five copies of such amended labeling should be submitted for our records.
>
> HARRY W. HAYS, *Ph. D.*,
> *Director.*

There are two major provisions of disclaimer statements which have been used by many registrants that are considered to be false or misleading. These are the claims "that the information is provided gratuitously," and disclaiming on any responsibility "whether used in accordance with the directions or not."

[5] Mrak report, p. 148.

There is no objection to statements that are aimed at protecting the seller against damages from careless or improper handling, or use, so long as they are not false or misleading.

EXAMPLES OF DISCLAIMER STATEMENTS WHICH ARE NOT OBJECTIONABLE

(1) Seller makes no warranty, expressed or implied, concerning the use of this product other than indicated on the label. Buyer assumes all risk of use and/or handling of this material when such use and/or handling is contrary to label instructions.

(2) Follow directions carefully. Timing and method of application, weather and crop conditions, mixtures with other chemicals not specifically recommended, and other influencing factors in the use of this product are beyond the control of the seller. Buyer assumes all risks of use, storage or handling of this material not in strict accordance with directions given herewith.

(3) Buyer assumes all risks of use, storage or handling of this material not in strict accordance with directions given herewith.

(4) Seller's guarantee shall be limited to the terms of the label, and subject thereto the buyer assumes any risk to persons or property arising out of use or handling and accepts the product on these conditions.

(5) Our recommendations for use of this product are based upon tests believed to be reliable. The use of this product being beyond the control of the manufacturer, no guarantee, expressed or implied, is made as to the effects of such or the results to be obtained if not used in accordance with directions or established safe practice. The buyer must assume all responsibility, including injury or damage, resulting from its misuse as such, or in combination with other materials.

Mr. WELLFORD. This order stated that the following disclaimer would be considered both false and misleading, and a product which had this kind of disclaimer on it would be rendered misbranded:

> The information furnished hereon is provided gratuitously by the manufacturer who assumes no responsibility whatsoever for the effectiveness or safety of this product regardless or whether or not it is used as directed.

Here I have two identical cans of Ortho Poison Ivy Killer, both purchased in the last 2 days. One of them has the proper disclaimer. It says "Notice, buyers assume all responsibility for safety and use not in accordance with directions."

Senator HART. Would you restate that?

Mr. WELLFORD. Yes, sir. The buyer assumes all responsibility for safety and use not in accordance with directions. That is a proper disclaimer. This other product was purchased at the same time. It is the same substance, but it has the illegal disclaimer on it:

> Because of critical, unforeseeable factors beyond the manufacturer's control prevent it from eliminating all risks in connection with the use of chemicals even though reasonably fit for such use, buyer and user acknowledge and assume all risks and liability resulting from handling, storage, and use of this material. These risks include, but are not limited to, damage to plants, crops and animals to which the material is applied, failure to control pests, damage caused by drift to other plants or crops and personal injury. Buyer and user accept and use this material on these conditions whether or not such use is in accordance with directions.

Now, either these products were being marketed in violation of the order of the Pesticide Regulation Division or they have been on the shelves since 1966. The manager of the store at which we bought the one with the illegal disclaimer stated that the spray had been purchased 1 month ago and that the distributor got it 4 months before that.

So, we would assume that these products are still being manufactured with that disclaimer.

Sixty percent of all the herbicides we examined in all the stores, contained either 2,4-D or 2,4,5-T or a combination of both.

By the way, if the product—Ortho Poison Ivy Killer—which you have just examined has been on the shelves since 1966, it raises questions of when the new quality control standards introduced by Dow Chemical Co. and by probably other chemical companies which have reduced the level of the dioxin contaminant took effect, it may be that many products that we are now able to buy off the shelves of local hardware stores were produced before the new quality control standards were introduced.

If that label is not illegal, it was produced at least 3 years ago, maybe more, and this raises one of the basic questions we want to stress this morning, to bring to your attention this morning, and that is we really do not know at all how much of the dioxin contaminant is in the herbicides which are so widely available at the local hardware stores. Some may be very old. We do not know whether all the chemical companies that produce 2,4,5-T are adhering to the same quality standards that Dow uses. This is a major area that needs investigation.

In short, the general population is being exposed to 2,4,5-T herbicides in use in residential areas. Over 60 percent of all herbicides we examined in local stores contained either 2,4-D, 2,4,5-T or a combination of both.

Estimating the precise hazard posed by weedkillers is difficult due to the scantiness of scientific information and the difficulty of extrapolating whatever information there is from animals to humans. For instance, the level of dioxins in these products is totally unknown. In many of the products 2,4,5-T is slightly modified chemically, although this modification would probably not effect the dioxin. Therefore, without further data there is no good way to extrapolate the effects of high doses on small numbers of animals to large populations exposed to low doses. Moreover, human sensitivity to 2,4,5-T probably varies greatly from person to person. Finally, it is worth recalling that thalidomide proved 50 to 200 times more teratogenic in humans than in the test rats and animals which had been used to demonstrate its safety.

The potential harmfulness of the 2,4,5-T products marketed for individual consumer use depends in part on the amount of dioxin contaminant they contain.

I have just made my point on that, the fact that we do not know how much they do contain.

Dow Chemical Co. has reassured the Department of Agriculture that it can now produce 2,4,5-T containing as little as 0.4 parts per million dioxin, but not all of its 2,4,5-T may be that pure. No one knows how much dioxin is in Dow's older products still on the market, which may have been made before it instituted its present quality controls. Moreover, no one knows how much dioxin is in 2,4,5-T products produced by other chemical companies which may not have the standards of quality control employed by Dow Chemical. Moreover, even Dow's current production of 2,4,5-T contains over 5 percent of other impurities, including some other kinds of dioxins. The effects of these impurities are totally unkown. Dr. Moselson, appointed last year by the American Association for the Advancement of Science to head a 2,4,5-T evaluation project, says:

> The tetrachlore dioxin represents just one of 12 or 13 ways the chlorine atoms can arrange themselves on a benzene ring to form dioxin molecules. How do we know about the hexa, hepta, and octyclors, or about how persistent the tetrachlor itself is? Moreover, I'm very concerned about the dioxins that might be formed by unreacted trichlorphenol (*2,4,5-T precursor*) when the product is exposed to heat. If it were taken up by plants or wood and these were burned, you'd get more dioxin. Finally, I'm bothered by the bizarre mental effects suffered by German workers making 2,4,5-T. I say when in doubt,stop it.[6]

A mystery with disturbing implications for public health is whether the dioxin contaminant in 2,4,5-T accumulates in human tissue. There is as yet very little scientific evidence on this point. If the dioxins do accumulate, they pose a danger, not only to the children of women who were pregnant when exposed, but also to women who become pregnant subsequent to exposure. If the dioxin or other teratogenic substances in 2,4,5-T are cumulative, it becomes academic whether a single or even several exposures to herbicides used on residential lawns and gardens are of sufficient magnitude to cause birth defects.

CLINICAL EVIDENCE OF 2,4,5-T POISONING

The clinical evidence of injury to persons coming into contact with herbicides containing 2,4,5-T is difficult to compile because techniques for monitoring pesticide poisonings are nearly as inadequate as those for measuring the incidence of birth defects. Poison control centers in many cities regularly treat victims of pesticide poisoning, but only rarely are their records of poisoning broken down as to particular pesticides.

Nevertheless, there are enough case histories on record to substantiate the laboratory findings that the herbicide 2,4,5-T, in addition to being a possible teratogen, can be severely toxic to human beings, causing nausea, diarrhea, chloracne (a serious skin disease), mental distress, kidney damage, and other maladies.

(1) Thomas Whiteside, in an article in the New York magazine, reported that chloracne has been found among workers in plants manufacturing 2,4,5-T. In the mid-1960's the Dow Chemical Co. closed down part of a 2,4,5-T plant in Midland, Mich., because workers there contracted chloracne apparently as a result of contact with the dioxin contaminant in 2,4,5-T. The symptoms of this disease included extensive skin erruption, central nervous system disorders, fatigue, lassitude, and depression. Similar symptoms were reported in German workers in plants producing 2,4,5-T as early as 1955.

(2) Professor Arthur Galston, at a hearing on herbicides conducted by Congressman Richard McCarthy in Globe, Ariz., discussed a scientific report entitled "Dermatitis and Kidney Damage Ascribed to Weedkiller 2,4,5-T." This report told of two girls, aged 4 and 6 years old, who had played for several hours in a yard which had been sprayed heavily a short time before with Ortho Brush Killer. The girls developed general reddening of the skin and swell-

[6] Medical World News, Feb. 27, 1970, p. 17.

ing of the oral and vaginal mucous membranes. The limbs and eyelids were also slightly swollen. On the third day after exposure, kidney damage was indicated by the discovery of albumen in the urine which persisted for about 2 weeks.

(3) Several suspected cases of 2,4,5-T and 2,4-D poisoning are being studied in Globe, Ariz.

(4) The Montgomery County Health Department has received at least one report from a Bethesda woman whose child suffered nausea, diarrhea, swelling of the lymph glands, and prolonged mental distress, as a result of exposure to a spray containing 2,4-D, 2,4,5-T and petroleum distillates, which had drifted from a neighbor's lawn being treated with the herbicide.

More clinical evidence might easily be compiled if an epidemiological study were made of particularly vulnerable groups. For example, it would be interesting to know if Dow and other companies producing 2,4,5-T have surveyed their female workers to see if they have a higher incidence of birth abnormalities. Similar studies should be made of farmworkers, especially those associated with rice and sugar production where 2,4,5-T is used. A special concern should be females who work in the timber industry, where 2,4,5-T spray is used to kill trees.

I worked for several summers spraying trees myself, and my colleagues among the sprayers were very frequently young women who carried spray cans on their backs and sprayed the spray into cuts on the trees made by men.

There is simply no way of, after using that spray for more than 30 minutes that you would not be literally drenched with it.

It would seem very likely that many of these women, being young, were in the early stages of pregnancy at some point in their work, and that they may have received very heavy doses of herbicide from vapors and wetting of the skin.

In this work, herbicides containing 2,4,5-T or 2,4-D plus 2,4,5-T in concentrations ranging from 8 to 16 pounds of acid equivalent per 100 gallons of fuel oil are commonly used.[1] These herbicides are sprayed into cuts made from girdling trees. It is common practice in Virginia, for example, for women to be hired to carry spray cans and apply herbicides on the trees after they have been cut by men. The sprayer is invariably heavily exposed to the herbicide, through inhilation of the vapors and wetting of the skin. Scores of women, many of them in the early stages of pregnancy, have probably been exposed to heavy concentrations of 2,4,5-T and 2,4-D in this manner. The Government should take immediate steps to warn women engaged in this work of the risks they are running, especially since many of them are working on projects sponsored or advised by the Forest Service of the U.S. Department of Agriculture.

In conclusion, Mr. Chairman, it seems an unnecessary risk to public health that a herbicide with the dangerous potential of 2,4,5-T should be widely available for casual use by the individual consumer. In a recent letter, Dr. Steven Lipson of the Montgomery County Health Department, stated this view very well.

(The letter follows:)

MARCH 17, 1970.

MR. HARRISON WELLFORD,
Center for Study of Responsive Law,
Washington, D.C.

DEAR MR. WELLFORD: In response to your request, I am writing you with regard to my personal and professional feelings about the use of 2,4-D, 2,4,5-T and related herbicides. I should like to preface my remarks with the note that these comments are made as an individual and not as an official of the Montgomery County Health Department.

Over the past years this Department has received a number of inquiries as to the possible toxicity of these chemicals. Our information relating to this is drawn from standard sources and it appears that there is no question but that these are dangerous substances when used improperly. As you know, exposure to these chemicals may cause central nervous system depression, weakness, loss of appetite, diarrhea, coma and sudden death. There is also a risk of skin irritation and damage to peripheral nerves.

Extensive discussion of this problem within the Health Department leads us to the conclusion that local control is impractical. One may pass any number of regulations regarding the use of herbicides in suburban and residential areas but enforcement is completely impractical. Public education appears to be the only feasible local alternative but as the continuing smoking problem demonstrates daily, this is a long and slow process. Our conclusion then was that the only reasonable means of control is at the point of sale. While again one could limit this locally, the movement of the consumer to different marketing areas is great, particularly in the Washington Metropolitan area, and limitation of sale of a substance by any single jurisdiction would have little effect.

Accordingly, as a parent, physician and public health worker, I would strongly support the limitation of use of herbicides to those situations in which it is economically and scientifically indicated. To my mind, this would not include suburban residential use. If the question is taken even more broadly, I would agree fully that no foreign substance, irrespective of whether toxicity has been demonstrated, should be introduced into the environment unless there is a clear and evident need for its use. This argument becomes even stronger when we talk about the exposure of children to potential hazards.

I strongly support and concur in your attempt to remove 2,4-D and 2,4,5-T from the individual consumer market.

Sincerely yours,

STEVEN LIPSON, *M.D., M.P.H.*
Chief, Division of Epidemiology and Surveillance,
Montgomery County Health Department.

Mr. WELLFORD. The economic value of using these herbicides to defoliate residential backyards of dandelions and chickweed is manifestly outweighed by their potential hazards. Until the manufacturers establish that exposure of the population to 2,4,5-T and 2,4-D is not dangerous, they should be banned from the individual consumer market. We would also support efforts to ban the use of these herbicides on food crops. Thank you very much for inviting us to make this statement.

My colleague, Mr. James Turner, now has a statement to make.

[1] Weed Control, National Academy of Sciences, 1968, p. 330.

STATEMENT OF JAMES TURNER, CENTER FOR STUDY OF RESPONSIVE LAW, WASHINGTON, D.C.

Mr. TURNER. I too would like to thank you for your kind invitation to be present here today to discuss the way in which pesticide safety is regulated by the Departments of Agriculture, and Health, Education, and Welfare.

My colleague, Mr. Wellford, has outlined to you the details of the very serious potential health problem presented by the widespread use—particularly around the homes of many American families—of the pesticides 2,4,5-T and 2,4-D.

There are possibly many other widely used pesticides which pose health hazards to the public, because under the current pesticide control system effectiveness is considered more important than safety. It is also important to note that pesticides are only part of the chemical problem.

An increasing number of scientists believe that many of the thousands of chemicals to which the average American is exposed—food additives, drugs, industrial wastes, cosmetics and others, as well as pesticides—present a massive threat to future health and well-being.

The potential health hazard presented by chemicals in the environment is difficult to exaggerate. Americans have been much too secure in the belief that their health is the best in the world. In fact, the health of Americans is not good. The life expectancy of an American female ranks 21st in the world; that of an American male, 37th. That is for people who have reached the age of 20. Infant mortality figures, called by many statisticians the best measure of a nation's overall medical ability place the United States approximately 15th in the world, a drop from fifth in 1950.

Since 1900 the average American reaching the age of 40 has had only 4 years added to his life expectancy. The National Foundation of the March of Dimes reports that one in seven births ends in death or deformity of the infant.

An increasing number of scientists believe that much of this deterioration of American Health can at least in part be traced to the strain placed on individuals by chemicals in the environment. Heart disease, the major killer, may be made more likely by chemicals such as caffeine. Cancer accounting for 20 percent of all deaths is being increasingly related to chemical causes.

But more important is the potential for genetic damage that many chemicals present. At present, the knowledge gained about potential mutagenic perils from research efforts by scientists in the field of genetics has far outreached the efforts of the Federal Government to establish procedures to insure that the general population is not exposed to mutagenic chemicals.

The regulatory history of 2,4,5-T and 2,4-D serves as an example of the governmental failure to protect the public from potentially dangerous chemicals. There are some general antidotes that might possibly begin to reverse the Government failure to deal with what is potentially a very serious health hazard.

It might be of interest to this committee to examine the current relationship and overlap between the Food and Drug Administration and the Department of Agriculture in the pesticide field. Currently the law requires all pesticides to be certified by the Pesticide Regulation Division of the Department of Agriculture.

The main criteria for registration is the effectiveness of the pesticide for its intended purpose. At one stage in the process of registering a new pesticide, the Agriculture Department is required to seek an advisory opinion on safety of the pesticide product from the Food and Drug Administration.

As the situation now stands the recommendations of the FDA that certain products are unsafe and should not be marketed as intended have been rejected nearly 100 percent of the time by the Agriculture Department.

In addition, thousands of FDA recommendations concerning the proper labeling of pesticide products have been rejected. As the law now stands this procedure is perfectly legal. Agriculture is merely required to seek advice. It is not required to act upon it. In the present time when the safety of pesticides has become a more important concern than their economic usefulness, it might be wise to readjust the relationship between these two agencies.

Perhaps pesticide registration should be officially the responsibility of the Food and Drug Administration, with the Department of Agriculture providing only an advisory opinion as to the potential usefulness and effectiveness of the chemical. Such a readjustment would direct Government attention toward the problem of safety and environmental contamination which have become the major concerns in the pesticide field and away from the much simpler task of determining the potential usefulness of pesticides in killing particular bugs and weeds.

It might also be of interest to this committee to explore the relationship between the producers of chemical pesticides and the agencies that must certify and offer opinions on them. Currently the overwhelming majority of scientific information relied upon to establish the effects of pesticide use is provided by industry sources. This, by the way, is true in all chemical fields—foot additives, cosmetics, drugs, as well as pesticides.

Often the manufacturers of these chemicals work out relationships with independent testing facilities which tend to seek the answer most advantageous to their clients. An illustration of the situation is what recently happened in the Food and Drug Administration itself, to a scientist who sought some testing results from an independent laboratory. He waited for several weeks for the results of what should have been a relatively easy experiment. Finally he called the researcher and was told that there was trouble with the experiment. The Food and Drug Administration official asked what the problem was, and was told that as hard as he tried, the scientist conducting the experiment could not find anything wrong with the chemical he was testing. He was under the impression that he had to find something wrong, or the Food and Drug Administration would be dissatisfied with the work.

This kind of bias exists in both directions throughout much of the community that is currently testing the effects of chemicals. To solve this problem in the drug field, Senator Nelson has proposed a large Government-run testing facility that would establish the safety or

data of all drugs submitted to the Food and Drug Administration for approval.

Such a program, if feasible in the drug area, could easily be extended to pesticides, as well as the food additives and cosmetics.

Another approach to the problem of how to separate the manufacturer from the researcher would be the establishment of a Government referral board. Such a board would receive requests from the manufacturer that wishes to have a certain pesticide or food additive certified. This board would refer these requests—by a prearranged lottery system—to one of a series of independent laboratories previously certified by the board for such testing. The rules for such referrals could include a prohibition against any laboratory testing two or more chemicals from the same manufacturer in succession, thereby breaking up the economic dependence that some laboratories have on one manufacturer.

The rules could also outline in some detail the methods that must be followed. And the laboratories could be inspected to insure that they do in fact have the facilities and the expertise to conduct the experiments necessary. Such a board could be useful in maintaining the economic independence of scientific research facilities as well as in upgrading the caliber of research done on environmental chemicals.

It might also be of interest to this committee to examine the interlocking relationships of the various groups that make up the scientific establishment in the pesticide field. Currently the FDA runs three separate programs in the pesticide field. It sets tolerances and then conducts investigations to insure that tolerances once set are not exceeded.

Second, it conducts a total diet survey which is the major program to determine whether or not too much pesticide is finding its way into food.

Third, it advises the Agriculture Department on pesticide safety.

I have already indicated that the FDA program of advice to Agriculture is not particularly effective. Unfortunately, the other programs pursued by FDA are not much more effective. The total diet program has been seriously criticized by one former FDA science advisor—"former," apparently as a direct result of the criticism leveled at this program—because of its failures to be chemically relevant.

Apparently claims were made for scientific test methods that were scientifically untrue, operators were sloppy in the use of scientific procedures and equipment, and officials did not wish to alarm the public. As a result, it appears that the reports of the total diet study which strongly suggest that pesticides in food present little danger seem to be over optimistic.

Presently the FDA is conducting a review of the program based on these criticisms. The tolerance setting program has been subject to the twin problems of limited scientific ability and information and a desire not to alarm.

What is most important about the FDA programs in these fields, however, is that they tend to seek support for their effectiveness from the Association of Official Agricultural Chemists and the

National Academy of Science's National Research Council committee on pesticides.

Both of these groups are presented as independent evaluators of FDA methods and programs. In fact, however, there is a significant overlap between the groups. FDA officials are also officials of the AOAC, and sit on the NAS-NRC committee. In fact, FDA itself is proud of their claim that it would be difficult to find any experts in pesticide tolerance and residue monitoring techniques outside of the agency. Interlocking relationships of this kind, however, are notorious for their ability to undermine scientific excellence. Without vigorous and challenging debate, little scientific advance takes place. The empirical evidence on pesticides suggests that a problem of major magnitude threatening the health and well-being of thousands of Americans may exist from exposure to pesticides.

The FDA, supported by the AOAC and NAS-NRC tend to discount this evidence. It would seem that information about these groups and how they relate to each other would be useful in evaluating the meaning of their pronouncements.

More critical examination of official reassurances about the effect of pesticides on the environment, an evaluation of the usefulness of either greater Federal testing for safety of pesticides or the establishment of a Government referral board and the shifting of the Government focus away from pesticide effectiveness and toward pesticide safety could begin to place the serious potential dangers from pesticides into the proper focus.

Thank you for your consideration of these thoughts and the opportunity to appear before you.

Senator HART. Gentlemen, thank you for expressing it so effectively.

Shortly after you began your testimony, Mr. Wellford, we were joined by the Senator from Hawaii, Mr. Inouye, and the Senator from Tennessee, Mr. Baker.

I have just a few questions.

First, you talk about two potential disasters in your testimony. The first is one that may have already occurred, relating to past birth deformities. You suggest that these perhaps are attributable to the use of 2,4,5-T or related chemicals.

The second I think is implicit in your suggestion that 2,4,5-T and 2,4-D and the dioxin contaminant of these and other chemicals may accumulate in the human tissue and so build up in each of us on sort of a regular basis. The end result of an accumulation of such highly toxic or teratogenic material is depressingly clear.

Now, there are many—if past experience is our guide—who will criticize you, and perhaps me, for talking about these possibilities. They will suggest that it is scare tactics, or some other label.

Anticipating that kind of reaction, what comment do you make? I'm sure you have been exposed to it before.

Mr. WELLFORD. If people think we sound alarmist, they should talk to the scientists we talk to, because they are very alarmed.

I think our main point is, there is just too much that we do not know about these herbicides, and, secondly, we're saying that the burden of proof should be placed on the manufacturers to establish their safety.

In the meantime, the public should not continue to be the unwilling guinea pigs of their casual, and I think frequently unnecessary use.

There is substantial evidence that these herbicides are potentially dangerous. There will be *many more* tests to find out exactly how dangerous they are, and maybe in the end there will be a determination that the amount of exposure that the average American receives is nothing to worry about.

But at this point, there is no evidence that we shouldn't be concerned, and in light of what we feel that the use of these herbicides should be restricted.

Senator HART. In your prepared statement—and I think as you summarized it you repeated it—you make reference to the Whiteside [1] articles in the New Yorker. If there is no objection, I would ask that they be made a part of the record at this time.

Mr. WELLFORD. I would also say, in further response to your question, that there has been a serious credibility gap on the part of the Government in the whole herbicide case. The Bionetics report was withheld for a long time, even from scientists. Dr. DuBridge made a statement in October. The public was reassured that something was being done. And yet the statement was ignored.

I think in this situation there is a particular necessity for people to speak out. And that is one of the reasons we have appeared this morning.

Senator HART. One specific reason I asked that the Whiteside articles go in is to acknowledge that they are responsible in major part for our hearings.

The first of those two articles mentions the important part played in the release of the Bionetics report by Nader's Raiders. Though you were not introduced as such, can you respond by telling us the story of the involvement of your organization in the release of the information?

Mr. TURNER. Yes, Senator. That activity regarding the Bionetics paper revolved around the group that was investigating the activities of the Food and Drug Administration, of which I was the project director. Before going into the specific story, I would like to add one comment to the statement in answer to your first question.

If anything, we have understated the scientific concern about the problems of chemicals in the environment, of which pesticides is one part. But what we have tried to talk about here is how to bring the legal system into some kind of meaningful response to what we admit to be a very unclear field of scientific endeavor.

We don't know, nor do many other people, what, exactly, is happening in the chemical environment. How, then, are we as a society, as the Congress and as people—how are we going to respond to the legal challenge that scientific uncertainty presents? And the kinds of things we have been talking about today are designed to suggest answers to these questions.

Concerning the Bionetics paper, to me it has represented since we first came across it, oh, sometime in August of last summer, one of the real tragedies and one of the sad parts of the kind of a job we

[1] See p. 107.

try to do on a day to day basis. And that is, in our [illegible]vities we constantly find scientists and regulatory officials in the Government who have very important concerns which they would like to bring to the attention of someone, to have some kind of resolution made about the issue that they are concerned with.

This was the case in the Bionetics paper. This, by the way, is not the only paper of that nature that we have run across in the course of the last year. We received the paper from two sources, basically, that were unhappy scientists who saw a very serious potential problem and said that no one would really listen to what was being said—internally, this is. We talked to the MRAK Commission and asked them if they were aware of the report, and they had been aware of the announcement and aware of the withdrawal, but had not seen the report. So we did then try to make it known to certain officials gradually, not knowing the full impact or not knowing the full meaning of it. It is not all that clear, although it does raise some serious problems. What was clear to us, though, were a number of scientists in Government who were concerned about a specific problem that they wanted an answer to, or wanted attention focused on, and could not find any way to bring such attention to focus.

Then we began to talk to people who we thought would raise the issue in the proper circles. That is essentially the way the paper began to emerge.

I would like to emphasize this is not the only one of those situations which we discovered.

Senator HART. You discovered other situations that bore on pesticides as potential dangers?

Mr. TURNER. There are some other problems on pesticides which we have alluded to which are disturbing. The question of trying to bring to the attention of regulatory officials the problems of Folpet and Captan has been one that I have been concerned with for over 18 months, and have tried to alert various people in the regulatory agency, the Food and Drug Administration, to. The scientists in that agency are concerned about these two particular pesticides, and they are asking why are they on the market? The first time that question was asked of me was in the summer of 1968, and we are not approximately—well, almost 2 years later, still selling these same pesticides.

Senator HART. In your testimony you have talked about the tests that several federal agencies have undertaken which clearly show in Mr. Wellford's prepared statement that even the purest 2,4,5-T still produces birth defects in test animals at significant levels.

You go on to say that a recent test on 2,4-D confirmed that this herbicide which was very often mixed with 2,4,5-T, is teratogenic.

I have been told, however, that the tests which have been conducted are merely preliminary and that although they suggest certain conclusions they cannot confirm them. Is that correct? In other words, as of now, we can't say that we know that the currently produced 2,4,5-T is teratogenic, can we?

Mr. WELLFORD. Certainly, as far as the effect on human beings, you are entirely right. There is no clear evidence—we haven't been able to find evidence through epidemiological surveys for reasons

that described, that these herbicides are definitely a danger to human beings that come in contact with them.

It is also true that there has not been time for the tests, which raise the suspicion to be checked and rechecked by many other scientists.

But I think one thing that is certain is the tests do raise very serious questions, and we can take no comfort whatsoever from them, that these herbicides are not dangerous as they are not being used.

Again, it is a question of burden of proof.

Senator HART. You would turn it around and say—well, yes, you can't say they are harmful, but you can't say they aren't?

Mr. WELLFORD. I would say the more recent tests have expended the burden of proof upon those who wish to prove that they are not harmful.

Senator HART. You suggest, or mention, that the advice on registration that Food and Drug has given the Department of Agriculture frequently, or sometimes, or however you phrase it, has been ignored.

Mr. TURNER. What was said was 100 percent of the time, Senator.

Senator HART. Almost.

Mr. TURNER. Almost.

Senator HART. Almost 100 percent of the time. Isn't it the fact, however, that Food and Drug could make its advice stick by setting residual tolerances which a pesticide, if registered, would exceed? Of course, that is a shorthand way, and perhaps arbitrary.

Mr. TURNER. It is somewhat arbitrary. The problem is, the kind of recommendations that are made are directed to products that have some usefulness, and to arbitrarily say there should be no residue could seriously affect the use of these pesticides which—in themselves—may not be particularly dangerous.

The kinds of things we have been talking about, on a number of occasions FDA recommended that certain marketed home use products that are used to kill rats should not be directed for use on bread crumbs or bread particles to be left where rats can get them, because then children can get them.

On a number of occasions this particular recommendation has been ignored by Agriculture, and they have gone ahead with that kind of a direction.

There are others. For example, the Food and Drug Administration has recommended on a number of occasions that seeds which will find their way back into the food supply, after they have been treated with pesticide, be dyed with a color, so that you can see them—blue or red. This is the general procedure in this kind of pesticide program.

But there are some products to which this is not done. When FDA on several occasions has said this should be done before there is any more marketing of that product, they have been rebuffed by Agriculture on those particular products.

Senator HART. In those situations where FDA has fixed a tolerance, do they have the facility to enforce adequately the tolerance that is established?

Mr. TURNER. It is my judgment that they do not. There are two ways that the residue problem is dealt with by FDA. One is by trying to enforce established tolerances, and the other is by the general survey called the total diet study, which I mention

In the former program, the FDA conducts approximately 7/100ths of 1 percent of an investigation process. That is how many interstate shipments they examine for pesticides—seven-one hundredths of a percent.

The way that they do many of these investigations is by being alerted beforehand that there is some kind of problem with the particular shipment. The situation, as the statistics emerge from FDA—by the way, that is one-half of their announced program for the years of 1963 to 1966; they did one-half of what they thought they were going to do—shows that they find a 3 percent violation of tolerance in the seven hundredths of a percent investigation that they conduct, and this would indicate—if the samples are statistically sound—a minimum of 70,000 shipments going in interstate commerce with pesticide residues that are not detected each year.

My judgment is that that is not enough to provide the kind of incentive to a person using pesticides, who must then ship his products, to be deterred from using too much.

I also would like to point out that the second program, the total diet program, which is the one that is done to survey the entire food supply, also has various weaknesses. The way the program works is that the total diet of a 19-year-old boy—he is considered to be the major eater in the country—is bought at a supermarket at various areas in the country. Then it is in various ways broken down and tested to see how much pesticide exists in the 2 weeks diet.

On the basis of this particular program, the Food and Drug Administration has assured the American people for several years that there is no real problem of pesticide residue in food.

When the science advisor began to look closely at this particular program, he came up with a series of very disturbing findings. There were no verified test methods for evaluating what, in fact, was being found by FDA when they conducted these tests. FDA had reported residue amounts that were lower than the error that was granted to the system. The system could find down to perhaps 10 parts per million without error, and they were finding residues as low as five parts, or below, per million. The science adviser in Baltimore made a detailed presentation to the agency and he shortly thereafter found that his contract for that year was not renewed.

It was at that point that I found another one of these tragic cases in Government, and began talking to the Food and Drug Administration about this particular problem. And at our insistence and our negotiation, a hearing within the agency was impaneled and a discussion was held, and a review of the program was decided upon.

As yet, I don't know what the results of their review are, but my feeling is we do not have good pesticide residue monitoring from that program either, at this point.

Senator HART. I have indicated, and you have agreed, that for FDA simply to fix a tolerance level not to be exceeded as a device to keep an item off the market, or a particular use, would be arbitrary. And you indicate that FDA's ability to ride herd on tolerances that are established is inadequate.

You wind up, I take it, by making the case that the key to the [illegible] that we are seeking to insure some protection involves the business of registration. You don't quite put it this way, but what you are telling us is that registration is presently under the control of the inappropriate agency.

Mr. TURNER. That is my feeling, Senator.

Senator HART. This is not necessarily to be critical of Agriculture, because, as you point out, they have always been pretty occupied, understandably, determining the potential usefulness of pesticides on bugs, weeds and what have you, and FDA more likely would be concerned with the problems of environmental contamination and safety.

My last question. You have told us how the lable content is regulated. What agency would have jurisdiction to require more adequate packaging to avoid breakage? Maybe I should say what agency, if any?

Mr. WELLFORD. It is clearly a public health problem, and to be perfectly frank, I do not know precisely who has direct jurisdiction over packaging. I would presume, though, that the first responsibility is the pesticide regulation division of the Department of Agriculture, but Mr. Bayley will be testifying and you can ask him that question.

Senator HART. That does not require a great deal of scientific interchange to figure that out.

Mr. WELLFORD. No, it does not.

Senator HART. If we have given anybody the authority to fix the package so it does not bust, they ought to be able to figure it out, and if we have not, we ought to fix the law.

Senator Inouye?

Senator INOUYE. Thank you very much, Mr. Chairman.

Gentlemen, are you convinced that the presently available data on the potential dangers of 2,4,5-T and 2,4-D are sufficient to outlaw the use and sale of these herbicides and pesticides?

Mr. WELLFORD. I think that the data is sufficient to at the very least suspend their use until the tests that need to be undertaken to answer the questions we have raised have been performed. I think it is an unnecessary risk for the public to have these substances so widely used when we know so little about them.

Senator INOUYE. In other words, you are suggesting that further and more intensive studies be made before a final decision be issued by the government?

Mr. WELLFORD. No, I think that one decision can be taken by the Government right away, and that is to suspend the use of these products until more tests are done. I think to delay that, to wait until tests and retests are performed might unnecessarily expose people to hazards which you will eventually regret. That is the point.

Senator INOUYE. I am convinced of the potential dangers of these pesticides and herbicides, but I cannot help recalling the recent cyclamate scare that we had here in which our shelves were rid of all these canned fruit cocktails with cyclamate. Now recently I recall reading an article in which scientists indicated that in order to suffer the dangers involved in the use of cyclamate, a child would

have to consume about 120 servings of fruit cocktails per day for 10 years. As a result, I believe cyclamate fruits can be sold on the shelf with a warning of some sort.

That is why I asked this question. Are you convinced that the data are sufficient to suspend present use?

Mr. TURNER. I would like to comment on the analogy to the cyclamate situation. There has been a great deal of misinformation circulated about cyclamates, and it should be brought back into perspective.

First of all, it is my belief that the present marketing of cyclamates as it is being conducted is a violation of the law, and we will proceed to undertake some kind of action to either bring that to the attention of Congress or to try to do something in the courts about it.

In fact, when the Food and Drug Administration gave its warning about cyclamates in April of 1969, before they knew that there was any involvement with cancer, they announced that people, in order to be safe, should consume only a certain amount of cyclamate. That amount for children was two-thirds of a package of presweetened Kool-Aid.

When the final cancer tests were done by Abbott Laboratories, the ones that Secretary Finch relied on to take the substance off the market, the National Academy of Sciences reported that applying the hundredfold safety measures that is used generally in food additive areas and applying the general methods that are used scientifically to deal with food additives would show certain levels of cyclamate to be safe.

The amount that they were talking about as safe was the amount that would go into three cups of coffee in a day. Subsequent to that time, the Food and Drug Administration conducted experiments which showed that the same kind of cancer problem that was found by Abbott Laboratories was also found at FDA at one-sixth of the level of intake over an 18-week period rather than a 2-year period, thereby greatly increasing the amount of problem that a person is perhaps subjected to when he takes cyclamate.

In addition to that, there is on file at the Food and Drug Administration or in the Health, Education, and Welfare Department a memorandum which specifically points out that there is a serious question that diabetics may be more susceptible to the dangers of cyclamate than other people.

The question that you raise is very apropos to both of these chemicals. We cannot really say at this point that there is anything that we can prove that will definitely say people are going to get these results. The problem we have is how do we structure the legal system to deal with the problems of uncertain science.

What we have done in the food additive area is to say that a food additive must be proven to be safe before it goes into the food except for what was to be a very small loophole in the law. Unfortunately, as that law has been administered, that list has nearly become as large as the additives proven safe.

In dealing with 2,4,5-T, we would like to provide, that is, those of us testifying today, would like to provide the same kind of standard in the pesticide area that should be prevalent in the food additive

area, and that is until the substance is proven to be not harmful it should not come on the market.

Once it is on the market. if there are questions raised and they cannot be disposed of, then it should be removed from the market until those questions are disposed of.

Senator INOUYE. Your testimony indicates that you have done a lot of study in this area, and I commend you for this. I am certain you are aware that the State of Hawaii uses a lot of this herbicide. In the year 1968 to cover an area of 120,000 acres we used 197,000 pounds of 2,4-D and 6,000 pounds of 2,4,5-T.

Obviously, it is an important part of our economy, because this constitutes the major portion of our income. In your studies, have you come up with any substitute for 2,4,5-T or 2,4-D?

Mr. WELLFORD. There are other herbicides on the market. I am not really qualified to explain the virtues of one against the other as far as the uses that 2,4-D and 2,4,5-T now have.

Again, I think that if there are not adequate substitutes that there probably could be with more research and investigation. I think that at the very least having these herbicides suspended for use in populated and residential areas could help bring that search about.

Mr. TURNER. I think there is another point to make in regard to your question, and that is in much of this area of chemical environment we are presented with some minds set or misconceptions that lead us down wrong tracks. One of the problems with using pesticides such as 2,4,5-T and 2,4-D and many others is that they are what we might call broad spectrum pesticides. They can be used on many different pests.

If we are going to find alternatives for them we cannot think in terms of finding a pesticide that can do as much as these will. We have to think of what sort of pest is sought to be controlled and find a method for it.

The Agriculture Department has found many methods of controlling problems, for example, alfalfa and cotton. These are nonchemical methods. Sterilization of pests is one of the approaches. It depends on what kind of particular thing you happen to be directing the pesticide at.

There can be alternatives. I think we have not found them largely because we thought in terms of these massively effective chemicals rather than thinking in terms of how do we control this pest with the least possible strain on the population around it.

Senator INOUYE. Last week when I did my shopping to prepare myself for the spring, I bought some of the items on your desk there. Do you suggest that I get rid of them?

Mr. WELLFORD. I suggest you be careful how you get rid of them. I think one of the real problems with these substances is the difficulty of knowing how to dispose of them if you decide you do not want to use them. You are not supposed to put them in a trash can; you are not supposed to bury them; you cannot put them down the sink.

I think if you call the various public health authorities to try to find out the best way to dispose of them, they eventually tell you, take them out to an incinerator on the outskirts of Washington, but they do not really have any other answer.

I think that problem indicates the toxicity of the substances that we are dealing with, and in direct answer to your question, I would not apply them to my lawn if there was any chance that pregnant women or small children could come into contact with them.

Senator INOUYE. Thank you very much.

Thank you, Mr. Chairman.

Senator HART. Senator Baker?

Senator BAKER. Thank you, Mr. Chairman.

I must say I, too, commend you for a rather extensive and well prepared presentation this morning on Mr. Wellford's part and Mr. Turner's part.

There are two or three things that occur to me about which I would like to inquire that relate to Senator Inouye's concern, and that is what do we do if we discard these pesticides and herbicides? I wonder, for instance, if there is a study or if you have any statistical evidence on what the economic loss would be, say, to the State of Hawaii in the case of a suspension of 2,4,5-T and 2,4-D? Before you answer, do not assume I am arguing against such suspension.

I am rather concerned about a complex life, and to be facetious for a moment, none of us really will live through it.

Is there some way to judge the relative good or the relative bad that flows from the use or nonuse of any of these pesticides?

Mr. WELLFORD. One quick point on that. There is a report commissioned by the Defense Department which is on the ecological effects of continued herbicide use, and in that report they state that in many cases the chemical weed clearance methods are often no more expensive and just as good as the use of herbicides.

It has been my experience that herbicides or their massive and popular use reflects the American penchant for technological gadgetry and marvels and perhaps we could find many more ordinary ways to get the same job done.

Senator BAKER. I agree with that. There are other ways. Take the extreme case of the mosquito and malaria. Clearly there must be some way to control the breeding grounds of the mosquitoes. It is done in some areas by raising and lowering the lake levels. It is done in other areas only by the use of pesticides.

Once again there is a trade-off. In those cases and in remote areas of Vietnam or the United States, it might be demonstrably better to run whatever risk there is of mutagenic danger in order to dispose of the more immediate and more imperiling danger of malaria.

Is there any sort of study? Is there any sort of weighing of these equities by your group?

Mr. TURNER. There is not anything done by our group and I do not know if there has been anything done by any other group. Our approach to this problem is this, and I think it bears directly on this question: I am concerned about the cyclamate case, for example, because of the tremendous economic impact that it had, but that economic impact came largely from the delay and the lack of effective action over the period of 4 or 5 years when cyclamate was developing.

What concerns us in the area of 2,4,5-T, is that it was announced its use would be curtailed. We in the public were under the impres-

sion it was being curtailed. We began to look at what was happening, and it was not being curtailed.

We have urged specifically that the use in the home, in and around the home be the first one to be specifically curtailed immediately.

My feeling is that since we have known for approximately 18 months that as a sprayed pesticide or herbicide it (2,4,5-T) has been a problem,—that since someone has known for probably the last 15 years that it did present a problem, action should have already been taken.

The workers at Dow were having serious occupational problems from the manufacture of the substance. Now, admittedly when a substance appears in the public eye for the first time it does create the kind of crisis situation where you perhaps must balance economics versus the potential harm from the substance. However, what I am concerned about is how we prevent that kind of crisis situation from developing on other substances and on this particular one.

In my area, which is more directed at the food chemicals, I would be more than willing to say that we should have an interim period to solve the kinds of problems you are suggesting if there was any kind of guarantee that that interim period would be lived up to by the producers of these chemicals.

The problem is in any area where the interim period has been allowed in the food area it has been used to erode the provisions of the law, so at the end of the interim period there is not any kind of authority or force to keep the product in control. That is what concerns me about trying to accommodate the economic situations which you are raising, although I consider them to be rather serious.

Senator BAKER. They are not only economic conditions. What I am referring to is health. I am asking if there has been a study by anyone in your group or any other group on the relative merit of the discontinuance of the use of pesticides on the one hand and the prevention of mosquitoes and malaria on the other.

Mr. TURNER. For example, you mentioned the mosquito problem. I notice that one major company has just developed a nontoxic method of controlling mosquitoes. This would be an example. I think with the proper kind of direction and the proper kind of focus, these alternatives can be developed.

The reason I was talking about economic importance, is the real impact of these particular pesticides we are talking about are economic. I do not believe they have use as a health control method.

Senator BAKER. Can you tell me whether FDA or anyone else is conducting any extensive research on substitute agents for pest controls?

Mr. TURNER. As far as I know they are not, they are not conducting extensive research, but Agriculture does have a research program on this subject, but I do not believe it is extensive.

Senator BAKER. Are you familiar with the plan by the Forest Service and, of all people, by the Atomic Energy Commission to develop sterilization techniques for certain pests that attack forests?

Mr. TURNER. Presently, as I understand the Agriculture Department, it is dealing with this area and it believes at present they are

able to control about ten percent of the pest problem with those kinds of methods.

Senator BAKER. To control completely ten percent of the pests rather than ten percent control?

Mr. TURNER. Right.

Senator BAKER. Would it seem to you that further research in these fields might very well produce acceptable substitutes, acceptable as we know them by these standards? I freely predict that 10 years from now they will not be the standards?

Mr. TURNER. That is right. Agriculture is working on research methods whereby they can use much smaller amounts of pesticides to achieve the same results that are achieved by the very large use of chemical pesticides. In some cases more than a million or so pounds of 2,4-D is used on certain submerged weed areas to control the weeds per acre. This is an incredible amount of pesticide use.

Now they are down in some cases to using chemicals to one one hundredth of an ounce on an acre. This kind of a balance is going to go a long way toward controlling some of the environmental hazards that we have.

There are experiments being conducted at the University of Georgia which indicate that perhaps as much as 99 percent of all sprayed pesticides come out of nozzles in droplets which are so large that they are useless for this purpose and they are absorbed by the environment.

Senator BAKER. Are you familiar with the use of underground watering for microporous piping?

Mr. TURNER. No, I am not.

Senator BAKER. That, of course, would stop air contamination by pesticides and herbicides and it would increase the concentrations and the effectiveness and eliminate the resin problem.

Mr. TURNER. Right.

Senator BAKER. I really commend that to further study.

Senator HART. Thank you, Mr. Baker. I think the committee is fortunate to have Senator Baker on it. He either has done homework or somehow or other is involved in the field.

Mr. Bickwit?

Mr. BICKWIT. I would just like to clear up one point on my own mind. On several occasions in your testimony you have complained that Dr. DuBridge's statement made in October of 1969 was not complied with. However, in other parts of your testimony you have suggested that mere compliance with that statement would not be sufficient in your view. Am I right in understanding that you feel that Dr. DuBridge's statement does not go far enough?

Mr. WELLFORD. Absolutely. I think its biggest gap is the fact it does not discuss pesticides which are used in residential lawns and gardens. I think there he was under the misapprehension that 2,4,5-T was not widely used for this purpose. In fact it is. I suspect if he had known it, he would have included that in his statement.

Senator HART. Gentlemen, thank you very much, for a very helpful presentation. You have raised some questions that clearly will have to be resolved.

Next, we welcome Ned Bayley, the Director of Science and Education of the Department of Agriculture.

STATEMENT OF DR. NED D. BAYLEY, DIRECTOR OF SCIENCE AND EDUCATION, DEPARTMENT OF AGRICULTURE; ACCOMPANIED BY DR. T. C. BYERLY, ASSISTANT DIRECTOR OF SCIENCE AND EDUCATION

Dr. BAYLEY. Mr. Chairman, I am Ned Bayley, Director of Science and Education, Office of the Secretary, Department of Agriculture.

I have with me Dr. T. C. Byerly, Assistant Director of Science and Education.

Before I proceed with the formal statement, I would like to respond to some extent to the information which has been presented already this morning.

Senator HART. Let me make it easier. Let me encourage you to do it, and any succeeding witnesses, too.

To make the record as useful as possible, we would welcome exchanges in the nature of reply.

Dr. BAYLEY. I appreciate in doing this I am putting myself in an impromptu position and, therefore, would appreciate the privilege to provide to the committee, for the record if they desire, fuller statements regarding the activities of the Department of Agriculture in regard to pesticides.

Senator HART. Very well.

Dr. BAYLEY. I testified before this committee previously regarding the broad policies and positions of the Department in the pesticide area, and stated that we recognize that all pesticides are economic poisons. They are only one group of the tremendous number of economic poisons which we use for a large number of useful reasons, not only for economic purposes but also to take care of public health.

We recognize also, as part of our civilization and as part of the standard of living and the food supply that we already have, that without these economic poisons and their judicious use, we would be in a very serious situation from the standpoint of our ability to produce food and fiber for this country.

Now, I also want to point out very briefly the references to the activities of the Department of Agriculture regarding the registration of pesticides. I will be the first to agree that there have been some problems in regard to these registration procedures and I will be the first to agree that we haven't resolved all of them.

We have, however, particularly during the past year, taken a number of steps towards eliminating some of the complexities and bureaucratic difficulties which have existed in the area of registration.

The references which have been made here this morning primarily reflect the relationship among the departments that did exist, but, I think I am safe in saying, do not exist now.

Senator HART. I remember in those earlier hearings we discussed this problem.

Dr. BAYLEY. Yes, we did.

Senator HART. And I had the impression that there was a transition period.

Dr. BAYLEY. I will very briefly refer to that. The crux of the problem was that there was an interagency agreement for resolving differences in regard to registration. There was a procedure within the agreement of bringing differences to the attention of the three Secretaries involved.

Unfortunately, however, over the years this agreement was in existence, not one of the departments ever used this procedure to resolve their differences.

We in the Department of Agriculture must share the major responsibility for not getting the differences resolved primarily because the enforcement of FIFRA was primarily our responsibility. But I am glad to say that since that time, with the initiative of Secretary Hardin and Secretary Finch and Secretary Hickel, there has been a new agreement worked out between the departments. This agreement specifically provides the basis whereby differences in judgments regarding pesticide regulation can be brought up through the decisionmaking procedure and the three Secretaries can share in this as needed.

We believe this is a sound basis for increasing the interdepartmental relationships and providing a basis for all three departments to have a rightful input into this.

I think you are also acquainted with the fact——

Senator HART. Doctor, if you are going to leave that new agreement, I would like to ask one question. In the event of disagreement, when the three Secretaries' attention is invited to the competing claims, is the decision made by majority vote, or does the Secretary of Agriculture retain the final voice?

Dr. BAYLEY. It is my understanding that they will pursue the disagreement until they agree. The Secretary of Agriculture does retain the final voice according to the law, however. We believe that based on the way we are operating today this procedure can be effective.

Senator HART. A meeting in Paris?

Dr. BAYLEY. Well, we are not dealing with that. I think we recognize that three Cabinet officers have the public interest in mind when they get together and can make a decision along these lines.

Senator HART. Just to push you a little harder on it, and I suppose this is academic at the moment since no such dispute has yet reached the three Secretaries?

Dr. BAYLEY. This is correct.

Senator HART. If one does get there, it will involve the tricky balance that Senator Baker was talking about, the economic claims, the public health claims, and the environmental concerns. HEW will tend, I assume, to emphasize the health factor. Is Interior the third department?

Dr. BAYLEY. Yes.

Senator HART. They would I suppose, be concerned principally with effects on fish and wildlife, and Agriculture would think primarily of the utility to the agricultural economy.

To put it harshly, why shouldn't the fellow who says it has not yet been established as safe for humans have the ultimate vote and voice?

Dr. BAYLEY. May I say this, that from the standpoint of the Department of Agriculture, we recognize that issues involving human health should have priority over all other issues.

The reference which was made earlier that the emphasis in USDA had been primarily on effectiveness is not only incorrect regarding the past but it is utterly incorrect regarding our position now.

Senator HART. Whatever the past, I would hope that human health does have the overriding concern of three or any other numbers of men that meet on this kind of claim.

I interrupted you.

Dr. BAYLEY. Surely.

With those preliminary comments, I will be glad to turn to the issue of 2,4,5-T and the facts as we see them at the present time.

The herbicide 2,4,5-T has been recognized as the most effective herbicide registered for use for control of certain weeds and brush species for more than 20 years. About four-fifths of the domestic use of 2,4,5-T is for nonfarm use, the largest such use being for control of brush on rights-of-way. It is also used extensively to control brush on forest lands and certain weeds in turf. 2,4,5-T has been used in the production of fruit crops, cereal grains, and sugarcane. It is the most effective herbicide for control of brush on several million acres of rangeland in the Southwestern United States.

2,4,5-T is degraded in the environment within a few months after application so that residues do not persist from one season to the next. Residues on foods are unusual. Among 5,300 food samples analyzed by FDA for 2,4,5-T during the past 4 years, 25 were reported to contain trace amounts; i.e., amounts less than the 0.1 p.p.m. limit of accuracy of present analytical procedures for foods. Two samples showed residues of 0.19 and 0.29 p.p.m., respectively.

No finite tolerance has been established for 2,4,5-T in food. In the absence of such tolerances, any detectable amount of 2,4,5-T in food would make such food subject to seizure if found in the channels of interstate commerce. From the data cited above——

Senator PERCY. Do I understand your statement to mean, Doctor, that in the absence of the establishment of human tolerances for 2,4,5-T, it is the present policy of the Department of Agriculture to seize any shipments that show any measurable trace of 2,4,5-T on food shelves?

Dr. BAYLEY. It is the responsibility of the Food and Drug Administration to enforce the procedures and make the seizures.

Senator PERCY. I understood you to say there are no tolerances established; therefore in the absence of any established human tolerances for 2,4,5-T, it is the present operation of the U.S. Department of Agriculture to seize any food stuffs that contain any measurable trace amounts of 2,4,5-T.

Dr. BAYLEY. It is the present obligation of the Food and Drug Administration to do so.

Senator PERCY. Thank you very much.

Dr. BAYLEY. From the data cited above, it is apparent that contamination of food with 2,4,5-T is very infrequent and then only at very low levels.

There is current concern over the continued use of 2,4,5-T arising from the report of a research study completed under contract by the National Cancer Institute by Bionetics, Inc. This study was based on a commercial lot of 2,4,5-T acquired for the study in 1965. It was fed to pregnant mice and rats. Many of their developing young had birth defects.

After review of this information and after consultation with Federal agencies concerned, Dr. Lee A. DuBridge, the President's Science Adviser, announced on October 29, 1969, a coordinated series of actions being taken by those agencies with respect to the use of 2,4,5-T.

Among them was the announcement that: "The Department of Agriculture will cancel registrations of 2,4,5-T for use on food crops effective January 1, 1970, unless by that time the Food and Drug Administration has found a basis for establishing a safe legal tolerance in and on foods."

USDA was informed in January that the lot of 2,4,5-T used in the Bionetics study contained significant amounts of a highly toxic contaminant, tetrachlorodibenzo paradioxin. The Department was further informed that lots of 2,4,5-T of current and recent manufacture were reported to contain less than 1 p.p.m. of this contaminant in contrast to the 27 p.p.m. reported for the lot used in the Bionetic study.

Extensive studies are underway to determine whether 2,4,5-T is itself teratogenic. Preliminary reports are consistent with the hypothesis that the teratogenic results reported in the Bionetics study were due to the contaminant dioxins or to interactions of such contaminants with the 2,4,5-T rather than to 2,4,5-T per se.

The Department announced on February 6 that it would undertake examination of 2,4,5-T and 17 related compounds registered for pesticidal use to determine whether or not they are contaminated with dioxins. Preliminary results on 2,4,5-T show that those lots examined of current manufacture and those now in channels of trade gave the following results—I can summarize these quickly—the amounts ranged from a trace to 2.9 parts per million, and they were conducted both by the Department of Agriculture and the Food and Drug Administration.

(The table follows:)

TABLE I.—AMOUNTS OF TCDD FOUND IN COMMERCIAL 2, 4, 5-T BY TWO METHODS

Sample	Manufacturer	Lot	Grade[1]	Collected	TCDD content p.p.m.[2] USDA	FDA
2, 4, 5-T	Dow	120110	TG	2/70	trace	0.07
2, 4, 5-T	Monsanto	07-020	TG	2/70	1.1	2.9
2, 4, 5-T	Hercules	X-17394-21-5	TG	2/70	N.D.[3]	N.D
2, 4, 5-T[4]	Dow	MM-120449	TG	2/70	.48	.47-.52

[1] TG=Technical grade.
[2] TCDD Refers to the 2, 3, 7, 8-tetrachloro-dibenzo-p-dioxin (TCDD).
[3] N.D.=Levels of TCDD are below the limits of detection or below 0.05 p.p.m.
[4] Sample supplied by Dow as a reference check and reported to contain about 0.5 p.p.m. TDCD.

These data are preliminary and are obtained from first drafts of methods developed by chemists in the Crops Research Division of the USDA and in the Pesticide Chemistry and Toxicology Division of the FDA. The dioxin values refer only to the 2,3,7,8-tetrachloro-

dibenzo-p-dioxins (TCDD) and do not indicate levels of other halogenated dioxins (containing 5, 6, 7, or 8 chlorines) in the 2,4,5-T samples.

In view of all the information now available, we have not found that registered use of 2,4,5-T without a finite tolerance on food crops constitutes a hazard requiring cancellation or suspension of such registered uses.

There has been and is concern over the ecological effects of 2,4,5-T used as a defoliant in Vietnam. Dr. Fred Tschirley, Assistant Chief of our Crops Protection Research Branch, has reported the results of his examination of areas treated in Vietnam. He has reported no evidence of irreversible ecological damage. Allegations that defoliation will lead to extensive laterization of Vietnamese soils, that Mangrove areas will not recover, that fish production in wetland areas will be reduced were not verified.

Dr. Tschirley also headed a team of scientists who investigated allegations of injury to humans and animals due to herbicide treatment for control of Chapparal by the Forest Service on the Tonto National Forest near Globe, Ariz. They found that apparent damage consisted of damage to susceptible plants near the treated area from drift of the herbicides used. The alleged injuries to a duck and a goat were found to be groundless. Human illnesses were those expected in a normal population with the possible exception of one man with skin irritation on his eyelids. Clinical chemistry on specimens obtained during the investigation is in process.

Mr. Chairman, that is the completion of my formal statement. We primarily presented it to provide you with the latest findings that we have in the particular area.

Senator HART. Thank you very much, Doctor.

To summarize with respect to the sequence of events on the DuBridge announcement of October 29, 1969, the Department did not in fact deregister 2,4,5-T as DuBridge indicated would occur unless these affirmed findings came along. But you tell us your action was based upon information that the tests by Bionetics used samples that contained the contaminant dioxin, and that the current production of that product was free of the dioxin; is that right?

Dr. BAYLEY. Not completely free. The dioxin was at a sufficiently low level that we believed that——

Senator HART. That the test material had sufficiently more dioxin than the normal production amount?

Dr. BAYLEY. Yes.

Senator HART. Are you aware that the preliminary results of tests conducted by Food and Drug, Dow, and by the National Instititute of Dental Research and by the National Institute of Environmental Health Scientists all indicate that 2,4,5-T contaminated with no more dioxin than is found in the currently produced 2,4,5-T is teratogenic?

Dr. Bayley. We are fully aware of this. The critical facts in regard to those experiments is that those low level dioxin contaminated 2,4,5-T samples were fed at sufficiently high dosages that they would be comparable to the dosages used for the 27 parts per million or nearly so.

Therefore, we do not believe this in any way changes the hypothesis that the low level of dioxin is safe.

Senator HART. You say in your statement "Should the teratogenic nature of 2,4,5-T be confirmed, registration for use on food crops will be canceled."

I am attempting to establish what will confirm it. What events do you look to to determine whether these preliminary indications which you say resulted from the contamination in fact did? Is there something in particular that you look to?

Dr. BAYLEY. Yes, the important considerations here are the usages for which the 2,4,5-T are permitted or which in actual practice are carried out.

The difference between the possibility of teratogenicity of the contaminate and the teratogenicity of the material that is used in the field is based on the rates of application, the losses which occur. All these effect the possibility of contamination of human beings.

Incidentally, my advice is that the one part per million level is at least tenfold below what they would consider a safe level in terms of allowances. In other words, that is the safety margin in this estimate.

Senator HART. In other words, there would have to be a finding of 10 times more?

Dr. BAYLEY. That is what I am told, yes. This is a statement based on scientific information provided to me.

Senator HART. Do you have any opinion as to whether Food and Drug might set a safe tolerance level in food?

Dr. Bayley. The action we have taken is to extend the time in which information can be provided or application made with Food and Drug Administration regarding the establishment of tolerances on food. I would not in any way want to prejudge what their actions should be, because it should be based on the data provided.

If I may elaborate on that, a petition was filed with the Food and Drug Administration in December of 1967 requesting the establishment of tolerances of 0.2 parts per million for residues of 2,4,5-T on apples, barley, blueberries, corn, oats, rye, sugarcane, and wheat. Those were the only crops to which that petition would apply.

The petitioner withdrew his petition on December 29, 1969, as provided under the pesticide regulations. We have extended to December 1970 the opportunity for him to provide the data needed to reach a decision on this.

There is one thing I think is important here, and that is the earlier reference to the concept of first, the burden of proof, and secondly, that we should not believe their data. We have to watch out for this paradox.

We in the Department of Agriculture, as you know, with the cooperation of the Food and Drug Administration, are not simply accepting the proof from industry in these cases. We are going out to obtain samples and testing them ourselves in order to verify the kind of information that is coming in.

Senator HART. I am not sure it is a paradox to say that the burden of proof is on the fellow that wants to expose the public to a product and some saying you cannot trust his data. They are two separate problems.

Dr. BAYLEY. I recognize that.

Senator HART. Let us be assured that the data is reliable and objective, and let us insist that the burden of proof may be on those who may or may not be proposing the introduction of a chemical.

Dr. BAYLEY. I suspect I am sensitive to this because some people challenge why we test products. We think it should be done in the public interest when it is needed.

Senator HART. On the matter of the dioxin that was found to exist in the samples in a much higher percentage than the normal production thereof——

Dr. BAYLEY. You mean the samples that the Bionectics group had?

Senator HART. Yes, that the Bionetics group used. That was the reason, was it not, that led you not to follow through on the DuBridge pronouncement of October? Is that the meat and potatoes of it?

Dr. BAYLEY. This is an often misunderstood situation. I think it is important to realize that the date we chose in regard to the possibility of taking such action was chosen because the Food and Drug Administration had agreed to complete their action on the petition by that time. When they had not completed their action, I wrote Dr. Roger Egeberg a letter on January 7, asking for a statement regarding the status of their considerations on the petition.

On January 21, I received a response indicating that they had further data which changed the position in regard to the need for immediate cancellation.

We will be glad to provide these letters for the record.

Senator HART. I think it may be helpful.

(The letters follow:)

JANUARY 7, 1970.

DR. ROGER O. EGEBERG, *Assistant Secretary for Health and Scientific Affairs, Office of the Secretary, Department of Health, Education, and Welfare, Washington, D.C.*

DEAR DR. EGEBERG: On December 13, 1967, a petition was filed with the Food and Drug Administration to establish tolerances for 2,4,5,T on specific food crops. In accordance with the interdepartmental agreement reached in Dr. DuBridge's office on October 29, 1969, we announced that we would issue notice of cancellation of the registered uses of 2,4,5,T on these crops unless the Food and Drug Administration found a basis for establishing tolerances by January 1, 1970. This date was chosen because the Food and Drug Administration agreed to complete action on the petition by that time.

We would appreciate receiving without delay a statement from the Food and Drug Administration regarding the status of their considerations on the petition in order that we may take appropriate action. This request is made in accordance with our mutual interest to take responsible action on this matter and also in full cognizance of the exchange of letters between Secretary Hardin and Secretary Finch regarding public health responsibilities in pesticide registration.

Sincerely,

NED D. BAYLEY, *Director, Science and Education.*

SURGEON GENERAL OF THE PUBLIC HEALTH SERVICE,
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE,
Washington, D.C., January 21, 1970.

DR. NED N. BAYLEY, *Director of Science and Education, Office of the Secretary, U.S. Department of Agriculture, Washington, D.C.*

DEAR DR. BAYLEY: In reply to your letter of January 7, 1970, inquiring as to the status of Pesticides Petition 8F0669 (2,4,5-T), submitted by the National Agricultural Chemicals Association, the following is the current status.

We have been advised by Dow Chemical Company:

(1) That a sample of the 2,4,5,-T herbicide used in the Bionetics study contained 27±8 ppm of a highly active (biologically) contaminant, tetrachlordibenzo-para-dioxin. (This has been confirmed by the National Institute for Environmental Health Sciences.)

(2) That this material produced serious inflammation in rabbit ear tests and that the presence of the contaminant had been confirmed by chromatography.

(3) That standard production 2,4,5-T contains less than 1 ppm of this contaminant and does not produce inflammation in the rabbit ear test.

(4) That Sprague-Dawley female rats as dams have been under test in their laboratory at Zionsville, Indiana, at five levels (25 rats each) of the standard production line material. They came to term January 7, 8 and 9, 1970, at which time they were killed by carbon dioxide inhalation and fetuses were removed by cesarean section and subjected to standard examinations for malformations and anatomical anomalies of various kinds; further examination will include clearing, staining and histopathologic procedures.

Dr. Howard L. Richardson, Chief, Pathology Branch, FDA, participated in the evaluation which related to full-term rat embryos subjected to 2,4,5-T during gestation, as well as a number of full-term rabbit embryos. He reports that no signs of malformations were found in gross and microscopic dissection of these embryos, but that histologic examinations are yet to be made. Personnel from the National Institute of Environmental Health Sciences were unable to participate, but will be involved in the examination of this and other informational material.

Further characterization of the contaminant tetrachlordibenzo-para-dioxin is currently under way and Dr. Leo Friedman will welcome participation by your research staff in this effort. We would point out the resemblance if not the "practical identity" of the tetrachlordibenzo-para-dioxin with the "chick edema factor." This substance is of extremely high toxicity to all species of animals that have been exposed, and until now, its source in contaminated fatty materials has been a mystery.

Considering the imminence of the availability of this additional information and the legitimate question as to whether or not the teratology reported by the Bionetics study was due to the 2,4,5-T or to the contaminant, we have elected to delay action on the petition for a few more days. As you know, the petitioner had requested, on December 5, 1969, an extension of 3 additional months.

We will advise you of our decision as soon as our scientific staff assays the results of this nearly completed test and considers them together with the results of other current research on 2,4,5-T at the National Institute for Dental Research and in the Food and Drug Administration. Thus far, no one has confirmed the Bionetics results although 2,4,5-T (with 27±8 ppm contaminant) has been found to be embryotoxic.

Sincerely yours,

JESSE L. STEINFELD, M.D.,
Surgeon General.

Senator HART. At the beginning of your testimony you say residues on food are unusual. When they do occur, are they the result of unauthorized use of 2,4,5-T or authorized use, or both?

Dr. BAYLEY. Mr. Chairman, I am going to ask Dr. Byerly to respond to that.

Dr. BYERLY. Sir, one of the two significant values, 0.19, was on milk, and the other was on sugar beets. As far as sugar beet use, I would have to verify whether or not there is a registered use on sugar beets. There is on sugarcane. There is certainly none on milk. This would be unauthorized use in the case of milk, certainly.

Senator HART. Is it authorized for use on grass?

Dr. BAYLEY. Yes, sir.

Senator HART. It is?

Dr. BAYLEY. It is authorized for use on grass.

Senator HART. And clearly not on milk?

Dr. BYERLY. No.

Senator HART. What do you say to the suggestion that if you know that there is a regular unauthorized use of a pesticide going on you ought not permit it to be registered? How can you register a pesticide even for safe usage when regularly it is used in an unsafe, unauthorized manner?

Dr. BAYLEY. The law provides, and I will not pretend to quote it exactly, that if despite the registration restrictions, including use, the Department finds there is injury to people and to the environment or desirable environmental organisms, that we can consider this as misbranded and cancel the registration.

So the pattern of the enforcement of that part of the law is for a surveillance program to determine the extent to which their unauthorized uses are providing injury and then we take action.

This is very clearly shown in the action we took recently involving a mercurial seed treatment program compound. It was this type of action, where unauthorized use was creating an injury and we immediately suspended it.

Senator HART. You say the use is authorized on grass, not milk. Could not cows eat grass, thus producing residues in our milk.

Dr. BAYLEY. This is based on the recommendations for use and also based on the degradation properties of 2,4,5-T itself. Good practice would require withholding the grazing of cows from these pastures until such time as we can be sure there will be no residue in the animal product.

The widespread use of this as herbicide on ranges and pastures indicates that farmers are following these practices with the possible one exception that we know of at this point.

Senator HART. What information can you add to this record substantiating the statement that 2,4,5-T degrades in a matter of—how did you put it?

Dr. BAYLEY. We will be glad to supply for the record the scientific information indicating the degradation time of 2,4,5-T as well as the circumstances under which this will vary.

Senator HART. That will be printed in the record.

(The information follows:)

There is a voluminous body of published literature on the degradation and persistence of herbicides. Enclosed are five reprints available to us that deal specifically with 2,4,5-T. For a more comprehensive discussion of 2,4,5-T and other pesticides, we recommend the following publications:

(1) Kearney, P. C. and D. D. Kaufman (Editors). 1969. Degradation of Herbicides. Marcel Dekker, Inc., New York.

(2) Miller, M. W. and G. G. Berg (Editors). 1969. Chemical Fallout—Current Research in Persistence of Herbicides. Charles C. Thomas Publishers.

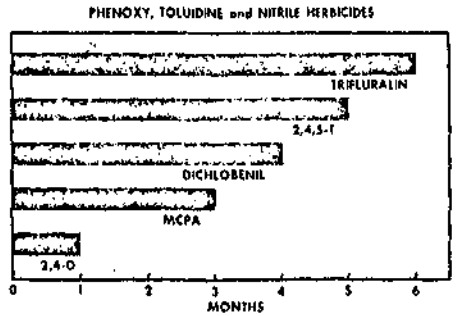
(3) Nature and Fate of Chemicals Applied to Soils. 1960. U.S. Department of Agriculture, ARS 20-9.

The literature supplied herewith substantiates our statement that 2,4,5-T is degraded in the soil in a few months. There will, of course, be some exception to any general statement. Greater persistence would be expected in a cool, dry environment having a low microfloral activity.

[From: Chemical Fallout—Current Research on Persistent Pesticides Ed. by Morton W. Miller & G. G. Berg. Charles C. Thomas, Publisher. 1969]

Soil Persistence of 2,4,5-T

The persistence of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), when applied at recommended rates (0.25 to 8 lb/A) is 4 to 5 months as compared to about 1 month for 2,4-dichlorophenoxyacetic acid (2,4-D) as shown in the figure below.



DeRose (3) found that a 3 lb/A field application of 2,4,5-T was no longer phytotoxic to soybeans 3 months later. However, rates of 10 and 20 lb/A remained highly phytotoxic after 3 months. Newman, et al (4) found that 2,4,5-T lost its phytotoxicity after 4 months when applied at rates up to 26 lb/A. Burger, et al (1) found a similar loss of phytotoxicity to alfalfa 4 months after the application of 25 ppm (50 lb/A). However, these were both laboratory studies in which the soils were maintained moist and warm, two conditions that facilitate the microbial inactivation of phenoxyacetic acid herbicides. The 6 month persistence of 2,4,5-T in soil, DeRose and Newman (3), appears to be one of the largest persistences reported.

Microorganisms are primarily responsible for degrading phenoxyacetic acid herbicides in soils. The kinetics of 2,4-D, 2-methyl-4-chlorophenoxyacetic acid (MCPA), and 2,4,5-T detoxification in soil-perfusion experiments were exactly what would be expected if microorganisms were the detoxicating agents (4); in addition, detoxication was blocked by the bacterial inhibitor sodium azide. The bacteria and actinomycetes responsible for degrading phenoxyacetic acids are shown in Tables 1-2 (4).

The metabolism of the phenoxyacetic acid herbicides has been studied extensively (4). There appear to be two major pathways of degradation, i.e., via a hydroxyphenoxyacetic acid intermediate and degradation via the corresponding phenol. Some of the important steps in microbial metabolism of representative phenoxyacetic acids are shown in Figures 1-6 (4).

TABLE I-2

Bacteria and Actinomycetes which Degrade Phenoxyacetic Acids

Organism	References	Phenoxyacetic acids metabolized: Phenoxyacetic acid	2-Chlorophenoxyacetic acid	4-Chlorophenoxyacetic acid	2,4-Dichlorophenoxyacetic acid	2,6-Dichlorophenoxyacetic acid	2,4-Dibromophenoxyacetic acid	4-Bromo-2-chlorophenoxyacetic acid	2-Methyl-4-chlorophenoxyacetic acid	4-Hydroxyphenoxyacetic acid	2-Hydroxy-4-chlorophenoxyacetic acid	6-Hydroxy-2,4-dichlorophenoxyacetic acid	2,4,5-Trichlorophenoxyacetic acid
Bacteria													
Pseudomonas sp.	84,85				+*								
Pseudomonas sp.	84,86			+*							+		
Mycoplana sp.	87	+	+	+	+*				+		+		+
Achromobacter sp.	88			+*							+		
Achromobacter sp.	88,89		+	+	+		+	+	+*				
Achromobacter sp.	90,91	+	+	+	+*	+			+			+	+
Flavobacterium peregrinum	88,89		+	+	+*		+						
F. peregrinum	92,93				+*								
F. peregrinum	99								+*				
Corynebacterium sp.	94				+*								
Corynebacterium-like organism	95,96				+*				+				
Arthrobacter globiformis (*Bacterium globiforme*)	78,79				+*								
Arthrobacter sp.	97,98	+	+	+	+*				+	+			
Sporocytophaga congregata (*Flavobacterium aquatile*)	95,96,100				+*								
Actinomycetes													
Nocardia sp.	82			+	+								
Streptomyces viridichromogenes	101				+								+

* Substrate in enrichment and isolation media.

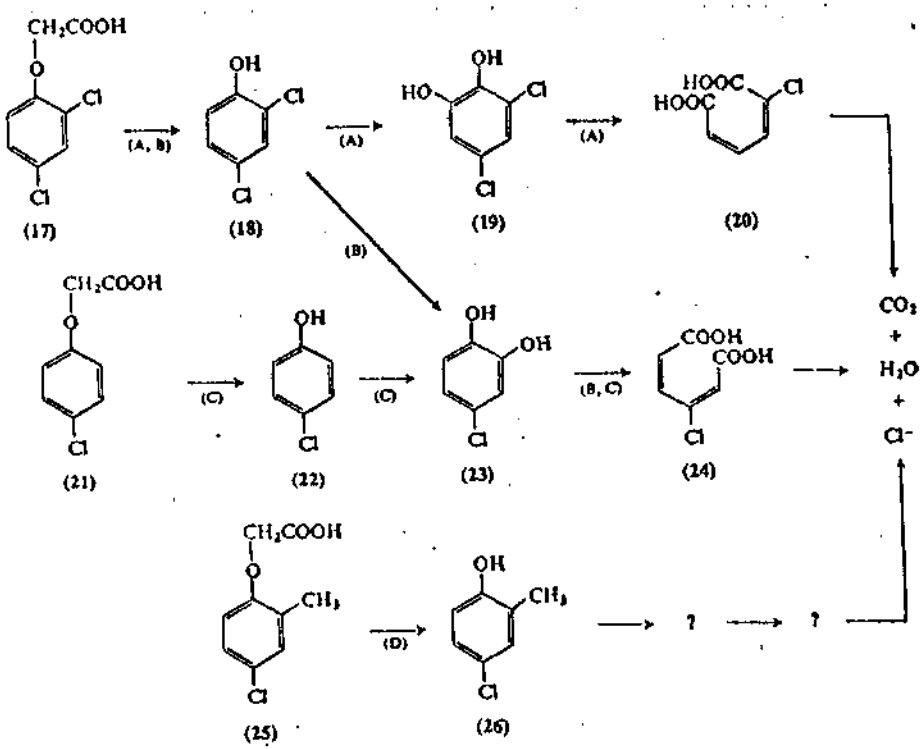


Fig. 1-6. Microbial degradation of phenoxyacetic acids via the corresponding phenols. (A) 2,4-D (17) degradation via 2,4-dichlorophenol (18), 3,5-dichlorocatechol (19), and α-chloromuconic acid (20); (B) 2,4-D degradation via 2,4-dichlorophenol, 4-chlorocatechol (23), and β-chloromuconic acid (24); (C) 4-CPA (21) degradation via 4-chlorophenol (22), 4-chlorocatechol, and β-chloromuconic acid; and (D) MCPA (25) degradation via 2-methyl-4-chlorophenol (26). [After (83,89).]

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Growth of Crops in Soils After Herbicidal Treatments for Brush Control in the Tropics[1]

R. W. Bovey, F. R. Miller, and J. Diaz-Colon[2]

ABSTRACT

Herbicides 4-amino-3,5,6-trichloropicolinic acid (picloram), a 1:1 mixture of the butyl esters of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid (2,4-D:2,4,5-T), and a 2:2:1 mixture of the isooctyl esters of 2,4-D:2,4,5-T:picloram at 6.7, 26.9, and 16.8 kg/ha (6, 24, and 15 lb/A) respectively, were applied as foliar sprays to control guava (*Psidium guajava* L.). Six crop species were planted in soil collected from each plot, 1, 2, 3, 6½, 9½, and 15½ months after treatment, to detect herbicide residues and to determine crop tolerance. Corn, sorghum, wheat, rice and cotton could be grown without reduction in fresh weight as early as 3 months after application. Soybeans were the most susceptible crop to herbicide residues.

Additional index words: herbicide residues, picloram, 2,4-D, 2,4,5-T, guava.

A COMBINATION of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) is an established herbicide for woody plant control (2,4,5). Herbicide 4-amino-3,5,6-trichloropicolinic acid (picloram) and combinations of picloram with 2,4,5-T show promise for control of woody species which exhibit resistance to phenoxy herbicides (1). Since high herbicide dosages are required for adequate brush control in the tropics (10, 11) damage to agronomic and forage crops seeded following treatment could result from herbicide residues.

Disappearance of 2,4-D and 2,4,5-T from warm moist soils in the temperate zone is rapid. Accumulation of harmful residues is unlikely from year to year if recommended rates for weed control are followed (4,5). Factors responsible for decomposition of phenoxy herbicides include microbial activity, leaching and volatility (9). It is assumed that phenoxy compounds would have an accelerated rate of disappearance in tropical soils compared to temperate climates because of higher temperatures and greater rainfall. Available information suggests that picloram remains in soils much longer than 2,4-D (3) (R. W. Bovey, 1968. Unpublished data). Decomposition by microorganisms is very slow (12). Bioassay studies indicated that the half-life of picloram in soils throughout the United States varied with location, but some persisted 1 year after treatment (3). Similar studies in Texas (6) and Puerto Rico indicated that less than 0.05 ppm was present 1 year after treatment of picloram at 8 lb/A (8.96 kg/ha) and 9 lb/A (10 kg/ha), respectively (C. C. Dowler, 1968. Unpublished data). However, the Texas determinations were made from sandy loam soils and samples were taken to a depth of 24 inches (61 cm). Additional work has indicated that the main routes of picloram disappearance and decomposition from soil profiles are by leaching and photo-decomposition (7).

Jungle areas in Hawaii were aerially treated with 2-methoxy-3,6-dichlorobenzoic acid (dicamba), 2-(2,4,5-trichlorophenoxy) propionic acid (silvex), combination of paraquat plus dicamba and 2,4-D plus picloram (8). When treated plots were planted to Monkey pod trees (*Samanea saman* (Jacq.) Merrill) 1 month after application, the trees developed no injury from herbicide residues in the soil.

This paper reports the growth of six crop species in a tropical soil treated with picloram and combinations of 2,4-D:2,4,5-T and 2,4,D:2,4,5-T:picloram collected from a guava control area, to determine the longevity of herbicide residues and the tolerance of individual crop species to each herbicide.

MATERIALS AND METHODS

Herbicidal treatments were made on mature stands of guava (*Psidium guajava* L.) near Mayaguez, Puerto Rico. Characteristics of guava, the physical environment, and responses to herbicides have been described (11). Herbicides were applied to guava with a pole sprayer designed to cover a 12.18-m (40-ft) diameter circle (Dowler, C. C., and F. H. Tschirley, 1966. Defoliation Project Mayaguez, Puerto Rico. Ann. Rep., USDA). Herbicides applied included the potassium salt of picloram at 6.72 kg/ha (6 lb/A), a 1:1 mixture of the butyl esters of 2,4-D:2,4,5-T at 26.88 kg/ha (24 lb/A and a 2:2:1 mixture of the isooctyl esters of 2,4-D:2,4,5-T:picloram (M-5140) at 16.80 kg/ha (15 lb/A).

Soil samples were taken from herbicide treated plots 1, 2, 3, 6½, 9½ and 15½ months after treatment by collecting the top 1 ft (32.48 cm) of soil at three or four locations in each plot. (Soil samples 1 and 2 months after treatment were not taken for M-5140.) To prevent contamination, a clean shovel was used for each plot. Soil was placed in plastic bags, sealed, and immediately transported to the greenhouse. Each soil was pulverized by hand and placed in 38 × 30.5 × 13-cm (15 × 12 × 5-inch) boxes lined with plastic to prevent leaching of the herbicide. Each worker was required to wash his hands thoroughly between each soil treatment. Untreated soil samples were prepared first, followed by longest field applied treatment (15½ months) to the shortest (1 month). Four replications were prepared for each treatment. Soil from the treated area was a Mucara silty clay-loam (11).

Twenty-five seeds of corn (*Zea mays* L.) var. 'USDA-34,' sorghum (*Sorghum bicolor* L.) var. 'Combine Kafir-60,' wheat (*Triticum aestivum* L.) vac. 'Alentana,' rice (*Oryza sativa* L.) var. 'Taichung Native No. 1,' cotton (*Gossypium hirsutum* L.) var. 'Blightmaster,' and soybeans (*Glycine max* (L.) Merrill) var. 'Clark' were planted in each replicatim, covered with a 0.6 to 1.2-cm (¼ to ½-inch) layer of soil, and watered. The crops were grown for 21 days. Aerial portions of all plants in each replication were weighed on an electronic balance and recorded as fresh weight. Numerical values presented in the tables that follow are percentage of the control.

RESULTS

Rainfall is important in the decomposition and disappearance of herbicides from soil profiles. Rainfall data are presented in Table 1 for each of the treatment periods.

Picloram. Growth of six crops in soil treated with picloram at 6.72 kg/ha (6 lb/A) is given in Table

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Table 1. Amount of rainfall from each date of herbicide application to date of sampling soil (Dec. 6, 1967).

Months after application	Date herbicide applied	No. of days	Rainfall inches	Rainfall cm
1	Nov. [illegible], 1967	[illegible]	4.70	11.9
2	Oct. 3, 1967	63	16.65	[illegible]
3	Aug. 30, 1967	[illegible]	22.84	58.4
6½	May 1[illegible], 1967	20[illegible]	52.77	134.0
9½	Feb. [illegible], 1967	30[illegible]	[illegible]	[illegible]
13½	Oct. 2[illegible], 1966	40[illegible]	[illegible]	[illegible]

Table 2. Percentage growth of six crops in soils from foliar applied herbicides on guava 1, 2, 3, 6½, 9½ and 13½ months after treatment (growth of control plants = 100*).

Months after treatment	Corn	Sorghum	Wheat	Rice	Soy beans	Cotton	Avg (months)
			Picloram applied, 6.72 kg/ha				
1	[illegible]	8 c	18 b	4 c	1 c	1 c	8 c
2	44 b	45 b	56 bc	46 b	1 c	56 b	35 b
3	60 b	92 a	86 ab	99 a	42 b	101 a	80 a
6½	78 ab	97 a	140 a	88 a	90 a	110 a	97 a
9½	91 a	97 a	90 abc	96 a	102 a	127 a	96 a
13½	74 ab	88 a	72 a	100 a	81 ab	90 b	84 a
Avg (Crops)	66 a	86 a	74 a	71 a	54 b	88 a	
			2,4-D; 2,4,5-T applied, [illegible] kg/ha				
1	83 b	84 a	78 a	68 b	16 b	60 b	68 a
2	90 b	81 bc	98 bc	91 b	46 a	113 a	90 bc
3	143 a	127 a	148 a	200 a	44 a	138 a	139 a
6½	100 b	112 ab	128 ab	88 b	81 a	132 a	112 ab
9½	94 b	83 bc	99 bc	80 b	72 a	99 ab	88 abc
13½	78 b	73 bc	71 a	70 b	64 a	83 ab	78 bc
Avg (Crops)	99 a	88 a	103 a	94 a	44 a	108 a	
			M-3140 applied, 16.8 kg/ha				
3	133 a	103 a	138 a	113 a	30 c	40 a	104 a
6½	85 b	94 ab	107 ab	88 a	80 ab	140 a	91 a
9½	138 ab	118 ab	237 a	137 a	60 bc	109 a	131 a
13½	88 b	99 b	96 b	121 a	103 a	112 a	103 a
Avg (Crops)	115 a	101 a	110 a	110 a	65 b	101 a	

* Numbers followed by the same letter do not differ significantly at the 5% level using Duncan's multiple range test.

2. All crops showed a significant reduction in growth when seeded 1 and 2 months after application of the herbicide. Rice, soybeans, and cotton were severely affected and showed essentially no growth when seeded in soils 1 month after treatment. Soybeans and corn continued to be adversely affected when seeded in soils 3 months after treatment; while sorghum, wheat, rice and cotton were not significantly affected. Since the disappearance of picloram is much dependent upon leaching the 58.4 cm (23 inches) of rainfall received in the 3 months after treatment (Table 1) undoubtedly removed lethal amounts of picloram. These data suggest that all crop species studied could be safely seeded in soils 6 months after picloram treatment without adversely affecting growth.

2,4-D:2,4,5-T. Crop growth was retarded in soil treated with the 2,4-D:2,4,5-T combination 1 and in some cases 2 months before seeding (Table 2). At 1 and 2 months this combination of herbicides was less inhibitory than picloram although the rate of treatment was four times as great. This suggests that the phenoxy compounds either dissipated more rapidly than picloram or that the crops were more tolerant to the herbicide combination or both. Wheat and cotton produced growth comparable to the control when seeded in soils 2 months after treatment. Soybeans were the most readily inhibited of all crops grown. Reasons for reduced growth in soils treated for 13½ months are unknown. That this may be a chance variation is indicated by over 100% production in samples of soil taken in plots having a shorter time after these same treatments (excepting soybeans). Average (means) for all crops did not differ significantly at the 5% level of Duncan's Multiple Range Test.

M-3140. Blended rates of treatment for M-3140 were 6.72, 6.72, and 3.36 kg/ha (6, 6, and 3 lb/A) of 2,4-D, 2,4,5-T, and picloram, respectively. No significant differences (5%) in growth occurred among averages for months but did for crop species when M-3140 was applied. Soybeans showed injury in soils treated for 9½ months or less before seeding (Table 2). Cotton seeded 3 months after treatment was slightly injured. Apparently, sufficient picloram persisted for the described intervals to cause damage to soybeans and cotton; but other crops were unaffected.

DISCUSSION

These studies indicate that high rates of herbicides used to control vegetation in tropical and subtropical areas disappear rapidly, even though persistent compounds such as picloram are used. Hence, most crops could be safely grown within 6 months after application with few if any adverse effects.

Soybeans were the crop most sensitive to herbicidal residues in soil. Use of an alternative crop should be considered, if seedings are made during the first few months after treatment. Graminae species such as corn, sorghum or wheat may be used first in treated areas, because these are more tolerant to the residues of herbicides used for controlling brush.

Rainfall (Table 1) is important in leaching herbicides from soil profiles. In areas of more abundant precipitation one could expect more rapid disappearance than was encountered in this study, providing that other soil and environmental factors are equal. We have concluded that harmful residues of herbicides in the soil would not occur in tropical areas after chemical brush removal if a reasonable deferment of land was observed after application.

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Effect and Persistence of Herbicides Applied to Soil in Puerto Rican Forests[1]

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Abstract. Six herbicides were applied at 3, 9, and 27 lb/A to the soil in forests of three types in Puerto Rico. The forests differed in soil type, amount and pattern of rainfall, and species composition. Defoliation occurred slowly, but the correlation between percentage defoliation and plants killed was significant at the 1% level of probability. Some defoliation was evident 1 month after treatment and increased slowly during the next 6 to 8 months. The most effective herbicide was 4-amino-3,5,6-trichloropicolinic acid (picloram). Generally, 2-methoxy-3,6-dichlorobenzoic acid (dicamba), 2-methoxy-4,6-bis(isopropylamino)-s-triazine (prometone), and 5-bromo-3-sec-butyl-6-methyluracil (bromacil) were less effective than picloram. Both 2,3,6-trichlorophenylacetic acid (fenac) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) were ineffective. Defoliation was greatest and more plants were killed in the driest site.

One year after treatment, fenac was the most persistent herbicide in the soil. Herbicides were found to a 36 to 48-in depth within 3 months after application.

Herbaceous and woody seedlings were present at all sites within 14 months after herbicide application. There was no definite relationship between herbicidal treatment and secondary succession, except that the number and frequency of successional species were greater on plots having the highest percentage of defoliation.

INTRODUCTION

The effect of herbicides applied to the soil for control of woody plants in tropical forests has not been studied intensively or extensively. A short review by Mayo-Melendez (7) indicated that frill treatment to trees was the most effective method of application. Beveridge (2), Sposta (11), and Wyatt-Smith (17, 18) used frill treatments successfully on a large number of tropical species but noted differential susceptibility among species. Dawkins (4) reported that a basal spraying or painting was as effective as frill treatment.

Many hardwoods can be controlled effectively with phenoxyacetic or picolinic acids applied in basal frills or as tree injections (6, 15). Many compounds are effective as foliar treatments for controlling woody plants (3, 16). Nation (9) reported that 4-amino-3,5,6-trichloropicolinic acid (picloram), when applied as a soil treatment, was an effective herbicide for controlling woody plants. He also noted differential susceptibility among species.

Inseparable from the biological effect of herbicides applied to the soil are their movement and persistence. Factors such as rainfall, physical and chemical characteristics of the soil, microorganisms, chemical characteristics of the herbicides, and method of application may influence herbicidal movement and persistence (5, 8, 10, 12).

Our objectives were (a) to determine the toxicity of selected herbicides on tropical arboreal vegetation, (b) to determine the movement and persistence of these chemicals in the soil, and (c) to observe the short-term effects of these chemicals on secondary plant succession.

MATERIALS AND METHODS

Our studies were located in Guanica Commonwealth Forest, Maricao Commonwealth Forest, and Luquillo National Forest. The soil type at the Guanica Commonwealth Forest is Jacana clay. It is an alluvial soil normally less than 36 in deep, with very low permeability. The vegetation is xerophytic. There were 35 woody species within the test area, but *Leucaena leucocephala* (Lam.) DeWit and *Haematoxylon campechianum* L. comprised more than 90% of the woody plant population. Mean height of the vegetation was 15 ft. Annual rainfall in the Guanica area, estimated at approximately 30 in, occurs largely from July to October. The recorded annual rainfall at the site for 1964 and 1965 was 27.88 and 25.00 in, respectively.

The soil type at the Maricao Commonwealth Forest site is Nipe clay, a permeable, well-drained, lateritic soil derived from serpentine. The vegetation on this site is classified by Beard (1) as moist tropical forest. There were 106 woody species within the test area. The mean canopy level was approximately 50 ft. Mean annual rainfall at the Maricao site is estimated to be about 90 in. Rainfall is normally distributed throughout the year, but December to May is the driest period. The recorded annual rainfall at the site for 1964 and 1965 was 84.64 and 109.88 in, respectively.

The soil type at the Luquillo National Forest site is Los Guineos clay loam, a plastic clay with poor internal drainage. The vegetation is a tropical rain forest (1) with a mean canopy level of about 60 ft. There were 88 tree species on the test site. Mean annual rainfall is estimated to be over 100 in. The highest rainfall normally occurs from July to October, but droughts are unknown. The recorded annual rainfall near the site was 85.78 in for

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1964 and 126.12 in for 1965. Tschirley *et al.* (14) characterized the Maricao and Luquillo sites in greater detail.

The herbicides used in this study were 5-bromo-3-*sec*-butyl-6-methyluracil (bromacil), dimethylamine salt of 2-methoxy-3,6-dichlorobenzoic acid (dicamba), 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron), Na salt of 2,3,6-trichlorophenylacetic acid (fenac), K salt of picloram, and 2-methoxy-4,6-bis(isopropylamino)-*s*-triazine (prometone). Each herbicide was applied at 3, 9, and 27 lb/A.

A randomized complete block design was used at each location. The treatments were replicated three times at Guanica and Luquillo, but only twice at Maricao because of topographic limitations. Each plot was 60 by 80 ft. with a 20-ft buffer separating adjacent plots. Data were collected from a centrally located 40 by 60-ft subplot. Narrow walkways were cut through the long axis of each plot to facilitate treatment and data collection.

All woody plants having a diameter breast high (hereinafter referred to as dbh) measurement of 0.75 in or greater were marked, mapped, and identified (at least to genus) at the Guanica site. At Maricao and Luquillo, only plants having a dbh greater than 3 in were marked, mapped, and identified. All defoliation data were collected only from the marked plants.

The herbicides were applied with a cyclone hand spreader as granules, pellets, wettable powder, or liquid adsorbed on vermiculite. Vermiculite was used as a carrier to provide additional bulk for all treatments. The application dates were: Guanica—October, 1963; Maricao—December, 1963; and Luquillo—January, 1964. Percentage of defoliation of each marked plant was estimated periodically after treatment. Plot defoliation, calculated from individual plant defoliation, represents a mean percentage weighted according to the relative prevalence of various species.

The downward movement of herbicides and their persistence in the soil were studied by sampling 3, 6, and 12 months after treatment. Duplicate soil samples were collected at random from centrally located 20 by 40-ft subplots. Soil from depths of 0 to 6, 6 to 12, 12 to 24, 24 to 36, and 36 to 48 in was analyzed separately. If the soil was not 48 in deep, samples were collected to the maximum possible depth. The samples were placed into 1-qt styrene cups, then sealed and transferred to the greenhouse where a bioassay was immediately initiated. Cucumber (*Cucumis sativus* L., var. Puerto Rico 39) was used as an indicator plant. Approximately six cucumber seeds were planted in each sample. After emergence, the cucumbers were thinned to two seedlings per sample. A mean injury rating of each sample was made after 28 days of growth and compared to an established standard curve. Abnormal growth characteristics were expressed on a 10-point scale, where 0 = no effect and 10 = plants killed.

Succession data were collected from one replication at each site. Observations on the species present and their relative importance were made at Maricao, Guanica, and Luquillo in April, May, and September, 1965, respectively. The successional species on each plot were identified to family, genus, or species and grouped according to tree seedlings, grasses and sedges, or herbaceous plants and vines.

Results

Herbicidal effect on woody plants. The correlation coefficient of percentage defoliation and plants killed was significant at the 1% level of probability; consequently, results are based on defoliation only.

Guanica. Picloram was clearly the most effective herbicide (Table 1). Bromacil and prometone caused high

Table 1. Percentage of defoliation and plants killed of all arboreal species in the Guanica, Maricao, and Luquillo forests approximately 2 years after herbicidal treatments.

Herbicide and rate, lb/A		Guanica[a] Def	Guanica[a] PK	Maricao[a] Def	Maricao[a] PK	Luquillo[a] Def	Luquillo[a] PK
Picloram	3	30	37	32	21	24	23
	9	92	91	73	57	55	52
	27	99	99	93	89	79	69
Dicamba	3	9	3	12	6	9	6
	9	13	4	18	10	19	14
	27	22	9	50	41	52	52
Bromacil	3	28	16	11	3	18	15
	9	92	78	29	21	24	22
	27	100	100	38	29	55	94
Prometone	3	9	8	11	3	0	0
	9	62	56	13	5	10	9
	27	99	97	29	21	18	12
Diuron	3	5	2	11	0	0	0
	9	15	11	5	1	3	2
	27	16	10	9	3	8	9
Fenac	3	5	2	15	5	8	0
	9	28	20	11	2	4	2
	27	29	14	19	4	22	9
Check		12	6	5	2	1	1

[a]Def = percentage defoliation; PK = percentage plants killed.

defoliation at the higher rates but were less effective when only 3 lb/A was used. Dicamba, diuron, and fenac caused only slight defoliation even at the highest rate.

Differential susceptibility of the five principal species at Guanica is shown in Table 2. Picloram, considering all

Table 2. Percentage of defoliation of the five principal species in Guanica test site 2 years after herbicidal treatments to the soil.

Herbicide and rate, lb/A		1	2	3	4	5
Picloram	3	61	81	56	40	0
	9	82	100	100	0	20
	27	100	100	100	80	100
Dicamba	3	11	0	7	-	0
	9	14	2	22	15	33
	27	11	33	45	5	0
Bromacil	3	24	48	15	6	0
	9	90	96	100	61	51
	27	100	100	100	49	72
Prometone	3	27	1	25	7	2
	9	79	25	30	91	100
	27	100	98	100	100	100
Diuron	3	8	0	40	0	0
	9	18	5	43	14	0
	27	17	6	90	60	-
Fenac	3	6	2	7	28	0
	9	30	22	47	65	13
	27	27	33	18	20	43
Check		15	6	5	22	3

[a]1. *Leucaena leucocephala* (Lam.) DeWit. 2. *Haematoxylon campechianum* L. 3. *Stenopalla bertiana* (DC.) Britton & Rose. 4. *Prosopis juliflora* (Sw.) DC. 5. *Trichilia hirta* L.

rates, had the broadest spectrum of herbicidal activity. Prometone was more effective than was any other herbicide for defoliating *Prosopis juliflora* (Sw.) DC. and *Trichilia hirta* L.

At Guanica, the maximum percentage of defoliation resulting from treatments with picloram was obtained about 3 months after treatment (Figure 1). Defoliation

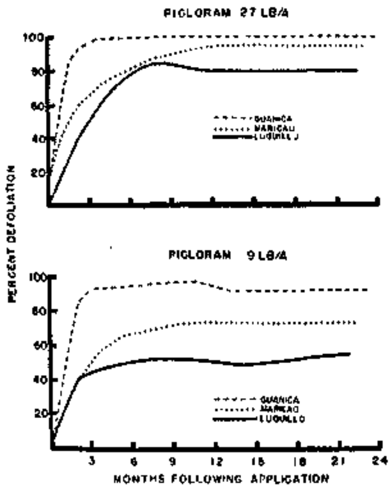

Figure 1. Percentage of defoliation at specified intervals after picloram was applied to the soil in three forested areas in Puerto Rico.

remained essentially constant for the rest of the 24-month period.

Total vegetation control was short-lived, even on plots that had been treated with 27 lb/A. No treatment prevented plant succession for more than 18 months. Two years after treatment, *Ipomoea* spp., *Indigofera suffruticosa* Mill., and cacti (*Opuntia* spp.) were abundant. Grass and tree seedlings were less numerous. *Leucaena leucocephala* (Lam.) DeWit was the most abundant tree seedling. Seedling density was directly related to percentage of defoliation.

Maricao. Picloram caused the highest percentage plot defoliation (Table 1). Dicamba at 27 lb/A caused 50% defoliation, but this was considerably lower than that caused by picloram at 9 lb/A. Bromacil, prometone, diuron, and fenac were considerably less effective.

Picloram affected the broadest spectrum of tree species (Table 3). *Miconia sintenisii* Cogn. was more susceptible to picloram than were the other eight species. *Rapanea ferruginea* (R. & P.) Mez appeared to be most susceptible to bromacil. *Ocotea leucoxylon* (Sw.) Mez tolerated all the herbicides except picloram at 9 and 27 lb/A.

Maximum defoliation resulting from treatment with picloram was obtained at Maricao about 1 year after treatment (Figure 1). There was essentially no change during the second year of observation.

No herbicidal treatment prevented the succession of tree seedlings, grasses and sedges, or vines and herbaceous plants at Maricao. Tree seedlings were sparse on plots treated with picloram at 9 and 27 lb/A, but composition

Table 3. Percentage of defoliation of the nine principal species in Maricao test site after herbicidal treatments to the soil. Treated December, 1963 and rated October, 1965.

Herbicide and rate, lb/A		1	2	3	4	5	6	7	8	9
Picloram	3	0	45	0	0	32	24	90	33	—
	9	33	70	38	63	94	87	100	100	57
	27	96	100	90	83	65	87	100	100	80
Dicamba	3	12	10	0	3	50	0	43	0	—
	9	2	25	41	0	19	3	50	8	—
	27	7	33	100	8	30	0	100	77	—
Bromacil	3	10	0	85	17	20	6	25	0	—
	9	13	10	45	47	73	0	100	4	90
	27	67	8	100	24	74	19	73	60	27
Prometone	3	18	1	37	0	—	0	0	—	8
	9	25	0	19	0	17	0	27	0	53
	27	2	75	63	0	57	0	100	0	7
Diuron	3	3	8	14	35	—	—	9	0	—
	9	3	8	10	6	15	0	30	0	—
	27	3	—	9	24	0	0	35	0	15
Fenac	3	3	15	15	43	20	27	0	0	31
	9	—	0	33	32	0	0	0	0	0
	27	22	—	23	0	0	0	1	—	—
Check		4	5	16	8	0	6	37	0	8

[a]1. *Calophyllum calaba* Jacq. 2. *Casearia-Dypetes*. 3. *Rapanea ferruginea* (R. & P.) Mez. 4. *Alchornea aquilina* Chrim. 5. *Terebraria resinosa* (Vahl) Sprague. 6. *Ocotea leucoxylon* (Sw.) Mez. 7. *Miconia sintenisii* Cogn. 8. *Coccoloba* sp. 9. *Linociera domingensis* (Lam.) Knobl.

and density of the seedling complex could not be definitely associated with herbicidal treatment. The most common succession species in the Maricao site were *Terebraria resinosa* (Vahl) Sprague, *Clusia* spp., *Ichnanthus pallens* (Sw.) Munro, *Panicum glutinosum* Sw., and *Polypodium* spp.

Luquillo. Picloram caused the highest percentage of defoliation at the Luquillo site (Table 1). The highest rates of bromacil and dicamba caused appreciable defoliation, but both herbicides were much less effective when only 3 or 9 lb/A were used. Prometone, diuron, and fenac caused only slight defoliation at all rates. Picloram was as effective at 9 lb/A as dicamba and bromacil at 27 lb/A.

Differential susceptibility was apparent among the eight most numerous species (Table 4). *Tabebuia heterophylla* (DC.) Britton was the most resistant species in that no treatment caused more than 31% defoliation. *Psychotria berteriana* DC. on the other hand, was completely de-

Table 4. Percentage of defoliation of the eight principal species in Luquillo test site 21 months after herbicidal treatments to the soil.

Herbicide and rate, lb/A		1	2	3	4	5	6	7	8
Picloram	3	3	11	17	57	29	11	33	0
	9	14	46	18	100	100	33	100	17
	27	30	100	72	100	100	100	100	100
Dicamba	3	4	0	0	8	2	0	0	8
	9	10	71	4	40	16	6	100	8
	27	31	96	19	56	0	61	100	100
Bromacil	3	10	18	7	0	23	0	71	0
	9	8	17	0	13	50	12	100	13
	27	27	76	40	100	75	0	100	0
Prometone	3	0	0	0	0	0	0	0	4
	9	0	9	0	0	0	18	21	8
	27	0	5	0	0	20	32	55	0
Diuron	3	0	0	0	2	0	0	0	0
	9	0	4	0	0	11	0	0	0
	27	4	0	5	0	37	10	11	0
Fenac	3	0	5	53	0	0	6	43	1
	9	0	0	4	0	0	0	33	0
	27	21	2	14	0	19	17	71	0
Check		0	3	2	0	0	0	0	0

[a]1. *Tabebuia heterophylla* (DC.) Britton. 2. *Cyathea arborea* (L.) J. E. Smith. 3. *Cordia borinquensis* Urban. 4. *Prestoea montana* (Graham) Nicholson. 5. *Miconia prasina* (Sw.) DC. 6. *Casearia-Drypetes*. 7. *Psychotria berteriana* DC. 8. *Inga fagifolia* (L.) Willd.

foliated by 9 and 27 lb/A of picloram, dicamba, and bromacil. Picloram had the broadest spectrum of herbicidal activity. At the 27 lb/A rate, six of the eight species were completely defoliated and the other two species, *Tabebuia heterophylla* (DC.) Britton and *Cordia borinquensis* Urban, were partially defoliated. The 27 lb/A rate of dicamba caused 100% defoliation of *Psychotria berteriana* DC. and *Inga fagifolia* (L.) Willd. but no defoliation of *Miconia prasina* (Sw.) DC. The 27 lb/A rate of bromacil caused 100% defoliation of *Prestoea montana* (Graham) Nicholson and *Psychotria berteriana* DC. but no defoliation of *Casearia-Drypetes* and *Inga fagifolia* (L.) Willd.

The speed at which trees defoliated was essentially the same for all herbicides applied. Data for the rate of defoliation caused by picloram indicate that maximum effectiveness was obtained about 6 to 8 months after treatment (Figure 1). The maximum defoliation occurred sooner from picloram at 9 lb/A than picloram at 27 lb/A. Defoliation remained essentially constant for the remainder of the 24-month period.

The relative density of new seedlings at the Luquillo site could not be correlated with the herbicidal treatment. The most common tree seedlings were *Psychotria berteriana* DC., *Ocotea leucoxylon* (Sw.) Mez, and *Prestoea montana* (Graham) Nicholson. Grasses were best represented by *Paulcum adspersum* Trin. and sedges by Scleria *secans* (L.). Many vines and herbaceous plants were present, *Ipomoea* spp. being the most common. There was a direct relation between percentage of defoliation and number of new seedlings. One year after treatment, the forest floor of plots that had been completely defoliated was covered with vegetation. The density of new plants is shown in Figure 2.

Herbicidal residue in the soil. Three months after application, the herbicides had moved downward in the soil to the 36 to 48-in depth. The bioassay data for all sampling depths indicated persistence of the herbicides in the soil 1 year after treatment for all locations was in the order of fenac > prometone > picloram > diuron > bromacil > dicamba. An example of the residue data is shown in Figure 3. Dicamba had almost completely disappeared 1 year after treatment. Two years after treatment, fenac was still the most persistent herbicide, followed by prometone and picloram.

The persistence of the herbicides generally was greatest in the driest area (Guanica) and least in the wettest area (Luquillo) (Figure 4). One year after application, the residue of picloram in plots treated at 27 lb/A remained in relatively high concentrations at all test sites, as determined by the cucumber bioassay test. The presence of picloram in plots treated at 9 lb/A could be easily detected 1 year after treatment, but the concentrations were about 10 times less than in plots treated with 27 lb/A. The residue data for all locations indicated a trend for all the herbicides to dissipate more rapidly in the top 12 in of soil.

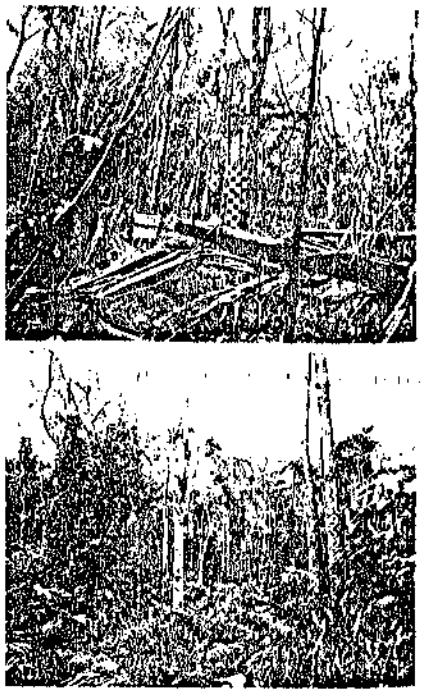

Figure 2. Forest floor of plot treated with picloram at 27 lb/A at Luquillo. Top: Eight months after treatment; bottom: Two years after treatment. Note secondary succession.

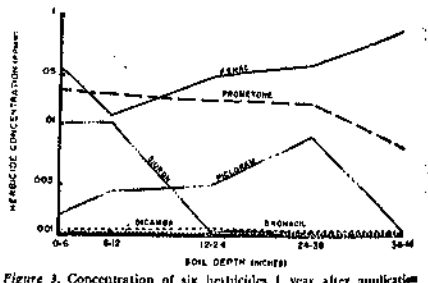

Figure 3. Concentration of six herbicides 1 year after application to Jacana clay (Guanica site) at 9 lb/A.

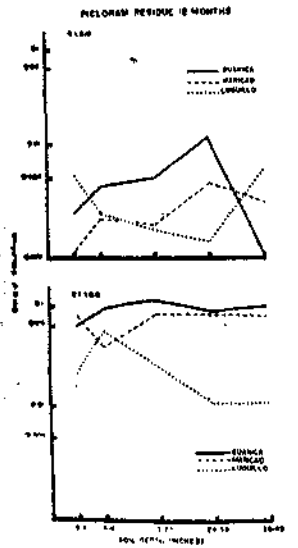

Figure 4. Picloram residue at various depths in three forest areas of Puerto Rico 12 months after application.

Discussion

Over 200 woody species were represented on the three test sites, but several species were represented by only a few individuals. Tschirley (13) enumerated some of the problems involved in evaluating herbicides in tropical forests. Other workers (2, 9, 11, 16) have shown distinct differential susceptibility of woody plants to various herbicides. Differential species susceptibility also was evident at our test sites. When all the treatments at all locations are considered, *Tabebuia heterophylla* (DC.) Britton, *Cordia borinquensis* Urban, *Inga fagifolia* (L.) Willd., and *Ocotea leucoxylon* (Sw.) Mez were most resistant and *Psychotria berteriana* DC., *Miconia sintenisii* Cogn., *Senegalia* westiana (DC.) Britton & Rose, and *Leucaena leucocephala* (Lam.) DeWit were most susceptible. *Casearia-Drypetes*, *Miconia sintenisii* Cogn. and *Miconia prasina* (Sw.) DC. were represented at both Maricao and Luquillo. The reaction of these species to the herbicides followed the same general trend at both locations, but defoliation was greater at Maricao.

The effect of climatic and edaphic factors on herbicidal activity cannot be clearly elucidated in this study because of differences in species composition. At all three test sites, rainfall before application was sufficient to maintain adequate plant growth. Rainfall for 2 months after treatment was 2.41 in at Guanica, 5.16 in at Maricao, and 5.94 in at Luquillo. Sufficient rain to leach the herbicides into the soil fell at all three locations within a few days after application.

The rapid increase in defoliation shown for dicamba and check plots in the Guanica site is the result of refoliation occurring during the rainy season (Figure 5).

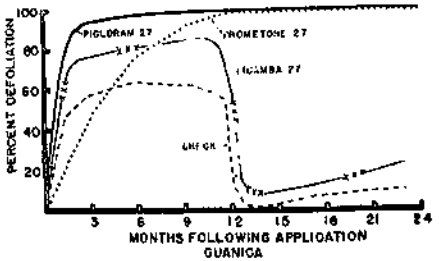

Figure 5. Percentage of woody defoliation in the dry, Guanica Forest site of Puerto Rico after treatment with three herbicides applied to the soil.

The rainy season usually occurs during July to October. Most of the woody plants grow vigorously during the rainy season and are deciduous during the long dry season. Refoliation on plots treated with 27 lb/A of dicamba attests to its herbicidal ineffectiveness at the Guanica site. The lack of refoliation of woody plants on plots treated with picloram and prometone is indicative of their effectiveness.

In this study, sufficient rainfall occurred after treatment to leach the herbicides into the soil and prevent large losses from volatilization and photodecomposition. The highest concentration of a herbicide in the soil profile was consistently found at the low rainfall Guanica site. On the other hand, the lowest concentrations of herbicides occurred at the continually moist Luquillo site.

Persistence was related to the amount of herbicide applied, but the effectiveness of a herbicide on woody plants was not related to its persistence. Fenac, the most persistent herbicide, was ineffective for defoliating woody plants at all test sites. Prometone was more persistent than was picloram but effectively defoliated plants only at the Guanica site. Picloram effectively defoliated woody plants at all three test sites.

Although a high degree of woody plant defoliation was obtained from several treatments, total vegetation control was short-lived. Secondary succession occurred within 18 months on all defoliated plots at all test sites. Grasses, herbaceous plants, and vines generally were more numerous than were woody tree seedlings.

The amount of rainfall and increased light penetration appeared to influence secondary succession more than did the herbicidal treatment. The number and density of successional species were greatest on the wet Luquillo site and smallest at the dry Guanica site. In general, the number and density of successional species were greater on plots that had been defoliated. This suggested that

increased light penetration was one of the major factors influencing secondary succession. There did not appear to be any relation between herbicidal residue and invading species. For example, several species such as *Psychotria berteriana* DC. were extremely susceptible to initial application of herbicides, but they were found on all treated plots 18 months after application.

Acknowledgments

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Persistence of 2,4-D, 2,4,5-T, and Dicamba in Range Forage Grasses[1]

Howard L. Morton, E. D. Robison, and Robert E. Meyer[2]

Abstract. The herbicides 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2-methoxy-3,6-dichlorobenzoic acid (dicamba) each labeled in the carboxyl position were sprayed on a pasture consisting of a mixture of silver beardgrass (*Andropogon saccharoides* Swartz.), little bluestem (*A. scoparius* Michx.), and dallisgrass (*Paspalum dilatatum* Poir.) and a sideoats grama (*Bouteloua curtipendula* [Michx.] Torr.) pasture over a 3-year period. Plant samples were harvested at intervals between 1 hr and 16 weeks after treatment and residues determined by radioassay. No important differences were found in the persistence of herbicides or of different formulations of the same herbicide. Rainfall was the most important factor influencing the persistence of the herbicides. The little bluestem-silver beardgrass-dallisgrass samples harvested 1 hr after treatment with the butoxyethyl ester of 2,4,5-T contained both this ester and the acid of 2,4,5-T. One week after treatment, the acid of 2,4,5-T and unknown metabolites were found but no ester.

Introduction

A variety of herbaceous and woody plants are controlled by 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2-methoxy-3,6-dichlorobenzoic acid (dicamba). Although the phenoxy acids have been registered and are used for weed control on lands devoted to forage production, the substituted benzoic acids have restricted usage on these areas. Little direct evidence of the persistence of these compounds in forage grasses has been published. Glastonbury *et al.* (3) sprayed peas (*Pisum sativum* L. var. Onward) with the sodium salt of 4-(2-methyl-4-chlorophenoxy)butyric acid (MCPB) and found that the half-life of the retained chemical was 3 days. Gutenmann and Lisk (4) sprayed the diethylamine salt of 4-(2,4-dichlorophenoxy)butyric acid (2,4-DB) on a pasture containing birdsfoot trefoil (*Lotus corniculatus* L.) and timothy (*Phleum pratense* L.) and found a rapid decrease in herbicide concentration in the forage after rainfall. Concentrations of 2,4-DB in the forage immediately after application of 1.5 and 3.0 lb/A rates were about 70 and 160 ppm, respectively, but were 0.32 and 0.80 ppm, respectively, after 48 days.

Klingman *et al.* (5) sprayed a Kentucky bluegrass (*Poa pratensis* L.) pasture with either the butyl ester or the 2-ethylhexyl ester of 2,4-D and found that most of the butyl and about 75% of the 2-ethylhexyl ester were hydrolyzed to the 2,4-D acid within ½ hr after spraying. Total concentrations of 2,4-D residues from the butyl and 2-ethylhexyl esters dropped from 58.4 and 48.4 ppm ½ hr after treatment to 5.0 and 15.1 ppm, respectively, 7 days after treatment.

The investigation reported herein was conducted to determine the persistence of 2,4-D, 2,4,5-T, and dicamba in range forage grasses, to compare the persistence of amine and acid formulations of 2,4,5-T, and to determine the influence of rate of application on the persistence of 2,4-D and 2,4,5-T.

Materials and Methods

Two field sites were fenced for the study. One was at College Station, Texas, in a pasture in which silver beardgrass (*Andropogon saccharoides* Swartz.), little bluestem (*A. scoparius* Michx.), and dallisgrass (*Paspalum dilatatum Poir.*) were the dominant species. The other was at Spur, Texas, in a pasture in which sideoats grama (*Bouteloua curtipendula* [Michx.] Torr.) was the dominant species. Different areas were treated at each site each year.

Herbicides labeled in the carboxyl position with carbon-14 were mixed with technical grade herbicides in the proportions necessary to give the specified radioactive levels as well as the specified rate of herbicide per acre. In all experiments, sprays were applied at volumes equivalent to 20 gpa with a compressed air sprayer. Two replications of each treatment were used. In 1962, the plots were 2 by 10 ft and they were 2 by 12 ft in 1963 and 1964.

In 1962, we applied butoxyethyl ester of 2,4,5-T at rates equivalent to ½ and 2 lb/A. Sprays were applied June 11 and June 19 at Spur and College Station, respectively, which contained 5 μc of radioactivity per plot. The carrier consisted of 7 parts water and 1 part diesel fuel (v/v).

In 1963, we applied 2,4-D and 2,4,5-T acids to the silver beardgrass-little bluestem-dallisgrass pasture June 14. Each solution contained 50 μc of radioactivity and sufficient herbicide to provide ½ or 2 lb/A rate. The carrier was acetone-water (1:1) containing 0.5% (v/v) surfactant[3].

In 1964, we applied dimethylamine salt of 2,4-D, dimethylamine salt of dicamba, and triethylamine salt of 2,4,5-T to the silver beardgrass-little bluestem-dallisgrass pasture July 1. We applied both the amine and acid of 2,4,5-T and dicamba to the sideoats grama pasture July 7. The carrier was water containing 0.5% (v/v) surfactant[3] for amine salt formulations and acetone-water (1:1 v/v) for the acid of 2,4,5-T. Each solution contained 60 μc of radioactivity and sufficient herbicide to provide a rate of 1 lb/A.

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[3]Surfactant contained alkylarylpolyoxyethylene glycols, free fatty acids and isopropanol.

In all years, we sampled the treated plots 1 hr (0 week), 1, 2, 4, and 8 weeks after treatment. An additional sampling was obtained on the fourteenth and sixteenth weeks in 1963 and 1964, respectively. We harvested 2-sq-ft subplots from each main plot by clipping the grass plants at ground level. The clipped plants were separated into those tissues produced during the current year, designated green tissues, and those tissues produced during previous seasons, designated litter tissues. Partially decomposed plant tissues were gathered from the soil surface of the subplots and were added to the litter tissues. After separation, the samples were placed in polyethylene bags, sealed with rubber bands, weighed, and stored at −10 C. Samples harvested as Spur were transported to College Station in an ice chest for analysis.

The harvested samples were shredded, and a 20-g portion was homogenized in a blender with 80% ethanol and filtered. The homogenization was repeated until the radioactivity of the residue was less than two times background. The filtrates were combined, reduced in volume under vacuum in a rotary evaporator, and brought to volume in a 25-ml volumetric flask. Duplicate 1-ml samples of each concentrated filtrate were dried in 1-in planchets, weighed, and the radioactivity assayed with a Geiger-Müller tube. Counts were converted to weight of herbicide from standard curves with appropriate corrections for background and self-absorption. The quantity of herbicide recovered on and in the forage was calculated for each subplot and converted to parts per million equivalents of fresh weight.

In 1962 and 1963, identification and characterization of the radioactive compounds in the concentrated ethanolic extracts were made by descending chromatography on Whatman No. 1 filter paper. An isopropanol:ammonium hydroxide:water (10:1:1 v/v/v) developer was used. After development and drying, each chromatogram was scanned with an autoscanner to determine the location of radioactive substance or substances on the chromatogram. Chromatograms of the ester of 2,4,5-T treating solutions contained radioactive butoxyethyl ester of 2,4,5-T and small amounts of acid. Identifications of the butoxyethyl ester of 2,4,5-T, and acids of 2,4,5-T and 2,4-D were made by co-chromatography of the ethanolic extracts and standard solutions of these compounds.

Results and Discussion

Recovery of herbicides from sprayed plots. The amount of herbicide recovered from grass tissues harvested 1 hr after treatment, calculated as a percentage of the amount applied, varied from 28% (20% green tissue and 8% litter tissue) to 102% (42% green tissue and 60% litter tissue) (Table 1). In all but two plots, greater quantities of the herbicides were recovered from the green tissues than from the litter tissues. Although the silver beardgrass-little bluestem-dallisgrass stands were relatively uniform, the plants and litter did not cover all of the plot areas. The low recovery percentages were due to sparse stands and the higher recoveries to dense plant and litter cover.

Experiments in 1962. Figure 1A presents a semilogarithmic graph of the concentrations of the ester of 2,4,5-T in green tissues of silver beardgrass, little bluestem and dallisgrass harvested at five dates after treatment. The lines for the two rates are essentially parallel, indicating that the rate of disappearance was not affected by rate of application. Concentrations of the ester of 2,4,5-T residues decreased rapidly during the second week after treatment when 2.18 in of rainfall occurred. The apparent half-life of the ester of 2,4,5-T (half-life equals average length of time necessary for one-half of herbicidal residue to disappear) under the conditions of this experiment averaged 2.6 weeks. Concentrations of ester of 2,4,5-T in the green tissues 8 weeks after treatment were 25 and 7 ppm, respectively, at the 2.0 and 0.5 lb/A rates.

Table 1. Percentage of herbicides recovered in green and litter tissues of silver beardgrass, little bluestem, and dallisgrass and sideoats grama harvested 1 hr after treatment.*

Herbicide	Treatment rate	Silver beardgrass-little bluestem-dallisgrass		Sideoats grama	
		Green tissue	Litter tissue	Green tissue	Litter tissue
	lb/A	1962			
Ester of 2,4,5-T	0.5	12	5	33	6
Ester of 2,4,5-T	2.0	34	16	30	18
		1963			
Acid of 2,4-D	0.5	32	17	—	—
Acid of 2,4-D	2.0	59	24	—	—
Acid of 2,4,5-T	0.5	48	26	—	—
Acid of 2,4,5-T	2.0	20	8	—	—
		1964			
Amine salt of 2,4-D	1.0	44	34	—	—
Amine salt of 2,4,5-T	1.0	42	60	54	24
Acid of 2,4,5-T	1.0	—	—	64	20
Dicamba	1.0	34	44	52	20

*Average of two replications.

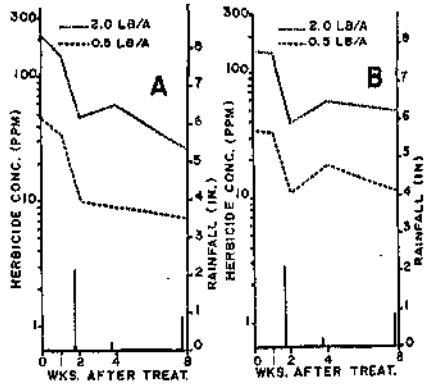

Figure 1. Concentrations of ester 2,4,5-T residues found in silver beardgrass-little bluestem-dallisgrass tissues harvested at five dates after treatment June 19, 1962 at 0.5 and 2.0 lb/A at College Station. (A) Green tissues, (B) litter tissues. Solid vertical lines indicate rainfall which occurred during the indicated interval after treatment.

Figure 1B is a semilogarithmic plot of the ester of 2,4,5-T concentrations in the litter tissues of silver beardgrass, little bluestem, and dallisgrass. Disappearance was most rapid during the second week after treatment. The rate of disappearance in litter tissues was slower than in the green tissues. The apparent half-life of ester of 2,4,5-T in the litter tissues was about 4 weeks under the conditions of this experiment. Two factors were important in the slower rate of disappearance. First, growth of the green tissues would have diluted the herbicide, but the litter samples were composed of non-living tissues and growth was not a factor in lowering the concentrations in these samples. Second, conditions for microbial decomposition of the herbicide were unfavorable due to the low rainfall.

Figure 2 presents a semilogarithmic plot of the apparent ester of 2,4,5-T concentrations in the green and litter tissues of sideoats grama. The ester of 2,4,5-T disappeared more rapidly from sideoats grama than from silver beardgrass, little bluestem, and dallisgrass. More rain fell on the sideoats grama than on the silver beardgrass, little bluestem, and dallisgrass. The apparent half-life of the herbicide averaged 1.6 weeks in the green tissues and 1.7 weeks in the litter tissues. The amount and frequency of the rainfall were conducive to leaching, microbial decomposition of the herbicide, and growth of sideoats grama plants. All of these factors contributed to a rapid reduction in herbicide concentrations.

Experiment in 1963. The concentrations of 2,4-D and 2,4,5-T residues found in green and litter tissues of silver beardgrass, little bluestem and dallisgrass harvested at six dates after treatment June 18 are shown in Figure 3. A 0.69-in rain occurred during the first week after treatment, and the concentrations of both herbicides in green and litter tissues decreased rapidly. No rainfall occurred during the second week after treatment and the rate of herbicide disappearance was slower in most of the plots than it was during the first week. During the third and fourth weeks after treatment, 1.58 in of rain occurred and the rate of herbicide disappearance was more rapid in most of the plots than during the second week. During the fourth through the eighth weeks after treatment, only 0.08 in of rainfall occurred and relatively small decreases in herbicide concentrations were found. The 2.78 in of rainfall which occurred during the eighth through the fourteenth weeks after treatment probably was the primary factor responsible for the rapid rate of herbicide disappearance during this interval. The average half-life for 2,4-D in green and litter tissues was 2.3 and 2.8 weeks, respectively. The average half-life of 2,4,5-T in green and litter tissues was 2.9 and 3.4 weeks, respectively.

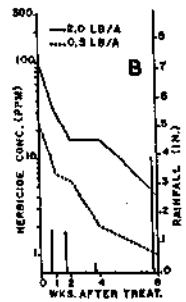

Figure 2. Concentrations of ester of 2,4,5-T residues found in sideoats grama tissues harvested at five dates after treatment June 11, 1962, at 0.5 and 2.0 lb/A at Spur. (A) Green tissues, (B) litter tissues. Solid vertical lines indicate rainfall which occurred during the indicated interval after treatment.

Experiments in 1964. Residues of amine salts of 2,4-D, 2,4,5-T, and dicamba disappeared from silver beardgrass, little bluestem, and dallisgrass tissues at about the same rate (Figure 4). The apparent average half-life for each of the three compounds in green tissues was 2.9 weeks under the conditions which existed during the experiment. Because of heavy rainfall during the fifteenth week after treatment, the concentrations of the three compounds were reduced to 1 or 2 ppm in the green

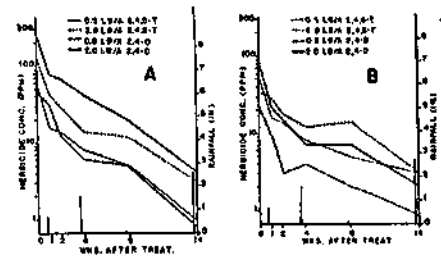

Figure 3. Concentrations of 2,4-D and 2,4,5-T residues found in silver beardgrass-little bluestem-dallisgrass tissues harvested at six dates after treatment June 14, 1963 at 0.5 and 2.0 lb/A at College Station. (A) Green tissues, (B) litter tissues. Solid vertical lines indicate rainfall which occurred during the indicated interval after treatment.

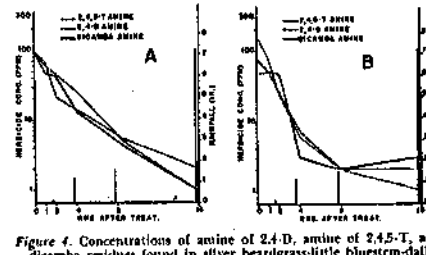

Figure 4. Concentrations of amine of 2,4-D, amine of 2,4,5-T, and dicamba residues found in silver beardgrass-little bluestem-dallisgrass tissues harvested at six dates after treatment July 1, 1964, at 1 lb/A at College Station. (A) Green tissues, (B) litter tissues. Solid vertical lines indicate rainfall which occurred during the indicated interval after treatment.

tissues. Concentrations of the three herbicides decreased rapidly in the litter tissues during the first 8 weeks after treatment (Figure 4B) when frequent rainfall kept the soil and litter tissues moist. The average half-lives of 2,4-D, 2,4,5-T, and dicamba in the litter tissues were 2.8, 2.7, and 2.6 weeks, respectively.

A relatively slow disappearance rate was found for all three herbicides in the green and litter tissues of sideoats grama (Figure 5). This slow disappearance oc-

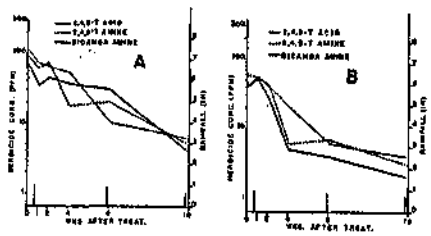

Figure 5. Concentrations of acid of 2,4,5-T, amine of 2,4,5-T, and dicamba residues found in sideoats grama tissues harvested at six dates after treatment July 7, 1964, at 1 lb/A at Spur. (A) Green tissues. (B) litter tissues. Solid vertical lines indicate rainfall which occurred during the indicated interval after treatment.

curred during a period of low rainfall. Although considerable variability occurred in the concentrations of the three herbicides at each sampling date, all herbicides had essentially the same rate of disappearance during the 16-week duration of the experiment. Concentrations of the acid of 2,4,5-T, amine of 2,4,5-T, and dicamba were 6, 5, and 4 ppm, respectively, in the green tissues at the time of final sampling 16 weeks after treatment. The concentrations of the three herbicides in litter tissues of sideoats grama are shown in Figure 5B. The acid of 2,4,5-T disappeared more slowly than the amine of 2,4,5-T and dicamba in litter tissues during weeks 2 to 4, but all three compounds were present after 8 weeks in approximately equal concentrations.

Data indicate that formulation had no significant effect upon the persistence of 2,4,5-T in the tissues of silver beardgrass, little bluestem, dallisgrass, and sideoats grama. While there were minor differences in the rates of disappearance of the three herbicides applied at College Station and Spur, their persistence in forage tissues appears to be essentially the same after several weeks. The most important factor influencing the persistence of these herbicides was rainfall. Both amount and frequency of rainfall were important.

Even when rainfall did not occur, there was a gradual reduction in the herbicide concentrations in the green tissues, particularly if rainfall had occurred prior to the interval when herbicide concentration was being measured. Dilution of the herbicides by plant growth was an important factor during the intervals after rainfall had occurred and soil moisture was adequate for growth of the plants. Important reductions in the concentrations of the herbicides were not found in the litter tissues when no rainfall occurred. This is evident in Figures 1B, 3B, and 5B. The exception to this statement is found in Figure 4B when a reduction in the concentration of the amine of 2,4,5-T from 146 ppm to 78 ppm occurred during the first week after treatment.

It is not surprising that formulations had no influence on the persistence of 2,4,5-T. Phenoxy herbicides deposited on the surfaces of plant leaves as ester formulations are hydrolyzed to the acid in a relatively short period of time (1, 2, 5).

Identification of herbicide residues. Attempts to identify the radioactive components in the ethanolic extracts by paper chromatography were only partly successful. All extracts from silver beardgrass, little bluestem, and dallisgrass green tissues harvested 1 hr after treatment with ester of 2,4,5-T contained the applied herbicide and the acid of 2,4,5-T. The Rf values ranged from 0.69 to 0.75 and 0.85 to 0.89, respectively, for the acid and ester of 2,4,5-T. Approximately 10% of the radioactivity was attributed to the ester and 90% to the acid of 2,4,5-T. These data confirm the results of Klingman *et al.* (5) who found rapid hydrolysis of the ester of 2,4-D by Kentucky bluegrass. Extracts of green tissues of silver beardgrass, little bluestem, and dallisgrass harvested 1 week after treatment contained the acid of 2,4,5-T and unidentified metabolites which had Rf values ranging from 0.10 to 0.30 but no ester of 2,4,5-T. Approximately 50% of the radioactivity was attributed to the acid of 2,4,5-T and 50% to the unknown metabolites.

The extracts of green tissues of silver beardgrass, little bluestem, and dallisgrass harvested 1 hr after treatment with acid of 2,4-D or acid of 2,4,5-T yielded only the acids of 2,4-D or 2,4,5-T. Tissues harvested 1 week after treatment contained both the acid and unknown metabolites. The metabolites of 2,4,5-T had Rf values ranging from 0.10 to 0.30, and those of 2,4-D had Rf values ranging from 0.07 to 0.25.

Acknowledgment

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Herbicides in Soils[1]

T. J. Sheets and L. L. Danielson[2]

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Introduction

Many herbicides are applied directly to the soil surface as selective preemergence sprays and as nonselective soil sterilants. Other chemicals are applied subsurface or are thoroughly mixed with the soil after surface application. Residues remaining on leaves after foliar applications are carried to the soil in rainwater or fall to the soil when injured leaves abscise and fall. Therefore at least part of all herbicidal sprays eventually reach the soil.

Soils vary greatly in composition and reactivity. Many complex and ever-changing processes occur continuously in most soils. Soils are composed of mineral matter, organic matter, water, and air. The mineral fraction varies in amounts of sand, silt, and clay, and in types and amounts of clay minerals. The hydration and base saturation of the clay minerals also vary. The organic-matter fraction consists of decaying plant and animal residues and active soil flora and fauna. The organic and mineral colloids present in the soil contribute directly and indirectly to the extremely active nature of soil systems.

This mixture of mineral and organic matter is permeated by pore spaces of various sizes. These spaces are filled with water and air in a more or less reciprocal relation. The soil water contains many soluble compounds and serves as an essential medium for many physical and chemical processes. The soil atmosphere is composed of oxygen, carbon dioxide, nitrogen, and several minor gases. The composition of the soil atmosphere varies, particularly the oxygen and carbon dioxide contents. The complexity and variation of soil systems make the study of the fate of herbicides therein complicated and time consuming.

Methods of Assaying Herbicide Residues in Soils

In most investigations on the persistence of herbicide in soils, researchers determine the presence of the active entity of the herbicide by growing sensitive plants. The influence of time on herbicidal residues has been measured by the growth of successive crops of test plants. This method, which has been used in both field and greenhouse experiments, is qualitative only.

Quantitative bioassays have been developed for some herbicides. Holstun and Loomis (*35*) measured the elongation of young shoots of germinated millet seeds to determine the concentration of the sodium salt of 2,2-dichloropropionic acid [dalapon] in soils. Burschel and Freed (*11*) used heights and weights of seedling oats to determine the concentrations of isopropyl N-phenylcarbamate [IPC], isopropyl N-(3-chlorophenyl) carbamate [CIPC], and 3-amino-1,2,4-triazole [amitrole] in soils. Rahn and Baynard (*45*) used weight of oat seedlings to assay quantitatively 3-(p-chlorophenyl)-1,1-dimethylurea [monuron] in soils. Biological assays developed for solutions and vapors of herbicides could be adapted for use with soils (*17*, *46*, *55*).

Some herbicides have been extracted from soils and their concentrations determined by physical or chemical methods. Methods are available for monuron, amitrole, CIPC, and pentachlorophenol [PCP] (*10*, *11*, *30*, *62*). Whiteside and Alexander (*61*) followed the breakdown of several chlorinated phenoxy aliphatic acid herbicides in solutions inoculated with soil by the disappearance of the specific ultraviolet absorption.

A physical or chemical assay may be most suitable in one situation and a biological assay in another. Both types of analyses are useful in some cases. Rahn and Baynard (*45*) reported that the chemical method for the determination of monuron in soils was accurate if the assay was made within a few weeks after application. If soils were chemically assayed more than 1 month after treatment, values for monuron concentration were greater than those obtained by bioassay. Rahn and Baynard (*45*) suggested that this apparent disagreement could be explained since the chemical assay for monuron was based on *p*-chloroanaline, a nonphytotoxic, hydrolytic product of monuron.

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FACTORS ALTERING HERBICIDES IN SOILS

Factors affecting the movement and persistence of herbicides in soils have been reviewed by several workers (*1, 8, 25, 26, 32, 41, 43*). Leaching, fixation by soil colloids, chemical and microbial decomposition, and volatilization were stressed in one or more of these papers. In this discussion microbial action, volatilization, adsorption, leaching chemical reaction, photodecomposition, and absorption by plants will be considered for their significance in the performance and fate of soil-applied herbicides.

Microbial action.—Most organic herbicides subjected to appropriate tests have been inactivated more rapidly in soil under conditions favoring growth and proliferation of soil microorganisms. Absorption by microorganisms is one of the major pathways by which organic herbicides are detoxified. Perhaps 2-(2,4-dichlorophenoxy) propionic acid [2-(2,4-DP)], 2,4,5-trichlorophenoxyacetic acid [2,4,5-T], 2-(2,4,5-trichlorophenoxy) propionic acid [silvex], and 4-(2,4,5-trichlorophenoxy) butyric acid [4-(2,4,5-TB)] are exceptions (*7, 61*). Optimum oxygen, moisture, temperature, and nutrients favor microbial activity and also herbicidal detoxication. Numbers of soil microorganisms capable of inactivating 2,4-dichlorophenoxy acid [2,4-D] apparently increase when 2,4-D is present in the soil (*8, 42, 43, 61*). Thus repeat applications of 2,4-D were less persistent in soil and therefore may be less effective herbicidally than the initial application. With the phenylureas and *s*-triazines such an increase in microbial activity apparently does not occur, because soils appear to exhibit about the same capacity for inactivation for long periods. Therefore it seems that with the phenylureas and the *s*-triazines the action of soil microorganisms utilize them but not selectively or preferentially. Another explanation of this effect is that inactivation of these chemicals is catalyzed by heat-sensitive substances occurring in the soil as products of microbial activity and that the herbicides are not utilized directly by microorganisms as energy sources.

Bacteria, *Bacterium globiforme* and *Flaviobacterium aquatile*, which were capable of inactivating 2,4-D were isolated from soil and grown in pure culture (*6, 8, 36*). Evans and Smith (*27*) isolated a small, Gram-negative, motile soil organism which grew freely in a mineral-salt medium containing *p*-chlorophenoxyacetic acid as the only organic-carbon source. They separated 2-hydroxy-4-chlorophenoxyacetic acid and 4-chlorocatechol from the culture. The same investigators isolated a Gram-negative, motile rod which grew on a mineral-salt, 2,4-D medium. From this culture they separated a phenolic acid and presented evidence to suggest that the compound was 6-hydroxy-2,4-dichlorophenoxy-acetic acid. They hypothesized that hydroxylation of the ring was followed by ring cleavage.

Whiteside and Alexander (*61*) presented evidence suggesting that 4-(2,4-dichlorophenoxy)butyric acid [4-(2,4-DB)] was converted to 2,4-D by microorganisms in the soil and that a microflora capable of quickly inactivating both 2,4-D and 4-(2,4-DB) was present in soils which had received 4-(2,4-DB) previously.

Hill, *et al.* (*33*) reported that a soil bacterium of the *Pseudomonas* group was capable of oxidizing monuron particularly in the presence of yeast extract.

One group of herbicides, the esters of chlorophenoxy alcohols, becomes herbicidally active only on contact with the soil. In warm, moist soil sodium, 2,4-dichlorophenoxyethyl sulfate [sesone] is hydrolyzed to 2,4-dichlorophenoxyethanol in the presence of either microorganisms or acids (*12, 13*). The hydrolysis by microorganisms was attributed to acids secreted during their metabolism. The ethanol product is oxidized in the soil to 2,4-D, the active entity.

In experiments conducted by the senior author, 2-chloro-4,6-bis(diethylamino)-*s*-triazine [chlorazine] mixed in the soil became more toxic to seedling oats with time. This trend reversed after several months, the time depending on the soil type and concentration, and thereafter the herbicidal activity of cultures containing chlorazine decreased with time. The increase in toxicity could not be accounted for completely as a response to growing conditions in the greenhouse. Autoclaving the soil prior to treatment retarded the rate of onset of increased toxicity. If one ethyl group was lost from either or both of the amino substitutions, compounds much more toxic than chlorazine would be formed. Perhaps formation of one of these compounds did occur in the soil.

The rates of inactivation of IPC, CIPC, and amitrole in the soil depended on the initial concentration of the herbicides; and the inactivation of these

herbicides appeared to follow a first-order reaction (*34*). The rate of disappearance of monuron from soil was proportional to the concentration (*33*). Hill, *et al.* (*33*) concluded that although soil moisture and temperature often altered the rates of inactivation of monuron and 3-(3,4-dichlorophenyl)-1,1-dimethylurea [diuron], the first-order equation was probably applicable under natural field conditions. When monuron and diuron were applied at rates of 1 and 2 pounds per acre in more humid regions of the United States, major parts of the herbicides were inactivated each year. Accumulation from applications on the same soil 2 years in succession was negligible. Rahn and Baynard (*45*) found that monuron applied at 3.6 pounds per acre in two applications for 3 years in succession did not persist from one year to the next. When applied at 0.4 pounds per acre, monuron toxicity persisted from one year to the next, but no accumulation occurred. Research conducted in the arid Southwest during the last 7 years indicated that monuron and diuron did not accumulate significantly from successive annual applications at rates used for selective weed control in cotton. Some carryover often occurred, and the amount of carryover appeared to be related to weather conditions.

Current research indicates that the solvent used in the application of an herbicide may have a profound influence on the persistence of herbicidal activity (*18*). Ethel N,N-di-*n*-propylthiolcarbamate [EPTC] was applied in several solvents and incorporated into the soil. At weekly intervals up to 6 weeks after treatment, the soils were assayed by the used of oat plants as indicators of toxicity. When the commercial formulation of EPTC was applied in water, growth of oat plants seeded 6 weeks after treatment were markedly inhibited on flats which received 1 and 2 lb./A. The persistence of technical EPTC applied in acetone was comparable to that of the commercial formulation applied in water. However the rate of inactivation of technical EPTC applied in kerosene was much more rapid than that of the commercial formulation applied in water. Four weeks after treatment the 2 lb./A rate of technical EPTC applied in kerosene did not inhibit growth of oats.

Persistence of several groups of herbicides in the soil is related to halogenation of the benzene ring. This relation was demonstrated for certain chlorinated phenoxyacetic acids, carbamates, and phenylureas (*21, 22, 23, 50*). The results of Alexander and Aleem (*3*) indicated that resistance of chlorinated phenoxyalkyl carboxlic acid herbicides or their derivatives to microbial degradation was governed by the position of the halogen rather than by the number of halogens on the ring and that the linkage of aliphatic side chain also influenced susceptibility to microbial breakdown.

Volatilization.—All compounds are volatile to some degree. Volatility of some herbicides is very low and of little significance. However, measurable loss of others occurs from soil surfaces by vaporizations.

The volatilities of formulations of the same basic herbicide structure may be quite different. The isopropyl ester of 2,4-D is more volatile than the octadecyl ester, which in turn is more volatile than the sodium salt (*39*). Many other esters and salts of 2,4-D exhibit various rates of vaporization.

Vapors of soil-applied herbicides have caused severe injury to treated crop plants in some instances. Vapors of 4,6-dinitro-*o*-*sec*-butylphenol [DNBP] after preemergence applications caused extensive injury to cotton in the Mississippi Delta in 1952 (*25, 26, 34*). DNBP injury was associated with high temperatures. Hollingsworth and Ennis (*34*) found that vapor injury to young cotton plants increased as soil moisture increased.

DNBP injury to cotton was reduced by application of lime and other basic materials to the treated soil surface (*9, 19*). Upon addition of a base, the phenol-phenate equilibrium was probably shifted to the phenate, which is less volatile than the phenol (*9*).

Volatilization of the carbamates has been related to their effectiveness as preemergence herbicides. IPC and CIPC volatilized rapidly from tinfoil and glass surfaces at high temperatures (*4*). IPC volatilized more rapidly than CIPC, particularly at temperatures of 60° to 85° F. The most volatile carbamates were found to be most phytotoxic (*38*); however, loss by volatility following preemergence application reduced the concentration of the more volatile compounds to nonherbicidal levels more rapidly than the less phytotoxic, less volatile compounds.

Many herbicides are formulated on granular carriers to reduce loss by volatility and leaching after application to the soil surface. The vapor and contact activities of CIPC-impregnated granular carriers were investigated by

Danielson (*17*). The vapor activity of CIPC was related to the physical structure and adsorptive capacity of the granular carriers. When the physical structure of attapulgite granules was changed by moistening with water, vapor activity increased. Carriers that did not change in physical structure on contact with water exhibited unchanged or reduced vapor activity. Danielson proposed the use of impervious granular carriers to obtain immediate short-term activity of CIPC and more adsorptive carriers for long-term activity.

Adsorption.—The activity of most herbicides varies with soil composition. Since many herbicides are adsorbed by colloidal particles and since the amounts of mineral and organic colloids vary among soils, much of the variation in herbicidal activity is attributed to differences in the adsorptive capacity.

The adsorption of six growth-regulator herbicides by several ion-exchange resins was demonstrated by Weaver (*59*). Weaver (*60*) and Smith and Ennis (*53*) used activated charcoal as a soil amendment to protect germination seeds from 2,4-D applied to the soil surface.

In a greenhouse experiment the initial toxicity of 2,4-D was greater in sandy soils than in most clay soils (*16*). A butylester of 2,4-D was fixed in a clay-sand mixture more strongly than a triethanolamine salt form (*2*). Both amine and polypropylene ester formulations of 2,4-D were adsorbed by montmorillonite, illite, and kaolinite clays (*32*). The adsorption of 2,4-D increased as the cation-exchange capacity and specific surface increased.

CIPC was adsorbed by activated charcoal and certain other materials (*17*). In laboratory and greenhouse experiments EPTC was adsorbed least by those soils in which it was most phytotoxic (*5*).

Sherburne and Freed (*51*) demonstrated adsorption of monuron by activated charcoal, sawdust, straw, and soil. The amount of monuron adsorbed by soils was correlated with organic matter and clay content. Studies by Hill (*32*) showed that the clay content, type of clay, and organic matter of soils influenced the amount of monuron adsorbed. Adsorption increased as clay content or organic matter increased. Approximately 150 p.p.m. was required on a bentonite clay to give 1 p.p.m. in the soil solution whereas less than 1 p.p.m was required on a kaolinitic clay to give 1 p.p.m. in solution.

The herbicidal activity of the phenylureas was correlated inversely with soil organic matter, total clay, and cation-exchange capacity (*47, 56*). Multiple regression analyses suggested that soil organic matter was most important in toxicity reduction of monuron, diuron, 3-phenyl-1, 1,1-dimethylurea [fenuron], and 3-(3,4-dichlorophenyl)-1-methylurea [DMU].

Variations in the effective dosage ranges among several soils suggested greatest adsorption of diuron and DMU and least adsorption of monuron and fenuron (*47*). Coggins and Crafts (*15*) showed that clay suspended in solutions of the phenylurea herbicides reduced the toxicity to barley. The toxicity of 1-*n*-butyl-3-(3,4-dichlorophenyl)-1-methylurea [neburon] was altered most, and alteration of toxicities of DMU, diuron, monuron, and fenuron followed. In general, water solubility and adsorption were inversely related. In a recent report Leopold, *et al.* (*37*) demonstrated on inverse relation between solubility of several chlorinated phenoxyacetic acids and their adsorption on charcoal.

In an aqueous medium 2-chloro-4,6-bis(ethylamino)-*s*-triazine [simazine] was adsorbed to a cation exchanger and to activated charcoal but not to an anion exchanger (*48*). Soil toxicity tests with the *s*-triazines suggested considerable variation in soil adsorption of these compounds. The effect of soil organic matter, clay content, cation-exchange capacity, and pH on the phytotoxicity of simazine was investigated in detail. Soil organic mater appeared to alter the initial toxicity of simazine in soils most.

In soil systems adsorbed herbicides are probably gradually desorbed as leaching, chemical and biological degradation, and absorption by plants reduce the concentration in the soil solution. However, the adsorption-desorption relations of herbicide molecules in soils and the importance of these phenomena in the movement of herbicides in soils have not been adequately investigated.

Leaching.—The movement of herbicides in soils depends on or is influenced by several factors. Upchurch and Pierce (*57, 58*) indicated that at least two steps are involved in the movement of an herbicide downward in soil: (a) Entrance of the herbicide into solution and (b) adsorption of the herbicide to soil particles. Entrance into solution could occur from solid particles of the herbicide or from colloidal particles with adsorbed herbicide molecules. These two processes, solution and adsorption, may be affected by several variables.

The solubilities of herbicides and of salts of herbicides that may form in the soil are important properties affecting leaching (*32, 41, 43*). Minarik (*41*) discussed the leachability of 2,4-D and its salts. He pointed out that the calcium, magnesium, potassium, sodium, and ammonium salts of 2,4-D are more soluble in water than the acid, whereas salts of heavy metals such as iron and copper are less soluble than the acid. The equilibrium status of the several forms of 2,4-D in the soil probably affects the leaching rate of 2,4-D. However, Smith and Ennis (*53*) did not measure a difference in the movement of the acid, the triethanolamine salt, and the sodium salt of 2,4-D in soils. Hill (*32*) concluded that the lower water solubility of diuron compared to monuron resulted in slower movement of diuron that monuron in soils. Diuron is adsorbed more strongly than monuron and differences in adsorption probably contribute to differential leaching of these two compounds.

An herbicide that is strongly fixed in soils should leach less readily than one that is not so tenaciously fixed (*2*). The adsorptive capacity of soil is influenced by soil organic matter and the amounts and types of clay minerals. The adsorption process is influenced by temperature and the nature of the solvent. The adsorptive characteristics of a compound are influenced by pH of the solution. Since these factors influence adsorption, they must influence movement of herbicides in soils. The organic matter content and soil texture are known to influence leaching (*20, 24, 25, 26, 32, 35, 44, 47, 52, 53, 58*).

The leachability of DNBP appeared to be influenced by pH. Dowler, *et al.* (*24*) concluded that movement of DNBP in soils was as much a function of soil type and soil reaction as of the amount of rainfall. In experiments by Upchurch and Pierce (*58*) soil temperatures of 50° to 45° C. had little effect on the monuron leached from the upper 2-inch layer of soil columns. However, greater amounts of monuron were retained by the 2- to 8-inch layer at 25°, 35°, and 45°C. than at 5° and 15° C.

That the amount of rainfall or of water applied as irrigation influences the movement of herbicides has been demonstrated by many research workers. Sherburne, *et al.* (*52*) compared the movement of monuron in soil columns to the movement of compounds in chromatography and concluded that the depth of the highest concentration of the herbicide in soil columns was a function of the amount of water added to the soil surface. Upchurch and Pierce (*57*) studied the effect of amount, intensity, and frequency of simulated rainfall on the leaching of monuron. The greater the amount of simulated rain the greater the movement of monuron. Rainfall intensities of 1/16 to 4 inches per application had little influence on the amount of monuron retained in the top 2-inch layer. In the 2- to 8-inch zone greater accumulation of monuron occured with low intensities than with high. A greater movement of monuron from the upper soil layers occurred as frequency of rainfall increased. Approximately half of the frequency effect was attributed to evaporation of water from the surface of soil columns that received less frequent applications. These workers concluded that of the three variables studied, the amount of rainfall would be most directly correlated with the distribution of herbicides in soil profiles under field conditions although they maintained that intensity and frequency might also be of practical importance.

The effect of the amount of monuron applied on the amount moved by simulated rainfall was also investigated by Upchurch and Pierce (*58*). Monuron was applied to surfaces of soil columns at rates of 0.5, 1, 2, 4, 8, 16, 32, 64, 128, and 256 lb./A, and the applications were followed by 4 inches of simulated rainfall. The lowest percent retention (34 percent) in the 0- to 2-inch layer was found in columns treated with 32 lb./A. Retention by the 2-inch layer was increasingly greater as the rate increased and decreased from 32 lb./A. These workers suggested that the increasing percent retention when the rate of application was increased from 32 to 256 lb./A was attributable to the low solubility of monuron.

Molecular size may also be a factor in the movement of herbicides in soils (*15, 44*).

The exact pattern of movement of an herbicide in a particular soil would be impossible to predict presently. However, relative rates of movement can be predicted for many herbicides.

Chemical reaction.—The formation of salts of 2,4-D and DNBP and a possible reaction scheme for inactivation of 2,4-D in soils have been discussed.

Relatively little is known of the chemical reactions that most herbicides undergo in soils. Hydrolysis, oxidation, and formation of complexes are known reactions for certain herbicides.

Amitrole forms stable complexes with cobalt, copper, nickel, iron, and magnesium. Sund (*54*) suggested that complexing with metal ions in the soil solution was one mechanism by which amitrole was detoxified.

The 2-chloroacetamides, for example 2-chloro-N,N-diallylacetamide [CDAA], can be hydrolyzed in the soil (*31*). The chlorine atom and the amide linkage are sites on the molecule where hydrolysis may occur. Regardless of the site where hydrolysis begins, the end products are glycolic acid and secondary amines.

The dithiocarbamates, for example 2-chloroallyl diethyldithiocarbamate [CDEC], can be broken down in the soil by oxidation and hydrolysis (*31*). If hydrolysis of CDEC precedes oxidation, allyl alcohol is an intermediate; whereas if oxidation precedes hydrolysis, 2-(diethyldithiocarbamyl)acetice acid is an intermediate. The two reactions can occur separately or concurrently in the soil. End products of breakdown are formic acid, glycolic acid, carbon disulfide, and secondary amines for both reaction pathways. The breakdown products may undergo further reaction in the soil.

Freed, *et al.* (*29*) suggested that EPTC was hydrolyzed in water; and according to the reaction scheme which they proposed, a secondary amine, carbon dioxide, and ethylmercaptan were end products.

Soil treated with 3,5-dimethyltetrahydro-1,3,5,2 *H*-thiadiazine-2-thione [DMTT] evolves formaldehyde, which is thought to be the first product of DMTT breakdown in the soil. (*40*). Methylaminomethyldithiocarbamate forms next and activation continues by forming monoethylamine, methyl isothiocyanate, and hydrogen sulfide. Monoethylamine and hydrogen sulfide react with formaldehyde and form methylaminoethanol, dimethylaminomethane, and 1,3,5-trithiocyclohexane. Eventually this reaction proceeds to carbon dioxide, ammonia, sulfur dioxide, and water. The methyl isothiocyanate and water react to give carbon dioxide, hydrogen sulfide, and methylamine; and the methylamine degrades into carbon dioxide and ammonia.

Monuron is thought to be hydrolyzed slowly in the soil to *p*-chloroaniline (*45*).

In the presence of moisture tris-(2,4-dichlorophenoxyethyl) phosphite [2,4-DEP] is slowly hydrolyzed step-wise to form one mole of phosphorous acid and three moles of 2,4-dichlorophenoxyethanol (*28*).

The reactions which herbicides and agricultural pesticides in general undergo in soils and the products formed are important with respect to residues in soils. Weed research scientists should emphasize this phase of herbicide research.

Photodecomposition.—Less is known about the direct effect of light on the breakdown of herbicides than other factors suspected of being involved. However, photodecomposition of monuron was demonstrated by Hill, *et al.* (*33*). When a solution containing 88.3 p.p.m. of monuron sealed in quartz tubes was exposed for 48 days to sunlight, an 83-percent loss of monuron occurred. Hill, *et al.* (*33*) concluded that in dry areas of the Western United States monuron may be inactivated by ultraviolet irradiation. They suggested that this factor would account for disappearance of only a small part of the herbicide in humid regions where frequent rains move it into soils.

Neburon, diuron, monuron, fenuron, and DMU were applied as alcohol solutions to filter paper (*14*). After the paper dried, it was exposed to ultraviolet light for several hours. The herbicides were not visible prior to exposure, but in white light they were readily visible after exposure as light tan spots. The compounds were apparently changed during exposure.

The effects of shade, moisture, and position in the soil on the residual activity of monuron, diuron, and simazine were investigated in cooperation with the California Agricultural Experiment Station. The activity of monuron and diuron disappeared more rapidly from soil exposed to the sun from shaded soil. The activity of monuron and simazine disappeared more rapidly from moist soil than from dry soil. Monuron, diuron, and simazine were not affected to the same degree by these variables. Soil temperature was measured but not controlled in this experiment, and soil temperatures varied considerably among the treatments during the day. Temperature markedly influences vapor pressure and chemical reactions. Therefore the difference in the rate of disappearance in shaded soil and soil exposed to the sun cannot be attributed unquestionably to light inactivation.

Absorption by plants.—Herbicides are absorbed by plant roots and are usually translocated to the aerial parts. Within the plant the herbicide molecules are subjected to various physical and chemical processes. Crop plants may be removed from the land or they may be returned to the soil along with weed growth. Most plant roots remain in the soil. Therefore a portion of herbicides absorbed by plants and the metabolic products of herbicides in plant tissues may eventually be returned to the soil.

It has been stressed previously that soils reduce the initial effectiveness of herbicides and that the degree of effect varied among soil types and herbicides. Unpublished data showed that at least four times as much simazine was required in a clay loam soil as in solution culture to produce the same weight reductions of seedling oats. In another experiment the dry-weight increase of oat tops following simazine treatment through the roots was reduced 50 percent by 7.2 mg. of the herbicide (C^{14} expressed as simazine) per gram of dry tissue at harvest 9 days after initial exposure (*49*). The amount of simazine (C^{14} expressed as simazine) required in seedling oat plants to reduce plant weight was less than 2 percent of that present in 400 ml. of the 0.05 p.p.m. by weight culture solution initially. Although the conditions of this experiment were markedly different from those which occur in the field, plants probably absorb only a small fraction of the total amount of an herbicide applied to the soil.

CONCLUSIONS

Although considerable progress has been made, much additional information is needed on the fate of herbicides in soil. Weed research scientists need to know more about the persistence of herbicides in soils under varying environmental conditions so that they can establish safe rotational practices. Mammalian toxicity of some soil degradation products should be determined, because these products can also be absorbed by plants. Information on adsorption-desorption relationships; on the interrelationships of adsorption, volatility, solubility, and leaching of herbicides; on the nature and extent of microbial and chemical inactivation; on the importance of photodecomposition; and on the influence of various environmental factors on these processes is essential to an understanding of the behavior of herbicides in soils.

Weed scientists should determine the component or components of the soil from which dosage requirements of soil-applied herbicides can be predicted. Some of this information is available (*48, 56*), but more is necessary. Eventually specific recommendations of rates, times, and methods of application of herbicides may be based on weather forecasts and analyses of soil samples from farmers' fields (*25*).

One of the most urgent needs for research on the fate of herbicides in soils is methods of isolation and identification of herbicides and breakdown products. Biological and chemical assays must be improved and new ones derived. Radioactive isotopes have been used very little to study the decomposition of soil-applied herbicides. Soil samples could be treated with different lots of an herbicide with each lot tagged with C^{14} at different positions in the molecule. By known analytical techniques the unchanged herbicide and many reaction products could be separated and identified. Radioisotopes should be most useful tools in future research of this nature.

As new herbicides are developed, their behavior in soils in response to variable soil characteristics, weather conditions, and cultural practices must be investigated concurrently with some of the more fundamental aspects prior to recommendations for use by farmers.

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Senator HART. Do you have any comparable information about dioxin?

Dr. BAYLEY. The answer at this point is no.

Senator HART. Are any tests being run?

Dr. BAYLEY. Not yet. One of the problems we had here is developing the methodology for testing these in products. As soon as this is settled, we will be able to expand our efforts and find this information.

Senator HART. And you hope you will find that it has not been building up in our bodies every day?

Dr. BAYLEY. We sincerely do yes, sir. But that will not bias our results, I assure you.

Senator HART. Perhaps Dr. Byerly might help us with this. I am told by my notes here that dioxins are chlorinated hydrocarbons and that these tend to be stable, and more significantly, in view of the fact that other dioxins are known to be absorbed and retained in the tissue of animals, isn't it likely that there is a build-up in the human of the dioxins found in 2,4,5?

Dr. BYERLY. I will give you an opinion first. You stated since they are chlorinated hydrocarbons, they therefore would be persistent. This does not necessarily follow. There is a very wide range from almost complete persistence, if you like, long-time persistence, to a very short-time persistence on the part of some other chlorinated hydrocarbons.

With respect to these which are in the family of chlorinated hydrocarbons, of which 2,4,5-T is a member, the time of disappearance substantiated by empirical evidence, it is a matter of a few months. With respect to the dioxins themselves, Dr. Bayley answered you quite frankly, we do not have the information. We will seek it when the methods are complete.

Senator HART. In your report you state that you agree that more rigorous tests on teratogenicity should be imposed before registration. If you favor such test as a requirement for registration, isn't there an inconsistency in allowing 2,4,5-T, which is a pesticide with suspected teratogenicity to be allowed to continue in a registered status pending the outcome of the tests? In other words, should you not deregister it now and then if the tests prove negative, register it?

Dr. BAYLEY. The basis on which we would take such action would have to be on the consideration that we believe a hazard now exists. Based on the information which has been provided to us from the Department of Health, Education, and Welfare, and on our own analyses of the levels of the contaminent we do not believe such a hazard exists at this time.

Senator HART. You cite the content of four lots of [illegible]-T in terms of the content of, as you put it, TCDD. How many more producers of that product are there?

Dr. BYERLY. There are only three primary producers, sir. There are many formulators.

Senator HART. Is a formulator engaged in a process which changes the generic business?

Dr. BYERLY. No.

Senator HART. Almost the total of production is from three sources, right?

Dr. BYERLY. Primary production. I understand the check has been made and the importation is very small.

Senator HART. After you have registered the product, how frequently do you check up on the amounts of dioxins in the products that are being produced?

Dr. BAYLEY. Mr. Chairman, one of the requests which we are making of the Congress this year is to strengthen our law so that we can have plant inspection and insist on quality control within these particular plants. We have asked the Congress to help strengthen our activity in this area. It is not adequate at the present time. We very definitely need legislation to improve it.

Senator HART. Is that sort of a way to say that you do not check the dioxins?

Dr. BAYLEY. This is the first time. One of the reasons, of course, is that the methodology has only been newly developed. The results presented here were developed through check procedures with our laboratories, the Food and Drug Administration, and the industry group to see that our methodology was technically correct. So we are just getting started.

Senator HART. You will include in your budget request moneys to provide what?

Dr. BAYLEY. The President's budget includes an increase of approximately $2.4 million for the pesticide regulatory division. This is between 50-and 100-percent increase in the funding for that organization.

Senator HART. Do you believe that with that sum you would be in a position to have plant inspections on a regular basis? Would you be able to have an enforcement staff which would be able to move in the event a violation was discovered? Is this the sort of thing that you visualize?

Dr. BAYLEY. Yes. We based that estimate in our budget request on what we currently thought was necessary to do this.

Now, we all recognize that the problems of concern about chemicals are expanding, and I would not want to suggest that is the final request that we would make in order to improve our operations. We put those in believing they currently were adequate from the standpoint of the enforcement and registration procedures.

Senator HART. Is the table which shows the amount of dioxin of this particular type—is there a test and do you have facility and personnel to attempt to identify the existence of any of the other seven possible dioxins?

Dr. BYERLY. This, sir, is in the process of development in cooperation with the Department of Health, Education, and Welfare and

industry. These methods are in the process of development. For some of them the methods are quite adequate; for others, the methods have to be developed.

Senator HART. For some you feel you can?

Dr. BYERLY. Yes, sir.

Senator HART. In that case you do.

Dr. BYERLY. We will.

Senator HART. Hinged on the money problem?

Dr. BYERLY. No, it depends primarily on the development of the competence of personnel. This is the thing that has to grow. We have to have a cadre of people who are highly competent. These are most sensitive methods and we cannot just create people who can handle them overnight.

Senator HART. In the testimony of the first two witnesses, and I do not recall whether it was that of Mr. Wellford or Mr. Turner, it is my impression there was a reference to a chick embryo study. Do you recall the comment they made?

Dr. BYERLY. Mr. Chairman, I do not recall the specific comment. There is a chick embryo test. It is highly sensitive. I would point out that it has, as a screening test, possibilities. Again, its sensitivity would make me want to suggest that we be very careful with respect into whose hands the tests were put.

With respect to the teratogenicity, with respect to the chick embryo, the application in this test is hardly like the application either to the skin or in the food of a mammal. So a direct comparison of the effects in the chick embryo with rats and mice, the traditional ones, would require a great deal of review.

Dr. BAYLEY. It would really require a correlated study to ascertain the relationship.

Dr. BYERLY. It would indeed.

Senator HART. I think just from what we have heard this morning, everyone on this subcommittee will be eager to assist you in obtaining the additional moneys, whether it is 2.4 million or more to insure the development just as rapidly as possible of the technical data on which to base tests and the human hands to administer them.

Let me get into this burden of proof again—we sort of dismissed it—very briefly.

The basic conclusion of your testimony is you have not found that registered uses of 2,4,5-T without a finite tolerance on food crops constitutes a hazard requiring cancellation or suspension of such registered use.

Dr. BAYLEY. That is correct.

Senator HART. And yet this morning we have heard testimony that preliminary tests suggest that 2,4,5-T when contaminated by dioxin comparable to that found in currently produced 2,4,5-T is teratogenic in three species; that the Mrak Commission or a panel advisory to it said that the teratogenic effects in one or more such species should be grounds for immediate restriction of pesticide use; that residues of 2,4,5-T are now found on approximately one out of every 200 food samples analyzed by FDA; that we can't be sure of the amounts of tetradioxin in 2,4,5-T now being sold, nor do we

have as yet clear ideas on the amount of other dioxins in the pesticide, some of which may be more potent than tetra; that no evidence suggests that these dioxins are not persistent or cumulative in human tissue, and that some evidence which would indicate perhaps they are.

If you accept that as a premise, in view of all of this, would you say that you are sure that registration of 2,4,5-T for use directly on food crops does not constitute a hazard to man?

Dr. BAYLEY. I would say that the information we have does not give us indication that it is a hazard to man in accordance with the registered uses.

I think we have to recognize that—and I am sure the committee and we are in agreement—these are all economic poisons, and the purpose of registration is to provide for their use in such a way that they are not a hazard. That is the basis on which we make our judgment.

Senator HART. Your position is that they do not constitute a hazard?

Dr. BAYLEY. Yes, sir. And our position is based not only on our own data but that provided to us from the medical authorities of the Department of Health, Education, and Welfare, and to add. 20 years of safe use.

Senator HART. The first two witnesses described the difficulty of finding the brand name on a deformed infant.

There are lots of birth defects. How can anyone say over 20 years that this has not been a factor in some of these private tragedies.

Dr. BAYLEY. I do not in any way want to be facetious, but I think we have to recognize that one of the compounds closely related to this contaminant is lysol, a rather common household disinfectant, and I hear no suggestion that we take this off the market. We in the Department of Agriculture recognize that there is a large group of chlorophenols that we are going to have to examine to find out whether there is a real hazard or not. I am not here to raise a scare, but I think we recognize that in dealing with these compounds we must have evidence that they are a hazard or we will be dealing with emotional conjecture based on inference from various scattered data.

Senator HART. To make clear what is meant, what is your position with respect to lysol?

Dr. BAYLEY. We have no reason to take action at this time.

Senator HART. Do you have in process studies or evaluations to see whether you modify that?

Dr. BAYLEY. Within our capability, for example, we have already moved out to ascertain the dioxin content of 17 other pesticides in this area, and we recognize that this is a field in which we want to make intensive study.

I do not single out this particular product as one which I would consider as more hazardous than any of the rest, but merely as an example of the total problem that we have in meeting these pesticide issues.

It seems to me that from the standpoint of protecting the public health, the important thing for us to do is to take those which, based

on scientific data appear to have the greatest potential hazard and put our resources on evaluating these as we go ahead.

This is true of the mercurials. We are looking very closely at all of these compounds. I think we must recognize that we are going to have to do this on a priority basis as we go. We are giving attention to 2,4,5-T, but we need to have the appropriate facts as we proceed.

Mr. BICKWIT. I think what has come out here is that really it all boils down to a question of burden of proof.

While you say there is no evidence that 2,4,5-T is hazardous, I would have to dispute that. Assuming that you are right, that there is no evidence that it is hazardous, and yet it cannot be shown that it is not hazardous; on what do you base your inaction?

Dr. BAYLEY. Let's recognize first of all that these are economic poisons. We should all agree to that to start with. And when I use the word "hazardous" I use it in terms of sufficiently hazardous to take action. This is bound to be a judgment based on scientific—including medical scientific data. If there is a disagreement between us, then it is in this judgment, not in anything else.

Mr. BICKWIT. I recognize my inability to make adequate scientific judgments and, as a result, defer to those who I regard are capable of making such judgments.

The panel which reported to the Mrak Commission has recommended whenever teratogenic effects of a given pesticide are shown in one or more mammalian species, that immediate steps should be taken to restrict the use of that pesticide.

Are you rejecting that advice?

Dr. BAYLEY. No. The use of this pesticide is already restricted because of the registered uses.

Mr. BICKWIT. I suspect that the thrust of their statement would require that it be further restricted but perhaps we would have some difficulty pursuing what the exact intention of their statement was.

You say that you must believe that a hazard exists before you can take a pesticide off the market and that in the case of 2,4,5-T you do not believe that a hazard exists.

Dr. BAYLEY. We do not believe that a hazard exists which would authorize us to take it off the market, yes.

Mr. BICKWIT. Are you sure that a hazard does not exist?

Dr. BAYLEY. One can never be absolutely sure that a hazard does not exist, even if we are talking about table salt.

In fact, we know that table salt is hazardous if taken improperly, and we don't even register it.

Mr. BICKWIT. There is a distinction from table salt in this case, and that is that there has been evidence that suggests, and to my mind rather strongly suggests, that there is a hazard here.

Dr. BAYLEY. I do not see the difference that you are trying to point out.

Mr. BICKWIT. You do not think that the studies that have been done by FDA, by NIEHS, although preliminary, establish that there is any greater hazard than the hazard of table salt?

Dr. BAYLEY. No; I did not say that.

Mr. BICKWIT. I misunderstood you.

Dr. BAYLEY. I did not say that. But again I come back to the point that we are dealing with economic poisons. There are hazards

in the use of all of them. The decision that has to be made is are the hazards sufficiently great to take action at this particular time. That is the difference.

Mr. BICKWIT. You do say issues involving human health should have priority over all other issues.

Dr. BAYLEY. Yes, sir.

Mr. BICKWIT. What I am not clear on is whether you have to actively believe there is a hazard before you take a pesticide off the market.

Is that a legal requirement?

Dr. BAYLEY. I don't know what you mean by actively believe.

Mr. BICKWIT. I conceive of relative states of mind as being belief, state of suspension, and state of disbelief.

Do you think you have to be on the belief side of state of suspension in order to take a pesticide off the market?

If you are in a state of suspension, would that authorize you legally to take it off the market?

Dr. BAYLEY. The information provided to us has not shown that there is sufficient hazard for us to take action, and the information provided to us from the Department of Health, Education, and Welfare is the information primarily that we have used.

Mr. BICKWIT. And you do not feel that if you are not sure one way or the other that that would authorize you to take it off the market?

Dr. BAYLEY. The data that we have at this time are not adequate to show us that there is a hazard, and the data to the contrary are sufficiently adequate to suggest that there is no hazard as 2,4,5-T is presently registered.

Mr. BICKWIT. I guess what it all does boil down to are two differences between us: one, in evaluation of the evidence——

Dr. BAYLEY. Yes, sir; and these types of judgments are inherent to the decisions being made.

Mr. BICKWIT. (continuing). And, two, differences in feelings about burden of proof?

Dr. BAYLEY. And we believe that the relationships between the departments have been fully utilized in working out this type of a basis of position.

Mr. BICKWIT. At least we have emphasized what the differences are.

Senator Baker earlier asked whether any studies have been run to weigh the benefits of poisons as against the detriments.

Have any actually been run?

Dr. BAYLEY. Are you talking about specific compounds or about all of them?

Mr. BICKWIT. All of them.

Dr. BAYLEY. Are there any data on it, Dr. Byerly?

Dr. BYERLY. There is one study done by Velmar Davis.

Whether it would be suspended without the suspension of other phenoxy herbicides or whether only it would be suspended makes a substantial difference.

If all of them were suspended, it might amount to more than $100 million of added cost. If other phenoxys remained available to us, it would only be a fraction of that amount.

Let me emphasize that this kind of study is very treacherous in drawing any conclusion at all, because if you make a substantial difference in the amount produced or the quality produced, that which remains may sell at a higher price, and this again brings in a matter of value judgments.

What we can say, I think, with respect to all herbicides and all pesticides in general, is that if we had to do without them and had available the hoes and the people to do the hoeing and the other things to produce our same crop, you would add a cost of production of more than $2 billion a year.

Let me emphasize, however, that our primary concern is not the economic cost in the aggregate.

Important as this may be, our primary concern, as Mr. Bayley has said, is that we shall control pests and we shall do it safely and without hazard to human health or the public welfare.

Mr. BICKWIT. We have emphasized that we have some differences in evaluation of the evidence. I am trying to discern what evidence would convince you that this pesticide was in fact hazardous.

The one thing that you have said about what would convince you is that you specify that should the teratogenic nature of 2,4,5-T be confirmed, registration for use on food crops will be canceled.

My understanding, correct me if I am wrong, is that the four studies cited earlier; although all preliminary, demonstrate that 2,4,5-T is teratogenic.

Dr. BYERLY. We do not accept that statement.

Mr. BICKWIT. In what way do you not accept it?

Dr. BYERLY. I think the statement that Dr. Bayley read is correct, that all of the evidence known to me is compatible with the hypothesis that these results were due to contaminant dioxin or the interaction of that dioxin and 2,4,5-T.

Mr. BICKWIT. The evidence which I have read shows that 2,4,5-T when contaminated with dioxin in amounts similar to or less than those in currently produced 2,4,5-T does produce teratogenic effects.

Now, I am on the basis of that evidence, unwilling to say it is because of the dioxin or the 2,4,5-T or the relationship between the two. Are you?

Dr. BYERLY. I believe that our previous dialogue indicated that these are preliminary results, so preliminary, sir, that I have not seen the published figures nor have I seen all of the figures to which you allude in the record.

Mr. BICKWIT. I agree they are preliminary. What I am asking you is if they are confirmed, will you deregister 2,4,5-T for use on food products?

Dr. BYERLY. This depends upon the dosage at which they are effective.

Mr. BICKWIT. You will have to modify your statement then.

Dr. BYERLY. In what way?

Mr. BICKWIT. Well, you have said that should the teratogenic nature of 2,4,5-T be confirmed, you would deregister the pesticide.

Dr. BYERLY. I do not modify the statement. I said if 2,4,5-T.

Mr. BICKWIT. What does it matter whether pure 2,4,5-T is teratogenic if there is no such product as pure 2,4,5-T on the market? I

assumed that by your reference to "2,4,5-T," you meant pure 2,4,5-T as currently produced on the market.

Dr. BAYLEY. I think you ought to recognize that he was answering your question precisely.

Dr. BYERLY. I believe good manufacturing practice can restrict the amount of contaminant dioxin in the product. I believe it should be done; I believe it is now being done and that it will be done.

Mr. BICKWIT. Then, I take it that your statement was not in reference to the teratogenic nature of 2,4,5-T when contaminated with any dioxin whatsoever?

Dr. BAYLEY. No.

Mr. BICKWIT. Have we ever produced 2,4,5-T without any dioxin whatsoever?

Dr. BYERLY. This is not a statement that can be answered absolutely, but it can be answered within the limits of the method in Dr. Bayleys statement. It indicates there was one in which there was no detectable amount of dioxin.

Mr. BICKWIT. Would you be willing to say that if the teratogenic nature of 2,4,5-T with the amount of dioxin that is contained in currently produced 2,4,5-T is found to be teratogenic that you would deregister it for food use?

Dr. BYERLY. I would be willing to say, sir, if the 2,4,5-T with no detectable amount of dioxin, of tetrachlorodibenzo paradioxin, would prove to be teratogenic, I would recommend to the Department that actions to cancel uses on food crops be taken.

Mr. BICKWIT. Yet, what is really relevant here is the effect of currently produced 2,4,5-T. Why then are you basing your decision on the effects of 2,4,5-T in a form that we do not know it commercially?

Dr. BYERLY. I think you are misconstruing my reply. Again, pending the fact that neither you nor I have before us published figures which would sustain your statement that all four of these things do in fact show teratogenic effects, if we accept what you say may be true, but it has not been published nor publicly disclosed, then let me say further that so far as I know, the dosage at the current level of 150 milligrams per kilo is equivalent to the amount of the dioxin therein contained at one part per million which would be expected to give a teratogenic effect if there were no 2,4,5-T present, and 150 milligrams per kilo is astronomically higher than any amount to which any person would normally be exposed in the normal course of usage.

Mr. BICKWIT. How long is the usage to which you refer?

Dr. BYERLY. I did not make a limit.

Mr. BICKWIT. In one's lifetime?

Dr. BYERLY. In a lifetime.

Mr. BICKWIT. You are willing to say this is more dioxin than one is likely to be exposed to in his entire lifetime?

Dr. BYERLY. That is my opinion.

Mr. BICKWIT. You will have to admit it is not based on much.

Dr. BYERLY. You are forcing me into the realm of conjecture and I do not choose to go into that very far. We do not have the empirical evidence on which to state whether or not it is degradable. We have no evidence, sir, that it is not degradable.

Mr. BICKWIT. Here we are again; we do not know whether it is or is not.

Dr. BYERLY. We intend to find out.

Dr. BAYLEY. Mr. Chairman, this dialogue is the same type of dialogue that we have with industry people who come in and want to know exactly what evidence we have to have in order to assure them that they have provided us with sufficient proof. These judgments are not so simple that you can conjecture ahead of time of seeing the data exactly what position you are going to take. It is characteristic of them; it is inherent to them. And I suggest this is characteristic not only when we are dealing with industry but when we are also concerned about the public health.

Senator HART. Gentlemen, did you have anything you would like to add?

Dr. BAYLEY. We do not, sir. We would be glad to enclose the additional statements for the record that we have discussed.

Senator HART. Thank you very much.

I had hoped we could continue through the lunch hour, but I am stuck with a Policy Committee lunch.

(The statement follows:)

STATEMENT BY NED D. BAYLEY, DIRECTOR OF SCIENCE AND EDUCATION, U.S. DEPARTMENT OF AGRICULTURE

MR. CHAIRMAN: I am Ned Bayley, Director of Science and Education, Office of the Secretary, USDA. I have with me T. C. Byerly, Assistant Director of Science and Education.

We are pleased to be here to comment on the current state of knowledge with respect to the herticide 2,4,5-T. We will be glad to respond to questions relevant to its usage as fully as information available to us enables us to do so.

The herbicide 2,4,5-T has been recognized a the most effective herbicide registered for use for control of certain weeds and brush species for more than 20 years. About four-fifths of the domestic use of 2,4,5-T is for nonfarm use, the largest such use being for control of brush on rights-of-way. It is also used extensively to control brush on forest lands and certain weeds in turf. 2,4,5-T has been used in the production of fruit crops, cereal grains, and sugarcane. It is the most effective herbicide for control of brush on several million acres of rangeland in the Southwestern United States.

2,4,5-T is degraded in the environment within a few months after application so that residues do not persist from one season to the next. Residues on foods are unusual. Among 5800 food samples analyzed by FDA for 2,4,6-T during the past four years, 25 were reported to contain trace amounts; i.e., amounts less than the 0.1 ppm limit of accuracy of present analytical procedures for foods. Two samples showed residues of 0.19 ppm and 0.29 ppm, respectively.

No finite tolerance has been established for 2,4,5-T in food. In the absence of such tolerances, any detectable amount of 2,4,5-T in food would make such food subject to seizure if found in the channels of interstate commerce. From the data cited above, it is apparent that contamination of food with 2,4,5-T is very infrequent and then only at very low levels.

There is current concern over the continued use of 2,4,5-T arising from the report of a research study completed under contract by the National Cancer Institute by Bionetics Inc. This study was based on a commercial lot of 2,4,5-T acquired for the study in 1965. It was fed to pregnant mice and rats. Many of their developing young had birth defects.

After review of this information and after consultation with Federal agencies concerned, Dr. Lee A. DuBridge, the President's Science Advisor, announced on October 29, 1969, a coordinated series of actions being taken by those agencies with respect to the use of 2,4,5-T.

Among them was the announcement that: "The Department of Agriculture will cancel registrations of 2,4,5-T for use on food crops effective January 1, 1970, unless by that time the Food and Drug Administration has found a basis for establishing a safe legal tolerance in and on foods."

USDA was informed in January that the lot of 2,4,5-T used in the Bionetics study contained significant amounts of a highly toxic contaminant, tetrachlorodibenzo paradioxin. The Department was further informed that lots of 2,4,5-T of current and recent manufacture were reported to contain less than 1 ppm of this contaminant in contrast to the 27 ppm reported for the lot used in the Bionetics study.

Extensive studies are under way to determine whether 2,4,5-T is itself teratogenic. Preliminary reports are consistent with the hypothesis that the teratogenic results reported in the Bionetics study were due to the contaminant dioxins or to interactions of such contaminants with the 2,4,5-T rather than to 2,4,5-T per se.

The Department announced on February 6 that it would undertake examinations of 2,4,5-T and 17 related compounds registered for pesticidal use to determine whether or not they are contaminated with dioxins. Preliminary results on 2,4,5-T show that those lots examined of current manufacture and those now in channels of trade gave the following results:

TABLE 1.—AMOUNTS OF TCDD FOUND IN COMMERCIAL 2,4,5-T BY TWO METHODS

Sample	Manufacturer	Lot	Grade[1]	Collected	TCDD Content p.p.m.[2] USDA	FDA
2,4,5-T	Dow	120110	TG	2/70	trace	0.07
2,4,5-T	Monsanto	07-020	TG	2/70	1.1	2.9
2,4,5-T	Hercules	X-17394-21-5	TG	2/70	N.D.[3]	N.D.
2,4,5-T	Dow	MM-120449	TG	2/70	.48	.47-.52

[1] TG = Technical grade.
[2] TCDD Refers to the 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD).
[3] N.D. = Levels of TCDD are below the limits of detection or below 0.05 p.p.m.
[4] Sample supplied by Dow as a reference check and reported to contain about 0.5 p.p.m. TCDD.

These data are preliminary and are obtained from first drafts of methods developed by chemists in the Crops Research Division (USDA) and in the Pesticide Chemistry and Toxicology Division (FDA). The dioxin values refer only to the 2,3,7,8-tetrachloro-dibenzo-p-dioxins (TCDD) and do not indicate levels of other halogenated dioxins (containing 5, 6, 7, or 8 chlorines) in the 2,4,5-T samples.

In view of all the information now available, we have not found that registered use of 2,4,5-T without a finite tolerance on food crops constitutes a hazard requiring cancellation or suspension of such registered uses.

There has been and is concern over the ecological effects of 2,4,5-T used as a defoliant in Viet Nam. Dr. Fred Tschirley, Assistant Chief of our Crops Protection Research Branch, has reported the results of his examination of areas treated in Viet Nam. He has reported no evidence of irreversible ecological damage. Allegations that defoliation will lead to extensive laterization of Vietnamese soils, that Mangrove areas will not recover, that fish production in wetland areas will be reduced were not verified.

Dr. Tschirley also headed a team of scientists who investigated allegations of injury to humans and animals due to herbicide treatment for control of Chaparral by the Forest Service on the Tonto National Forest near Globe, Arizona. They found that apparent damage consisted of damage to susceptible plants near the treated area from drift of the herbicides used. The alleged injuries to a duck and a goat were found to be groundless. Human illnesses were those expected in a normal population with the possible exception of one man with skin irritation on his eyelids. Clinical chemistry on specimens obtained during the investigation is in process.

Senator HART. I think in fairness to all we should recess to resume at 2:15.

(Whereupon, at 12:45 p.m., the subcommittee was recessed, to reconvene at 2:15 p.m., this same day.)

AFTERNOON SESSION

Senator HART. The committee will be in order.

Resuming this afternoon, our first witness is Dr. Arthur H. Westing. Dr. Westing is chairman of the Biology Department of Windham College in Putney, Vt.

STATEMENT OF DR. ARTHUR H. WESTING, CHAIRMAN, BIOLOGY DEPARTMENT, WINDHAM COLLEGE, PUTNEY, VT.

Dr. WESTING. Senator Hart, I consider it a privilege to be able to testify before your committee. Actually, I am very pleasantly surprised that you and your staff show such tolerance toward me despite a fairly questionable record with respect to your state.

First of all, most of the year I lived in Michigan, I devoted to spraying your forests with 2,4,5-T, and perhaps even worse, when I left Michigan I took with me one of your most desirable natives as my wife.

Senator HART. I don't know whether that makes an even trade or not.

Dr. WESTING. All the time I was listening to this morning's testimony and realizing how muddled the situation was with respect to the medical and public health aspects and the legal and administrative aspects, I kept thinking that those aspects were crystal-clear in relation to the aspects that I am going to try to talk about, and that is the impact of 2,4,5-T on the environment.

It is such a difficult field to cope with because ecology is still really in its infancy, particularly when it comes to the study of the full impact of a toxic introduction to the environment.

To judge from the popular press, our nation is on the brink of environmental disaster. Ecology has achieved some sort of a mystical significance to many people, and a whole new vocabulary has emerged overnight utilizing that wonderful avant-garde prefix "eco."

Over and over again we are being reminded of our collision course with "ecocatastrophe" leading to "ecodeath." We are told that we have to really use drastic "ecotactics;" a Senator like you should be using aggressive "ecopolitics." The whole world is being challenged to accept a protocol on "ecocide." And I suspect that psychiatrists are very soon going to be diagnosing "econeuroses."

Actually, the unhappy truth of the matter is that there may well be good cause for concern over the future of man's environment. It is being assaulted from all quarters with a gusto that is hard to grasp. Man has habitually ignored the impact that he has had on the environment, the environment that all of us depend upon for our well-being and survival. Western man has always considered himself master of his surroundings. Until the present, with far smaller numbers and very ineffectual technology, this self-delusion made very little difference.

But today we are introducing a great diversity of chemicals into our environment in vast, continuing, and exponentially increasing quantities. Among these chemicals, pesticides are worthy of particular scrutiny because of their potential ability to de[illegible]ate certain classes of living organisms, and thereby to upset the balance of nature—to disrupt what the ecologist refers to as the "ecosystem."

I shall here limit my remarks to the potential dangers to the environment that might be expected from the excessive or otherwise incorrect use of one such substance: 2,4,5-T.

As we have heard this morning, this compound has recently gained a degree of notoriety owing to its massive military use in Vietnam despite the suspected ability of it, or an associated impurity, to cause birth defects.

I need not refer you to Thomas Whiteside's article on this subject which really is a beautiful expose of the current legal, administrative, and other associated problems. (New Yorker, 7 February and 14 March 1970). Now, to speak briefly on the current use of 2,4,5-T, it is one of a class of potent herbicides or plant killers, the one preferred by utility employees, foresters, range managers, and farmers, and by our armed forces in Vietnam for the destruction of unwanted woody vegetation. It is one of a class of growth-hormone-mimicking herbicides whose close chemical relatives include 2,4-D, MCPA, and Silvex. 2,4,5-T was developed during the early 1940's (as a possible chemical warfare agent) and came into widespread domestic use during the mid-1950's. In 1964, some 13 million pounds of 2,4,5-T were manufactured in the United States. About a million of these pounds were applied to about 3 million acres of U.S. croplands, another million pounds were sprayed on perhaps 80,000 acres of forest lands in Vietnam, and most of the remaining 11 million pounds were presumably used domestically on an undetermined number of acres of noncroplands. This morning we were vividly reminded that a small portion of this is also used by home gardeners.

Now, these are the 1964 figures. Although I am not sure of the current ones, I understand that the domestic use of herbicides in general has been increasing at a compounded growth rate of 10 percent per annum.

2,4,5-T is commercially available in a number of formulations of which the most important are the oil-soluble esters and the slightly less effective water-soluble amines. Whereas the amine formulations are very low in volatility, some of the ester formulations are relatively high and others are relatively low. The low volatility esters are actually somewhat more effective than the high volatility ones, but they are also slightly more expensive. 2,4,5-T is also available in combination with 2,4-D, a mixture which is known domestically as "brush killer" and by the military as "agent orange."

The 2,4,5-T is effectively applied either to the foliage of unwanted woody vegetation from ground- or aircraft-mounted spray rigs, or to their stems by a variety of techniques.

Domestically, it is very often applied highly diluted by oil or water, although some domestic techniques of individual application call for strong concentrations.

In Vietnam, it is aerially applied in totaly undiluted form.

Recommended broadcast dosages—these are domestic recommendations—range from one-half to three pounds of active ingredient per

acre. At these levels of one-half to three pounds per acre, the 2,4,5-T is quite selective, killing many species of broad-leafed woody plants and sparing most grasses and conifers. At the high rates the military use in Vietnam—which is about 13 pounds per acre, together with as much again of 2,4-D—it becomes far less selective and kills a high proportion of the vegetation.

In their silvicultural applications, foresters do some aerial spraying, but often resort to individual application to unwanted trees. However, in range improvement and in the control of vegetation on rights-of-way, and in Vietnam, application is mainly or entirely from the air.

Overall, the domestic applications average out to about one-third to one-half pound per acre treated.

That is a very brief summary of the use of 2,4,5-T.

Now I would like to spend a few minutes on the potential dangers from the use of 2,4,5-T. I am limiting my remarks, by and large, to the dangers to the environment since the medical and public health aspects were covered previously, and I understand will be covered by subsequent speakers.

Senator HART. Doctor, as you leave the use section and before you get into these potential dangers, can you describe for the record—I think it has not yet been stated in layman's language for the record—what the bush or tree or grass or area of earth surface looks like when this is applied to it, you say 1 to 3 pounds an acre.

Dr. WESTING. That is right.

Senator HART. If you can in language describe for the reader and me what it looks like. I frankly have not seen it.

Dr. WESTING. Stretching my memory back to the Upper Peninsula.

Senator HART. The beautiful Upper Peninsula.

Dr. WESTING. I might interject here that a lot of pioneering work in aerial forest spraying was actually done in Michigan. The leaves on unwanted oaks or maples very rapidly turn brown, within a matter of 3 or 4 days. In 5 days they start showing signs of shriveling up. They usually hang on that way for 6 to 8 weeks, and perhaps longer; so, one sees a lot of trees that have brown, shriveled up leaves. If conifers are intermingled, they show no damage so they stand out like green thumbs, and a good bit of the forest floor stays green; grasses and so on stay green, ferns and so on will turn brown; some plants stay green and others do not, depending upon the type. What it looks like really in this country is as if fall had just decided to come a few months early.

Senator HART. How would you describe the same scene if there was applied to it the 13 pounds per acre which you say is the current application on the average in Vietnam?

Dr. WESTING. It is actually about 25 or 26 pounds. It is 13 of 2,4,5-T plus another 13 of 2,4-D.

Senator HART. The picture you described——

Dr. WESTING. Was for one to two pounds.

Senator HART. Of 2,4,5-T only?

Dr. WESTING. Right.

I have not seen an area myself that has been hit this heavily, but I have seen pictures. Within a very short period of time, all the leaves look brown and shriveled up and within a matter of perhaps two to three weeks most of the leaves drop off the trees, vines, and shrubs.

This, of course, is the reason why the military spray these herbicides and sprays them in such heavy dosages, in order to get as rapid a leaf defoliation as possible. But in the process of getting rapid defoliation, there is a high degree of kill, which is an unhappy corollary. I am not sure if this is really intended: it happens, particularly in certain types of vegetation.

Subsequently, grasses, bamboos, and a variety of other weeds grow back fairly rapidly. So, after several months you see lots of large dead trees and then a very heavy new undergrowth.

Senator HART. You say the tree does die?

Dr. WESTING. Well, it depends upon the species, Senator Hart. Mangroves would be killed by one application in Vietnam whereas some other trees might not be killed unless they were sprayed a second time. A single spraying seems to kill about 10 percent of the trees. There is a great diversity of tree species there.

I have flown over areas in southeast Asia that have been sprayed once and it seems that roughly one tree in eight or 10 is dead.

If these were sprayed a second time 6 months later, perhaps two out of three trees would be dead, or maybe even more.

Senator HART. Thank you.

Dr. WESTING. I wish now to touch upon some of the potential dangers to the environment from the use of 2,4,5-T, and I am speaking again primarily domestically. The dangers can arise not only from the 2,4,5-T itself, but also from its contaminants, (such as were discussed at great length this morning), from its additives, (and there are endless kinds of additives: wetting agents, emulsifiers, stickers, penetrants, thickeners, humectants, spreaders, etc.), from its carriers or diluents, (such as fuel oil, kerosene, seal oil), and from its degradation products (or perhaps degradation products arising from subsequent burning). All of these various possibilities I shall lump together for purposes of my comments here, just calling them 2,4,5-T.

The dangers from the use of 2,4,5-T need not be confined to the site of application, but can be carried elsewhere by wind, either as liquid or as vapor, or carried elsewhere by water, either surface water or ground water. Moreover, the potential dangers are not confined to the time of application, but last, of course, until the 2,4,5-T degrades to the level of insignificance. Under wet and warm field conditions, one of the advantages of 2,4,5-T is that it breaks down within a matter of several weeks, 6 or 8 weeks perhaps. But under dry and cool conditions, this may take well over a year. Furthermore, the rate of degradation in the groundwater may also be very slow.

The dangers from the use of 2,4,5-T can result from damage to plants, damage to animals, both higher and lower, possibly from damage to microorganisms, and from direct and indirect combinations of these effects.

I shall elaborate very briefly on some of these possibilities.

The most spectacular effect of 2,4,5-T—when used as recommended domestically—is, of course, on certain classes of plants, particularly

but not exclusively the broadleafed woody vegetation. In selectively destroying such plants and sparing others, the species composition of the treated area is altered, the overall diversity of species is reduced, and the total mass of living things is probably diminished. And such changes are considered by ecologists to be an unstabilizing and therefore detrimental influence on an ecosystem. In other words, they make the balance of nature more precarious.

A properly functioning, relatively undisturbed ecosystem owes its stability—indeed, its very integrity—to a highly complex set of interactions amongst all of its many living and nonliving components. Nutrients cycle and recycle from the soil up through the interlocking food chains and back again to the soil. Population levels of the many component plants, animals, and microorganisms are kept in balance by a staggering multitude of predator/prey, host/parasite, and other long-established interactions of mutual dependency.

As soon as a toxic factor such as 2,4,5-T intrudes upon this highly complex, totally interacting system, a certain amount of the so-called ecological buffering action (of the many inherent checks and balances) is lost, and things start going wrong. Erosion may be accelerated, particularly in hilly terrain and even more particularly when streamside vegetation is killed. This effect, together with a reduction in the total mass of the living component of an ecosystem inevitably leads to a loss to the area of vital nutrient materials. Especially following heavy or repeated applications, the result is a steady decline in the productivity of the treated ecosystem—something that may take it centuries from which to recover.

On top of this there are all sorts of subtle things that can go wrong. For example, a continuing supply of available nitrogen—one of the elements essential to all life, and often in short supply—depends to a large extent on the presence of certain 2,4,5-T sensitive plants, whose roots play host to various microorganisms crucial to this process.

Actually, there has been some evidence of this occurring in the Pacific Northwest, where ponderosa pines are the crop tree and alders are being removed by 2,4,5-T as weeds, with a resulting loss to the area of available nitrogen.

Additionally, the birds and other animals that depend upon the 2,4,5-T decimated plants for food or cover are placed at a great disadvantage and may be partially or even completely eliminated from a treated area.

The direct toxicity of 2,4,5-T to most higher animals is known not to be very severe, particularly at the recommended rates of application. However, that there is also potential danger in this regard is suggested by its known effects on humans. The U.S. Department of Agriculture categorize 2,4,5-T as "mildly" irritating to the skin in a standard dermal response rating, and as "moderately" toxic when ingested. In fact, one can quote the following precaution from the product label: "Do not contaminate irrigation ditches or water used for domestic purposes;" and also the following warning: "Causes irritation of skin and eyes."

Moreover, in aquatic habitats, the death of trout and some other fish has been reported when 2,4,5-T is applied at recommended rates for weed killing. Certain crabs, shrimps, and mollusks are also harmed by low concentrations of 2,4,5-T.

The adverse effects on wildlife are not limited to the ones already alluded to. Some plants exposed to sublethal doses of 2,4,5-T (or 2,4-D) start producing abnormally high levels of nitrates (and in some cases there has been a suggestion of even cyanide). It has been noted with livestock that when such plants are ingested, the excess nitrates are converted to nitrites, toxic or even lethal to the animals.

Another occasional result of 2,4,5-T application is that naturally poisonous, and usually avoided, plants are made attractive to animals as a result of 2,4,5-T spraying; and then the animals feed on these newly attractive plants and are poisoned.

The known ability of 2,4,5-T to cause chromosomal damage in some plants and the fact that in some animals it, or an associated impurity, results in deformed offspring when ingested during pregnancy, suggest that the plant and animal populations thus affected will be less able to cope with their environment.

All of these debilitations that I have been cataloging, and additional ones that I have not, do harm not only to the affected species, but, of course, thereby also to the ecosystems of which they are a part.

Since man is also a part of nature, I can bring out here once again for emphasis that there is strong reason to suspect that 2,4,5-T or an unavoidably associated impurity, the dioxin we have been hearing about this morning, 2,3,7,8-tetrachlorodibenzo-p-dioxin (or, by the way, a dozen or so closely related compounds all coming under the name of dioxin), are now known to be highly teratogenic. In other words, they result in malformed offspring when ingested during pregnancy. Until this issue is clarified, I think it should go without saying that the use of 2,4,5-T both domestically and in Vietnam be restricted to locations and amounts that would preclude its possible human ingestion.

Well, let me now make a few concluding remarks.

Senator HART. Doctor, I think it would be wise if we interrupt briefly for a recess. That was a signal that sounded for a vote. I think this is the time to suspend.

(Short recess.)

Senator HART. Doctor, with luck we will finish before there is another vote.

You were just about to begin with your conclusions.

Dr. WESTING. It is possible that I have been painting somewhat too grim a picture of the domestic use of 2,4,5-T. But I have no particular fears that detailed exposition of its safety and benefits can be left to the herbicide manufacturers and others. So, I figure that what I am describing here from the environmental standpoint is one side of the picture, and let the manufacturers tell us the other side.

Senator HART. Let me react to that, but very briefly. It is not inappropriate or a matter of surprise, nor in my book, should it be the basis of criticism, if the manufacturer of the product describes it in glowing terms if society and its government permits him to market it. If those responsible for the protection of the health of the society conclude that he can market the product with those claims, then why get mad at him? Why don't we get mad at the society's institutions?

Dr. WESTING. I agree.

Senator HART. You can't have it both ways, if I make myself clear.

Dr. WESTING. Yes, I certainly am in full sympathy with this. I think the burden falls upon our regulatory agencies.

Senator HART. Clearly.

Dr. WESTING. I don't think Dow is the culprit here at all. It is FDA and USDA, and so on.

Senator HART. This goes beyond the immediate product line we are talking about. This goes to the marketplace and the role of society in protecting itself, establishing regulations where needed, and enforcing them as established.

Dr. WESTING. I would certainly have to admit that the vast successes of productivity upon which our nation's current affluence hinges, depend to a large extent upon the use of pesticides such as 2,4,5-T. And it seems clear that the use of pesticides will continue, perhaps even unabated, without a highly unlikely downward trend in our population, and, even more particularly, in our collective desires and demands.

However, the time seems to be fast arriving when certain precautions must be taken so as not to overload our environment with such potent pollutants. A number of suggestions are thus in order to forestall the need for a basic change in our way of life.

First of all, research efforts should be expanded on several fronts. Effective cultural and biological controls of pest species should be sought and developed with renewed vigor. With respect to the pesticides themselves, highly selective and rapidly degrading ones should be aimed for.

In the light of the current 2,4,5-T affair, I must add here that all pesticides, existing and potential, must be rigorously tested prior to their general release for possible toxicity, carcinogenicity, teratogenicity, and mutagenicity to humans; and additionally, for possible adverse effects on livestock, on wildlife, on game, on fish, and on other components of the ecosystem.

With respect to 2,4,5-T, its use—in my considered opinion—must be limited to areas remote from human habitation. Control of vegetation on rights-of-way must be regulated with particular care since utility, transportation, and other rights-of-way are by their very nature frequently close to civilization. I want to emphasize here, Senator Hart, that one of the major uses of 2,4,5-T—one of its preferred uses—is in woody vegetation control along rights-of-way. This is a major place where 2,4,5-T is likely to impinge upon human habitation, to come in contact with civilization.

Broadcast applications, where safely remote from human habitation, should not exceed 3 pounds per acre; and where spraying covers extensive areas, unsprayed zones should be left as oases for wildlife, and so forth.

Repeat applications should be controlled, perhaps to intervals of 3 years or more. Aerial broadcast spraying should be avoided where possible, and always avoided near bodies of water, in favor of spot applications, or individual applications.

In those areas where aerial spraying is permissible, the highly volatile (though cheaper) formulations should be banned completely.

The low volatile formulations are not only more effective as herbicides, but they are also much safer with respect to the problem of drift and volatilization.

Aerial spraying should be confined to relatively windless periods (wind speeds of less than 5 mph) and to air temperatures of less than 85 degrees. Only nozzles equipped with course sprays should be used. The cleaning of spray equipment or the dumping of excesses near lakes or streams must be avoided; and getting rid of the empty cans and so on should be limited to sanitary land-fill dumps or similarly safe locations.

To insure all of the above, State and Federal regulations should be tightened both for manufacturers and users, and educational efforts increased with the aim of minimizing unnecessary or excess application. Our flagrant misuse in Vietnam should be halted immediately (see, e.g., my article in the Friends Journal of 1 April 1970).

Finally, I wish to stress once again the complex and as yet little understood nature of our environment. The study of ecosystems as such is still in its infancy. And since hormonal herbicides have been in general use now for only two decades or less, we simply are not yet able to predict the full range of potential disasters that their unrestricted use may inflict upon us and all other living creatures with which we share this small world.

Senator HART. Doctor, for all its brevity, this is a very helpful statement.

I have a couple of questions that I would like for you to react to.

You tell us in dry and cool conditions it may take well over a year for 2,4,5-T to degrade. I think you were here this morning. The Department of Agriculture is not in agreement with that statement.

Can you give us some evidence for your statement, or refer us to sources that are in agreement with your statement?

Dr. WESTING. To my knowledge, there has been precious little research done on the life of 2,4,5-T in the environment. I am aware of one study that was done in a forest environment in which it was shown that 2,4,5-T degraded to insignificance in a matter of several months, as I recall.

On the other hand, it has been well established, and it is clearly known, that 2,4-D—a compound similar to 2,4,5-T—degrades much more rapidly than 2,4,5-T. It has been demonstrated a number of times that under dry conditions, 2,4-D can persist in the environment and have detrimental effects for as long as a year or a year and a half after application. From this I infer that 2,4,5-T, which is more persistent than 2,4,5-D, would have at least a similar life under dry conditions.

Senator HART. Then, adopting your reasoning, it would mean that under those conditions, 2,4,5-T might be found on food that is served months after the spraying of the crop; is that correct?

Dr. WESTING. I have no direct information, but one could surmise that this could happen. This is a possibility.

Senator HART. What would you think the possibility of 2,4,5-T's capacity is to persist within the organism, plant or animal, which had ingested it, including the human?

Dr. WESTING. I have no first-hand knowledge on this whatsoever; so I prefer not to try to answer it.

Senator HART. You would agree that it is impossible to say it is not possible?

Dr. WESTING. The likelihood is there. As far as I know, it may persist, or even build up in the human body. Some other chemicals that are fat soluble (as are the ester formulations of 2,4,5-T) are known to deposit and be stored in the fatty tissue of humans; so, it is highly possible that 2,4,5-T does this, but I simply do not know whether it does or not.

Senator HART. You suggest that use of 2,4,5-T be limited to areas remote from human habitation, and that it should be restricted in other respects. Does that mean that you would feel that Dr. DuBridge's suggestion that pesticides be deregistered for food use, assuming there can be no tolerance level set by FDA doesn't go far enough?

Dr. WESTING. 2,4,5-T as it is commercially available with its impurities, is a substance that should not have any food tolerance at all. It should have zero tolerance, at least given the current state of knowledge.

Certainly, the suggestion made this morning by—I think it was Mr. Wellford—that its use should be curtailed severely, or suspended until we clarify this whole issue is one that I fully support. I think that 2,4,5-T is probably a safe chemical to use at the low, recommended doses in areas remote from human habitation. I don't think it need be banned under such conditions in the forest environment, or on range lands.

On the other hand, along power line rights-of-way, railroad rights-of-way, and so on, that get near houses, I think there should be severe restrictions.

Senator HART. What about proximity to crops?

Dr. WESTING. Food crops?

Senator HART. Yes.

Dr. WESTING. I think that certainly for the time being, it should not be registered for use on food crops and not be used near them.

Senator HART. In these areas that you have described where 2,4,5-T has been applied you have said that some of the birds and animals that depend on the plants that have been destroyed may be eliminated. Which birds and which animals are likely to be affected?

One way to answer that I suppose is any that are in that area, but I am trying to find out if some are and others are not affected.

Dr. WESTING. I wish I could give you some spectacular answer about bird *X* or *Y* having become extinct as a result of the use of 2,4,5-T, but I cannot. I can quote a recent statement made by a British authority on pesticides, Dr. N. W. Moore, director of the Monks Wood Experimental Station in England:

> The use of 2,4-D and 2,4,5-T to control scrub by roads and in woods reduces the essential habitat of almost all British land birds, which, because they are survivors of the original forest fauna, are still dependent on trees and bushes. (Advances in Ecological Research 4:108; 1967)

To judge from this statement Dr. Moore is concerned over the fate of the native British birds as a result of the routine use of these herbicides.

In this country there is an extensive program over many tens of thousands of acres in the West of sagebrush control in which herbicides of this nature are used, primarily 2,4-D. There is some evidence that the sage grouse population has been depleted at least the hunters are not as happy as they used to be.

I have to warn you, Senator Hart, this is one area where the herbicide proponents will jump up and say that there are a number of clear cases where the use of herbicides has actually benefitted wildlife populations.

Senator HART. I made an interjection earlier to say that if we are going to get mad at somebody let's get mad at ourselves first of all as a people for not recognizing dangers and setting down the laws that will prohibit the marketing of certain things, but equally true, of course, is that the producer is obligated, absent any explicit regulation, to make truthful representation about its product—again, I am thinking not of chemicals alone but anything—and report factually the experience that has come to his attention to whatever public agency there is that is expected to make the judgment for all of us as to whether that product in fact should be marketed. So, if they jump up and explain it is good for us, I hope, they will not do so unless they can explain why.

You noted, among other things, in your conclusion that we should expand research, attempting to develop other controls of pests. What development do you imagine would be fruitful?

Dr. WESTING. Well, the main thrust of alternatives to the use of insecticides has been to introduce predators or diseases of the insect pests. This same approach can also be used with herbicides such as 2,4,5-T. Plant pests are a little less amenable to this sort of an approach, but one could push ahead on research on possible virus diseases or fungus diseases or insect enemies of weed species.

I am familiar with one success story in this regard. A serious weed in the Northwest is St. Johnswort, and a beetle (*chrysolina*) has been introduced from Australia that feeds on the St. Johnswort, in a highly successful alternative to chemical herbicides. This general type of approach should be exploited to the greatest extent possible.

There are all kinds of other possibilities. Just in forestry, for example, closer spacing of crop trees shades out certain weeds. You can go back to a greater emphasis on some of the mechanical methods that are now avoided because of the high cost of labor: mowing, weeding (pulling out the weeds or cutting them down), burning. Flamethrowers are used in certain instances and even controlled fires. These methods have a much more selective effect on the actual weed and a minimum of lasting untoward side effects.

If chemicals are to be used, the forester's approach of individual application is far preferable to the utility and range manager's approach of broadcast spraying from the air.

So, there are a variety of alternatives available. With just the slightest amount of urging, the slightest realization that there is a necessity to worry, these alternatives would at least be explored. In the past it had never even been realized that there were possible ill side effects to the use of herbicides.

Senator HART. Now, you have lectured us quite thoroughly on the dangers inherent in changing the ecological pattern. Yet every one

of these alternatives that you talk about suggests similar dangers and some additional ones.

The Australian beetle is not native to the Northwest, I take it, but you are going to bring Australian beetles in. The flamethrower is not really an altogether acceptable——

Dr. WESTING. There is a history of introducing something to combat a pest and thereby introducing a worse pest, so there has to be some very careful preliminary testing and evaluating before this approach is used. With this in mind, it is safer to use something like a virus than it is to use something like an insect or a fungus because the virus one will be far more host-specific and therefore will not switch to an alternate host after it does its job and then become a pest in its own right. This is a danger that has to be kept in mind.

With regard to the pesticides, I suggested that we keep searching for much more highly selective ones. The problem with 2,4,5-T is that although selective in a certain sense it still is relatively unselective and kills lots of things that you do not want it to kill. This is the sort of thing that has to be watched out for.

Senator HART. I must admit that I got the impression clearly this morning that the existence of a realistic alternative to some of these things might help to convince the Department of Agriculture to take action; that is if they knew they had a realistic alternative, maybe the evidence which the Department now says is not sufficient to alarm them might have higher credibility.

I don't know whether I make myself clear.

Dr. WESTING. Yes; that is why it is important to mention that there are possible alternatives or at least that a goodly research effort should be aimed in that direction, to provide possible alternatives. We have come to depend upon the chemicals to such an extent that I think other possible control methods have become less interesting.

Senator HART. I think it should be said, and not necessarily as a direct criticism of anybody, but humans are humans and if there is some acceptable alternative for what would otherwise be a decision that would put a lot of heat on the fellow making the decision, it would be much easier to make and somewhat unconsciously perhaps the existence of an alternative might change the attitude of some of these individuals.

Mr. Bickwit.

Mr. BICKWIT. Part of your evidence for the persistence of 2,4,5-T under certain conditions for over a year stems from experiments establishing the persistence of 2,4-D. I think for the record we ought to have some reason why you can jump from evidence of the persistence of 2,4-D to conclusions about the persistence of 2,4,5-T. Can you meet the argument that the 2,4-D evidence might show that 2,4-D is just more persistent than 2,4,5-T?

Dr. WESTING. No, I think one could be on completely safe ground in saying that 2,4-D is considerably less persistent than 2,4,5-T. 2,4-D will degrade under normal, moist environmental conditions in a matter of weeks. 2,4,5-T is perhaps twice as persistent. There are a lot of studies to show that 2,4-D degrades more readily than 2,4,5-T; lots of short-term experiments have shown this. I am not familiar with any definitive long-term 2,4,5-T studies. It is very reasonable to assume that as long as 2,4-D will, under dry conditions such as you find in Idaho, have harmful effects on crops a year or more after use that 2,4,5-T would also.

Senator HART. Doctor, thank you very much. It was a helpful paper.

The signal a few moments ago indicated another roll being called in the Senate. I apologize to Dr. Kotin, but we will have to take another recess, and I will be back just as soon as I can get on the roll.

(Recess.)

Senator HART. The Committee will be in order.

Our concluding witness on this first day of hearing is the Director of the National Institute of Environmental Health Sciences, Dr. Paul Kotin.

STATEMENT OF DR. PAUL KOTIN, DIRECTOR, NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES

Dr. KOTIN. Mr. Chairman, I am privileged to be here today engaging in the practice of one of my most pleasant responsibilities—that of discussing the programs and plans of the National Institute of Environmental Health Sciences of which I am Director.

Our Institute is a newcomer in the executive branch; we have been in existence since 1966, achieving the status of National Institute of Environmental Health Sciences only in January 1969.

This activity started as a small segment of the Department of Health, Education, and Welfare's effort in environmental health in response to recommendations made by several public advisory committees during the late 1950's and early 1960's. These committees—starting with one chaired in 1958 by Dr. Stanhope Bayne-Jones and concluding with one headed in 1965 by Dr. Detlev W. Bronk—repeatedly emphasized the necessity of establishing within the Public Health Service an organization dedicated to performing fundamental research into the real and potential effects of human health wrought by a rapidly changing environment.

The decision of the Surgon General in 1966 that this research program be located within the National Institutes of Health—that Federal agency responsible for building the Nation's base of fundamental biomedical, health-related research—made clear the mission envisioned for our program. That mission was and is:

First, to determine the magnitude and significance of the hazard to man's health from long-term exposures to low-level concentrations of chemical, physical, and biological agents in the environment; and second, to elucidate the underlying mechanisms of adverse response with the hope that principles and generalizations would be identified to provide a scientific base for criteria upon which control agencies could set standards for protective and preventive measures.

During the present (1970) fiscal year, Congress and the President have authorized $17,730,000 to be expended in the conduct of this program.

Since you may be familiar with other programs of the National Institutes of Health, I would like to take just a moment to point out

to you some ways in which we are similar to other parts of NIH and some ways in which we differ. I might preface this by saying that we are similar to other NIH research components in more ways that we are different.

Like the other research institutes of NIH, our mission reflects two very important principles of operation: (1) We are in business primarily to add to the fundamental knowledge and understanding of environmental agents which as biomedical hazards immediately or ultimately affect human health.

In other words, we are concerned about the what and how of health effects first and foremost in human beings. That we must also understand that what and how of the complex constituents of our environment in order to perform the primary task is obvious.

Nevertheless, it is the results in humans which is of overriding concern to us. (2) The responsibility for taking direct action to control or eliminate the hazards which we must identify resides in other components of HEW.

I hasten to emphasize that we do not consider our job done until our findings are made available to the appropriate components of Government. To accomplish this, we maintain effective, close, and continuing relationships with the Environmental Health Service, the Food and Drug Administration, the Department of Interior, the Department of Agriculture, the Federal Trade Commission, and other agencies with control responsibilities.

The reasons for the distinction between fundamental research and control powers are, I think, important. First, the urgency in the need for control measures requires research directed to answering today's questions with today's techniques.

There is, however, an equally, or perhaps more, important need for research directed to questions having long-range implications extending for decades and perhaps even generations into the future. It is in response to this need that our Institute's program is designed.

While techniques frequently used in attacking these two sets of questions are similar, the orientation and end points stand in sharp contrast.

Second, freedom from control activities permits us to devote our total effort to research.

Third, control activities are performed by experts in an environment in which the guidelines for operation are completely dedicated to this responsibility.

Fourth, our relationship with industries, communities, and individuals is one based exclusively on scientific grounds rather than one of regulation, monitoring, and enforcing.

Finally, our inputs to control agencies are objective and provide an impartial basis for the very real practical considerations which must be faced in formulating and inaugurating control measures.

As noted, the fruits of our work are promptly forwarded to appropriate Government agencies for use in the pursuit of their mission with virtual simultaneous publication in professional journals rather than in the popular press.

This practice assures that our findings are subject to the scrutiny and critical review of other researchers who have an opportunity to question our methods and conclusions by usual stringent standards.

I hope that the preceding discussion has placed the National Institute of Environmental Health Sciences in perspective for you.

I would now like briefly to tell you in somewhat greater detail some of the things we are doing, why we are doing them, and how we come to be involved in the resolution of the problem which is the subject of these hearings.

Speaking quite broadly, the NIEHS program attempts to employ a wide spectrum of scientific disciplines and bring them to bear on real and potential human health problems resulting from:

1. Changes in the makeup of the environment in consequence of technological progress and industrialization;
2. Changes in the size and characteristics of the population; and
3. Changes in the character of interactions between these two.

In order to best understand the significance of changes in the makeup of the environment, we employ the disciplines of analytical and synthetic chemistry, pharmacology, and of biophysics.

In order to better understand our changing population and the subtle interactions of new and changing environments on people, we employ the sciences of epidemiology, biometry, pathology, and toxicology.

In order to establish the mode and mechanisms of interactions, we employ all categorical divisions of scientific inquiry with special emphasis on comparative biology to assure maximum relevance of research data to man.

These varied resources and methods have so far been brought to bear in programs studying the potential health hazards of:

Natural products including fungal contaminants of food; fibers and polymer dusts, asbestos and fiberglas; alpha radiation; trace metals (such as lead) and their compounds; hydrocarbons and their reaction products; tobacco smoke; and pesticides and pesticide synergists (including herbicides).

In all of these studies we are concerned with the effects of long-term exposures to low levels of concentration because these are the usual characteristics of exposure during life in the environment we have created for ourselves.

Effects are likely to be gradual in appearance, and most commonly the result of interactions of numberous agents combining in additive, synergistic, or antagonistic manners.

To dissect these complexities we must identify interactions at all levels from the intracellular organelle to the whole organism.

Our goals include determinations of threshold for response, effects of repetitive exposures, effects of storage of the agents in living organisms, and the roles of such host factors as age, sex, antecedent or concurrent illness, nutrition, behavioral characteristics, and genetic make-up.

It may seem that our approach is somewhat complex, but it must be so in order to resolve the complex problems wrought by the changes in our environment intrinsic to technological progress.

We have attempted, in the process of establishing the program of the Institute during the past 3 years, to maintain a measure of flexibility amid this essential complexity to provide for response to

unanticipated problems. Our current efforts in response to concern over the widening use of herbicides is in a way a case in point.

You are aware, I am now certain, that the recently completed study which revealed information about the toxicity of the herbicide 2,4,5-T, in fact, was initiated by the National Cancer Institute in 1963.

As indicated earlier, our Institute was not in existence at that time. However, I was the scientific director for etiology in the Cancer Institute at that time, and along with members then and now on my staff played a leading role in the initiation of the research contract with Bionetics Research Laboratories, Inc., which yielded the information under discussion.

Very briefly, that study was undertaken primarily to identify any potential carcinogenic (cancer causing) or teratogenic (birth defect causing) agents in a wide variety of pesticides and allied compounds in commercial use.

We also anticipated that the study would provide data on which to develop improvements in our methods for identifying carcinogenic agents and hopefully identify any correlations that might exist between the carcinogenic and teratogenic capabilities of single specific compounds.

Pesticides were selected for inclusion in the study on either of two bases; First, a projection of the potential extent of their use in terms of their utility in the community; and; second, a judgment as to potential carcinogenicity by virtue of chemical structure or metabolic fate.

In consequence, some 86 pesticidal products—including insecticides, fungicides, and herbicides—were subjected to controlled, long-term studies on mice. As had been intended from the start, the study continued through the 1960's.

In the interim, the then Division of Environmental Health Sciences was established, and I was asked to become its first director. In agreeing, I was granted approval to take with me one or two key staffmembers—scientists, as it happened—who had also been associated with the Bionetics contract.

Since intensive programing and developmental responsibilities faced my staff and me during the first years of our Institute, we were quite satisfied to leave the management of the Bionetics pesticide study in the able hands of our successors in the Cancer Institute. Furthermore, it should be recalled that the one major basis for the study was quite clearly related to the mission of the Cancer Institute, the identification of cancer-causing agents in the environment.

Upon completion of the study in early 1969, the Cancer Institute released the results of the study. The results of the teratogenic studies were released to the Mrak Commission immediately as they became available. The popular press took intense interest in the findings reported, and pressures developed for more complete information on several of the pesticides included in the study.

The herbicide 2,4,5-T came under special scrutiny because its use is especially widespread, particularly in military operations in Vietnam. Word that the Bionetics study had shown this chemical compound as "causing significantly more deformities than expected" was especially alarming in some quarters.

Dr. Endicott, then director of National Cancer Institute, requested that NIEHS staff familiar with the study in question, and also familiar with teratogenicity and pesticide chemistry generally, be assigned to data analysis and interpretation. NIEHS assumed sole responsibility for the statistical analysis of the very large volume of data.

During the early stages of the now public discussion, it was pointed out by the Dow Chemical Co., a major supplier of 2,4,5-T, that the materials used in the Bionetics study were significantly different than those which had been supplied by Dow since 1965.

It is certainly true that the 2,4,5-T used in the study contained significantly larger amounts of an impurity, dioxin. This impurity is highly toxic and its presence occurs incidental to minor alterations in the reaction conditions during the manufacture of 2,4,5-T.

Dow Chemical Co. scientists contended that it was the dioxin derivative rather than the 2,4,5-T which had caused the deformities in test animals. A sample of the original 2,4,5-T used in the Bionetics study was analyzed and was found to contain 30 parts per million of this dioxin compound.

In consequence, it became necessary to restudy the situation to see whether the virtually no-longer-existing impurity in 2,4,5-T could be held responsible for the adverse effects.

In order to verify the possible role of dioxin, NIEHS brought its available resources to bear and undertook an accelerated program of research.

Pure 2,4,5-T—and by pure, I mean that which is now in the marketplace with a dioxin concentration of less than one tenth of a part per million—has been made available to us and recently we received the dioxin in pure state so that experiments can be repeated with the pure material, as well as with a combination of the two ingredients.

These studies are now underway. As indicated in prior discussions with the subcommittee staff, the results of this research are not yet complete. At such time as they are, in the very near future, we will be pleased to supply them to this committee.

I would be happy at this time to answer any questions of the committee regarding the mission of NIEHS or the circumstances leading to our current study of 2,4,5-T.

Senator HART. Thank you, Doctor. It was thoughtless of me—I should have suggested, since you commented on having a sore throat before, that you not read the statement, but merely put it into the record.

But I think as long as you were able to get through it, it helps all of us to hear it, rather than waiting for the printed record.

On this business of the study, do you know when the National Cancer Institute received its first data from Bionetics suggesting that 2,4,5-T was teratogenic?

Dr. KOTIN. I can't tell you offhand, but I would be very happy to get it for the record, sir.

(The information was subsequently received for the record:)

"In June of 1966, we received the first data indicating that 2,4,5-T administered by injection at a dose of 113 mg/kg of body weight produced teratogenic effects. In May of 1968, data indicated teratogenic results from oral administration of 2,4,5-T at a dose of 113 mg/kg of body weight."

Senator HART. We would appreciate that, and it will be made a part of the record. I am under the impression that it was sometime in 1966. In a sense I guess that's about the time you departed the premises?

Dr. KOTIN. Exactly.

Senator HART. Let's assume that the date is June 1966, that being the time the first data was received from Bionetics by the Institute. Do you recall when the final report came out?

Dr. KOTIN. Yes, the final report, in 1969—late 1968 and early 1969, as I recall. A little over a year ago, as I recall.

Again, I can't be sure of that, but I would be pleased to get the exact date. I had left the Institute.

(The information was subsequently received for the record:)

"Bionetics supplied a draft "final" report in September of 1968. Questions raised by NIH required additional work by Bionetics and subsequent revisions of the report. Bionetics completed this work and submitted a truly "final" report in September of 1969."

Senator HART. The NIEHS report—when did that come out?

Dr. KOTIN. The final report was last fall, when we were providing the results of our statistical analysis, and the data on the teratogenicity to the Mrak Commission.

Senator HART. If it develops that the June 1966 date is the time that the National Cancer Institute got its first data from Bionetics, and the final report by NIEHS came out in the fall of 1969, why in the world did it take so long to come up with the information for that final report?

Dr. KOTIN. I really can't answer that, other than to say that at the time the National Institute of Environmental Health Sciences was asked by Dr. Endicott to provide the statistical and analytical competency for the review of the data, the work was done very promptly. In fact, we didn't even wait until the end of the report to make the information available to the Mrac Commission.

As each little increment of information that represented a part of the total became available, this was made immediately available to the Mrak Commission, and the Food and Drug Administration.

Senator HART. I am trying to get these dates clearly fixed, if I can. You state that NCI released the results of the study in early 1969. Was this the preliminary report of Bionetic's findings?

Dr. KOTIN. No, sir; this, I think, represented the first report in which conclusions were published, both in the scientific literature and in the Journal of the National Cancer Institute, as well as made available to the various responsible government agencies.

The really important aspects of the conclusions, the necessity for voluminous work—there were some 86 compounds—the National Cancer Institute justifiably felt that in-house staff should at least on a random basis review the data. There was much, much new information that heretofore had been unknown. And just the histological review of the slides from the autopsied animals, the statistical analysis of data from a series of experiments in which multiple species were used, multiple doses were used, were terribly time-consuming.

So that all I can do is vouch for the commitment of resources it took from the National Institute of Environmental Health Sciences to do its little share, provide its little share of the total.

Senator HART. Doctor, I am going to ask Mr. Bickwit to continue with these questions. We have reviewed them prior to the hearing, and I will remain, using the time to read a memorandum that explains what this vote that was just signaled is all about. I hope by the time he finishes, and I finish this, we will have the answers.

Dr. KOTIN. I hope I don't disturb you.

Mr. BICKWIT. I'm frankly not clear on the major dates that are involved here, the dates that you received the Bionetics information, the date that you came out with your first report on it, and the date that you came out with your final report on it.

Now, if I'm right in thinking that those are relevant dates, could you tell me what those dates are?

Dr. KOTIN. Right. Well, the dates are relevant. I think it was, again, the date I offered for the record, which I don't remember offhand, is the date the Cancer Institute received the Bionetics report.

You will recall Dr. Falk and our associates instigated the Bionetics study, and it wasn't a personal contract with us. It was with the Cancer Institute.

So the report went to the Cancer Institute and I don't know when they received that.

Fundamentally, the only reason I suspect that we would have gotten involved at all in terms of the Bionetics report, as distinct from our own commitment by virtue of our mission in this, was the fact that Dr. Endicott did have a need for a tremendous amount of statistical and chemical analytical competency, and it was more than he had available in the Cancer Institute.

So I can give the date at which the material was forwarded to us. This was in 1968, and again, I will get the date for the record. But it was—actually, the material was forwarded to us coincidental with the request to get involved with some of the analyses.

Mr. BICKWIT. About when in 1968?

Dr. KOTIN. I will be happy to give you the exact date for the record, sir.

(The information was subsequently received for the record:)

NIEHS performed analyses of the raw data between January and June 1969.

Mr. BICKWIT. Then you released reports periodically?

Dr. KOTIN. To the Mrak Commission only, and to the relevant Government agencies.

Mr. BICKWIT. About how many reports were there?

Dr. KOTIN. These were not formal reports, but they were presented quite informally—we finished the analysis of the White Swiss Mouse data, the C–57 black data, the DBA data.

We checked the statistical significance of the differences between test and controls, between the various dose levls, between the various modes of admission. So that, rather than adorn the data with

[redacted]se, we just gave them the statistical material with the listing [redacted] the conclusions.

Mr. BICKWIT. So, whenever you had anything of any importance, it went to Mrak.

Dr. KOTIN. Promptly.

Mr. BICKWIT. Your final report came out in the fall of 1969, is that right?

Dr. KOTIN. Yes, we have submitted a paper for publication in the journal *Science* which relates our analysis on the teratogenicity of 2,4,5-T, and it should be appearing shortly.

Again I would be happy to make a preprint copy of the manuscript available for the Committee if you desire.[1]

Mr. BICKWIT. Thank you. That would be fine.

Now, if you got your information sometime in 1968, and we don't know when, let's assume it was late 1968, and it took until the fall of 1969 to come up with a final report, why did it take that length of time?

Dr. KOTIN. Just the difference between the magnitude of the job and the availability of professional resources within our institute. At that time, our Biometry branch consisted of two professional biometricians at the doctorate level. This staff was involved in a series of studies including one on the relationship of asbestos to lung cancer, and another on a quantification of the hazard to uranium miners. This limited staff had to be literally redeployed in order to perform the necessary analyses of the Bionetics data.

Mr. BICKWIT. On the carcinogenicity studies, when did you get the information from Bionetics?

Dr. KOTIN. We really didn't, other than as information. It came as part of the same report. But the analysis of the carcinogenicity study remained entirely within the Cancer Institute, since it was clearly relevant to their mission and responsibility as the National Cancer Institute.

Mr. BICKWIT. You were not responsible for analyzing that?

Dr. KOTIN. No, sir.

Mr. BICKWIT. You have stated the results of the teratogenicity studies were released to the Mrak Commission immediately when they became available. I am sure you are familiar with Mr. Whiteside's allegation that Dr. Samuel Epstein of the Mrak Commission had a great deal of difficulty acquiring information on the studies.

I wonder if you could reply to this allegation? If you are not familiar with it——

Dr. KOTIN. I am familiar with the allegation. I read it in the story in the New Yorker, of course.

No, I think that we are probably speaking of two different things. There was, at no time, the necessity for the requesting of any information from us. There was a mechanism for the forwarding of the information to the Mrak Commission; the best evidence that this allegation is not so in another sense is that the head of our Biometry Branch, Dr. David Gaylor, was on the Mrak Teratogenicity Committee, the very committee to which the data were being supplied.

[1] See p. 98.

So, essentially it would be denying his own data to [redacted]own committee if this were so.

Do you follow me.

Mr. BICKWIT. I am sorry, I don't.

Senator HART. I am going to have to interrupt again, I am sorry. I hoped we could avoid the necessity of holding you, but I will miss the vote.

I will not be able to return as promptly as I like, because I must remain on the floor to get something done, a matter that will be voted on tomorrow.

So, we will have to recess in the very unhappy condition of not knowing exactly when I will get back, but as quickly as I can.

(Recess.)

Senator HART. We will resume, and with better luck than we have been having in the last hour or so, maybe we can conclude before the next vote is signaled.

Mr. BICKWIT. I believe the last statement which you made I had some difficulty with.

Dr. KOTIN. What I was saying was that Dr. Epstein and Dr. Gaylor were on the same teratogenicity panel of the Mrak Commission, and each meeting they held Dr. Gaylor brought the data up.

So the only information Dr. Epstein might have asked for that he did not get were data that just were not complete. But certainly in relation to the teratogenicity, I cannot conceive of any available data that would not have been made available.

Mr. BICKWIT. Was the final Bionetics report made available to the Mrak Commission when they asked for it?

Dr. KOTIN. It is my impression that it was. And again they would not have come to us, because the final report was the property of the National Cancer Institute, as the contracting institution.

Mr. BICKWIT. If they did come to you, would you have had authority to give it to them?

Dr. KOTIN. Actually, I suspect I would have picked up the phone and asked Dr. Endicott who was responsible, and I would have gotten authority for it because the information contained in it was germane to the Mrak Commission. But again I would emphasize that the final report of any contractor would not include the interpretation and the analysis of the data. This was not part of the purchase.

Mr. BICKWIT. I realize that, but if Dr. Epstein of the Mrak Commission had asked you for the final Bionetics report, without an analysis from NIEHS, you would have furnished it to him immediately?

Dr. KOTIN. I would have furnished it to the Mrak Commission.

Mr. BICKWIT. Would you not have furnished it to Dr. Epstein?

Dr. KOTIN. The data itself?

Mr. BICKWIT. Yes.

Dr. KOTIN. Uninterpreted?

Mr. BICKWIT. Yes.

Dr. KOTIN. Oh, I probably would not have, no.

Mr. BICKWIT. Why not?

Dr. KOTIN. Essentially the data are crude data that require interpretation, and essentially the implications, the results of the report, are the conclusions, and the responsibility for those conclusions would have been ours,—that is, the responsibility of the NIH.

Mr. BICKWIT. These data, I understand, did raise doubts, about the teratogenicity of 2,4,5-ff.

Dr. KOTIN. You mean, rather than raise doubts, established the experimental teratogenicity of this. After the data were analyzed, yes.

Mr. BICKWIT. You are saying that you do not believe that a member of the teratology panel of the Mrak Commission should have the right to examine those data unalyzed?

Dr. KOTIN. Oh, not at all. All I am trying to say is the data themselves, short of total package, once the data were analyzed, and conclusions made, then by no stretch of the imagination would the date be withheld from anybody.

Mr. BICKWIT. But unanalyzed, he should not be entitled to look at them?

Dr. KOTIN. I do not think so, no, sir.

Mr. BICKWIT. Should anybody other than the organization entrusted with the analysis of the data be entitled to look at them?

Dr. KOTIN. Oh, surely. Mr. Hart's Committee, or there are a whole spectrum of responsible agencies.

Mr. BICKWIT. Could you list those agencies that would be entitled to look at this data?

Senator HART. You are inquiring about before analysis?

Mr. BICKWIT. Yes.

Dr. KOTIN. The hierarchy above me, as a lowly director of an institute, the director of NIH, The Surgeon General, the Secretary of HEW, all of the way up, any member of the legislature, any member of the executive branch, with the authority, surely.

Mr. BICKWIT. But you would not want to allow a nongovernmental scientist with some expertise in the field to look at this data?

Dr. KOTIN. Again, there is no flat yes and no. There are many instances when we call people in nongovernmentally to look.

Mr. BICKWIT. What I am asking you to do is draw the line. I know it is hard, but you have excluded one nongovernmental scientist. I would like to know how you formulate your opinion in deciding who should be excluded and who should be included.

Dr. KOTIN. That is a matter of judgment. How much help I think we can get from them, how much help we can provide them.

Mr. BICKWIT. Is that the only basis for your decision?

Dr. KOTIN. I would have to think. I suspect that is the major one. We have crude data and what we try to do is get the best expertise. We have everything from advisory committees to councils to study sections to consultants to the institutes, who are not Government employees, who are on call at all times and who are used rather consistently, particularly by a young institute like our own, (we are 3 years old; our $17 million budget, when contrasted with the $150-plus million budget of the larger well established institutes is probably as good an indication of our size as anything.)

I think a corollary of our small size is the great consistency with which we get outside help in terms of consultation. We just had a task force that spent 3 weeks preparing a consultative guide, as it were, for the Institute. So there is no tendency on our part at all to treat anything that we get as either clandestine or in any way not open to scrutiny. In fact, as I said in my testimony, I made a special point that scientific scrutiny is something that we insist on in all of our data before we accept it as fact.

As our critical mass at NIEHS enlarges, we will probably be more certain. But we are a small outfit and we use outside consultants a lot. So in answer to your question specifically do I feel categorically that data should not be seen by outside scientists, not at all. There are instances where you call them in and they see it initially with us, as it were, around the table for the first time.

Mr. BICKWIT. On the pro side you are weighing the potential helpfulness of the scientist who would be asking to see the data.

Dr. KOTIN. Oh, no. Also what he can contribute to the maximum utilization of the data. In the years I have been in NIH when there are implications of the data that affect other executive branches, or have socioeconomic implications, the people who you try to get help from and provide help to are judged on an individual basis. This is really so.

Mr. BICKWIT. What is on the other side? In formulating your opinion what is it that would keep you from giving the information out?

Dr. KOTIN. Number one, concern over data where the interpretation would be such that we would want our interpretation to be on the record at the time the data were made available. That would be one example.

Another example, where there is some question we have about the data ourselves, so we want to go back and verify techniques, verify the workbooks from which the reports were made. And in fact this was done in this case. So there are lots of reasons. Not as many as on the other side, but you just have to do it on an individual basis, decide what is the best way to get maximum returns from the data.

Mr. BICKWIT. With respect to Dr. Epstein, a member of the teratology panel of the Mrak Commission, would you rule out the possibility of his being able to contribute to the utilization of this material?

Dr. KOTIN. Yes.

Mr. BICKWIT. Could you elaborate on that?

Dr. KOTIN. Fundamentally it is a matter of judgment. I felt at that time that the data themselves needed analysis for the reasons I mentioned, that the conclusions were integral to the data because again the mere fact that you had chi square indicated there that the significance was in large measure determined by statistical methods.

It wasn't a situation where, as the data amply attest, an all or none response occurred, where all of the controls did one thing, all of the test animals did the other. There were statistical differences. There were differences in degree and intensity and in time. These had to be determined by statistical techniques.

Senator HART. Doctor, I will be brief in my thanks, since I am under the compulsion of another vote signal.

(The information referred to on p. 94 follows:)

Teratogenic Evaluation of 2,4,5-T

Abstract

The herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) has been shown to be teratogenic and fetocidal in two strains of mice using either subcutaneous or oral routes of administration, and in one strain of rats by oral administration. The incidences of both cystic kidney and cleft palate were increased in the C57BL/6 mice as well as the incidence of cleft palate in the AKR mice. The incidence of cystic kidney was also increased in the rats. In addition, an increase in liver to body weight ratio in the mouse fetus and the occurrence of hemorrhagic gastrointestinal tract in the rat fetus suggest that this compound also has fetotoxic properties.

The chlorinated herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) is used extensively for weed control (1). However, there have been relatively few reports concerning its pharmacologic and toxicologic properties in animals (2,3). Indeed, there are no data available concerning the effects of this compound on the developing embryo and fetus. Therefore, this report evaluates the teratogenic and fetotoxic potential of 2,4,5-T in mice and rats (4).

Breeding colonies of C57BL/6 and AKR strains of mice were established at Bioneties Research Laboratories, Inc., to supply the mice. For the study, breeding was by random mating. Detection of a vaginal plug indicated day zero of pregnancy. Rats were procured from the Holtzman Co., with known insemination dates. Detection of sperm indicated day zero of pregnancy. All animals received chow and water *ad libitum*.

2,4,5-T (5) was administered by one of two routes, subcutaneously or orally. A solution of 2,4,6-T in 100% dimethylsulfoxide (DMSO) in a volume of 100 µl/mouse was used for each subcutaneous administration. For oral administration by gastric intubation, 2,4,5-T was suspended in a honey solution (honey: water, 1:1) and volumes of 100 µl/mouse and 200 µl/rat were used.

In the studies with the C57BL/6 strain, 2,4,5-T was administered daily beginning on the sixth day of pregnancy and continuing through the 14th day or from the 9th through the 17th day. The mice were sacrificed on the 18th day of gestation for examination. In the studies with the AKR strain, 2,4,5-T was administered daily beginning on the 6th day of pregnancy and continuing through the 15th. These mice were sacrificed on the 19th day of gestation. The rats were treated on the 10th through the 15th and sacrificed on the 20th day of gestation.

Upon sacrifice both mothers and fetuses were examined carefully. In addition, about one-third of the mouse fetuses were stained with alizarin red S in order to detect skeletal anomalies.

Tables 1 through 3 contain data on fetal mortality, abnormal litters, abnormal fetuses per litter, fetuses with cleft palate, fetuses with cystic kidney, maternal weight gain, and maternal and fetal liver to body weight ratios. The following conventions were observed in compiling these data. If a fetus was either dead or resorbed, it was regarded as a dead fetus. A fetus was classified abnormal if it was alive and had at least one anomaly (regardless of type). Similarly, a litter was classified as abnormal if it contained one or more abnormal retuses. A fetus was said to have a cystic kidney if at least one of its kidneys was affected. In calculating the maternal liver/body weight ratio, maternal body weight was defined as the difference between the weight of the animal on the day it was sacrificed and the gravid uterus weight. Finally, the maternal weight gain was defined as the difference in the corrected maternal body weight on the day it was sacrificed and its weight on day zero of pregnancy.

The percentages for fetal mortality, abnormal fetuses, fetuses with cleft palate and fetuses with cystic kidney were computed by first obtaining the percent for each litter and then calculating the average of these percentages.

The percentage of abnormal litters provides a measure of the prevalence of abnormal fetuses across litters, while the percentage of abnormal fetuses per litter gives an indication of the prevalence of abnormal fetuses within litters.

In this report, the control animals are those that were on a large study during the 3-year time period in which 2,4,5-T was evaluated. The data from the DMSO and honey treated control groups were compared with the data for the non-treated control group. Then the results from animals treated with 2,4,5-T in either DMSO or honey were compared to the appropriate control data. Standard corrected 2x2 chi-square tests (6) were used to compare the results of 2,4,5-T treated animals with the appropriate control data for the proportion of litters containing abnormal fetuses.

The distribution of the percent of abnormal fetuses per litter for 2,4,5-T treated litters was compared with the appropriate control distribution by use of the non-parametric Mann-Whitney U-test (6). Also, this test was used for comparing the percent fetal mortality, cleft palate, cystic kidney, and enlarged renal pelvis per litter. This test requires that the proportion of dead or abnormal fetuses per litter is independent from litter to litter, but requires no assumption about the frequency distributions of these proportions.

Initial analyses of the data indicated that occurrences of anomalies among fetuses within litters were correlated. That is, anomalies were not randomly distributed across all litters but tended to cluster within litters. Many litters possessed no anomalies whereas all of the fetuses in some litters were abnormal. Since fetuses within the same litter tend to be more alike, pooling the data across litters before performing statistical tests is not appropriate. The experimental unit (7) is that entity to which treatments are applied, in this case the pregnant animal. Hence, all calculations of averages and all statistical tests were performed on the independent responses of the experimental units (litters).

The administration of DMSO or honey to mice or rats did not adversely affect the development of the fetuses. The incidence and type of naturally occurring anomalies observed in the DMSO and honey treated animals did not show an increase compared to the non-treated group. The alizarin stained fetuses of the control mice showed very few skeletal anomolies. No skeletal anomalies were detected by staining in the treated mice. For both mice and rats, there were no differences in the average number of implantations in the control and experimental litters. A few values for treated animals were less than those of their appropriate controls. None of these differences were statistically significant including the 3% fetal mortality observed in the C57BL/6 mice receiving a 21.5 mg/kg dose of 2,4,5-T reported in Table 1. This value of 3% reflects a period of low fetal mortality (9%) observed in the control mice during the initial few months of the study. This difference in mortality is not statistically significant. There were no other significant changes in these control data during the 3-year period.

As shown in Table 1, the administration of 2,4,5-T to C57BL/6 mice on days 6–14 at a dosage level of 113 mg/kg produced significant increases in percent of abnormal litters and percent of abnormal fetuses per litter. The anomalies produced by 2,4,5-T were almost exclusively cystic kidney and cleft palate. Similar results were obtained regardless of whether the compound was administered subcutaneously or orally. A dosage level of 46.4 mg/kg administered orally did not produce a significant increase in fetal mortality or an effect on palatal development, but did cause a significant increase in the percentages of fetuses with cystic kidney. Administration of 2,4,5-T subcutaneously at a dosage level of 21.5 mg/kg did not affect the visability or development of the fetuses. Thus, a dose-response relationship for the fetocidal and teratogenic properties of 2,4,5-T in mice is suggested for both routes of administration.

It was also observed that in mice treated with 2,4,6-T on days 6 through 14, there was a significant decrease in the incidence of naturally occurring anomalies. These consist of microphthalmia followed by anophthalmia and are in accord with other C57BL/6 colonies (8). Although the fetuses from mice treated on the 6–14th days had fewer naturally occurring anomalies, the fetuses from mice treated on the 9th to 17th days did exhibit these anomalies. Thus, it appears that the interval of days 6 to 9 of gestation is one of the sensitive periods of development with respect to 2,4,5-T. Two other sensitive periods are during development of the palate and kidney since they are so highly affected. The occurrence of these two anomalies are statistically unrelated.

In the study where 2,4,5-T was administered on the 9th to the 17th day of gestation with the C57BL/6 mice, maternal and fetal liver weights were determined. As seen in Table 2, this treatment produced a significant increase in maternal and fetal liver to body weight ratios. The significant increase in fetal liver to body weight ratio reflects both an increase in fetal liver weight and a decrease in fetal body weight. The significant increase in the liver to body weight ratio suggests a change in activity of drug metabolizing enzymes of the

endoplasmic reticulum which has been studied (9). Again, the Mann-W[illegible]ey U-test was used to compare the animals administered 2,4,5-T with the appropriate DMSO control mice.

Thus, in the C57BL/6 mice, 2,4,5-T is fetocidal, teratogenic and capable of producing an increase in the liver to body weight ratios.

Treatment of mice of the AKR strain with 2,4,5-T in honey produced a significant increase in fetal mortality. The incidence of cleft palate was increased with both routes of administration. However, 2,4,5-T did not produce an increased incidence of cystic kidney in this strain. There was no effect of 2,4,5-T administration in this strain on the maternal weight gain with either route of administration. However, the maternal liver to body weight ratio was increased using either route of administration.

In addition, hybrid litters resulting from mating C57BL/6 females with AKR males were evaluated. The administration of 113 mg./kg in DMSO from days 6 through 14 of gestation produced a high incidence of both cystic kidney and cleft palate. There was no effect on maternal weight gain.

The oral administration to rats of 2,4,5-T at a dosage level of 10.0 or 46.4 mg/kg on the 10th through the 15th day of gestation produced a significant increase in fetal mortality (Table 3). The two lower dosage levels, 4.6 and 10.0 mg/kg produced a significant increase in the percentage of abnormal fetuses. These fetuses displayed a high incidence of cystic kidney. At the highest dose level, 46.4 mg/kg, the marked increase in fetal mortality reduced the population of live fetuses to a small sample. However, cystic kidneys were observed. In a limited study, the administration of 2,4,5-T at dosage levels of 21.5 or 46.4 mg/kg from the 6th through the 15th day of gestation was highly fetocidal.

At all dosage levels studied in the rat, hemorrhagic gastrointestinal tracts were observed in the fetuses. The percentages of fetuses per litter with hemorrhagic gastrointestinal tracts showed a dose-reponse relationship; i.e., 3%, 50%, and 83% at doses of 4.6, 10.0 and 46.4 mg/kg, respectively. None were observed in the fetuses from the control animals. Drill and Hiratzka (2) have reported that dogs which received 2,4,5-T in the diet showed some necrosis and inflammation of the intestinal mucosa. The hemorrhagic gastrointestinal tracts observed in the rat fetuses is probably a toxic effect of 2,4,6-T on the fetal organ as opposed to a developmental defect.

In conclusion, these studies show that 2,4,5-T adversely affects the development and viability of the mouse and rat fetus.

TABLE 1.—TERATOGENIC EVALUATION OF 2,4,5-T IN MICE

Compound	Vehicle	Dose (mg/kg)	Number of litters	Average number live fetuses/ litter	Percent fetal mortality/ litter	Percent abnormal litters	Percent abnormal fetuses/ litter	Percent of fetuses per litter with— Cleft palate	Cystic kidney
C57VL/6 STRAIN TREATED DAYS 6–14									
Nontreated	None	None	72	5.8	26	38	11	<1	1
Control	DMSO	None	106	5.5	29	42	12	<1	2
Control	Honey	None	32	7.1	15	41	14	0	1
2,4,5-T	DMSO	21.5	6	7.7	3	50	12	0	0
2,4,5-T	DMSO	113.0	18	4.4	42	[3] 86	[3] 57	[3] 22	[3] 41
2,4,5-T	Honey	46.4	6	8.5	8	[2] 100	[2] 37	2	[3] 33
2,4,5-T	do	113.0	12	4.8	[1] 47	[3] 100	[3] 70	[3] 23	[3] 48
C57BL/6 STRAIN TREATED DAYS 9–17									
Nontreated	None	None	8	5.1	36	71	31	0	7
Control	DMSO	None	10	6.1	23	30	8	0	0
2,4,5-T	DMSO	113.0	10	7.7	11	[3] 100	[3] 77	[3] 29	[3] 60
AKR STRAIN TREATED DAYS 6–15									
Nontreated	None	None	58	7.1	16	19	5	<1	<1
Control	DMSO	None	72	6.9	15	24	4	<1	<1
Control	Honey	None	12	8.8	9	0	0	0	0
2,4,5-T	DMSO	113.0	14	6.9	23	[3] 71	[3] 29	[3] 28	1
2,4,5-T	Honey	113.0	7	5.3	[1] 42	[3] 100	[3] 55	[3] 55	0

[1] Statistical Significance Level = 0.10; [2] Statistical Significance Level = 0.05; [3] Statistical Significance Level = 0.01.

TABLE 2.—LIVER WEIGHT STUDY: 2,4,5-T ADMINISTERED DAILY AT 113 MG/KG SUBC[illegible]EOUSLY IN DMSO FROM THE 9TH THROUGH THE 17TH DAY OF GESTATION IN C57BL/6

Treatment	Fetal			Maternal	
	Liver wt. (gms)	Body wt. (gms)	Liver wt./ Body wt.	Wt. gain (gms)	Liver wt./ Body wt.
Nontreated	.047	.810	.058	6.00	.069
DMSO	.046	.818	.056	5.99	.068
2,4,5-T	[1] .057	[1] .738	[3] .076	4.65	[3] .120

[1] Statistical significance level = 0.10. [2] Statistical significance level = 0.05. [3] Statistical significance level = 0.01.

TABLE 3.—TERATOGENIC EVALUATION OF 2,4,5-T IN RATS

Compound	Vehicle	Dose (mg/kg)	Number of litters	Average number live fetuses/ litter	Percent fetal mortality/ litter	Percent abnormal litters	Percent abnormal fetuses/ litter	Percent of fetuses per litter with— Enlarged renal pelvis	Cystic kidney
Nontreated	None	None	7	9.9	11	43	9	9	0
Control	Honey	None	14	8.7	1	57	12	12	<1
2,4,5-T	do	4.6	8	8.2	12	88	[2] 36	18	[2] 21
2,4,5-T	do	10.0	7	7.1	[2] 28	86	[2] 46	17	[2] 30
2,4,5-T	do	46.4	6	2.7	[3] 59	67	60	27	33

[1] Statistical Significance Level = 0.10. [2] Statistical Significance Level = 0.05. [3] Statistical Significance Level = 0.01.

REFERENCES AND NOTES

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(2) Drill, V. A. and Hiratzka, T., *Arch. Industrial Hygiene Occupational Med.* 7, 61, 1953.

(3) Rowe, V. K. and Hymas, T. A., *Am. J. Vet. Res.* 15, 622, 1954.

(4) These results are from a large study designed to screen selected compounds for teratogenic effects in mice which was performed at the Bionetics Research Laboratories, Division of Litton Industries, under contract numbers PH 43–64–57 and PH 43–67–735 from the National Institutes of Health. During the performance of this study, Dr. Courtney was a staff member of the Bionetics Research Labs., Inc., and Dr. Falk was a member of the National Cancer Institute.

(5) 2,4,5-T was produced by the Diamond Alkali Co., 98%, Tech., m.p. 149–151°.

(6) Snedecor, G. W. and Cochran, W. G. *Statistical Methods, 6th ed.*, Iowa State Univ. Press, Ames, Iowa, 1967.

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(8) Kalter, H., *Teratology* 1, 193, 1968.

(9) Courtney, K. D. (In preparation).

Note added in proof:

The sample of 2,4,5-T used in this study contained approximately 30 ppm of 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) (10). Dioxin as well as purified 2,4,5-T are currently being evaluated for their teratogenic and fetotoxic potential.

(10) We thank Dow Chemical Co., for the analysis of 2,4,5-T.

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Senator HART. Are there any additional questions?

Mr. BICKWIT. No, Mr. Chairman.

Senator HART. If any arise we will submit them in writing and receive the replies in the record.

I appreciate the cooperation of everyone through the day, and apologize for the erratic scheduling this afternoon.

(The following was subsequently received for the record:)

Appendix 1

U.S. SHOWS SIGNS OF CONCERN OVER EFFECT IN VIETNAM OF 9-YEAR DEFOLIATION PROGRAM

(By Ralph Blumenthal, special to the New York Times)

SAIGON, South Vietnam, March 14—Many South Vietnamese who live adjacent to areas that are being defoliated by spray from United States planes are convinced that any ailments or misfortunes that they suffer are related to the sprayings.

There is no proof that they are right about the effect of the chemical sprays on the human body, but neither is there any assurance that they are wrong.

Although the defoliation program, organized and run by the United States, has been in operation for nearly nine years the full effect of the chemicals on animal and human life remains largely undetermined.

The United States military command says the program, which is designed to strip plant cover from areas occupied by the enemy and to destroy crops that might yield him food, has covered about 5,000 of South Vietnam's 66,350 square miles.

U.S. TERMS IT VALUABLE

The United States command says the program has proved its military worth. "It has contributed materially to the security of units operating in the field by increasing their visibility from the ground as well as the air," the command said.

About 13 per cent of the program has been directed against crops, presumably food grown by and for the enemy. Because of the drifting of defoliants and the difficulty of assessing the results on the ground, it is virtually impossible to say how much of the crop has been destroyed by the chemicals, but it would not appear to be a significant part of the country's capacity. It has brought hardships, however, to individual farmers.

After years of assuring the South Vietnamese that this extensive spraying was harmless to animals and humans, United States officials are showing signs of concern over recent reports the the chemical sprays may have some little-understood and alarming effects.

PANEL STUDYING EFFECTS

In the last several months, reportedly on instruction from Washington, the United States military command and the United States Embassy have formed a special committee to review the effects of the defoliation program, especially on humans.

The sensitivity of the issue has foreclosed official comment, but according to informed sources the science advisory office of the command is responsible for gathering data in interviews and tests that embassy officials will then evaluate.

The South Vietnamese Government regards the entire subject as taboo. Vietnamese newspapers have been suspended for publishing articles about birth defects allegedly attributed to the defoliants, and the public Health Ministry declines to provide any statistics on normal and abnormal births.

However, the concern felt among the Americans is shared by many South Vietnamese scientists, physicians, health officials and villagers interviewed in a three-week survey of the effects of the program.

Officers of the United States command are aware of the allegations of birth defects but they generally discount the reports.

Responsible South Vietnamese scientists and officials say they know virtually nothing about the effects of the chemical sprays.

Saigon's leading maternity hospital, Tudu, from which rumors of an increase of abnormal births emanate periodically, has not even compiled annual reports of statistics for the last three years. Recent monthly figures show an average of about 140 miscarriages and 150 premature births among approximately 2,800 pregnancies, but the hospital is not prepared to say whether this represents an increase and, if so, what the cause might be.

A high Agriculture Ministry official said: "I don't think the Americans would use the chemicals if they were harmful."

He conceded that his ministry had made no tests and asserted that his experts had been unable to get any information about the defoliants from the Defense Ministry, which considers such data secret. The main defoliant compounds and some information about them are available in the United States.

Last Oct. 29, President Nixon's science adviser, Dr. Lee A. Du Bridge, announced that as a result of a study showing that one of the defoliants used, 2,4,5-T, had caused an unexpectedly high incidence of fetal deformities in mice and rats, the compound would henceforth be restricted to areas remote from population.

That directive appears to be ambiguous in South Vietnam for military spokesmen assert that 2,4,5-T continues to be used only in "enemy staging areas"—by definition populated regions.

DEFOLIANTS WERE CONCEALED

Don That Trinh, Minister of Agriculture from November, 1967 to May, 1968, and for 10 years professor of agronomy at Saigon University, said that while he was minister, the Defense Ministry "would try to conceal the defoliant products from me."

"I did not believe in defoliation," he added.

According to one of the Vietnamese directors of a Government research laboratory in Saigon: "We didn't know anything before the United States started spraying. It was only when we received complaints from the livestock people that we started getting interested." But, he added, there are still no Vietnamese studies.

Even the village of Tanhiep, 20 miles north of Saigon, on which 1,000 gallons of defoliants were jettisoned on Dec. 1, 1968, has not been the object of attention or study.

An American C-123 flying out of Bienhoa air base, Northeast of Saigon, developed engine trouble shortly after takeoff. To lighten the craft, the pilot sprayed the full load of chemicals over Tanhiep and nearby Binhtri in 30 seconds instead of the usual 4 minutes 30 seconds, which spreads the defoliant at the rate of three gallons an acre in unpopulated areas.

The defoliant involved, according to the United States command, was a 50-50 mixture of 2,4-Dichlorophenoxyacetate, or 2,4-D, and 2,4,5-Trichlorophenoxyacetate, or 2,4,5-T, in an oil base. It is one of three compounds the military says it uses here, the others being a Dow chemical product called Tordon 101, a mixture of amine salts of 2,4-D and Picloram, and an arsenic compound of cacodylic acid.

No physicians visited Tanhiep to examine the people after their exposure, which, like eight similar emergency dumpings since 1968—some over unpopulated forests—was not made public by the United States command.

A United States Air Force medical team visited Binhtri shortly after the spraying and, according to American district officials, found the villagers had suffered no ill effects. There was no later inquiry.

Mrs. Tran Thi Tien of Tanhiep, hwo says she has four normal children, is convinced that the malfunction of her son, who still looks like a newborn at 14 months of age, "must be due to the chemicals I breathed."

Her neighbors, Mrs. Nguyen Thi Hai and Mrs. Tong Thi An, blame the spraying for the fact that their children, one year and 20 months old respectively, still crawl instead of walk.

Nguyen Van Nhap, a farmer, complains of suffering bouts of fever, sneezing and weakness.

"I was working in the field when the spray came down," Mrs. Tien said through an interpreter. "I felt dizzy, like vomiting and had to stay in bed three or four days."

Many other villagers reported feeling the same sensations as Mrs. Tien, but, except for the two children described as retarded in learning to walk, no other abnormal children were described to visitors at the village of 1,200 residents.

Tran Van Dang, a farmer in neighboring Binhtri, recalled that the days after the spraying two villagers, Tam Ten and Mrs. Hai Mua, died after suffering respiratory difficulties and trembling. The next day, he said, a third villager, Mrs. Hai Nuc, died after showing similar symptoms. Mr. Ten was an old man and could have been expected to die soon anyway but the two others, Mr. Dang said, were middle-aged and seemed healthy.

Such complaints are not limited to Tanhiep and Binhtri, where villagers were admittedly exposed to concentrated doses of defoliant—though just how concentrated has not been established.

In Bienhoa city, 10 miles from Tanhiep, any defoliant in the air drifts down from the heavily sprayed battle areas to the north.

Dr. Nguyen Son Cao says he finds a clear correlation between the days when there is spraying and the number of patients who come in with respiratory ailments, mostly sneezing and coughing.

Dr. Cao, who has been practicing in Bienhoa for 21 years, said he had also noticed that in the last two to three years the number of miscarriages among his patients had doubled.

"These women are convinced they are the victims of the chemicals," he said. "I only suspect there could be a relationship. This suspicion is very well known. The increase in miscarriages is very obvious, very significant."

However, the manager of another clinic reported no increase in miscarriages over the last several years.

Any increase in miscarriages has many possible explanations: perhaps the deterioration of the daily diet, the cumulative effect of the hardships of war, population and economic movements that register statistics of only certain groups, or air pollution, of which the defoliant chemicals are a part.

Appendix 2

Defoliants, Deformities: What Risk?

Dr. Jackie Verrett is fascinated and horrified by what has now become an everyday sight at her FDA toxicology lab in Washington, D.C.: several white leghorn chicks struggling to get to their feet and then finally walking—on their knees. Besides slipped tendons in their legs, some of the chicks have cleft palates and beak deformities. All this has been wrought by injecting fertilized eggs with an ethanol solution containing just 2.5 micromicrograms (or 50 parts per trillion) of 2,3,6,7-tetrachlorodibenzo-*p*-dioxin, a contaminant in 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

Over the past nine years, 40 million pounds of this defoliating herbicide have been sprayed in very heavy concentrations across at least five million acres of Vietnam to destroy crops and expose the enemy. By MWN's reckoning, some 30 million pounds have been spewed out in lesser concentrations during just the past five years across perhaps 30 million acres of range, forest, and farmland (not to mention home gardens) in the U.S—an area three times the size of Texas.

Thus, Dr. Verrett's preliminary findings are not just of interest to poultrymen. The 11 crippled chicks in her study were among 15 survivors of a clutch of 25 eggs. In the unhatched chicks, Dr. Verrett found pronounced evidence of chick edema—swollen tissues, cysts on the back, necrotic livers, and the same deformities the live birds have. The FDA researcher is diluting the dioxin content to try to find a "no effects" level. In another brood, she has produced a similar pattern of birth defects with just 2½ parts per trillion of dioxin, 1/400,000 the 1 ppm found in currently marketed products. Now she's experimenting with .25 parts per trillion. (The work is so politically sensitive that she doesn't even know the origin of the 2,4,5-T involved and feels "like I'm in the CIA.")

When told that HEW Secretary Robert Finch is doubtful about the applicability of the chick embryo work to human risk, Dr. Verrett snapped, "I know, I know, but the only time Bob Finch sees eggs is when he eats them for breakfast."

While Dr. Verrett labored in the lab early this month, Dr. Samuel Epstein, chief of toxicology at Children's Hospital Medical Center in Boston, was out in Globe, a foothill town in southeastern Arizona, to evaluate reports of toxic and

teratogenic effects attending the spraying of 2,4,5-T and its chemical cousin, 2-(2,4,5-Trichlorophenoxy) proprionic Acid (*Silvex*) in adjacent Tonto National Forest. These reports have disturbed the nation and drawn experts to the scene.

Odd Effects Around Globe

In Globe, Dr. Epstein saw two goats and a duck with leg deformities similar to those in Dr. Verrett's chickens, and studied the histories of sick people. "It's impossible to say for certain whether the claimed symptoms and effects are attributable to the spray," he said. But at the same time he lashed out at the U.S. Forest Service for risking the contamination of water sources against its own policy, for contributing to drift by using water as a 2,4,5-T solvent, and for failing to post the area before spraying.

MWN found that the Department of Agriculture keeps such casual tabs of 2,4,5-T spraying that it would take officials a week just to find which of the 33 national forests besides Tonto have been bombed with the two million pounds Forest Service has jetted out over the past six years. "But Interior uses more than we do," said one official. Replies an Interior spokesman, "We used only 44,232 pounds last year."

In the Globe area, the Forest Service has sprayed 2,4,5-T and *Silvex* four of the past five years to promote growth of grass in a burned-over section and to eliminate chaparral. But most 2,4,5-T use is unmonitored. The defoliant is bought by ranchers and private foresters and it's pretty much up to them what happens to it.

Human teratogenicity is the chief worry; it is fairly well known by now that Dr. Verrett's work is not the first study to dramatize the risk. Yet MWN learned that the U.S. doesn't keep nationwide birth-defect figures.

Dr. Edward Burger of the government's Office of Science and Technology does not seem worried by this absence of monitoring and supervision, nor, indeed, about the risk of 2,4,5-T teratogenicity. Dr. Burger, technical assistant to Presidential science advisor Lee A. DuBridge, acknowledges that a study done by Bionetics Research Laboratories for the National Cancer Institute showed last March (it was suppressed for six months) that nearly all offspring of mice and rats given 2,4,5-T early in gestation at the relatively high levels of 21.5 mg/kg or 46.4 mg/kg were born dead or deformed—in some cases with no eyes, with cleft palates, and cystic kidneys and enlarged livers. Even at 4.6 mg/kg dosage, 39% of the animals were born malformed.

The OST expert is more familiar than most with the high-level decision-making that went into Dr. DuBridge's declaration October 29 that on the basis of the Bionetics study, the use of 2,4,5-T in populated areas would be restricted. Dr. DuBridge said Agriculture would, by Jan. 1, 1970, withdraw licenses for its use on crops (corn, bluberries, peaches, pears, and several leafy vegetables) unless the FDA found that the residue was negligible and humans were tolerant of it.

Dr. Burger explains that the FDA missed this deadline for a number of reasons. First, Dow Chemical Co., a major maker of 2,4,5-T, discovered last December that the sample used by Bionetics contained 27 ppm of the tetrachloro dioxin instead of the "less than 1 ppm" Dow says is in its product. So the study is now being re-run with a Dow sample at Dow labs in Zionsville, Ind., and Midland, Mich., and at the National Institute Environmental Health Sciences.

Next, says Dr. Burger, even after the teratogenic potential is re-evaluated in a rodent model, the disappearance rate of the contaminant in the animal blood stream must be determined and calibrated with that in human volunteers. He concludes: "The possibility of exposure to 2,4,5-T, vis-a-vis the small teratogenic risk, is certainly not sufficient at this time to justify wiping the chemical off the market."

Comments Associate FDA Commissioner for Science Dale Lindsay: "Dr. DuBridge had no damned business setting a tolerance deadline. Our market-basket surveys for 1968 and 1969—thousands of samples of 120 foods and vegetables are constantly being assessed—show only five recoveries of 2,4,5-T—three from leafy vegetables at negligible levels, plus one from milk, and one from meat at the .01-mg level.

"Yet if we had to set a tolerance today it would be zero. The trouble with this very active dioxin contaminant is that while it may be a known quantity in a product, you can't extract it in the same quantity."

Harvard microbiologist Matthew Meselson is worried for the same reason—and many others. Dr. Meselson—appointed last year by the American Association for the Advancement of Science to head a 2,4,5-T evaluation project—says: "The tetrachloro dioxin represents just one of 12 or 13 ways the chlorine atoms can arrange themselves on a benzene ring to form dioxin molecules. How do we know about the hexa, hepta, and octychlors, or about how persistent the tetrachlor itself is? Moreover, I'm very concerned about the dioxins that might be formed by unreacted trichlorphenol [2,4,5-T precursor] when the product is exposed to heat. If it were taken up by plants or wood and these were burned, you'd get more dioxin. Finally, I'm bothered by the bizarre mental effects suffered by German workers making 2,4,5-T. I say when in doubt, stop it."

Dr. Julius Johnson, vice president and director of research for Dow, regards these concerns as speculative. "If we thought 2,4,5-T was harming anybody we'd take it off the market tomorrow," he says. "We've been dedicated to cleaning it up ever since 1964 [when the contaminant was linked to an outbreak of chlor-acne in Dow workers at Midland]." Dr. Johnson says it would take a 200-degree jolt to produce reaction of dioxin, and the contaminant disappears within hours under ultraviolet light. So far, he adds, Dow tests show that its 2,4,5-T has no teratogenic effect on rats at a dosage of 24 mg/kg and on rabbits at 40 mg/kg. But how about Dr. Verrett's new findings in the chick embryo test? The Dow executive confesses surprise. "But I'm confident," he says, "that we'll be safe when we propose a new specification for all 2,4,5-T products of .1 ppm of dioxin."

Safety also assumes gauges of teratogenicity in the population, however. FDA's Dr. Lindsay spoke with certitude when he told MWN that "the National Institute of Neurological Diseases and Stroke has recorded birth defects for some 15 years and would be telling us if they were on the rise." He's wrong. Dr. Heinz Berendes, chief of NINDS' perinatal research branch, admits dolefully that "no nationwide data are available on frequency or incidence of malformation."

Adds Yale biologist Arthur Galston: "It's shocking, but absolutely no studies have been made in Vietnam either. There have been reports of birth defects in Saigon papers since last June but hospital records haven't been made available."

State Department officials say they know of no policy whereby such data would be classified or withheld. Significantly, however, Dr. Malcolm Phelps, chief of the Vietnam medical section of the Agency for International Development, says he is acting on a recent White House request to collect figures on teratological occurrences in Vietnam civilian hospitals.

As for all the toxic effects reported by Globe residents after the June 8–11 spraying—a helicopter released 935 gallons of *Silvex*, 30 of 2,4,5-T, and 20 gallons of a combination called "Orange" over 1,900 acres of forest—an MWN reporter inquired into the histories of 18 patients with four of the five doctors who treated them, and checked on the two crippled goats, the crippled duck, a bleeding bull terrier, and two other dogs with pneumonia. Net result: two strongly suspected herbicide poisoning cases linked to the spraying, and one "definite." There's one-year-old Paul McCray, who lives on the edge of Tonto National Forest and whose father drove the family right up to the 'copter landing spot during spraying. The boy has had respiratory attacks and convulsions. Phoenix pediatrician W. Scott Chisholm finds Paul has lymphositosis, with a white cell count twice normal.

The second suspected case, a smeltery worker named James Andrews who has complained of a number of symptoms associated with herbicide poisoning —nausea, muscle weakness, vertigo, numbness, and stabbing pain—is vouchsafed by Dr. Granville Knight of Santa Monica, Calif. In the third case, that of Mrs. Billee Shoecraft, Dr. Knight says he has found 2-4-D in tissue.

Dr. Bernard Collopy would not label the muscle spasms and stabbing pain suffered by potter Robert McKusick, owner of the defective goats and ducks, as herbicide-related. Dr. William Bishop would not credit the chest pains of Bob McCray, father of little Paul, or his wife's tingling fingers and toes, as 2,4,5-T or *Silvex* poisoning. And veterinarian F. I. Skinner hadn't seen any of the animal cases.

Sums up Dr. Bishop: "There's a good possibility some of the human cases are related to spraying, but symptomatic connections aren't connections and

I'm no toxicologist. People here are emotional and each morning wake up with new nails pounded into their palms. What's needed is solid scientific investigation. All I hope is they don't leave us hanging in the air for the next 20 years."

Appendix 3

[From the New Yorker, Feb. 7, 1970]

A Reporter at Large: Defoliation

By Thomas Whiteside

Late in 1961, the United States Military Advisory Group in Vietnam began, as a minor test operation, the defoliation, by aerial spraying, of trees along the sides of roads and canals east of Saigon. The purpose of the operation was to increase visibility and thus safeguard against ambushes of allied troops and make more vulnerable any Vietcong who might be concealed under cover of the dense foliage. The number of acres sprayed does not appear to have been publicly recorded, but the test was adjudged a success militarily. In January, 1962 following a formal announcement by South Vietnamese and American officials that a program of such spraying was to be put into effect, and that it was intended "to improve the country's economy by permitting freer communication as well as to facilitate the Vietnamese Army's task of keeping these avenues free of Vietcong harassments," military defoliation operations really got under way. According to an article that month in the New York Times, "a high South Vietnamese official" announced that a seventy-mile stretch of road between Saigon and the coast was sprayed "to remove foliage hiding Communist guerrillas." The South Vietnamese spokesman also announced that defoliant chemicals would be sprayed on Vietcong plantations of manioc and sweet potatoes in the Highlands. The program was gathering momentum. It was doing so in spite of certain private misgivings among American officials, particularly in the State Department, who feared, first, that the operations might open the United States to charges of engaging in chemical and biological warfare, and second, that they were not all that militarily effective. Roger Hilsman, now a professor of government at Columbia University, and then Director of Intelligence and Research for the State Department, reported, after a trip to Vietnam, that defoliation operations "had political disadvantages" and, furthermore, that they were of questionable military value, particularly in accomplishing their supposed purpose of reducing cover for ambushes. Hilsman later recalled in his book, "To Move a Nation," his visit to Vietnam, in March, 1962: "I had flown down a stretch of road that had been used for a test and found that the results were not very impressive. . . . Later, the senior Australian military representative in Saigon, Colonel Serong, also pointed out that defoliation actually aided the ambushers—if the vegetation was close to the road those who were ambushed could take cover quickly; when it was removed the guerrillas had a better field of fire." According to Hilsman, "The National Security Council spent tense sessions debating the matter."

Nonetheless, the Joint Chiefs of Staff and their Chairman, General Maxwell Taylor, agreed that chemical defoliation was a useful military weapon. In 1962, the American military "treated" 4,940 acres of the Vietnamese countryside with herbicides. In 1963, the area sprayed increased five-fold to a total of 24,700 acres. In 1964, the defoliated area was more than tripled. In 1965, the 1964 figure was doubled, increasing to 155,610 acres. In 1966, the sprayed area was again increased fivefold, to 741,247 acres, and in 1967 it was doubled once again over the previous year, to 1,486,446 acres. Thus, the areas defoliated in Vietnam had increased approximately three hundredfold in five years, but now adverse opinion among scientists and other people who were concerned about the effects of defoliation on the Vietnamese ecology at last began to have a braking effect on the program. In 1968, 1,267,110 acres were sprayed, and in 1969 perhaps a million acres. Since 1962, the defoliation operations have covered almost five million acres, an area equivalent to about twelve per cent of the entire territory of South Vietnam, and about the size of the state of Massachusetts. Between 1962 and 1967, the deliberate destruction of plots of rice, manioc, beans, and other foodstuffs through herbicidal spraying—the word "deliberate" is used here to exclude the many reported instances of accidental

spraying of Vietnamese plots—increased three hundredfold, from an estimated 741 acres to 221,812 acres, and by the end of 1969 the Vietnamese cropgrowing area that since 1962 had been sprayed with herbicides totalled at least half a million acres. By then, in many areas the original purpose of the defoliation had been all but forgotten. The military had discovered that a more effective way of keeping roadsides clear was to bulldoze them. But by the time of that discovery defoliation had settled in as a general policy and taken on a life of its own—mainly justified on the ground that it made enemy infiltration from the North much more difficult by removing vegetation that concealed jungle roads and trails.

During all the time since the program began in 1961, no American military or civilian official has ever publicly characterized it as an operation of either chemical or biological warfare, although there can be no doubt that it is an operation of chemical warfare in that it involves the aerial spraying of chemical substances with the aim of gaining a military advantage, and that it is an operation of biological warfare in that it is aimed at a deliberate disruption of the biological conditions prevailing in a given area. Such distinctions simply do not appear in official United States statements or documents; they were long ago shrouded under heavy verbal cover. Thus, a State Department report, made public in March, 1966, saying that about twenty thousand acres of crops in South Vietnam had been destroyed by defoliation to deny food to guerrillas, described the areas involved as "remote and thinly populated," and gave a firm assurance that the materials sprayed on the crops were of a mild and transient potency: "The herbicides used are nontoxic and not dangerous to man or animal life. The land is not affected for future use."

However comforting the statements issued by our government during seven years of herbicidal operations in Vietnam, the fact is that the major development of defoliant chemicals (whose existence had been known in the thirties) and other herbicidal agents came about in military programs for biological warfare. The direction of this work was set during the Second World War, when Professor E. J. Kraus, who then headed the Botany Department of the University of Chicago, brought certain scientific possibilities to the attention of a committee that had been set up by Henry L. Stimson, the Secretary of War, under the National Research Council, to provide the military with advice on various aspects of biological warfare. Kraus, referring to the existence of hormone-like substances that experimentation had shown would kill certain plants or disrupt their growth, suggested to the committee in 1941 that it might be interested in "the toxic properties of growth-regulating substances for the destruction of crops or the limitation of crop production." Military research on herbicides thereupon got under way, principally at Camp (later Fort) Detrick, Maryland, the Army center for biological-warfare research. According to George Merck, a chemist, who headed Stimson's biological-warfare advisory committee, "Only the rapid ending of the war prevented field trials in an active theatre of synthetic agents that would, without injury to human or animal life, affect the growing crops and make them useless."

After the war, many of the herbicidal materials that had been developed and tested for biological-warfare use were marketed for civilian purposes and used by farmers and homeowners for killing weeds and controlling brush. The most powerful of the herbicides were the two chemicals 2,4-dichlorophenoxyacetic acid, generally known as 2,4-D, and 2,4,5-trichlorophenoxyacetic acid, known as 2,4,5-T. The direct toxicity levels of these chemicals as they affected experimental animals, and, by scientific estimates, men, appeared then to be low (although these estimates have later been challenged), and the United States Department of Agriculture, the Food and Drug Administration, and the Fish and Wildlife Service all sanctioned the widespread sale and use of both. The chemicals were also reported to be shortlived in soil after their application. 2,4-D was the bigger seller of the two, partly because it was cheaper, and suburbanites commonly used mixtures containing 2,4-D on their lawns to control dandelions and other weeds. Commercially, 2,4-D and 2,4,5-T were used to clear railroad rights-of-way and power-line routes, and, in cattle country, to get rid of woody brush, 2,4,5-T being favored for the last, because it was considered to have a more effective herbicidal action on woody plants. Very often, however, the two chemicals were used in combination. Between 1945 and 1963, the production of herbicides jumped from nine hundred and seventeen thousand pounds to about a hundred and fifty million pounds in this country;

since 1963, their use had risen two hundred and seventy-[illegible] percent—more than double the rate of increase in the use of pesticides, though pesticides are still far more extensively used. By 1960, an area equivalent to more than three per cent of the entire United States was being sprayed each year with herbicides.

Considering the rapidly growing civilian use of these products, it is perhaps not surprising that the defoliation operations in Vietnam escaped any significant comment in the press, and that the American public remained unaware of the extent to which these uses had their origin in planning for chemical and biological warfare. Nevertheless, between 1941 and the present, testing and experimentation in the use of 2,4-D, 2,4,5-T, and other herbicides as military weapons were going forward very actively at Fort Detrick. While homeowners were using herbicidal mixtures to keep their lawns free of weeds, the military were screening some twelve hundred compounds for their usefulness in biological-warfare operations. The most promising of these compounds were test-sprayed on tropical vegetation in Puerto Rico and Thailand, and by the time fullscale defoliation operations got under way in Vietnam the U.S. military had settled on the use of four herbicidal spray materials there. These went under the names Agent Orange, Agent Purple, Agent White, and Agent Blue—designations derived from color-coded stripes girdling the shipping drums of each type of material. Of these materials, Agent Orange, the most widely used as a general defoliant, consists of a fifty-fifty mixture of *n* butyl esters and of 2,4-D and 2,4,5-T. Agent Purple, which is interchangeable with Agent Orange, consists of the same substances with slight molecular variations. Agent White, which is used mostly for forest defoliation, is a combination of 2,4-D and Picloram, produced by the Dow Chemical Company. Unlike 2,4-D or 2,4,5-T, which, after application, is said to be decomposable by micro-organisms in soil over a period of weeks or months (one field test of 2,4,5-T in this country showed that significant quantities persisted in soil for ninety-three days after application), Picloram—whose use the Department of Agriculture has not authorized in the cultivation of any American crop—is one of the most persistent herbicides known. Dr. Arthur W. Galston, professor of biology at Yale, has described Picloram as "a herbicidal analog of DDT," and an article in a Dow Chemical Company publication called "Down to Earth" reported that in field trials of Picloram in various California soils between eighty and ninety-six and a half per cent of the substance remained in the soils four hundred and sixty-seven days after application. (The rate at which Picloram decomposes in tropical soils may, however, be higher.) Agent Blue consists of a solution of cacodylic acid, a substance that contains fifty-four per cent arsenic, and it is used in Vietnam to destroy rice crops. According to the authoritative "Merck Index," a source book on chemicals, this material is "poisonous." It can be used on agricultural crops in this country only under certain restrictions imposed by the Department of Agriculture. It is being used herbicidally on Vietnamese rice fields at seven and a half times the concentration permitted for weed-killing purposes in this country, and so far in Vietnam something like five thousand tons is estimated to have been sprayed on paddies and vegetable fields.

Defoliation operations in Vietnam are carried out by a special flight of the 12th Air Commando Squadron of the United States Air Force, from a base at Bien Hoa, just outside Saigon, with specially equipped C–123 cargo planes. Each of these aircraft has been fitted out with tanks capable of holding a thousand gallons. On defoliation missions, the herbicide carried in these tanks is sprayed from an altitude of around a hundred and fifty feet, under pressure, from thirty-six nozzles on the wings and tail of the plane, and usually several spray planes work in formation, laying down broad blankets of spray. The normal crew of a military herbicidal-spray plane consists of a pilot, a copilot, and a technician, who sits in the tail area and operates a console regulating the spray. The equipment is calibrated to spray a thousand gallons of herbicidal mixture at a rate that works out, when all goes well, to about three gallons per acre. Spraying a thousand-gallon tankload takes five minutes. In an emergency, the tank can be emptied in thirty seconds—a fact that has particular significance because of what has recently been learned about the nature of at least one of the herbicidal substances.

The official code name for the program is Operation Hades, but a more friendly code name, Operation Ranch Hand, is commonly used. In similar fashion, military public-relations men refer to the herbicidal spraying of crops sup-

edly grown for Vietcong use in Vietnam, when they refer to it at all [redacted] "food-denial program." By contrast, an American biologist who is less [redacted] enthusiastic about the effort has called it, in its current phase, "escalation to a program of starvation of the population in the affected area." Dr. Jean Mayer, the Harvard professor who now is President Nixon's special adviser on nutrition, contended in an article in *Science and Citizen* in 1967 that the ultimate target of herbicidal operations against rice and other crops in Vietnam was "the weakest element of the civilian population"—that is, women, children, and the elderly—because in the sprayed area "Vietcong soldiers may . . . be expected to get the fighter's share of whatever food there is." He pointed out that malnutrition is endemic in many parts of Southeast Asia but that in wartime South Vietnam, where diseases associated with malnutrition, such as beri-beri, anemia, kwashiorkor (the disease that has decimated the Biafran population), and tuberculosis, are particularly widespread, "there can be no doubt that if the (crop-destruction) program is continued, (the) problems will grow."

Whether a particular mission involves defoliation or crop destruction, American military spokesmen insist that a mission never takes place without careful consideration of all the factors involved, including the welfare of friendly inhabitants and the safety of American personnel. (There can be little doubt that defoliation missions are extremely hazardous to the members of the planes' crews, for the planes are required to fly very low and only slightly above stalling speed, and they are often targets of automatic-weapons fire from the ground.) The process of setting up targets and approving specific herbicidal operations is theoretically subject to elaborate review through two parallel chains of command; one chain consisting of South Vietnamese district and province chiefs—who can themselves initiate such missions—and South Vietnamese Army commanders at various levels; the other a United States chain, consisting of a district adviser, a sector adviser, a divisional senior adviser, a corps senior adviser, the United States Military Assistance Command in South Vietnam, and the American Embassy in Saigon, ending up with the American ambassador himself. Positive justification of the military advantage likely to be gained from each operation is theoretically required, and applications with such positive justification are theoretically disapproved. However, according to one of a series of articles by Elizabeth Pond that appeared toward the end of 1967 in the *Christian Science Monitor:*

"In practice, [American] corps advisers find it very difficult to turn down defoliation requests from province level because they simply do not have sufficient specific knowledge to call a proposed operation into question. And with the momentum of six years' use of defoliants, the practice, in the words of one source, has long since been "set in cement."

"The real burden of proof has long since shifted from the positive one of justifying an operation by its [military] gains to the negative one of denying an operation because of [specific] drawbacks. There is thus a great deal of pressure, especially above province level, to approve recommendations sent up from below as a matter of course."

Miss Pond reported that American military sources in Saigon were "enthusiastic" about the defoliation program, and that American commanders and spotter-plane pilots were "clamoring for more of the same." She was given firm assurances as to the mild nature of the chemicals used in the spray operations:

"The defoliants used, according to the military spokesman contacted, are the same herbicides . . . as those used commercially over some four million acres in the United States. In the strengths used in Vietnam they are not at all harmful to humans or animals, the spokesman pointed out, and in illustration of this he dabbed onto his tongue a bit of liquid from one of . . . three bottles sitting on his desk."

As the apparently inexorable advance of defoliation operations in South Vietnam continued, a number of scientists in the United States began to protest the military use of herbicides, contending that Vietnam was being used, in effect, as a proving ground for chemical and biological warfare. Early in 1966, a group of twenty-nine scientists, under the leadership of Dr. John Edsall, a professor of biochemistry at Harvard, appealed to President Johnson to prohibit the use of defoliants and crop-destroying herbicides, and called the use of these substances in Vietnam "barbarous because they are indiscriminate." In the late summer of 1966, this protest was followed by a letter of petition to

President Johnson from twenty-two scientists, including seven Nobel laureates. The petition pointed out that the "large-scale use of anticrop and 'nonlethal' antipersonnel chemical weapons in Vietnam" constituted "dangerous precedent" in chemical and biological warfare, and it asked the President to order it stopped. Before the end of that year, Dr. Edsall and Dr. Matthew S. Meselson, a Harvard professor of biology, obtained the signatures of five thousand scientists to co-sponsor the petition. Despite these protests, the area covered by defoliation operations in Vietnam in 1967 was double that covered in 1966, and the acreage of crops destroyed was nearly doubled.

These figures relate only to areas that were sprayed intentionally. There is no known way of spraying an area with herbicides from the air in a really accurate manner, because the material used is so highly volatile, especially under tropical conditions, that even light wind drift can cause extensive damage to foliage and crops outside the deliberately sprayed area. Crops are so sensitive to the herbicidal spray that it can cause damage to fields and gardens as much as fifteen miles away from the target zone. Particularly severe accidental damage is reported, from time to time, to so-called "friendly" crops in the III Corps area, which all but surrounds Saigon and extends in a rough square from the coastline to the Cambodian border. Most of the spraying in III Corps is now done in War Zones C and D, which are classified as free fire zones, where, as one American official has put it, "everything that moves in Zones C and D is considered Charlie." A press dispatch from Saigon in 1967 quoted another American official as saying that every Vietnamese farmer in that corps area knew of the defoliation program and disapproved of it. Dr. Galston, the Yale biologist, who is one of the most persistent critics of American policy concerning herbicidal operations in Vietnam, recently said in an interview, "We know that most of the truck crops grown along roads, canals, and trails and formerly brought into Saigon have been essentially abandoned because of the deliberate or inadvertent falling of these defoliant sprays; many crops in the Saigon area are simply not being harvested." He also cited reports that in some instances in which the inhabitants of Vietnamese villages have been suspected of being Vietcong sympathizers the destruction of food crops has brought about complete abandonment of the villages. In 1966, herbicidal operations caused extensive inadvertent damage, through wind drift, to a very large rubber plantation northwest of Saigon owned by the Michelin rubber interests. As the result of claims made for this damage, the South Vietnamese authorities paid the corporate owners, through the American military, nearly a million dollars. The extent of the known inadvertent damage to crops in Vietnam can be inferred from the South Vietnamese budget—in reality, the American military budget—*for settling such claims.* In 1967, the budget for this compensation was three million six hundred thousand dollars. This sum, however, probably reflects only the barest emergency claims of the people affected.

According to Representative Richard D. McCarthy, a Democrat from upstate New York who has been a strong critic of the program, the policy of allowing applications for defoliation operations to flow, usually without question, from the level of the South Vietnamese provincial or district chiefs has meant that these local functionaries would order repeated sprayings of areas that they had not visited in months, or even years. The thought that a Vietnamese district chief can initiate such wholesale spraying, in effect without much likelihood of serious hindrance by American military advisers, is a disquieting one to a number of biologists. Something that disquiets many of them even more is what they believe the long-range effects of nine years of defoliation operations will be on the ecology of South Vietnam. Dr. Galston, testifying recently before a congressional subcommittee on chemical and biological warfare, made these observations:

"It has already been well documented that some kinds of plant associations subject to spray, especially by Agent Orange, containing 2,4-D and 2,4,5-T, have been irreversibly damaged. I refer specifically to the mangrove associations that line the estuaries, especially around the Saigon River. Up to a hundred thousand acres of these mangroves have been sprayed. . . . Some (mangrove areas) had been sprayed as early as 1961 and have shown no substantial signs of recovery. . . . Ecologists have known for a long time that the mangroves lining estuaries furnish one of the most important ecological niches for the completion of the life cycle of certain shellfish and migratory fish. If these plant communities are not in a healthy state, secondary effects on the whole

interlocked web of organisms are bound to occur. . . . In the years ahead the Vietnamese, who do not have overabundant sources of proteins at present, are probably going to suffer dietarily because of the deprivation of food in the form of fish and shellfish.

"Damage to the soil is another possible consequence of extensive defoliation. . . . We know that the soil is not a dead, inert mass but, rather, that it is a vibrant, living community. . . . If you knock the leaves off of trees once, twice, or three times . . . you change the quality of the soil. . . . Certain tropical soils—and it has been estimated that in Vietnam up to fifty per cent of all the soils fall into this category—are laterizable; that is, they may be irreversibly converted to rock as a result of the deprivation of organic matter. . . . If . . . you deprive trees of leaves and photosynthesis stops, organic matter in the soil declines and laterization, the making of brick, may occur on a very extensive scale. I would emphasize that this brick is irreversibly hardened; it can't be made back into soil. . . .

"Another ecological consequence is the invasion of an area by undesirable plants. One of the main plants that invade an area that has been defoliated is bamboo. Bamboo is one of the most difficult of all plants to destroy once it becomes established where you don't want it. It is not amenable to killing by herbicides. Frequently it has to be burned over, and this causes tremendous dislocations to agriculture."

Dr. Fred H. Tschirley, assistant chief of the Crops Protection Research Branch of the Department of Agriculture, who made a month's visit to Vietnam in the spring of 1968 in behalf of the State Department to report on the ecological effects of herbicidal operations there, does not agree with Dr. Galston's view that laterization of the soil is a serious probability. However, he reported to the State Department that in the Rung Sat area, southeast of Saigon, where about a hundred thousand acres of mangrove trees had been sprayed with defoliant, each single application of Agent Orange had killed ninety to a hundred per cent of the mangroves touched by the spray, and he estimated that the regeneration of the mangroves in this area would take another twenty years, at least. Dr. Tschirley agrees with Dr. Galston that a biological danger attending the defoliation of mangroves is an invasion of virtually ineradicable bamboo.

A fairly well-documented example not only of the ecological consequences of defoliation operations but also of their disruptive effects on human life was provided last year by a rubber-plantation area in Kompong Cham Province, Cambodia, which lies just across the border from Vietnam's Tay Ninh Province. On June 2, 1969, the Cambodian government, in an angry diplomatic note to the United States government, charged the United States with major defoliation damage to rubber plantations, and also to farm and garden crops in the province, through herbicidal operations deliberately conducted on Cambodian soil. It demanded compensation of eight and a half million dollars for destruction or serious damage to twenty-four thousand acres of trees and crops. After some delay, the State Department conceded that the alleged damage might be connected with "accidental drift" of spray over the border from herbicidal operations in Tay Ninh Province. The Defense Department flatly denied that the Cambodian areas had been deliberately sprayed. Late in June, the State Department sent a team of four American scientists to Cambodia, and they confirmed the extent of the area of damage that the Cambodians had claimed. They found that although some evidence of spray drift across the Vietnamese border existed, the extent and severity of damage in the area worst affected were such that "it is highly unlikely that this quantity could have drifted over the border from the Tay Ninh defoliation operations." Their report added, "The evidence we have seen, though circumstantial, suggests strongly that damage was caused by direct overflight." A second report on herbicidal damage to the area was made after an unofficial party of American biologists, including Professor E. W. Pfeiffer, of the University of Montana, and Professor Arthur H. Westing, of Windham College, Vermont, visited Cambodia last December at the invitation of the Cambodian government. They found that about a third of all the rubber trees currently in production in Cambodia had been damaged, and this had happened in an area that normally had the highest latex yield per acre of any in the world. A high proportion of two varieties of rubber trees in the area had died as a result of the damage, and Dr. Westing estimated that the damage to the latex-producing capacity of some varieties might persist for twenty years. Between May and November of last year,

latex production in the affected plantations fell off by an average of between thirty-five and forty per cent. According to a report by the two scientists, "A large variety of garden crops were devastated in the seemingly endless number of small villages scattered throughout the affected area. Virtually all of the . . . local inhabitants . . . depend for their wellbeing upon their own local produce. These people saw their crops . . . literally wither before their eyes." The Cambodian claim is still pending.

Until the end of last year, the criticism by biologists of the dangers involved in the use of herbicides centered on their use in what were increasingly construed as biological-warfare operations, and on the disruptive effects of these chemicals upon civilian populations and upon the ecology of the regions in which they were used. Last year, however, certain biologists began to raise serious questions on another score—possible direct hazards to life from 2,4,5-T. On October 29th, as a result of these questions, a statement was publicly issued by Dr. Lee DuBridge, President Nixon's science adviser. In summary, the statement said that because a laboratory study of mice and rats that had been given relatively high oral doses of 2,4,5-T in early stages of pregnancy "showed a higher than expected number of deformities" in the offspring, the government would, as a precautionary measure, undertake a series of coordinated actions to restrict the use of 2,4,5-T in both domestic civilian applications and military herbicidal operations. The DuBridge statement identified the laboratory study as having been made by an organization called the Bionetics Research Laboratories, in Bethesda, Maryland, but gave no details of either the findings or the data on which they were based. This absence of specific information turned out to be characteristic of what has been made available to the public concerning this particular research project. From the beginning, it seems, there was an extraordinary reluctance to discuss details of the purported ill effects of 2,4,5-T on animals. Six weeks after the publication of the DuBridge statement, a journalist who was attempting to obtain a copy of the full report made by Bionetics and to discuss its details with some of the government officials concerned encountered hard going. At the Bionetics Laboratories, an official said that he couldn't talk about the study, because "we're under wraps to the National Institutes of Health"—the government agency that commissioned the study. Then, having been asked what the specific doses of 2,4,5-T were that were said to have increased birth defects in the fetuses of experimental animals, the Bionetics official cut off discussion by saying, "You're asking sophisticated questions that as a layman you don't have the equipment to understand the answers to." At the National Institutes of Health, an official who was asked for details of or a copy of the study on 2,4,5-T replied, "The position I'm in is that I have been requested not to distribute this information." He did say, however, that a continuing evaluation of the study was under way at the National Institute of Environmental Health Sciences, at Research Triangle Park, North Carolina. A telephone call to an officer of this organization brought a response whose tone varied from wariness of downright hostility and made it clear that the official had no intention of discussing details or results of the study with the press.

The Bionetics study on 2,4,5-T was part of a series carried out under contract to the National Cancer Institute, which is an arm of the National Institutes of Health, to investigate more than two hundred compounds, most of them pesticides, in order to determine whether they induced cancer-causing changes, fetus-deforming changes, or mutation-causing changes in experimental animals. The contract was a large one, involving more than two and a half million dollars' worth of research, and its primary purpose was to screen out suspicious-looking substances for further study. The first visible fruits of the Bionetics research were presented in March of last year before a convention of the American Association for the Advancement of Science, in the form of a study of possible carcinogenic properties of the fifty-three compounds; the findings on 2,4,5-T were that it did not appear to cause carcinogenic changes in the animals studied.

By the time the report on the carcinogenic properties of the substances was presented, the results of another part of the Bionetics studies, concerning the teratogenic, or fetus-deforming, properties of the substances, were being compiled, but these results were not immediately made available to biologists outside the government. The data remained—somewhat frustratingly, in the view of some scientists who had been most curious about the effects of herbicides—out of sight, and a number of attempts by biologists who had heard about the

teratological study of 2,4,5-T to get at its findings appear to have been thwarted by the authorities involved. Upon being asked to account for the apparent delay in making this information available to biologists, an official of the National Institute of Environmental Health Sciences (another branch of the National Institutes of Health) has declared, with some heat, that the results of the study itself and of a statistical summary of the findings prepared by the Institute were in fact passed on as they were completed to the Commission on Pesticides and Their Relationship to Environmental Health, a scientific group appointed by Secretary of Health, Education, and Welfare Robert Finch and known—after its chairman, Dr. E. M. Mrak, of the University of California—as the Mrak Commission. Dr. Samuel S. Epstein, chief of the Laboratories of Environmental Toxicology and Carcinogenesis at the Children's Cancer Research Foundation in Boston, who was co-chairman of the Mrak Commission panel considering the teratogenic potential of pesticides, tells a different story on the availability of the Bionetics study. He says that he first heard about it in February. At a meeting of his panel in August, he asked for a copy of the report. Ten days later, the panel was told that the National Institute of Environmental Health Sciences would be willing to provide a statistical summary but that the group could not have access to the full report on which the summary was based. Dr. Epstein says that the panel eventually got the full report on September 24th "by pulling teeth."

Actually, as far back as February, officials at the National Cancer Institute had known, on the basis of a preliminary written outline from Bionetics, the findings of the Bionetics scientists on the fetus-deforming role of 2,4,5-T. Dr. Richard Bates, the officer of the National Institutes of Health who was in charge of coordinating the Bionetics project, has said that during the same month this information was put into the hands of officials of the Food and Drug Administration, the Department of Agriculture, and the Department of Defense. "We had a meeting with a couple of scientists from Fort Detrick, and we informed them of what we had learned," Dr. Bates said recently. "I don't know whether they were the right people for us to see. We didn't hear from them again until after the DuBridge announcement at the White House. Then they called up and asked for a copy of the Bionetics report."

At the Department of Agriculture, which Dr. Bates said had been informed in February of the preliminary Bionetics findings, Dr. Tschirley, one of the officials most intimately concerned with the permissible uses of herbicidal compounds, says that he first heard about the report on 2,4,5-T through the DuBridge announcement. At the Food and Drug Administration, where appropriate officials had been informed in February of the teratogenic potential of 2,4,5-T, no new action was taken to safeguard the public against 2,4,5-T in foodstuffs. In fact, it appears that no action at all was taken by the Food and Drug Administration on the matter during the whole of last year. The explanation that F.D.A. officials have offered for this inaction is that they were under instructions to leave the whole question alone at least until December, because the matter was under definitive study by the Mrak Commission—the very group whose members, as it turns out, had such extraordinary difficulty in obtaining the Bionetics data. The Food Toxicology Branch of the F.D.A. did not have access to the full Bionetics report on 2,4,5-T until after Dr. DuBridge issued his statement, at the end of October.

Thus, after the first word went to various agencies about the fetus-deforming potential of 2,4,5-T, and warning lights could have flashed on in every branch of the government and in the headquarters of every company manufacturing or handling it, literally almost nothing was done by the officials charged with protecting the public from exposure to dangerous or potentially dangerous materials—by the officials in the F.D.A., in the Department of Agriculture, and in the Department of Defense. It is conceivable that the Bionetics findings might still be hidden from the public if they had not been pried loose in midsummer through the activities of a group of young law students. The students were members of a team put together by the consumer-protection activist Ralph Nader—and often referred to as Nader's Raiders—to explore the labyrinthine workings of the Food and Drug Administration. In the course of their investigations, one of the law students, a young woman named Anita Johnson, happened to see a copy of the preliminary report on the Bionetics findings that had been passed on to the F.D.A. in February, and its observations seemed quite disturbing to her. Miss Johnson wrote a report to Nader, and in September she showed a copy of the report to a friend who was a biology student at Harvard. In early October, Miss Johnson's friend, in a conversation with Professor Matthew Meselson, mentioned Miss Johnson's report on the preliminary Bionetics findings. This was the first that Dr. Meselson had heard of the existence of the Bionetics study. A few days previously, he had received a call from a scientist friend of his asking whether Dr. Meselson had heard of certain stories, originating with South Vietnamese journalists and other South Vietnamese, of an unusual incidence of birth defects in South Vietnam, which were alleged to be connected with defoliation operations there.

A few days later, after his friend sent him further information, Mr. Meselson decided to obtain a copy of the Bionetics report, and he called up an acquaintance in a government agency and asked for it. He was told that the report was "confidential and classified," and inaccessible to outsiders. Actually, in addition to the preliminary report there were now in existence the full Bionetics report and a statistical summary prepared by the National Institute of Environmental Health Sciences, and, by nagging various Washington friends, Dr. Meselson obtained bootlegged copies of the two latest reports. What he read seemed to him to have such serious implications that he got in touch with acquaintances in the White House and also with someone in the Army to alert them to the problems of 2,4,5-T, in the hope that some new restriction would be placed on its use. According to Dr. Meselson, the White House people *apparently didn't know until* that moment that the reports on the adverse effects of 2,4,5-T even existed. (Around that time, according to a member of Nader's Raiders, "a tremendous lid was put on this thing" within government agencies, and on the subject of the Bionetics work and 2,4,5-T "people in government whom we'd been talking to freely for years just shut up and wouldn't say a word.") While Dr. Meselson awaited word on the matter, a colleague of his informed the press about the findings of the Bionetics report. Very shortly thereafter, Dr. DuBridge made his public announcement of the proposed restrictions on the use of 2,4,5-T.

In certain respects, the DuBridge announcement is a curious document. In its approach to the facts about 2,4,5-T that were set forth in the Bionetics report, it reflects considerable sensitivity to the political and international issues that lie behind the widespread use of this powerful herbicide for civilian and military purposes, and the words in which it describes the reasons for restricting its use appear to have been very carefully chosen:

"The actions to control the use of the chemical were taken as a result of findings from a laboratory study conducted by Bionetics Research Laboratories which indicated that offspring of mice and rats given relatively large oral doses of the herbicide during early stages of pregnancy showed a higher than expected number of deformities.

"Although it seems improbable that any person could receive harmful amounts of this chemical from any of the existing uses of 2,4,5-T, and while the relationships of these effects in laboratory animals to effects in man are not entirely clear at this time, the actions taken will assure safety of the public while further evidence is being sought."

These actions, according to the statement, included decisions that the Department of Agriculture would cancel manufacturers' registrations of 2,4,5-T for use on food crops, effective at the beginning of 1970, "unless by that time the Food and Drug Administration has found a basis for establishing a safe legal tolerance in and on foods," and that the Departments of Agriculture and the Interior, in their own programs, would stop the use of 2,4,5-T in populated areas and in all other areas where residues of the substance could reach man. As for military uses of 2,4,5-T, the statement said, "The chemical is effective in defoliating trees and shrubs and its use in South Vietnam has resulted in reducing greatly the number of ambushes, thus saving lives." However, the statement continued, "the Department of Defense will [henceforth] restrict the use of 2,4,5-T to areas remote from the population."

All this sounds eminently fair and sensible, but whether it represents a candid exposition of the facts about 2,4,5-T and the Bionetics report is debatable. The White House statement that the Bionetics findings "indicated that offspring of mice and rats given relatively large oral doses of the herbicide during early stages of pregnancy showed a higher than expected number of deformities" is, in the words of one eminent biologist who has studied the Bionetics data, "an understatement." He went on to say that "if the effects on experimental animals are applicable to people it's a very sad and serious situa-

." The actual Bionetics report described 2,4,5-T as producing "sufficie[nt] prominent effects of seriously hazardous nature" in controlled experim[ents] with pregnant mice to lead the authors "to categorize [it] as *probably dangerous*." The report also found 2,4-D "potentially dangerous but needing further study." As for 2,4,5-T, the report noted that, with the exception of very small subcutaneous dosages, "all dosages, routes, and strains resulted in increased incidence of abnormal fetuses" after its administration. The abnormalities in the fetuses included lack of eyes, faulty eyes, cystic kidneys, cleft palates, and enlarged livers. The Bionetics report went on to report on further experimental applications of 2,4,5-T to another species:

"Because of the potential importance of the findings in mice, an additional study was carried out in rats of the Sprague-Dawley strain. Using dosages of 21.5 and 46.4 mg/kg [that is, dosages scaled to represent 21.5 and 46.4 milligrams of 2,4,5-T per kilogram of the experimental animal's body weight] suspended in 50 per cent honey and given by the oral route on the 6th through 15th days of gestation, we observed excessive fetal mortality almost 80 per cent) and a high incidence of abnormalities in the survivors. When the beginning of administration was delayed until the 10th day, fetal mortality was somewhat less but still quite high even when dosage was reduced to 4.6 mg/kg. The incidence of abnormal fetuses was threefold that in controls even with the smallest dosage and shortest period used. . . .

It seems inescapable the 2,4,5-T is teratogenic in this strain of rats when given orally at the dosage schedules used here."

Considering the fetus-deforming effects of the *lowest* oral dosage of 2,4,5-T used in Bionetics work on rats—to say nothing of the excessive fetal mortality—the White House statement that "relatively large oral doses of the herbicide . . . showed a higher than expected number of deformities" is hardly an accurate description of the results of the study. In fact, the statistical tables presented as part of the Bionetics report showed that at the lowest oral dosage of 2,4,5-T given to pregnant rats between the tenth and fifteenth days of gestation thirty-nine per cent of the fetuses produced were abnormal, or three times the figure for control animals. At what could without much question be described as "relatively large oral doses" of the herbicide—dosages of 21.5 and 46.4 milligrams per kilogram of body weight of rats, for example—the percentage of abnormal fetuses was ninety and a hundred per cent, respectively, or a good bit higher than one would be likely to deduce from the phrase "a higher than expected number of deformities." The assertion that "it seems improbable that any person could receive harmful amounts of this chemical from any of the existing uses of 2,4,5-T" also appears to be worth examining for this is precisely what many biologists are most worried about in relation to 2,4,5-T and allied substances.

It seems fair, before going further, to quote a cautionary note in the DuBridge statement: "The study involved relatively small numbers of laboratory rats and mice. More extensive studies are needed and will be undertaken. At best it is difficult to extrapolate results obtained with laboratory animals to man—sensitivity to a given compound may be different in man than in animal species. . . ." It would be difficult to get a biologist to disagree with these seemingly sound generalities. However, the first part of the statement does imply, at least to a layman, that the number of experimental animals used in the Bionetics study had been considerably smaller than the numbers used to test commercial compounds other than 2,4,5-T before they are approved by agencies such as the Food and Drug Administration and the Department of Agriculture. In this connection, the curious layman could reasonably begin with the recommendations, in 1963, of the President's Science Advisory Committee on the use of pesticides, which proposed that companies putting out pesticides should be required from then on to demonstrate the safety of their products by means of toxicity studies on two generations of at least two warm-blooded mammalian species. Subsequently, the F.D.A. set up new testing requirements, based on these recommendations, for companies producing pesticides. However, according to Dr. Joseph McLaughlin, of the Food Toxicology Branch of the F.D.A., the organization actually requires applicants for permission to sell pesticides to present the results of tests on only *one* species (usually, in practice, the rat). According to Dr. McLaughlin, the average number of experimental animals used in studies of pesticides is between eighty and a hundred and sixty, including animals used as controls but excluding litters produced. The Bionetics studies of 2,4,5-T used both mice and rats, and

their total number was, in fact, greater, not less, than this average. Including controls but excluding litters, the total number of animals u[sed] in the 2,4,5-T studies was two hundred and twenty-five. Analysis of the results by the National Institute of Environmental Health Sciences found them statistically "significant," and this is the real purpose of such a study: it is meant to act as a coarse screen to shake out of the data the larger lumps of bad news. Such a study is usually incapable of shaking out anything smaller; another kind of study is needed to do that.

Thus, the DuBridge statement seems to give rise to this question: If the Bionetics study, based on the effects of 2,4,5-T on two hundred and twenty-five experimental animals of two species, appears to be less than conclusive, on the ground that "the study involved relatively small numbers of laboratory rats and mice," what is one to think of the adequacy of the tests that the manufacturers of pesticides make? If, as the DuBridge statement says, "at best it is difficult to extrapolate results obtained with laboratory animals to man," what is one to say of the protection that the government affords the consumer when the results of tests of pesticidal substances on perhaps a hundred and twenty rats are officially extrapolated to justify the use of the substances by a population of two hundred million people—not to mention one to two million unborn babies being carried in their mothers' wombs?

The very coarseness of the screen used in all these tests—that is, the relatively small number of animals involved—means that the bad news that shows up in the data has to be taken with particular seriousness, because lesser effects tend not to be demonstrable at all. The inadequacy of the scale on which animal tests with, for instance, pesticides are currently being made in this country to gain F.D.A. approval is further indicated by the fact that a fetus-deforming effect that might show up if a thousand test animals were used is almost never picked up, since the studies are not conducted on that scale; yet if the material being tested turned out to have the same effect, quantitatively, on human beings, this would mean that it would cause between three and four thousand malformed babies to be produced each year. The teratogenic effects of 2,4,5-T on experimental animals used by the Bionetics people, however, were not on the order of one in a thousand. Even in the case of the lowest oral dose given rats, they were on the order of one in three.

Again, it is fair to say that what is applicable to rats in such tests may not be applicable to human beings. But it is also fair to say that studies involving rats are conducted not for the welfare of the rat kingdom but for the ultimate protection of human beings. In the opinion of Dr. Epstein, the fact that the 2,4,5-T used in the Bionetics study produced teratogenic effects in *both* mice and rats underlines the seriousness of the study's implications. In the opinion of Dr. McLaughlin, this is even further underlined by another circumstance—that the rat, as a test animal, tends to be relatively resistant to teratogenic effects of chemicals. For example, in the late nineteen-fifties, when thalidomide, that disastrously teratogenic compound, was being tested on rats in oral dosages ranging from low to very high, no discernible fetus-deforming effects were produced. And Dr. McLaughlin says that as far as thalidomide tests on rabbits were concerned, "You could give thalidomide to rabbits in oral doses at between fifty and two hundred times the comparable human level to show any comparable teratogenic effects." In babies born to women who took thalidomide, whether in small or large dosages and whether in single or multiple dosages, between the sixth and seventh weeks of pregnancy, the rate of deformation was estimated to be one in ten.

Because of the relatively coarse testing screen through which compounds like pesticides—and food additives as well—are sifted before they are approved for general or specialized use in this country, the Food and Drug Administration theoretically maintains a policy of stipulating, as a safety factor, that the maximum amount of such a substance allowable in the human diet range from one two-thousandth to one one-hundredth of the highest dosage level of the substance that produces no harmful effects in experimental animals. (In the case of pesticides, the World Health Organization takes a more conservative view, considering one two-thousandth of the "no-effect" level in animal studies to be a reasonable safety level for human exposure.) According to the standards of safety established by F.D.A. policy, then, no human being anywhere should ever have been exposed to 2,4,5-T, because in the Bionetics study of rats *every* dosage level produced deformed fetuses. A "no-effect" level was never achieved.

To make a reasonable guess about the general safety of 2,4,5-T for human beings, as the material has been used up to now, the most appropriate population area to observe is probably not the relatively healthy and well-fed United States, where human beings are perhaps better equipped to withstand the assault of toxic substances, but South Vietnam, where great numbers of civilians are half-starved, ravaged by disease, and racked by the innumerable horrors of war. In considering any potentially harmful effects of 2,4,5-T on human beings in Vietnam, some attempt has to be made to estimate the amount of 2,4,5-T to which people, and particularly pregnant women, may have been exposed as a result of the repeated defoliation operations. To do so, a comparison of known rates of application of 2,4,5-T in the United States and in Vietnam is in order. In this country, according to Dr. Tschirley, the average recommended application of 2,4,5-T in aerial spraying for woody-plant control is between three-quarters of a pound and a pound per acre. Therre are about five manufacturers of 2,4,5-T in this country, of which the Dow Chemical Company is one of the biggest. One of Dow Chemical's best-sellers in the 2,4,5-T line is Esteron 245 Concentrate, and the cautionary notes that a drum of Esteron bears on its label are hardly reassuring to someone lulled by prior allgeations that 2,4,5-T is a substance of low toxicity:

"Caution—may cause skin irriation, avoid contact with eyes, skin, and clothing keep out of the reach of children."

Under the word "warning" are a number of instructions concerning safe use of the material, and these include, presumably for good reason, the following admonition:

"Do not contaminate irrigation ditches or water used for domestic purposes

Then comes a "notice":

"Seller makes no warranty of any kind, express or implied, concerning the use of this product. Buyer assumes all risk of use or handling, whether in accordance with directions or not."

The concentration of Esteron recommended—subject to all these warnings, cautions, and disclaimers—for aerial spraying in the United States varies with the type of vegetation to be sprayed, but probably a fair average would be three-quarters to one pound acid equivalent of the raw 2,4,5-T per acre. In Vietnam, however, the concentration of 2,4,5-T for each acre sprayed has been far higher. In Agent Orange, the concentrations of 2,4,5-T have averaged *thirteen times* the recommended concentrations used in the United States. The principal route through which quantities of 2,4,5-T might be expected to enter the human system in Vietnam is through drinking water, and in the areas sprayed most drinking water comes either from rainwater cisterns fed from house roofs or from very shallow wells. It has been calculated that, taking into account the average amount of 2,4,5-T in Agent Orange sprayed per acre in Vietnam by the military, and assuming a one-inch rainfall (which is quite common in South Vietnam) after a spraying, a forty-kilo (about eighty-eight-pound) Vietnamese woman drinking two litres (about 1.8 quarts) of contaminated water a day could very well be absorbing into her system a hundred and twenty milligrams, or about one two-hundred-and-fiftieth of an ounce, of 2,4,5-T a day; that is, a daily oral dosage of three milligrams of 2,4,5-T per kilo of body weight. Thus, if a Vietnamese woman who was exposed to Agent Orange was pregnant, she might very well be absorbing into her system a percentage of 2,4,5-T only slightly less than the percentage that deformed one out of every three fetuses of the pregnant experimental rats. To pursue further the question of exposure of Vietnamese to 2,4,5-T concentrations in relation to concentrations officially considered safe for Americans, an advisory subcommittee to the Secretary of the Interior, in setting up guide-lines for maximum safe contamination of surface water by pesticides and allied substances some time ago, recommended a concentration of one-tenth of a milligram of 2,4,5-T in one litre of drinking water as the maximum safe concentration. Thus, a pregnant Vietnamese woman who ingested a hundred and twenty milligrams of 2,4,5-T in two litres of water a day would be exposed to 2,4,5-T at six hundred times the concentration officially considered safe for Americans.

Moreover, the level of exposure of Vietnamese people in sprayed areas is not necessarily limited to the concentrations shown in Dr. Meselson's calculations. Sometimes the level may be far higher. Dr. Pfeiffer, the University of Montana biologist, says that when difficulties arise with the spray planes or the spray apparatus, or when other accidents occur, an entire thousand-gallon load of herbicidal agent containing 2,4,5-T may be dumped in one area by means of the thirty-second emergency-dumping procedure. Dr. Pfeiffer has recalled going along as an observer on a United States defoliation mission last March, over the Plain of Reeds area of Vietnam, near the Cambodian border, during which the technician at the spray controls was unable to get the apparatus to work, and thereupon dumped his whole load. "This rained down a dose of 2,4,5-T that must have been fantastically concentrated," Dr. Pfeiffer has said. "It was released on a very watery spot that looked like headwaters draining into the Mekong River, which hundreds of thousands of people use? In another instance, he has recalled, a pilot going over the area of the supposedly "friendly" Catholic refugee villages of Ho Nai, near Bien Hoa, had serious engine trouble and dumped his whole spray load of herbicide on or near the village. In such instance, the concentration of 2,4,5-T dumped upon an inhabited area in Vietnam probably averaged about a hundred and thirty times the concentration reccommended by 2,4,5-T manufacturers as both effective and safe for use in the United States.

Theoretically, the dangers inherent in the use of 2,4,5-T should have been removed by means of the steps promised in the White House announcement last October. A quick reading of the statement by Dr. DuBridge (who is also the executive secretary of the President's Environmental Quality Council) certainly seemed to convey the impression that from that day onward there would be a change in Department of Defense policy on the use of 2,4,5-T in Vietnam, just as there would be a change in the policies of the Departments of Agriculture and the Interior on the domestic use of 2,4,5-T. But did the White House mean what it certainly seemed to be saying about the future military use of 2,4,5-T in Vietnam? The White House statement was issued on October 29th. On October 30th, the Pentagon announced that no change would be made in the policy governing the military use of 2,4,5-T in South Vietnam, because—so the Washington *Post* reported on October 31st—"the Defense Department feels its present policy conforms to the new Presidential directive." The *Post* article went on:

"A Pentagon spokesman's explanation of the policy, read at a morning press briefing, differed markedly from the written version given reporters later.

"When the written statement was distributed, reporters were told not to use the spokesman's [previous] comment that the defoliant . . . is used against enemy 'training and regroupment centers.'

"The statement was expunged after a reporter asked how use against such centers conformed to the Defense Department's stated policy of prohibiting its use in 'populated areas.'"

But the statement wasn't so easily expunged. A short time later, it was made again, in essence, by Rear Admiral William E. Lemos, of the Policy Plans and National Security Council Affairs Office of the Department of Defense, in testimony before a subcommittee of the House Foreign Affairs Committee, the only difference being that the phrase "training and regroupment centers" became "enemy base camps." And in testifying that the military was mounting herbicidal operations on alleged enemy base camps Rear Admiral Lemos said:

"We know . . . that the enemy will move from areas that have been sprayed. Therefore, enemy base camps or unit headquarters are sprayed in order to make him move to avoid exposing himself to aerial observation."

If one adds to the words "enemy base camps" the expunged words "training and regroupment centers"—centers that are unlikely to operate without an accompanying civilian population—what the Defense Department seems actually to be indicating is that the "areas remote from the population" against which the United States is conducting military herbicidal operations are "remote from the population" at least in part *because* of these operations.

As for the Bionetics findings on the teratogenic effects of 2,4,5-T on experimental animals, the Department of Defense indicated that it put little stock in the dangers suggested by the report. A reporter for the *Yale Daily News* who telephoned the Pentagon during the first week in December to inquire about the Defense Department's attitude toward its use of 2,4,5-T in the light of the Bionetics report was assured that "there is no cause for alarm about defoliants." A week or so later, he received a letter from the Directorate for Defense Information at the Pentagon which described the Bionetics results as based on "evidence that 2,4,5-T, when fed in large amounts to highly imbred and susceptible mice and rats, gave a higher incidence of birth defects than was normal for these animals." After reading this letter, the *Yale Daily News*

[illegible]rter again telephoned the Pentagon, and asked, "Does [the Department of [illegible]ense] think defoliants could be affecting embryo growth in any way [illegible] Vietnam?" The Pentagon spokesman said, "No." And that was that. The experimental animals were highly susceptible; the civilian Vietnamese population, which even under "normal" circumstances is the victim of a statistically incalculable but clearly very high abortion and infant-mortality rate, was not.

Nearly a month after Dr. DuBridge's statement, another was issued, this one by the President himself, on United States policy on chemical and biological warfare. The President, noting that "biological weapons have massive, unpredictable, and potentially uncontrollable consequences" that might "impair the health of future generations," announced it as his decision that thenceforward "the United States shall renounce the use of lethal biological agents and weapons, and all other methods of biological warfare." Later, a White House spokesman, in answer to questions by reporters whether this included the use of herbicidal, defoliant, or crop-killing chemicals in Vietnam, made it clear that the new policy did not encompass herbicides.

Since the President's statement did specifically renounce "all other methods of biological warfare," the reasonable assumption is that the United States government does not consider herbicidal, defoliant, and crop-killing operations against military and civilian populations to be part of biological warfare. The question therefore remains: What does the United States government consider biological warfare to consist of? The best place to look for an authoritative definition is a work known as the Joint Chiefs of Staff Dictionary, an official publication that governs proper word usage within the military establishment. In the current edition of the Joint Chiefs of Staff Dictionary, "biological warfare" is defined as the "employment of living organisms, toxic biological products, and plant-growth regulators to produce death or casualties in man, animals, or plants or defense against such action." But the term "plant-growth regulators" is nowhere defined in the Joint Chiefs of Staff Dictionary, and since a certain technical distinction might be made (by weed-control scientists, for example) between plant-growth regulators and defoliants, the question of whether the Joint Chiefs consider military defoliation operations part of biological warfare is left unclear. As for "defoliant agents," the Dictionary defines such an agent only as "a chemical which causes trees, shrubs, and the other plants to shed their leaves prematurely." All this is hardly a surprise to anyone familiar with the fast semantic legerdemain involved in all official statements on biological warfare, in which defoliation has the baffingly evanescent half-existence of a pea under a shell.

To find that pea in the official literature is not easy. But it is resonable to assume that if the Department of Defense were to concede officially that "defoliant agents" were in the same category as "plant-growth regulators" that "produce death . . . in plants," it would thereby also be conceding that it is in fact engaging in the biological warfare that President Nixon has renounced. And such a concession seems to have been run to earth in the current edition of a Department of the Army publication entitled "Manual on Use of Herbicides for Military Purposes," in which "antiplant agents" are defined as "chemical agents which possess a high offensive potential for destroying or seriously limiting the production of food and defoliating vegetation," and goes on "These compounds include herbicides that kill or inhibit the growth of plants; plant-growth regulators that either regulate or inhibit plant growth, sometimes causing plant death. . . ." The admission that the Department of Defense is indeed engaging, through its defoliation and herbicidal operations in Vietnam, in biological warfare, as this is defined by the Joint Chiefs and as it has been formally renounced by the President, seems inescapable.

Since the DuBridge statement, allegations, apparently originating in part with the Dow Chemical Company, have been made to the effect that the 2,4,5-T used in the Bionetics study was unrepresentative of the 2,4,5-T generally produced in this country, in that it contained comparatively large amounts of a certain contaminant, which, according to the Dow people, is ordinarily present in 2,4,5-T only in trace quantities. Accordingly, it has been suggested that the real cause of the teratogenic effects of the 2,4,5-T used in the Bionetics study may not have been the 2,4,5-T itself but, rather, the contaminant in the sample used. The chemical name of the contaminant thus suspected by the Dow people is 2,3,6,7-tetrachlorodibenzo-*p*-dioxin, often referred to simply as dioxin. The 2,4,5-T used by Bionetics was obtained in 1965 from the Diamond Alkali Company, now known as the Diamond-Shamrock Company and no longer in the business of manufacturing 2,4,5-T. It appears that the presence of a dioxin contaminant in the process of manufacturing 2,4,5-T is a constant problem among all manufacturers. Three years ago, Dow was obliged to close down its 2,4,5-T plant in Midland, Michigan, for several months and partly rebuild it because of what Dow people variously described as "a problem" and "an accident." The problem—or accident—was that workers exposed to the dioxin contaminant during the process of manufacture came down with an acute skin irritation known as chlor-acne. The Dow people, who speak with considerable pride of their toxicological work ("We established our toxicology lab the year Ralph Nader was born," a Dow public-relations man said recently, showing, at any rate, that Dow is keenly aware of Nader and his career), say that the chlor-acne problem has long since been cleared up, and that the current level of the dioxin contaminant in Dow's 2,4,5-T is less than one part per million, as opposed to the dioxin level in the 2,4,5-T used in the Bionetics study, which is alleged to have been between fifteen and thirty parts per million. A scientist at the DuBridge office, which has become a coordinating agency for information having to do with the 2,4,5-T question, says that the 2,4,5-T used by Bionetics was "probably representative" of 2,4,5-T being used in this country—and presumably in Vietnam—at the time it was obtained but that considerably less of the contaminant is present in the 2,4,5-T now being produced. Evidently, the degree of dioxin contamination present in 2,4,5-T varies from manufacturer to manufacturer. What degree of contamination high or low, was present in the quantities of 2,4,5-T shipped to South Vietnam at various times this spokesman didn't seem to know.

The point about the dioxin contamination of 2,4,5-T is an extremely important one, because if the suspicions of the Dow people are correct and the cause of the fetus deformities cited in the Bionetics study is not the 2,4,5-T but the dioxin contaminant, then this contaminant may be among the most teratogenically powerful agents ever known. Dr. McLaughlin has calculated that if the dioxin present in the Bionetics 2,4,5-T was indeed responsible for the teratogenic effects on the experimental animals, it looks as though the contaminant would have to be at least ten thousand times more teratogenically active in rats than thalidomide was found to be in rabbits. Furthermore, it raises alarming questions about the prevalence of the dioxin material in our environment. It appears that under high heat the dioxin material can be produced in a whole class of chemical substances known as trichlorophenols and pentachlorophenols. These substances include components of certain fatty acids used in detergents and in animal feed.

As a consequence of studies that have been made of the deaths of millions of young chicks in this country after the chicks had eaten certain kinds of chicken feed, government scientists are now seriously speculating on the possibility that the deaths were at the end of a chain that began with the spraying of corn crops with 2,4,5-T. The hypothesis is that residues of dioxin present in the 2,4,5-T remained in the harvested corn and were concentrated into certain byproducts that were then sold to manufacturers of chicken feed, and that the dioxin became absorbed into the system of the young chicks. One particularly disquieting sign of the potential of the dioxin material is the fact that bioassays made on chick embryos in another study revealed that all the embryos were killed by one twenty-millionth of a gram of dioxin per egg.

Perhaps an even more disquieting speculation about the dioxin is that 2,4,5-T may not be the only material in which it appears. Among the compounds that several experienced biologists and toxicologists suspect might contain or produce dioxin are the trichlorophenols and pentachlorophenols, which are rather widely present in the environment in various forms. For example, a number of the trichlorophenols and pentachlorophenols are used as slime-killing agents in paper-pulp manufacture, and are present in a wide range of consumer products, including adhesives, water-based and oil-based paints, varnishes and lacquers, and paper and paper coatings. They are used to prevent slime in pasteurizers and fungus on vats in breweries and are also used in hair shampoo. Along with the 2,4,5-T used in the Bionetics study, one trichlorophenol and one pentachlorophenol were tested without teratogenic results. But Dr. McLaughlin points out that since there are many such compounds put out by various companies, these particular samples might turn out to be —by the reasoning of the allegation that the 2,4,5-T used by Bionetics was unusually dirty—unusually clean.

Dr. McLaughlin tends to consider significant, in view of the now known extreme toxicity and possible extreme teratogenicity of dioxin, the existence of even very small amounts of the trichlorophenols and pentachlorophenol in food wrappings and other consumers products. Since the production of dioxin appears to be associated with high-temperature conditions, a question arises whether these thermal conditions are met at *any* stage of production or subsequent use or disposal of such materials, even in minute amounts. One of the problems here seems to be, as Dr. Epstein has put it, "The moment you introduce something into the environment it's likely to be burned sooner or later—that's the way we get rid of nearly everything." And most of these consumer products may wind up in municipal incinerators, and when they are burned, the thermal and other conditions for creating dioxin materials may quite possibly be met. If so, this could mean a release of dioxin material into the entire environment through the atmosphere.

Yet so far the dioxin material now suspected of causing the fetus-deforming effects in experimental animals has never been put through any formal teratological tests by any company or any government agency. If the speculation over the connection between dioxin in 2,4,5-T and the deaths of millions of baby chicks is borne out, it might mean that, quite contrary to the assumptions made up to now that 2,4,5-T is rapidly decomposable in soil, the dioxin material may be extremely persistent as well as extremely deadly.

So far, nobody knows—and it is probable that nobody will know for some time—whether the fetus deformities in the Bionetics study were caused by the 2,4,5-T itself, by the dioxin contaminant, or by some other substance or substances present in the 2,4,5-T, or whether human fetuses react to 2,4,5-T in the same way as the fetuses of the experimental animals in the Bionetics study. However, the experience so far with the employment of 2,4,5-T and substances chemically allied to it ought to be instructive. The history of 2,4,5-T is related to preparations for biological warfare, although nobody in the United States government seems to want to admit this, and it has wound up being used for purposes of biological warfare, although nobody in the United States government seems to want to admit *this*, either. Since 2,4,5-T was developed, the United States government has allowed it to be used on a very large scale on our own fields and countryside without adequate tests of its effects. In South Vietnam—a nation we are attempting to save—for seven full years the American military has sprayed or dumped this biological-warfare material on the countryside, on villages, and on South Vietnamese men and women in staggering amounts. In that time, the military has sprayed or dumped on Vietnam *fifty thousand* tons of herbicide, of which twenty thousand tons have apparently been straight 2,4,5-T. In addition, the American military has apparently made incursions into a neutral country, Cambodia, and rained down on an area inhabited by thirty thousand civilians a vast quantity of 2,4,5-T. Yet in the quarter of a century since the Department of Defense first developed the biological-warfare uses of this material it has not completed a single series of formal teratological tests on pregnant animals to determine whether it has an effect on their unborn offspring.

Similarly, officials of the Dow Chemical Company, one of the largest producers of 2,4,5-T, although they refuse to divulge how much 2,4,5-T they are and have been producing, admit that in all the years that they had produced the chemical before the DuBridge statement they had never made formal teratological tests on their 2,4,5-T, which they are now doing. The Monsanto Chemical Company, another big producer, had, as far as is known, never made such tests, either, nor, according to an official in the White House, had any other manufacturer. The Department of Agriculture has never required any such tests from manufacturers. The Food and Drug Administration has never required any such tests from manufacturers. The first tests to determine the teratogenic efforts of 2,4,5-T were not made until the National Institutes of Health contracted for them with Bionetics Laboratories. And even then, when the adverse results of the tests became apparent, it was, as Dr. Epstein said, like "pulling teeth" to get the data out of the institutions involved. And when the data were obtained and the White House was obliged, partly by outside pressure and publicity, to act, the President's science adviser publicly presented the facts in a less than candid manner, while the Department of Defense, for all practical purposes, ignored the whole business and announced its intention of going on doing what it had been doing all along.

There have been a number of reports from Vietnam both of animal abortions and of malformed human babies that are thought to have resulted from spraying operations in which 2,4,5-T was used. But such scattered reports, however well founded, cannot really shed much more light on the situation. The fact is that even in this country, the best-fed, richest, and certainly most statistics-minded of all countries on earth, the standards for testing materials that are put into the environment, into drugs, and into the human diet are grossly inadequate. The screening system is so coarse that, as a teratology panel of the Mrak Commission warned recently, in connection with thalidomide, "the teratogenicity of thalidomide might have been missed had it not produced malformations rarely encountered." In other words, had it not been for the fact that very unusual and particularly terrible malformations appeared in an obvious pattern—for example, similarly malformed babies in the same hospital at about the same time—pregnant women might still be using thalidomide, and lesser deformations would, so to speak, disappear into the general statistical background. As for more subtle effects, such as brain damage and damage to the central-nervous system, they would probably never show up as such at all. If such risks existed under orderly, normal medical conditions in a highly developed country, how is one ever to measure the harm that might be done to unborn children in rural Vietnam, in the midst of the malnutrition, the disease, the trauma, the poverty, and the general shambles of war?

DEPARTMENT OF AMPLIFICATION,
New York, March 5, 1970.

The EDITORS,
The New Yorker

DEAR SIRS: In an article that appeared in *The New Yorker* on February 7th, I wrote that Dr. Lee DuBridge, the President's science adviser, issued a statement last October at the White House saying that because a laboratory study had shown a "higher than expected number of deformities" in the fetuses of mice and rats exposed to the herbicide 2,4,5-T, agencies of the United States government would take action to restrict the use of that substance in this country and in Vietnam, where it was being used in extensive military defoliation operations. This action, Dr. DuBridge announced, would include the cancellation, by January 1st of this year, of Department of Agriculture permits for the use of 2,4,5-T on some American food crops unless the Food and Drug Administration had by then been able to determine a safe concentration of the herbicide in foods. Dr. DuBridge further announced that the Department of Defense would thenceforth "restrict the use of 2,4,5-T to areas remote from the population" in Vietnam. His statement added that these actions and others "will assure the safety of the public while further evidence [of the alleged harmful effects of 2,4,5-T] is being sought."

Four months have passed, and 2,4,5-T is still being used as widely as ever. The Department of Agriculture has yet to cancel its permits for the use of the herbicide on food crops in this country, and the Department of Defense is continuing to use it in populated areas of Vietnam. In the meantime, officials of the Dow Chemical Company, which is one of the largest producers of 2,4,5-T, have been maintaining that the samples of 2,4,5-T used in the study cited by Dr. DuBridge, which was done by the Bionetics Research Laboratories, of Bethesda, Maryland, were uncharacteristic of the 2,4,5-T currently being produced, because the material tested by Bionetics—which did not come from Dow—was contaminated to an unusual extent by a toxic substance identified as symmetrical 2,3,6,7-tetrachlorodibenzo-*p*-dioxin. This contaminant, usually called dioxin, was alleged by the Dow people to be present in the Bionetics samples at a concentration of approximately twenty-seven parts per million, and they claim that the 2,4,5-T that Dow is currently producing contains the dioxin contaminant in concentrations of less than one part per million. The Dow people maintain that their currently produced 2,4,5-T does not appear to have the effect of deforming rat fetuses. In January, a Dow official told the Department of Health, Education, and Welfare, "We strongly urge that action concerning the status of 2,4,5-T be held in abeyance until [Dow's] testing program is completed [in] April." The United States government's failure so far to place the promised restrictions on the use of 2,4,5-T in this country may in part be attributed to this plea.

Because of the seriousness of the issues involved, it seems to me that the government's failure to act on the use of 2,4,5-T here and in Vietnam calls for much fuller public discussion. Even though the dioxin contaminant may now be present in 2,4,5-T in what the Dow Chemical Company apparently considers to be no more than tolerable amounts, the substance is of such potency that its release even in small concentrations must prompt deep concern. In the presumably more heavily dioxin-contaminated samples of 2,4,5-T that were used in the Bionetics work, the smallest dosages of 2,4,5-T that the test animals were given caused extensive deformities in fetuses. In more recent studies of the dioxin contaminant, conducted by Dr. Jacqueline Verrett, of the Food and Drug Administration (who earlier was responsible for revealing the carcinogenicity of cyclamates), extensive teratogenic, or fetus-deforming, effects were discovered in chick embryos when the dioxin, or a distillate predominantly consisting of it, was present at concentrations of little more than a trillionth of a gram per gram of the egg. The magnitude of this effect on chick embryos may be gathered from the fact that, according to Dr. Verrett's studies, the dioxin appears to be a million times as potent a fetus-deforming agent as the notorious teratogen thalidomide was found to be in tests on chicks. Of course, chick embryos are far down the biological ladder from human fetuses, and they are also extremely sensitive to many substances. But even if, for theoretical purposes, we reduced the teratogenic power of the dioxin, as shown in Dr. Verrett's chick-embryo studies, approximately a million times, we would *still* have to consider that we were dealing with a substance as teratogenically potent as thalidomide. That the United States government permits the presence, even in minute amounts, of such a substance in herbicidal mixtures to be sold for spraying on food crops and on suburban lawns—where some of the chemical may enter shallow wells and other drinking-water supplies—is hardly reassuring. And it is particularly disturbing when one reflects that in the quarter of a century in which 2,4,5-T was used prior to Dr. DuBridge's announcement not a single regulatory agency of the United States government, not the Department of Defense—which has been spreading huge quantities of 2,4,5-T on vast areas of Vietnam—and not, as far as is known, the researchers for any one of the half-dozen large American chemical companies producing the material had ever so much as opened up a pregnant mouse to determine whether 2,4,5-T or the dioxin contaminant in it did any systemic or pathogenic harm to the fetus. Several studies of the sort are now under way, but the United States government still seems to take the position that the 2,4,5-T produced by Dow and other large chemical companies should be considered innocent until it is proved to be otherwise. Meanwhile, 2,4,5-T is being sprayed on certain crops and on areas where it may come into contact with human beings, cattle, and wildlife. In Vietnam, it is still being sprayed by the military in concentrations that average thirteen times as great as those that the manufacturers themselves recommend as safe and effective for use in this country.

It is true that the teratogenicity of dioxin—as distinct from dioxin-contaminated 2,4,5-T—has not yet been established in tests conducted on experimental animals of mammalian species. However, the direct toxic, or body-poisoning, effects—as distinct from fetus-deforming effects—of dioxin are known to be very high both in animals and in human beings. In past studies on rats, dosages of forty-five millionths of a gram per kilo of the mother's body weight have been found to kill fifty per cent of the offspring. When dioxin was given orally to pregnant rats in recent tests, it was found, on preliminary investigation, to kill all fetuses with dosages of eight millionths of a gram per kilo of the mother's body weight, and to damage fetuses with dosages of a half-millionth of a gram per kilo.

Further, the effects of dioxin on human beings, even in small dosages, are known to be serious. In the past, in plants manufacturing 2,4,5-T an illness called chloracne seems to have been widespread among the workers. In the mid-sixties, Dow was obliged to close down part of a 2,4,5-T plant in Midland, Michigan, for some time because about sixty workers contracted chloracne as a result of contact with dioxin, which seems to be always present in varying degrees during the process of manufacturing 2,4,5-T and in the finished 2,4,5-T itself. The symptoms of this disease include extensive skin eruptions, disorders of the central nervous system, chronic fatigue, lassitude, and depression. Workers at a 2,4,5-T plant in New Jersey run by another company suffered similar symptoms in the mid-sixties, and six years later some of them were reported to be still suffering from the effects of the disease. In Germany, since the

mid-fifties, workers in factory after factory producing 2,4,5-T and polychlorophenolic compounds have been afflicted with chloracne after absorbing apparently only minute amounts of the dioxin contaminant. Their symptoms have been described in several medical papers as including liver damage, nervous and mental disorders, depression, loss of appetite and weight, and markedly reduced sexual drive.

A few weeks ago, when a reporter approached an official in Dr. DuBridge's office for information on 2,4,5-T he was told that he would be given White House cooperation "only to a certain extent," because the official didn't want "wild speculation" stirred up. He cited as an example of "wild speculation" the recent controversy over the birth-control pill, which, he said, had "caused millions of women to get hysterical with worry." The reporter replied that he didn't think the analogy between 2,4,5-T and the Pill was a particularly good one, for the reason that a woman using the Pill could employ alternative methods of contraception, whereas a Vietnamese woman exposed to herbicidal spray put down by the American military had no choice in the matter.

But perhaps the comparison between 2,4,5-T (and its dioxin contaminant) and commonly used pills is worth pursuing. Suppose that such a dangerous substance as dioxin were found to be contained in a pill offered for human consumption in this country, and suppose that the contaminant were present in such minute amounts that an adult following the prescribed dosages might ingest a hundredth of a millionth of a gram of the contaminant per day. There is no doubt whatever that, according to existing Food and Drug Administration standards, the F.D.A. would immediately ban production and sale of the pill on the ground that it was highly dangerous to public health; in fact, the amount of such a potent contaminant that the F.D.A. would permit in a pill under the agency's present policy on toxicity would almost certainly be zero.

While 2,4,5-T, with or without the dioxin contaminant, doesn't come in pill form, it may be worthwhile to try to calculate, on the basis of a hypothetical pill, how much 2,4,5-T (and dioxin) a Vietnamese woman living in an area sprayed by the American military might ingest in a day. It has already been calculated by reputable biologists that, if one takes into account the average amount of 2,4,5-T sprayed per acre in Vietnam, and also takes into account a one-inch rainfall—such as is common there—after a spraying, a forty-kilo (about eighty-eight-pound) Vietnamese woman drinking two litres (about two quarts) of 2,4,5-T-contaminated water per day could be ingesting about a hundred and twenty milligrams (about a two-hundred-and-fiftieth of an ounce) of 2,4,5-T a day. If the 2,4,5-T contained the dioxin contaminant at a level of one part per million—which is what the Dow people say is the maximum amount present in the 2,4,5-T they are currently producing—the Vietnamese woman would be absorbing a little over a tenth of a microgram of dioxin per day, or ten times the amount of dioxin entering the system of an adult from the hypothetical pill that the F.D.A. would certainly find dangerous to human health. Further, if this Vietnamese woman were to conceive a child two weeks, say, after the spraying, the weight of the dioxin that by these same calculations would have then accumulated in her system (the evidence thus far is that dioxin accumulates in mammalian tissue in the same manner as the chlorinated hydrocarbons, such as DDT) would be more than the weight of the just-fertilized ovum. Considering the existing evidence of the frightening degree of teratogenicity of the dioxin in chick embryos and its highly toxic effects on mammalian fetuses, the presence of this much dioxin in a mother's body at the very beginning of a human life surely has ominous implications.

Now, what about the safety of 2,4,5-T itself? Admittedly, the dioxin contaminant seems to be a residue from one stage of its manufacture. But if by some future chemical miracle the very last trace of dioxin could be removed from the finished 2,4,5-T, would the resultant "pure" 2,4,5-T be harmless? The fact seems to be that even then 2,4,5-T, as produced in this country, would have to be viewed with suspicion, for the breakdown products of 2,4,5-T, when subjected to heat and other conditions, are themselves capable, according to a number of responsible biologists, of producing dioxin. Given this potential, the ultimate folly in our defoliation operations in Vietnam was possibly achieved during 1965 and 1966, when the military made large-scale efforts in two defoliated areas to create fire storms—that is, fires so huge that all the oxygen in those areas would be exhausted. The apparent intention was to render the soil barren. (A fire storm would also, of course, have the result of burning or suf-

[redacted]ating any living beings remaining in the area.) Operation Sherwood Forest, [redacted]nducted in 1965, was an attempt to burn a defoliated section of the Bo[redacted] Woods. In October, 1966, the military began Operation Pink Rose, a sim[redacted]r project. Neither of the projects, in which tons of napalm were thrown down on top of the residue of tons of sprayed 2,4,5-T, succeeded in creating the desired effect; whether they released into the atmosphere dioxin produced by the breakdown products of the 2,4,5-T will probably never be known.

There are also less spectacular ways in which conditions suitable for the release of dioxin in Vietnam may have been created. For example, after areas accessible by road have been defoliated, woodcutters move in to chop up the dead timber, which is then carted off to nearby towns and sold as firewood. Large quantities of it are said to have been entering Saigon for years. Since the fires are customarily tended by Vietnamese women, and since many of them are certainly pregnant, the hazards to health and to the lives of unborn children surely cannot be ignored.

In the United States, the potential hazards from the present use of 2,4,5-T are considerably less than they are in Vietnam. In the first place, the recommended concentrations of 2,4,5-T for spraying here are, as I have pointed out, about a thirteenth of what the Vietnamese population is sometimes subjected to. And, in the second place, a great deal, if not most, of the 2,4,5-T that would otherwise have been sprayed on American crops and grazing areas has for several years been sent to Vietnam. However, the shortage of 2,4,5-T in this country does not necessarily mean that the potential hazards are at a minimum. The substances known as the trichlorophenols and compounds of pentachlorophenol, which officials of the F.D.A. believe may be chemical precursors of dioxin under certain thermal and other conditions, are used widely in the manufacture of a large variety of consumer products, ranging from paper to laundry starch and hair shampoo. Dow Chemical puts out a whole line of polychlorophenolic chemicals known as Dowicide Products. Monsanto Chemical also puts out a line of pentachlorophenol substances, known as Penta Compounds. Since a very great many consumer products wind up being burned sooner or later, and since the polychlorophenolic compounds are suspected of being capable, under particular thermal and other conditions, of releasing dioxin, the alarming question arises whether, and to what extent, dioxin is being released into the environment through the atmosphere. Pentachlorophenol, used in certain herbicides, is readily decomposed in sunlight, and in its breakdown process a number of products, including chemical precursors of chlorodibenzo-*p*-dioxin compounds, are produced. Because of these factors, a whole range of pesticides, as well as of herbicides, now must come under suspicion of producing dioxin compounds.

Although the chemical companies that manufacture 2,4,5-T have long taken pride in pointing out that 2,4,5-T itself is quite readily decomposable in soil, the crucial matters of how stable the dioxin contaminant is and to what extent it is cumulative in animal tissue have apparently been neglected. Consequently, the fact that traces of compounds virtually indistinguishable from dioxin have already been detected in this country in the human food chain—in the livers of chickens and in edible oils—clearly indicates that dioxin should be considered a hazard to man. Why, under all these inauspicious circumstances, the production and the use here and in Vietnam of 2,4,5-T has not summarily been stopped by the United States government is hard to understand.

Sincerely,

THOMAS WHITESIDE.

Appendix 4

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE,
PUBLIC HEALTH SERVICE,
FOOD AND DRUG ADMINISTRATION,
Rockville, Md., March 12, 1970.

Hon. RICHARD D. MCCARTHY,
House of Representatives,
Washington, D.C.

DEAR MR. MCCARTHY: The Secretary has asked us to reply to your letter of February 3, 1970, requesting whether the Food and Drug Administration has information indicating that 2,4,5-T is now safe to use.

No tolerances have been established for residues of 2,4,5-T in food or feed crops. The whole matter of the safety of this herbicide, when its use results in a residue in or on a food crop, is currently under evaluation. This evaluation will be completed as expeditiously as possible. We are encl[redacted] a Fact Sheet explaining the status of 2,4,5-T at this time.

We shall promptly inform you of our decision upon completion of the evaluation of 2,4,5-T.

Sincerely yours,

M. J. RYAN, *Acting Director,*
Office of Legislative Services.

FDA FACT SHEET

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE,
PUBLIC HEALTH SERVICE,
FOOD AND DRUG ADMINISTRATION,
Washington, D.C.

* 2,4,5-T *

2,4,5-T (2,4,5-trichlorophenoxyacetic acid) has had extensive registered use as a defoliant and weed killer. It has also been registered by the U.S. Department of Agriculture as a pesticide chemical (herbicide) on a no residue basis on a few certain selected food crops for some years, primarily for weed control of pasture and rangeland.

TOXICITY

A research study recently completed under contract to the National Cancer Institute on a commercial lot of 2,4,5-T showed that the feeding of this material to rats and mice produced abnormal birth effects on the embryos.

Further investigation of the 2,4,5-T used in the feeding studies established that the material used contained a significant amount of one of more impurities called dioxins produced during the manufacture of 2,4,5-T. Improved manufacturing processes are claimed by one manufacturer to have reduced the dioxin impurities to insignificant amounts.

The dioxins are of concern because they are known to be extremely toxic to poultry and to have produced severe skin irritation to workers in plants exposed to dioxins inadvertently during the manufacture of other chemicals. At present a number of research studies are underway in both government and commercial laboratories to determine if the reported birth defects of the earlier study are due to 2,4,5-T itself, the dioxin impurities, or a combination of the 2,4,5-T and the dioxins.

Additional investigations are underway to improve our ability to detect very small amounts of dioxins in samples of 2,4,5-T and to determine whether other commonly used pesticides chemically related to 2,4,5-T contain significant amounts of the dioxin contaminants. Drinking water supplies are being tested for the presence of 2,4,5-T and other possible environmental sources of these chemicals studied, but no results are available at this time.

The USDA announced on February 6, 1970, that it is investigating 17 commonly used pesticides chemically related to 2,4,5-T to determine whether they contain hazardous amounts of these toxic contaminants.

FOOD IN THE UNITED STATES

The Food and Drug Administration is continually engaged in examining samples of individual foodstuffs for residues of pesticides above the safe tolerances established under the Miller Pesticide Amendment. In addition, FDA purchases food in the markets of several cities, prepares the food in the quantities and combinations typical of the diet of an average 19-year-old male, and determines the amounts of the several pesticides that might be actually ingested in the typical diet of a heavy eater.

Of 5300 food samples tested for 2,4,5-T residues during the last four-year period, 25 samples indicated trace amounts (less than the 0.1 p.p.m. limit of accuracy of present analytical procedures) and 2 samples showed higher residues. 0.19 p.p.m. 2,4,5-T was detected in one sample of milk taken in 1965 in New England, and one sample of sugarbeets from Ohio in 1966 showed 0.29

p.p.m. 2,4,5-T. The milk had been distributed before analysis was complete and processing of the sugar-beets removes the chemical. If food is found to contain finite residues of 2,4,5-T, it is subject to removal from the market.

STATUS OF 2,4,5-T UNDER THE FEDERAL FOOD, DRUG, AND COSMETIC ACT

No finite tolerances have been established for residues of 2,4,5-T or the dioxins in food. In the absence of established tolerances any detectable amount of either chemical in food would make the contaminated food illegal and subject to seizure if found in the channels of interstate commerce.

A petition was filed in December, 1967 requesting the establishment of tolerances of 0.2 p.p.m. for residues of 2,4,5-T on apples, barley, blueberries, corn, oats, rice, rye, sugarcane, and wheat. Neither the petition as originally submitted or as later supplemented provided data to support affirmative action and the petitioner withdrew his petition on December 29, 1969, as provided for under the pesticide regulations.

Petitions to establish a safe tolerance level for residues of 2,4,5-T in food may again be submitted to the FDA in the future. However, any such submission must include scientific research data to resolve the questions that have been raised concerning toxicity of 2,4,5-T and the dioxins.

CONCLUSION

The Department of Health, Education, and Welfare is continuing investigations to determine the potential hazards from the possible presence of residues of 2,4,5-T and dioxins in foods, water, and other environmental sources to which the public may be exposed.

It is to be emphasized that there is no tolerance for 2,4,5-T in food today; the testing of food over the past several years has revealed no significant problem of food contamination.

Appendix 5

PROBE INTO USE OF HERBICIDES BY CONGRESSMAN RICHARD D. MCCARTHY. D-N.Y.

Globe, Ariz., February 13, 1970

Ladies and gentlemen, I think we should begin. I am Congressman Richard D. McCarthy, and the hearings will come to order.

For more than a decade scientists have had serious misgivings about the widespread use of herbicides and pesticides in the environment. The late Rachael Carson warned of the risk of the use of herbicides, whose effects were either harmful or unknown.

In the United States 120 million acres each year are sprayed with herbicides for the clearing of railroads, for brush control, for watershed management, and for other purposes. One of these is known as 2,4,5-T. It was developed and perfected at Fort Detrick, Md., the army's chief Biological Warfare Research Center. The herbicide 2,4,5-T, and 2,4-D, a related herbicide, collectively account for some 83 million pounds of production per year—that was the figure in 1968.

I've long been concerned with the widespread use of these herbicides in Vietnam. Each day some 100 tons are dropped on South Vietnam, and scientists for many months have been concerned about the adverse ecological effects of this herbicidal inundation.

Last summer in the course of my inquiry into the Army's germ and gas warfare policies, I learned that a study, by the Bionetics Research Laboratories for the National Cancer Institute showed that the herbicide 2,4,5-T produced birth defects in rats and mice.

When the conclusions of this study were known, the President's science adviser, in October, announced a ban on the herbicide beginning January 1, 1970, unless the F.D.A. had found safe legal tolerances. I was distressed 11 days ago to learn that contrary to the White House's announcement, the Department of Agriculture continues to authorize the use of 2,4,5-T in the United States. It's incredible to me that someone, or some people should have succeeded in overruling the science adviser to the President of the United States.

We know from the thalidomide experience that if we are going to err, we should err on the side of caution, and not on the side of danger. It is my firm conviction that such chemicals should not be used unless full tests show that they are safe. It is also incredible to me that this herbicide, which has been in existence since its development some 25 years ago at the Germ Warfare Research Center, still has not been fully tested for its teratogenic effects on human beings—that is, its power to produce birth deformities.

We know that it produces birth deformities in test animals under laboratory conditions, and we continue to receive reports from Vietnam that civilian women living in this heavily defoliated area are bringing forth deformed offsprings.

The Saigon Press has reported on these in considerable detail.

Now, we have the allegations, and complaints emanating from here, Globe, Ariz. It is my hope that my investigation into these complaints and allegations will assist me in continuing my inquiry into this whole matter. I wish to determine how the White House was overruled, and why it is that we continue to use this herbicide despite the warning signals that have arisen.

As the great French scientist physiologist, Claude Banard, once said, "True science teaches us to doubt, and ignorance to refrain."

I want to welcome all the local State and Federal officials who are in attendance. I hope to have a chance to meet with you personally during our visit.

Our first witness is Prof. Arthur W. Galston, a professor of biology from Yale University.

Doctor Galston.

Professor Galston, I wonder if, for the record, you would identify yourself, and your background, and particular expertise in the matters under inquiry.

Dr. GALSTON. Very happy to do that, Congressman.

I'm currently a professor of biology at Yale University. I'm also lecturer in forestry, and director of the March Botanical Gardens at Yale. I've been a professor of plant physiology for about 27 years. I was trained at the New York State College of Agriculture at Cornell University.

I did my graduate work at the University of Illinois, where I earned a Ph.D. degree in 1943. I then went to work for the emergency rubber project for the U.S. Government, located at Cal-Tech. During World War I was agricultural officer for U.S. Navy Military Government on the Isle of Okinawa. I then worked at Cal-Tech for 10 years, and I've been at Yale for the last 15 years.

I've published books in the area of plant physiology, and I have over 100 articles in the subject.

Congressman MCCARTHY. For the record, Doctor Galston, I wonder if you could give us a scientific information about the herbicide under investigation.

Dr. GALSTON. Congressmen, what I'd like to do is to give you and the audience here some appreciation of the feeling of a large number of scientists as exemplified in this report recently delivered to the Secretary of Health, Education, and Welfare, Finch.

It is called, "The Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health." It's dated December 5, 1969, and was prepared by the distinguished panel shared by Doctor Emil Mrak, the chancellor emeritus of the University of California at Davis.

It included many academic people, and also the vice presidents of two important companies, Dow, and Eli Lilly, both of whom manufacture herbicides and other pesticides in wide use.

The Commission takes note of the fact that there are now more than 400 different kinds of chemicals which are being used as pesticides to combat insects, fungi, weeds, and other predators.

Our modern agriculture and highly technicalized food production activities demand that we do use chemicals in agriculture.

I'd like to make it clear that I'm not alining myself with people that say, "Stop all chemicals." That's ridiculous in this day and age. We are dependent upon chemicals, and we have to keep using them.

Nonetheless, some of these chemicals are terribly noxious when introduced into the environment.

All of us are now familiar with the fact that DDT may be more of a bane than a boom. It has become global. Even a penguin picked up on an ice flow in Antarctica is full of DDT, and that was 400 miles from the application of

DDT, and we know that DDT causes oversized livers, and alteration of the steroid metabolism in everyone's genes.

This Commission agrees unanimously that DDT must be phased out as quickly as possible as a pesticide.

With that as a background, I think it's perfectly clear that as scientific information develops, we are going to want to examine every pesticide for its possible harmful effects on man and his domestic animals, and his environment.

Here I must digress to tell you about the changes that have occurred in our concept of what constitutes adequate testing for a compound of this kind.

It used to be that simple toxicology tests were conducted. A laboratory animal, such as a mouse or a rat was fed a certain amount of chemical. If that animal showed serious symptoms, the teratogenicity was calculated on the base of how many milligrams per kilogram of body weight of this material produced the toxic effects.

We now have tables which tell us roughly how toxic given materials are.

Now, based on that kind of test, 2,4,5-T, for example, is not terribly toxic, it's only a mildly toxic compound in the order of 2 to 700 kilograms milligram of body weight cause toxicity.

If, however, you use more subtle tests, you find out that 2,4,5-T may be more dangerous.

Among these tests are: Does the compound cause cancer? That takes a much more serious look than simply feeding and watching the dying of animals.

Secondly, do the compounds cause genetic effects, that is, does it break chromosomes, or cause mutations.

Thirdly, does the compound cause birth abnormalities. The word to describe that is teratogenics; that is the formation of monsters.

Now, this report which I have alluded to has as its last chapter, a chapter on teratology, and I'd like to read you just a little bit out of this chapter, and out of the summary which is written here, which gives you my concern.

"All currently used pesticides should be tested for teratogenicity in the near future in two or more mammalian species chosen on the basis of the closest metabolic and pharmacologic similarity to human beings possible. Pesticides should be tested at various concentrations including levels substantially higher than those to which the human population are likely to be exposed. Test procedures should also reflect routes related to human exposures. Apart from the obvious route of ingestion, attention should be directed to other routes of exposure, including inhalation exposures from pesticide aerosols and vaporizing pesticide strips used domestically, and exposures from skin absorption. Parenteral administration is an appropriate test route for pesticides to which humans are exposed by inhalation, or for pesticides, which are systemically absorped following ingestion.

"The use of currently registered pesticides to which humans are exposed and which are found to be teratogenic by suitable test procedures in one or more mammalian species should be immediately restricted to prevent risk of human exposures."

I'd like to repeat that: "Currently registered pesticides to which humans are exposed and which are found to be teratogenic by suitable test procedures in one or more *mammalian* species should be immediately restricted to prevent risk of human exposure. Such pesticides, in current use, include—" I'll skip a lot of names, 2,4-D and 2,4,5-T are listed.

Here's the Government's most distinguished panel saying that there is evidence that 2,4,5-T has produced teratogenic effects in one or more mammalian species, its use should be restricted immediately. They also said no new pesticide found to be teratogenic, should be used only in circumstances where risk of human exposure is minimal.

Congressman MCCARTHY. What's the date of that report, Professor?

Dr. GALSTON. December 5, 1969, it's now only 2 months old, Congressman, and it says a scientific group, or commission should be charged with the responsibility for continued surveillance of the whole problem of pesticide teratogenesis.

Now, the problem of determining whether a problem is teratogenic, whether it's given rise to birth defects is terribly complicated. If you do a laboratory test where you have one group of mice getting the chemical, and one group not, there's no problem to determine teratogenicity. By this kind of test it has been determined that 2,4,5-T as tested is one of the most teratogenic chemicals

known. Even as little as 4½ milligrams per kilo of body weight have trebled the rate of abnormal production in mice and in rats a 113 milligrams per kilo of body weight has produced 100 percent abnormal litters, and 70 percent abnormal individuals in those litters.

Congressman MCCARTHY. I wonder if you could translate those figures into what a human being would be likely to receive in the United States, or in Vietnam.

Dr. GALSTON. Well, if you take the lowest of those figures, 4½ milligrams per kilogram of body weight, and you say you have a 50-kilogram woman, that's 110 pounds which is about the average weight of a Vietnamese woman, then she needs to digest only about 200 milligrams total to have a teratogenic dose, 100 milligrams per day. Now, we are spraying agent orange, which is a 1 to 1 mixture of 2,4-D, and 2,4,6-T, in Vietnam at the rate of 270 pounds per acre. I should note that is 10 times what we used locally.

Congressman MCCARTHY. What would it be in Arizona?

Dr. GALSTON. I think our Forestry friends could tell us, it is in the order of two pounds per acre.

Congressman MCCARTHY. We will get to that with them today or tomorrow, but that's about the range?

Dr. GALSTON. At the Vietnam dose rate, if you assume a 27-pound per acre sprayed, followed by a 1-inch rainfall, which is normal for that region and you know that the rainwater is collected off the roof, or stored in cisterns, or gotten from very shallow wells, then a woman need only consume less than 3 quarts of water per day in combined drinking and cooking operations to receive that teratogenic dose.

I have calculated on this basis that it's possible that in Vietnam people have been given this kind of teratogenic dose.

Congressman MCCARTHY. Doctor, let me ask you this. Here we have the Bionetics Research Laboratory test which showed that 2,4,5-T is teratogenic in test animals, mice and rats. Is it teratogenic in human beings—do we know?

Dr. GALSTON. One doesn't know for sure whether it's teratogenic in human beings, one doesn't experiment with pregnant women, feeding some of them 2,4,5-T, and not feeding others. That would be inhuman, we do not tolerate that kind of experimentation, but the paragraph I was about to read here in fact deals with this.

It says there are two ways that you can determine whether a chemical is teratogenic. "First, chemicals or other agents may be administered to experimental animals to determine whether they induce prenatal damage. Secondly, and on a post hoc basis, human populations may be epidemiologically surveyed to detect geographical, or temporal clusters of unusual types of frequencies of congenital malformaties. Combinations of these approaches are likely to insure early detection and identification of teratogenic hazards."

Congressman MCCARTHY. Now, to your knowledge, has that been done in Vietnam, or is it contemplated, is the American Association for the Advancement of Science going to do what you just read?

Dr. GALSTON. I think it's shocking that there are absolutely no studies on the possible teratogenicity of these chemicals either in Vietnam or in this country. That is why it's so important to gather data from places like globe, and from places like the Saigon area to attempt to correlate, if it's possible to do so, the use of any particular pesticide with the appearance of any birth abnormalities, or any physiological malfunctions.

Congressman MCCARTHY: Doesn't the commission's study recommend that no herbicides like this be used until we are sure that it doesn't produce effects in human beings?

Dr. GALSTON. That's correct, the Commission recommends that given the suspicion that these materials are teratogenic, given their widespread use, but given also our wide dependency on these things in agriculture, we should immediately restrict the use so that we only use these herbicides where it is absolutely necessary to do so, and where there is no possibility of contact with human organisms. I believe that is the safe policy when you think you may be doing harm. You stop until you find out whether you are in fact doing harm.

Congressman MCCARTHY. Do you have any information that you could give for the record here, which would suggest why The White House ban never went into effect? I have a letter here which I received just prior to leaving Washington, which needs further clarification. It is from Mr. Ned D. Bayley, director of science and education for the Department of Agriculture in

onse to a letter I'd addressed to Secretary Hardin, asking why The W[hite] House ban didn't go into effect. Among other things, here's what he s[aid]: "Now, data submitted to D.H.E.W., Department of Health, Education, and Welfare, relevant to this position is that the 2,4,5-T used in the bionetics study contained about 27ptm of——

Dr. GALSTON. Dioxin is the way it's usually referred to.

Congressman MCCARTHY. It's t-e-t-r-a-c-h-l-o-r-o-d-i-b-e-n-z-o p-a-r-a dioxin.

Dr. GALSTON. Tetrachlorodibenzo para dioxin.

Congressman MCCARTHY. A highly toxic contaminant.

Dr. GALSTON. Yes.

Congressman MCCARTHY. I'm going to seek further clarification that one of the reasons the ban was lifted was this discovery. Now, do you know anything about this in the course of your inquiry?

Dr. GALSTON. Yes, Congressman, I became aware of this new development—2,4,5-T is a chemical synthesized from the reactants that are put together in a vehicle. Depending on the method of synthesis, and the temperature of synthesis, you may or may not get certain impurities formed in that reaction that accompany the 2,4,5-T which is realized out of the reaction fixture. One of the impurities is tetrachlorodibenzo-p-dioxin.

Now, there's previous information that this compound is a highly noxious material. There have been several factory and laboratory accidents in which people exposed to this compound have developed very severe blistering, loss of sensation, and respiratory troubles. The Germans have had a similar experience.

So it's natural when you have a report of this kind about the toxicity of 2,4,5-T, to inquire whether the effect is due to the chemical itself, or to the impurity.

Congressman MCCARTHY. Does it matter?

Dr. GALSTON. I'll make this statement.

I think it does matter in the long run, Congressman, because if it's the impurity, then in the future we can learn possibly how to make the chemical without the impurity, and continue its use.

Congressman MCCARTHY. I've read in the long article by Mr. Whiteside in the latest issue of New Yorker Magazine, at least he made the point that you can't make 2,4,5-T without getting some dioxin.

Now, is that right?

Dr. GALSTON. That's correct, I don't know if any sample that has less than a part per million of dioxin, so all of the 2,4,5-T that has been sprayed both at home and abroad has some dioxin.

The question is: Can you lessen the dioxin level down to the point where it is no longer so dangerous?

Congressman MCCARTHY. Is there any other way that dioxin can be produced after it's sprayed?

Dr. GALSTON. Oh, yes, even if you sprayed 2,4,5-T without any dioxin it might form chemicals in this Arizona sunshine. Putting all that light energy in I could easily imagine compounds like the dioxin being formed.

If there were a little fire somewhere, that's just the condition which would form the dioxin from 2,4,5-T. The only hard data on the teratogenicity of 2,4,5-T are right in this book that I have. There are no date which tell me, or anybody else, that it's the dioxin and not the 2,4,5-T that's responsible for these teratogenic effects.

I've had telephone conversations with people who have alleged this,——

Congressman MCCARTHY. Who are they?

Dr. GALSTON. Well, one of them is a member of this Commission, Doctor Julius Johnson of Dow who is an old friend of mine, and I think he is very terribly concerned about this development. Naturally, he would be since Dow is the manufacturer of some of this, and he told me that there are tests going on now which are not finished. He said he would not care to quote the data as of the present moment.

Congressman MCCARTHY. Mr. James Hansen of the Dow Chemical Co. visited my office last week and alluded to, I assume, the same tests.

Dr. GALSTON. Yes.

Congressman MCCARTHY. That the Dow Co. itself was carrying out the following-up on this possibility that it is the dioxin.

Now, in this letter from Mr. Bayley he said new data submitted to D.H.E.W. relevant to this position indicates that the 2,4,5-T contained the dioxin.

Well, it sounds as if it's the same thing. What I don't understand is how the Dow Chemical Co. could, in effect, by intervening, counte[r]mand, or negate White House orders.

Now, have you discussed this with any other people in the Government, or outside the Government?

Dr. GALSTON. I have not, Congressman. I don't have any information on how this operation came about. I would only say that to me it's unthinkable that, in absence of hard data, and to protect the lives and welfare of people in the country, I don't see how this order could fail to be enforced.

We must be safe before we are sorry. I would say let's get the facts before we resume spraying with this 2,4,5-T and at the present time there are no published data that I, or any other scientists have seen, that would say that 2,4,5-T is not the culpable agent. I think it's very peculiar that the orders of Doctor DuBridge are not being followed by the Department of Agriculture and the Department of Interior. The Department of Defense, said it announced immediately it would not follow this directive.

Congressman MCCARTHY. That's right. The next day on October 30th, the spokesman for the Department of Defense contradicted the DuBridge order in a verbal briefing to newsmen. He said that the 2,4,5-T would continue to be sprayed in training and regroupment areas where obviously populated areas, and of course as you know it has been sprayed in rubber plantations in Cambodia, which are also populated.

Well, Professor Galston, I appreciate very much your testimony here.

Dr. GALSTON. Do you mind if I make one more brief statement?

Congressman MCCARTHY. No, please do.

Dr. GALSTON. As a biologist, I'm terribly concerned about this because I believe in herbicides, I want to see that they continue to be used. I'm afraid there may be overreaction on the part of the public. I would like to say that there are probably ways that we can safely use these compounds, and the first recommendation of this Commission—I would like to read you just two paragraphs, short ones, because they outline to me what would be a safe procedure.

It says: "A new interagency agreement is needed to strengthen cooperative action among the Department of Health, Education and Welfare, U.S. Department of Agriculture, and the U.S. Department of Interior, to protect public health, and the quality of environment from pesticides danger provided by the Secretaries of H.E.W. and Interior, as well as Agriculture, should be required for all pesticides registration, pesticide use determined by any of the three Secretaries to be hazardous should be restricted, or eliminated.

"The agreement should further require the continuous review of new scientific information on pesticides now in use with the formal reviews made 2 years after initial registration, and subsequent formal reviews by the three agencies at 5-year intervals."

That seems to be loudly, essential for the continued safe use of pesticides and it's coupled with the establishment of a national testing center for pesticides, which is also recommended, I would say that we would be well on our way for the safe use of pesticides.

Congressman MCCARTHY. Do you think it's proper to delegate to the manufacturer of such a chemical the responsibility for testing its teratogenicity and carcinogenicity?

Dr. GALSTON. Well, you can certainly accept the data that are contributed by the manufacturer as relevant to the solution of the problem. I think these people have shown necessary testing laboratories which give honest data, but I would not depend on those alone. I would want to see the FDA or some other agency independently test these same compounds also, under completely different conditions. That's only a scientific rule, you don't believe anything anybody tells you, it has to be confirmed once or twice before you can believe it.

I would certainly hope the FDA, or some other agency, HEW would continue conducting further tests on these toxic chemicals.

Congressman MCCARTHY. And not really solely on the research of Dow, or other manufacturers?

Dr. GALSTON. That's correct.

Congressman MCCARTHY. Professor, I wonder if you would be kind enough to sit with us here, I'd like to use you as a resource person when we have the other witnesses.

Our next witness is Mr. John Pierovich, Assistant Regional Forester, from Albuquerque.

Is he in the room?

If you would be seated and identify yourself for the record, and your responsibilities in areas under scrutiny here.

Mr. PIEROVICH. Yes, sir, I'm John Pierovich, Assistant Regional Forester in Albuquerque, N. Mex. My responsibilities related to this matter are in connection with the complaints we've received here at Globe, and the overall evaluation of our Chaparral program, and our Chaparral program guidelines.

The primary reasons the Forest Service is here today is because this is a Forest Service project. I think that we need to be cognizant of such hearings as this, and we do try to keep informed through the literature of regulatory rules and concerns.

In fact, we share quite deeply the concern of the people in this community with their environment, we wouldn't want to do anything that would jeopardize their safety.

They're our neighbors, we also live here.

At the same time, we've been asked repeatedly to announce that we would not spray again in the Globe area, and like Doctor Galston, I think that we wouldn't want to overreact at this time. So we've said that such an announcement would be premature, we have our own studies going forward, and that these studies must be resolved before we can reach decisions on herbicide's use, or on the Chaparral program.

In addition to that we believe that it would be also unwise to base decisions on herbicides used particularly from the current allegations, or suspicions here in this area.

These matters need to be studied deeply, and we hope to have them studied deeply, and frankly welcome this inquiry because it will help to daylight some of the areas of concern.

That's essentially our position, Mr. Congressman. I'd be glad to answer any questions you might have.

Congressman MCCARTHY. Thank you very much.

In the course of my study, I have come into possession of documents that have been exchanged between the Department of Agriculture and citizens in the area. Here is one from John A. Williams for the Task Group, U.S. Department of Agriculture, Forest Service. Are you familiar with Mr. Williams?

Mr. PIEROVICH. Yes, I am.

Congressman MCCARTHY. Is he an associate of yours?

Mr. PIEROVICH. He works in our regional office.

Congressman MCCARTHY. Is he here today?

Mr. PIEROVICH. No, he's not.

Congressman MCCARTHY. I'd like to read you some of the things that he says: "Paul Boffin (phonetic) called a Dow Chemical representative at Davis, Calif., and requested information about Silvex. This man called Supervisor Courtney later and indicated that a publicity release was being prepared for submission to the news media concerning the known toxicity of Silvex. This if accepted and used by the news media will go a long ways towards improving the situation, and dispelling the fear of Silvex as a highly toxic, or poisonous agent."

He then goes on to say in his conclusions, "We are fully convinced that many of the people in this area honestly believe they were being subjected to a highly toxic and extremely poisonous compound with a high degree of persistence and one which would increase in concentration in the water supplies, and in the bodies of humans, and animals. These ideas are not in any way supported by research findings."

Now, that is dated July 22, 1969, and if I just would ask Professor Galston when was the Bionetics study brought to light?

Dr. GALSTON. It was handed over to the Department of Health, Education, and Welfare in December of 1968, to the best of our knowledge.

Congressman MCCARTHY. So that to the best of your knowledge, the Department of Agriculture——

Dr. GALSTON. Might have had access to that information.

Congressman MCCARTHY. Actually, the tests were run in 1967. Now, Mr. Williams obviously either did not know about the Bionetics report, and I would—I would accept that, I don't think he did just from the tone of the letter, but I'll ask you to comment——

Now, which do you think it was?

Mr. PIEROVICH. First of all, Mr. Williams was heading a group for a general survey of the effects here in the Globe area at the request of the Forest Supervisor, and after the initial complaints. We've had subsequent studies go forward, one of these coming out as a second task force report which is somewhat more in depth. Mr. Williams' information was then of a general nature for an initial report for the forest supervisors. Williams himself is not an herbicide man. Mr. Boffin is, and his reason for talking with the Dow was to get more information.

The second question you've asked regarding the Bionetics study was not known to these people, and only known to a few people within the Forest Service but the word of mouth communication that took place following the review of the Bionetics study for publication.

This has precipitated a lot of discussion among the science community, and in the ——

Congressman MCCARTHY. Are you alluding to the Whiteside article in New Yorker Magazine?

Mr. PIEROVICH. No, that's the most recent and clarifying article, at least I found it very informative.

Congressman MCCARTHY. When did you first learn about the Bionetics findings on teratogenicity?

Mr. PIEROVICH. I personally learned about it in November when I was assigned to this problem area, and I learned about it through reading in the literature, seeing the discussions among others.

Congressman MCCARTHY. Was the present science advisors ordered ban ever transmitted to you, or here in the area?

Mr. PIEROVICH. We were furnished a policy statement from the Secretary of Agriculture in December which referred to the DuBridge statement.

Congressman MCCARTHY. Did you take that as a directive not to continue using 2,4,5-T?

Mr. PIEROVICH. We understood it to be directed towards crops, and that it was not at that time being restricted in range-land use. However, we could infer from this, and from discussions with our Washington counterparts, we learned that there were other studies underway on this compound, and as you perhaps have noted, we did defer our chaparral program in October. The last spraying on this project was in June, and these events have unfolded since that time.

It's currently our position here in this region not to use herbicides until some of these matters are researched. The studies that are underway should be most helpful to us in this regard.

Congressman MCCARTHY. I think there's a little confusion about just what the DuBridge announcement banned. Doctor DuBridge said—this is October 29, 1969.

That 2,4,5-T would be prohibited for use on American agricultural products after January 1, 1970, until the Food and Drug Administration could develop information showing that it could be used with safety.

Dr. DuBridge also announced that the use of 2,4,5-T in Vietnam would be restricted in areas remote from population.

Mr. PIEROVICH. This is where we found our references to the crop production area, and the Secretary has interpreted this way. As I said the ban on crops is in effect at this time, and as near as we can tell we are also examining the future of the 2,4,5-T as it is compounded today.

Dr. GALSTON. Congressman, could I make a comment here?

Congressman MCCARTHY. Yes.

Dr. GALSTON. I was unable to understand why when Dr. DuBridge issued this statement he did not also take care to specify prohibition of use in regions where 2,4,5-T might find its way into drinking water. For example, supposing you are using 2,4,5-T to clear shrubs from under a power line, and that power line is going through a town where people have wells, and they draw water from these wells. Don't we need to know if the 2,4,5-T is going to seep down in the water cable and get to these people? It seems to me applying the ban to the food crops is only a halfway measure.

Mr. PIEROVICH. I think we need to be concerned by this, and this is why we monitor water from treatment areas. It's significant in this Globe area. Our reference—or the Federal water quality control criterion of one-tenth part per million, this level has never been reached in any of the water analyzed that we've had run, or had been brought to our attention.

Congressman McCARTHY. You say you received the directive November——

Mr. PIEROVICH. We received the Secretary's explanatory information in December as I recall.

Congressman McCARTHY. Were you ever advised that the ban had been suspended?

Mr. PIEROVICH. No, sir.

Congressman McCARTHY. So the last you had was the DuBridge directive?

Mr. PIEROVICH. Yes, and a statement from our Secretary to agriculture agencies of which we are, telling us that 2,4,5-T was not to be used in crops, and incidentally, the Secretary has added to his statement that we would use alternative methods whenever these are available and practical, and is stressing within the department a use of nonchemical means where these are available to us.

Now, this is all developments since the last spraying here at Globe, I hope this is clear.

Congressman McCARTHY. Are you spraying in other parts of your region?

Mr. PIEROVICH. No, sir, and we have no plans to spray during current, or the coming fiscal year at this time.

Now, if we have some break-throughs, I'm sure we will be talking about this. Again, it would be premature to say.

Congressman McCARTHY. What's the basic rationale behind the spraying here at Globe?

Mr. PIEROVICH. You mean——

Congressman McCARTHY: What's the purpose of it?

Mr. PIEROVICH (continuing): The purpose of the project. This is the part of the region, and the Tonto National Forest chaparral management program. This program has many objectives for—if I may take a minute—fire is a very common ingredient in the life history of chaparral, and in trying to bring management to Chaparral Forest, we have excluded fire, or we are using fire by prescription, rather than have the chance of holocaust. In doing this, we attempt to bring a break to the fuels in large continuous masses by developing grassy ridge tops, or grassy openings. These have other advantages for people who want to use the forest, and for game.

It happens that the project here in the area was a water-yield project. We have learned through research at the 8-Bar experimental area, and particularly that we can substantially reach the flow of streams, particularly in the winter months where the vegetation is not using the amounts of water that chaparral vegetation does.

Now, herbicides were used here at Globe partially because of the known flooding potential of these streams, and that they also know that fire over a large area could cause floods. So rather than use prescribed fire as initial treatment, herbicides were used.

We have plans to use some small amount of fire to continue our work here.

Congressman McCARTHY. Doesn't it say right on the container that this should not be used over water?

Mr. PIEROVICH. That's correct, and as the project instructions were followed here, the applicator pilot was to interrupt his spray everytime he passed a major stream channel.

Congressman McCARTHY. "Interrupt his spray," you mean from a helicopter?

Mr. PIEROVICH. From his helicopter, yes.

Congressman McCARTHY. Do you think that is that the answer?

Mr. PIEROVICH. Well, I think it's quite practical, sir.

Congressman McCARTHY. Well, wind might carry. Aren't there restrictions under the circumstances in which you use it?

Mr. PIEROVICH. First, let me explain in spraying this area the primary pattern would be along, or parallel, or to a water course so that it isn't necessary to turn valves off as you may each time he crosses at the creek, but he was going to be crossing streams at the same time he has been spraying. So he would be than instructed to interrupt the spray before making such a crossing. Some drift did occur into the bayous, we have found some of the Sycamores in the Kellner area, the tops have been hit. We don't feel that a substantial amount of herbicide came to the water course, and the pilot was instructed not to apply this over water.

Water residues again haven't indicated any great amount of the herbicide in water.

Congressman McCARTHY. Are they instructed only to spray when the wind is blowing at a certain mile per hour?

Mr. PIEROVICH. Yes, that's right.

Congressman McCARTHY. What is it, eight?

Mr. PIEROVICH. In some projects it's 5-miles per hour, in the case it was 10.

Congressman McCARTHY. Ten?

Mr. PIEROVICH. Yes.

Congressman McCARTHY. Is that rigidly adhered to?

Mr. PIEROVICH. Well, I would hope that it is, here we are depending on other people to do our work, but we have a project area officer, and this project had a project area officer who works from the helispot where the copter is operating, using a pocket anemometer, and as he noticed the wind picking up he would take the pocket anemometer out and keep track of the gusts. Whenever it approaches 10-miles per hour, the project would be shut down.

I have records here with me of the shut-down on this project, if you are interested.

Congressman McCARTHY. You are undoubtedly aware that some of the residents in the area charge that spraying went on in much stronger wind velocities?

Mr. PIEROVICH. Yes, sir, I am, and I am aware that there has been drifts, and we are attempting to identify how far this drift went. In the task force 2 report, we identified a visual effects drift line, we are currently working on infrared interpretation, and I would be very happy to furnish you with a map which delineates how far the dead vegetation that shows up. That's not available to see by the naked eye.

Congressman McCARTHY. That would be very good to fill out the record. I would like to have that documentation very much.

Dr. GALSTON. Do you mind if I ask a question at this point?

As a scientist, I'm interested in following up one line of questioning here. The benefits that one wishes to derive from this program has to do with increased water flow?

Mr. PIEROVICH. In part.

Dr. GALSTON. And the other part is, I presume, to have a more accessible and manageable terrain where the Chaparral vegetation is?

Mr. PIEROVICH. That's a good generalization among other things. We would like the esthetic qualities of the area to be an indication.

Dr. GALSTON. Do you see any deleterious consequences of partial denudation of the hillsides where Chaparral is growing?

Mr. PIEROVICH. It's not our intent to denude the hillside.

Dr. GALSTON. I said partial.

Mr. PIEROVICH. In the course of making a conversion, one often has to take a compromise, and we do compromise to the extent that we will—say for example, in burning—taking out an area, we will burn only so long a slope here because any more we would have an overflow of plants and water, and erosion while it is bare from burning, it is an opportunity for a torrential thunderstorm, or wind to cause erosion. But this is also one of the compromises that a farmer must make when he plows his field.

Dr. GALSTON. And this is something you think you can keep under pretty good control with applied herbicides?

Mr. PIEROVICH. In this case we used herbicides for that reason, yes.

Dr. GALSTON. Was there any measurement for the relevant erosion rates before and after herbicide use in a given area?

Mr. PIEROVICH. In the 8-Bar area this is being noted at this time. The studies have been in progress for some time, I don't have those data with me, but I could find them for you.

Dr. GALSTON. I, personally, would be very interested in having those data. It's been my impression that some programs have been gone into fairly massively without the comfortable feeling that there's a lot of scientific data behind the original studies to tell us that this is really what we ought to do, and in calculating returns per acre, in terms of where we've applied, I think we have to have a negative quantity in there for possibly deleterious effects, that possibly are not measured.

Congressman McCARTHY. I'd be eager to see those.

Mr. PIEROVICH. I'd be happy to furnish them for you. I think something we have going right now, you may notice in the statement we've furnished you,

we are looking at alternatives, and tolerable levels, and we are approaching it very thing using projects that have been installed as a basis for arriving this.

Congressman McCarthy. On that I wonder if I could ask you, are you giving licenses for the use of Kuron?

Mr. Pierovich. We give no licenses for chemical uses. The answer would be no.

Congressman McCarthy. I see. From whom do they get these licenses?

Mr. Pierovich. The use of chemicals is done by—in our case, the approval of a project proposal by a regional and national pesticide committee. Once the forest officer who has a project wants to apply a herbicide he prepares a formal proposal, it's submitted to our regional committee, if they approve, to a national committee. And I'll tell you right at this point, our committee won't approve such a use, but we don't license.

Congressman McCarthy. Well, thank you very much.

Will you be available today and tomorrow?

Mr. Pierovich. Yes, sir, I will, as will the ranger and the acting supervisor here.

Congressman McCarthy. Thank you very much.

Our next witness is Dr. F. I. Skinner, veterinarian from Globe.

Is Dr. Skinner here?

Dr. Skinner, I'm pleased to have a veterinarian testify in light of recent indications that the use of 2,4,5-T spray may have had harmful effects on animal fetuses. I wonder if you would, for the record, identify yourself, your background and experience.

Dr. Skinner. I am Dr. Skinner, local veterinarian, I've been in the area 14 years, graduate of Kansas State University with a degree of T.B.M..

Now, these are my people, and I've lived amongst them. Now, any questions you'd like to ask I'll try to answer.

Congressman McCarthy. Would you recommend the use of this Silvex Kuron spray after tests have shown that is has teratogenic effects on animals?

Dr. Skinner. No, I wouldn't recommend it without further study, further research.

Congressman McCarthy. You think it should be stopped until——

Dr. Skinner. Yes, sir.

Congressman McCarthy. You have some question about the Bionetics findings of the effects of this on animals?

Dr. Skinner. I'm a clinician, I'm not research. I have not seen any effects of animals in this area—definitely, clinically. Now, as I say I'm not a research, I'm a clinician. I don't set myself up to be an expert on it, but I've not seen any abortions, malformations of fetuses in this area that I can clinically say it was caused by Silvex, or 2,4-D, or pesticides.

Congressman McCarthy. As I understand it, and we hope to hear from others, that there have been allegations made that the 2,4,5-T sprayed did cause malformation in animals.

Dr. Skinner. I cannot speak for those, I have not seen them myself.

Congressman McCarthy. You did not. Were you ever asked to examine the animals in question?

Dr. Skinner. No, sir.

Congressman McCarthy. You were not——

Dr. Skinner. No, sir.

Congressman McCarthy. So that you just don't know?

Dr. Skinner. I don't know, I don't pretend to know.

Congressman McCarthy. All right. Well, maybe they will be calling on you.

Dr. Skinner. I hope so.

Congressman McCarthy. Well, thank you very much, Doctor Skinner.

Dr. Skinner. Thank you, Congressman McCarthy.

Congressman McCarthy. Our next witness we'd like to call is Mr. Robert McKusiak.

Mr. McKusiak?

Mr. Skomp. Sir, I represent Mr. McKusiak as an attorney, and he's requested that he be called later. Can you pass him at this time? He wants to pass at this immediate time.

Congressman McCarthy. Surely.

In that event we'd like to call Mrs. Billee Shoecraft.

Mrs. Shoecraft, I wonder if you'd identify yourself for the record, and ——

Mrs. Shoecraft. Billee Shoecraft, Ice House Canyon, Globe, Ariz.

Congressman McCarthy. And if you would tell us a little bit about how long you've lived here, and your own experience with the chaparral spray program?

Mrs. Shoecraft. We have been in the area since 1947—Mr. Shoecraft a little longer than that.

Congressman McCarthy. I wonder if you could tell us about your experiences with the spray program, and some of the correspondence you've had with the various agencies of government in this connection.

Mrs. Shoecraft. I'd be glad to, thank you.

We first became aware that they were going to spray a chemical, which they asserted was harmless——

Congressman McCarthy. You say, "they"——

Mrs. Shoecraft. The Forest Service.

Congressman McCarthy. U.S. Forest Service?

Mrs. Shoecraft. Right, in 1965. They had published in the local paper a news item dated August the 19th, 1965, in which they said the herbicide will be 2,4-D, and 2,4,5-T mixed with diesel oil, and water. The diesel oil will serve as a weight factor to insure against wind drift. Neither 2,4-D or 2,4,5-T is harmful to birds, insects, fish, wildlife, or humans.

Congressman McCarthy. Do you have a date and name on that?

What was the publication, what newspaper is it?

Mrs. Shoecraft. From the Arizona Record.

Congressman McCarthy: Of what date?

Mrs. Shoecraft. Of August the 19th, 1965.

I also have the typed-up version when he initiated at that time from which he deleted the word. "I anticipate honest inquiry from many individuals and groups concerning the project I also anticipate adverse criticism and harassment from those who devote their lives to criticizing and harassing."

I forgot to read the part where he invited the general public to come and see them spray.

If you are as curious as I am, you will want to drive up and watch the operation. I hope you will.

Again, I read from the report No. 16, *Georgia Forest Research Counsel*, Macon, Ga., 1965. On page 28 it says, "Possible harmful effects: 2,4-D and 2,4,5-T have a low toxicity, although spray applications leave no toxic residue, a tolerance of five parts per million has been established on or in apples, citrus fruits, asparagus, pears, and quinces. We can find nothing in the Department of Agriculture to back this up."

Then, they further said, "Since some persons may be allergic to the oil in the herbicide mixture, skin contact should be avoided, and when treatments are used a respirator is also a desirable piece of safety equipment.

Congressman McCarthy. Who is saying this?

Mrs. Shoecraft. This is from the Southwestern Forest Experiment Station, Forest Service, U.S. Department of Agriculture, Asheville, N.C.

Congressman McCarthy. And the day on that, please?

Mrs. Shoecraft. The date on this was 1965. It further says—after mentioning the respirator, the odor, or vapors may bring on a case of nausea. The Forest Service Health and Safety cautioned that 2,4-D and 2,4,5-T are mildly poisonous, and flammable in an oil base. However, we were invited to come and see the spray.

Congressman McCarthy. Do you have any more documents that cast some——

Mrs. Shoecraft. Oh, I've many.

I have here this little item that was given to us, there were a few missing pages, it only had four, so I got in touch with Dr. Holston (phonetic) at Belleville, Md., because this is the U.S. Department of Agriculture, and I wondered where the rest of the pages were. So Dr. Holston from Belleville mailed me a package in which was included the rest of it, it totaled 25 pages, and this concerning the toxicity of some organic herbicide to cattle, sheep, and chickens. It tells about some of the things that they found in relation to the herbicides that we've been sprayed with. We don't know exactly because the reports have varied, but they have said they used 2,4-D, 2,4,5-T, and Silvex. They further said it one form, then the tests showed different forms. I quote: "We concluded—that the enlargements were caused by the chemical reaction of the diluted herbicide formulation. The ecropsy—the liver was enlarged and viable. The kidneys were congested. A small abcess was found in the parotid

lymph node. In one year that developed a swelling in the region related to the chemical reaction. Associated other lymph nodes of the body were often enlarged and hemologic."

Congressman McCarthy. Mrs. Shoecraft, I wonder if just for the record we might just interrupt you briefly. I would like to ask Professor Galston if he would explain the difference between Silvex Kuron, 2,4,5-T, and 2,4-D just for the record.

Dr. Galston. These are very closely related materials, and I think from the toxicology point of view, and from the points of view—the presence of any of these impurities like the dioxin we were talking about, they would all be in the same bag.

2,4-D is 2,4-dichlorophenoxyacetic acid, 2,4,5-T has one more, that is 2,4,5-trichlorophenoxyacetic acid, and Kuron is simply a trade name for a similar preparation that I believe is a Dow product.

Is that correct, I don't whether the foresters here would——

Mr. Pierovich. Yes, that's correct.

Congressman McCarthy. Is there anything significantly different between 2,4,5-T and Silvex?

Dr. Galston. I would say none whatsoever from the point of view we are talking about. The toxicity would not be due to the length of the chain, but due to the fluorinated aromatic nucleus, as a chemist would call it.

Congressman McCarthy. Mrs. Shoecraft, I realize you have many documents, and we would like if we could to have any of these you would care to submit for the record.

Mrs. Shoecraft. I'd be glad to.

Congressman McCarthy. Would you, this would help very much.

Mrs. Shoecraft. Yes.

Congressman McCarthy. However, now, if there are any particularly salient quotations that—without being overly lengthy, you think should go into the record at this point, we would like to have those.

Mrs. Shoecraft. May I submit Farmers Bulletin Number 2158, U.S. Department of Agriculture, issued April 1961, slightly revised, August 1969, referring to what their rules are on what the wind velocity should be.

Congressman McCarthy. What does that say?

Mrs. Shoecraft. It says, "Apply the spray when the wind velocity is less than 6 miles per hour, and the air temperature is 90° or less. Again use a coarse spray——"

They did not use a coarse spray, they used a fine spray. "Use a slowly vaporizing formulation."

They did not use a slowing vaporizing formulation, they substituted water for oil in a very small amount and released it at very high altitudes on a very hot and windy day, and they kept no records—weather records on the job.

Congressman McCarthy. Can you substantiate those points?

Mrs. Shoecraft. Yes, I can.

Congressman McCarthy. How?

Mrs. Shoecraft. I'm reading from file No. 2520, and it states in this left-hand corner to the file, it's from William H. Moehn, district ranger.

Congressman McCarthy. How do you spell that?

Mrs. Shoecraft. M-o-e-h-n, district ranger, date July 11, 1969, subject: Watershed protection, Kellner Russell chemical maintenance, fiscal year 1969.

This memo is a resume of the fiscal year 1969, maintenace project.

"The spraying done on June 8, 9, 10, and 11, 1969, were started at 6:40 a.m. on Sunday, June 8, and the hilltop located on the Icehouse Canyon Trail, at 6:51 a.m. after the third load was through, the pilot flew to the O.C.C. Camp to check his spray. When he landed Mrs. Shoecraft arrived and told him some of the spray had landed on her. The pilot returned at the hill at 7:14 a.m. and said someone should go talk to her.

"I left the spray job at that time and did not locate Mrs. Shoecraft."

In fact, I called Washington on the third day, but they didn't find me, but they could have if they had looked.

"I left the spray job and we continued to spray from the helispot until 10:57 a.m. when we landed at the helispot the wind was coming out of the East from 6:40 a.m. to 10:57 a.m., we left and went to the Pinal Road helispot and began to spray. We continued to spray until 15.05 a.m., at which time the wind reached 10 miles per hour plus, and we shut down. We resumed spraying at 5:03 p.m. when the wind dropped below 10 miles per hour and continued on until 7:35 p.m.

"On July 9, the first load was off the ground at 5:35 p.m. We continued to spray until 10:18 p.m., at which time we shut down because of winds in excess of 10 miles per hour. We did not spray anymore on the 9th.

"We started at 6:02 a.m. on June 10, 3 days after Mrs. Shoecraft had notified, and flew until 11:15 a.m., when wind forced us to shut down. We did not spray anymore on the 10th.

"On June 11, we started at 5:18 a.m., and flew until the project was completed. A total of 977 gallons of Silvex was used at a rate of 2 pounds acid, equivalent per acre. The total rate per acre was 8 gallons. 1,900 acres were treated. We did not keep weather records on this project.

"The wind speed and direction at the Globe Ranger Station at 1 p.m., each day of the spray job are listed on the next pages, and it shows on June 11, a speed of 16 miles per hour southwest.

"Signed and stamped by William H. Moehn."

Congressman McCarthy. So that even in his own records he acknowledges that he exceeded the limits that had been set?

Mrs. Shoecraft. Yes, he did. I refer further to the Department of the Army's Circular 33661. I have a letter here from Representative Steiger's office, to apply back in 120 days, but I didn't choose to apply in 120 days. I called the Adjutant General's office, I said we needed it now, I'm one of the victims. I was informed by the Department Office that they sent it out to the printer's. My suggestion was you either get it from the printer's, or you get a copy, I need it now. I received it in 3 days.

In this it refers to the formulation which they call, Orange, and it says that it is one part 2,4,5-T, and one part 2,4-D. I have before me a letter dated October 6, 1969, from the USDA, in Phoenix. The branch of the Forest Service, the Tonto National Forest Service, signed by Mr. Jenkins for Mr. R. E. Cortney, Forest Service. He says:

Dear Mrs. Shoecraft, following is a list of chemicals purchased by the Tonto Forest as requested by you. The mixture was two gallons chemical with seven and one-half gallons per acre. In a few cases more water was used, and all of them are 2,4-D and 2,4,5-T.

Since I was curious because there was no Silvex, I further proceeded to say who bought the Silvex, and I was finally informed by Mr. Moore at Salt River Project they made the decision to purchase the Silvex. They did not purchase it as they said in the Forest Service. They have lied, it's the only word I'd like to use because it's lying when it covers things when they know better.

Congressman McCarthy. I wonder if you could submit those documents to Mr. Riddleberger for our records?

Mrs. Shoecraft. All right.

Congressman McCarthy. And if you are available we hope to go out this afternoon and tour the area.

Mrs. Shoecraft. Be pleased to.

Congressman McCarthy. Thank you very much.

We would like to move on now and hear from Mr. McKusiak.

Mrs. Shoecraft. I had requested analysis that were done on our plant back in September before another task force is to arrive, which I understand is next week. I've spoken with Mr. Tschirley this morning, he called, I told him before I wanted anymore samples taken I would like the reports of what they took in September. They seem to be still evaluating these water samples we sent in, and for your information I just learned this morning the samples taken from our own drinking water last week are still highly contaminated, and I suppose I'm the first human to go on record to be able to say that they have now found 2,4-D in my pound of flesh, and that was as of this morning from two different laboratories.

Congressman McCarthy. That's important, could you elaborate on that? Do you have those laboratory findings?

Mrs. Shoecraft. These were found in the G.H.T. Laboratories in California, the other laboratory I'm not even aware of the name where the samples were sent.

Congressman McCarthy. What's that, G. H.——

Mrs. Shoecraft. That's the laboratory where the Department of Agriculture, Doctor Henton (phonetic) had recommended that the samples be sent on the plant life originally. There will be a longer report on it this afternoon.

Congressman McCarthy. We will check that out. Did you mean to imply that a biopsy has been applied on your tissues, and 2,4-D has been found in your——

Mrs. Shoecraft. As of this morning they were not complete.

Congressman McCarthy. Thank you very much.

We'd like to call Mr. McKusiak now.

Mr. McKusiak, do you care to be accompanied by counsel? If you do, it's perfectly all right.

Mr. Skomp. We have no objection.

Congressman McCarthy. All right. Mr. McKusiak, I wonder would you identify yourself for the record, please, your name and your background, and how long you've resided here.

Mr. McKusiak. I'm Robert McKusiak, and I've been an Artist in tile and mosaic for some 22 years. I have a background prior to that time, and since that time also in science. I majored in chemistry in college.

Congressman McCarthy. What was that?

Mr. McKusiak. University of Arizona, I do not hold a degree.

Congressman McCarthy. How long have you resided here?

Mr. McKusiak. I've lived in this area since 1932 with the exception of the time that I attended the University of Arizona.

Congressman McCarthy. Now, I wonder if you would verbally give us a generalization of your experience with the Forest Service spray program?

Mr. McKusiak. My experience with the Forest Service spray program really didn't come into being fully until 1969 following the June spraying. Well, let me back up, it came into being in about May 31, 1968. I was aware prior to that time that they had been spraying, but I was not aware that the things that they were spraying were particularly harmful. I had seen unusual effects taking place, but I didn't know what to attribute them to.

Congressman McCarthy. What unusual effects, could you cite a couple?

Mr. McKusiak. Yes, one in particular which I would prefer that Mrs. McKusiak documented for you because that's her field, and not mine, but specifically in 1966, in May of 1966, the brown pewee population, these are birds that live in our canyon area, suddenly started dying in great numbers in our yard. We have a waterer that birds come to, and there were birds all over during May which had matter in their eyes, and seemed to be having respiratory trouble, and were dying, and at that time we continued spraying it.

Congressman McCarthy. You don't happen to have any photographs of that, do you?

Mr. McKusiak. No, I don't, I would prefer on a discussion of birds to have Mrs. McKusiak go into this because that was her field. But, in 1968, on the 31st of May, I was up at my property where I get my clay, it's private land in the area that was sprayed, it was included in the area sprayed. I had my wife and three children, and the two dogs up there, and the spraying was taking place down canyon. The helicopter came up the canyon, we have a stock pond that was between us and the edge of the property, so to speak, and the helicopter came up the canyon and made a turn southerly, in other words, it made a right-angle turn toward the mountains, and it approached. We were waving our arms because we didn't want to be sprayed. He made a turn and he was so close to us, and the spray descended upon us, and upon the pond, and upon our kids and dogs, and so forth. At that time we weren't really aware that anything was wrong with it except we both rushed home, my wife and I have both had headches from it.

Congressman McCarthy. The pond, is that drinking water?

Mr. McKusiak. This is a pond which is used for livestock water, but it's on private land.

Congressman McCarthy. Now, you heard undoubtedly the Forest Service say that they stopped spraying when they would get over a stream, but they didn't over a pond. I suppose that would be obviously important?

Mr. McKusiak. It's incorrect that they stopped over streams, they sprayed directly over three different semipermanent streams that I know of, and one permanent.

Congressman McCarthy. Did you see that yourself?

Mr. McKusiak. I saw them spraying in this area over it, and the devastation continues right down to the edge of the stream, it's quite visible.

Congressman McCarthy. Will we be able to see that this afternoon?

Mr. McKusiak. I'm sure you will.

Congressman McCarthy. I think it's very important.

Mr. McKusiak. One canyon in particular in 1968 when I was sprayed with my family on our property, and we did have illnesses and have had illnesses thereafter, continued since this time. This particular little canyon, when they flew up toward us—which has a permanent stream in it, and they flew right up the canyon to the pond, it's a stream that seeps out from the pond, and has never been dry.

Congressman McCarthy. I wonder if you would, for the record, tell us about changes in livestock, and other animal life on your farm, which you would attribute to this spraying.

Mr. McKusiak. I really don't have a farm to correct the record, I have many different animals, my wife keeps ornamental fowl, she is an archeoornithologist, and she works with archeological birds, and she keeps files of various types for comparative work, and also for our own enjoyment.

We have 10 or 12 milking goats that we have had for 16, or 17 years. We've kept a small population of them, and in the last 2 years we have had a number of our milk goats bear kids, they have from two to three offspring a year, each goat, and a number of these have borne deformed offspring. When I say deformed, I'm referring generally to their heads, their heads were born malshaped, and malformed in some cases their bodies, but generally their heads.

We have one goat which is already been covered by the news media, but we have one goat which wasn't as malformed as the others. We have kept it alive simply because people were denying such things happening. I would say most of the offspring that were born were born either dead, or deformed, or both. Most of them who were born deformed were born dead. In other words, the animal miscarried deformed offspring.

Congressman McCarthy. Did you ever ask Dr. Skinner to come out and look at these animals?

Mr. McKusiak. No, I don't believe I've ever discussed these animals with Dr. Skinner until just recently, but Dr. Skinner and I are good friends, and we have from time to time called him to ask how much dosage to give an animal if we were going to give them a shot. Some of our animals from time to time have suffered from pneumonia, or things of this type. For example, many of our fowl in birth have died. I'm referring specifically to geese, and ducks, and some chickens, and many of them have died, and we found by giving them a shot of com-biotic, it's a penecillin streptomycin, I believe, combination, by giving them a shot, usually we could save them. These fowl would come down with what seemed to be pneumonia. There are many other people in the canyon whose fowl done the same thing.

We found by giving them a shot we could save them. We called Dr. Skinner to find out what the correct dosage would be, and we generally didn't call back telling him it came out.

Congressman McCarthy. Well, Mr. McKusiak, I know we could go on for some time, but we have to adjourn shortly, but we will be with you this afternoon.

Mr. McKusiak. I would like to make one other comment, if I could, for the record.

Congressman McCarthy. Surely.

Mr. McKusiak. I was talking about 1968 when we were sprayed on our own property, and our own dogs following this spraying, we went home and washed, but our own dogs that were with us, two of them became ill immediately with what we considered to be pneumonia, at that time we didn't associate it really with the spray, we didn't think about it, and we gave the dogs —we tried to call Dr. Skinner and he was out of town, and we gave the dogs com-biotics for this, and I believe it was the next day we called Dr. Skinner, he was back, and my wife checked with him and she checked the dosage she had given them, and he said it was twice too much, and give them half as much again, and we did, and the dog survived. It would have died if we had not given him the medication.

Congressman McCarthy. You still have the two dogs?

Mr. McKusiak. Neither are malformed or anything, one of them has never been quite well, it's never been well. It wheezes a lot.

One other thing, there are many families in the canyon and many families in Globe and Miami who have dogs that are bleeding from all body openings.

We have dogs of this type, and people who have had dogs die from this, we could put you in contact with.

Congressman McCARTHY. We would like to have that information.

Well, thank you, Mr. McKusiak. We'll look forward to seeing you this afternoon.

This hearing will stand adjourned.

Congressman McCARTHY. The hearings will come to order.

I've just received the following letter from the White House which I wish to read into the record at this point. It's from the Science Adviser to the President of the United States, Dr. Lee A. DuBridge.

"The White House, February 10, 1970.

"Dear Mr. McCarthy: This will acknowledge your February 3rd letter concerning 2,4,5-T, the October 29th announcement that you referred to was a statement of the actions that were planned to be taken by the various units of the Federal Government in relation to the 2,4,5-T. It was not a directive to agencies for the simple reason that statutory responsibility for these decisions rest in the separate agencies.

"I'm sure that by now you have heard from the Department of Agriculture. I appreciate your views on the desirability of an investigation of reports of birth, of malformed children in Vietnam. By copy of this letter I'm calling your views to Secretary Laird's attention since this area is primarily his responsibility.

"As to 2,4-D, this compound is being reviewed along with other compounds being singled out as requiring additional study in the Bionetics records to which you referred."

Signed, "Lee a DuBridge, Science Adviser to the President."

I'd like to contrast this with a statement as it was issued on October 29 where DuBridge said that the Defense Department will restrict use of 2,4,5-T to the areas remote from population, that the Agriculture Department will cancel registration of 2,4-D for food crops effective January 1, 1970. The Department of Agriculture and Interior will stop using 2,4,5-T in their own programs in populated areas, or where the residues from use could otherwise reach man. That the Department of Health, Education, and Welfare will complete action on a tolerance for 2,4,5-T, the residues on foods prior to January 1, 1970.

This is obviously a retreat from the position taken by the White House in October 29. As I read the statement at that time it was in the form of a directive that the departments will do such and such, now we find that the White House is backing off from this, and is saying that the statutory authority rests with the agencies.

It seems to me that the President of the United States has authority—the ultimate authority over these agencies, and I regret very much that the President's Science Adviser has seen fit to retreat from the decision of October 29, which I believe was the wise one. The use of this particular chemical should be banned pending tests.

On the plus side I'm delighted to be informed last night, and it's reported today in the press, that the distinguished Senator from Michigan, Philip Hart has announced he will hold hearings on 2,4,5-T. He asked Secretary Hardin, Secretary of Agriculture, Robert Finch, Secretary of Health, Education, and Welfare, and DuBridge to testify on March 11. This is further evidence to me that the compound's effects require additional evaluation, and I expect that I will testify myself before this Senate Subcommittee when they have hearings, I will make that request.

I should also announce that a report on my investigation will be prepared in consultation with Dr. Galston, and will be issued at the earliest practical point.

Now, we would like to hear again from Mr. Pierovich of the Forest Service. Is he here?

Mr. PIEROVICH: Yes, sir.

Congressman McCARTHY: I would like to say for the record, which I just said on the radio station here, that I have been very favorably impressed by the cooperation of the Forest Service. I think that anybody who has any smattering of knowledge about this whole thing must realize that this is something

transcending individual agencies out in the field, that we are t[illegible]g here about national policy, and what is done out in the field really is a result of decisions made at a much higher level, and to try to focus responsibility on a field unit I think is really to carry this too far. I've been most impressed with your cooperation, and that of your colleagues, Mr. Pierovich, and I want you to know that we appreciate it very much, and our report will so indicate.

I understand you would like to elaborate on the statements you made yesterday.

Mr. PIEROVICH. Thank you, Mr. Congressman, for your kind comments, and also for the way you've conducted this hearing. I think the Forest Service is pleased with the way the hearing has gone. There are some significant elements of Forest Service concern that I felt should be made a part of the record this morning, and I'll read essentially from that statement.

First of all, the Forest Service has used phenoxy herbicides, but not since the nationwide controversy broke last fall. In fact, the last use of herbicides on the Kellner Russell project was June 11, 1969, and to the best of my knowledge, the last use of any herbicide by the Southwestern National Forest was the August, 1969, on the Gila National Forest in New Mexico.

Second, it's apparent there are several persons in this area who believe there are unknown, or suspected characteristics of these herbicides which may have caused them damage, and this is of concern to us.

Three, it's apparent we must continue our efforts to ascertain the extent of drift levels of herbicide residues, and the definite relationships between herbicides over environmental factors and the responses of plants and animals in this area.

These studies are to be made public when they're completed.

Lastly, the extent of continued deferment of herbicide use in the Chaparral program is dependent upon the outcome of our studies and of the Department's investigation of these matters.

Congressman McCARTHY: Thank you very much. I wonder if you could for the record, repeat what you told me yesterday relative to the drift of the herbicide over streams, and into adjacent private property, and what steps, should this be resumed, assuming that it can be shown to be safe, what steps would be needed to correct that?

Mr. PIEROVICH. At this point, this will be my own opinion, but I first mentioned to you yesterday that our instructions to the applicator pilot were to interrupt his spray application when he crosses streams, we had definite plans for the project here to call for application away from the open water, and main stream courses. I do believe there was some drift into this stream course as evidenced by some top kill on the Sycamores on the stream bottom. There has been drift from the project area onto private property which we have established so far as the visual effects are concerned, and from this I'm certain that we will be developing new guidelines to both assure that the herbicides that we might apply in the future are confined to the project area, and to assure the safety of the public.

One definite indicator in this is that it would be desirable to use a much more restrictive windspeed in application.

Does that answer your question, sir?

Congressman McCARTHY: Yes, but what wind velocity do you think would be safe?

Mr. PIEROVICH: I wouldn't want to speculate at this time, but we do have a general rule of 5 miles per hour, and we know that herbicides were applied here to 10 miles per hour, and we see new development in the herbicide application field, the use of inverts has become more and more popular, and with some corrective work recently done is this area I feel this will help us a great deal.

Congressman McCARTHY: Another point that I definitely sympathize with you about is difficulty you have of getting information. I think the fact that you weren't apprised of the Bionetics Research Laboratory finding on teratogenicity until late last year suggests a problem in communications here, and if you have any suggestions for new legislation I'd be grateful. Do you feel you get enough information from Washington on such subjects?

Mr. PIEROVICH. I feel that in all of our—the exchange of information is a very complex thing today. We do make ourselves available to conferences, with

ople in these fields. Our technicians in herbicide work attend meetings ...rly on this matter. We are expected to keep ourselves informed. The l... ture has been quite full of the controversies on 2,4,5-T, and we have been aware of the developing controversies.

The most healthy thing that could happen in this area would be a definite summary of literature that our technicians could refer to. There are abstracts available now, but the combination of inputs from the universities and from the various departments of government in one abstract bulletin would be helpful to us.

Congressman McCarthy. Do you have anything to add, Mr. Pierovich?

Mr. Pierovich. No, I don't, sir.

Congressman McCarthy. Thank you very much, we appreciate it.

Mr. Pierovich. Thank you.

Congressman McCarthy. Our next witness is Dr. Paul Martin from the University of Arizona.

Dr. Martin, I understand you are accompanied by Dr. Russell?

Dr. Martin. That's right.

Congressman McCarthy. Would you like him to sit with you?

Dr. Martin. Yes.

Congressman McCarthy. Dr. Russell, would you care to join Dr. Martin?

Dr. Martin, we appreciate your being here. I wonder if you would identify yourself and Dr. Russell for the record, your background and your particular interest in this?

Dr. Martin. I'm Paul S. Martin, University of Arizona, Department of Geology. I had training as a professional ecologist, and with me is Dr. Stephen Russell who is a zoologist in the biology department in the University of Arizona. His special interest is in birds.

Congressman McCarthy. Thank you. Dr. Martin, I wonder before the record if you would tell us about your involvement with the spraying project, and any conclusions that you reached, based upon your analyses.

Dr. Martin. Well, I'm not involved in the spraying project, and I'm not a herbicide expert. I have no research experience with herbicides. I do have interest in the vegetation of Arizona. I've spent years studying its fossil pollen records, but the interest I had in Globe was in first seeing if indeed there was any effect on vegetation as a result of herbicide treatment that had been called to my attention, I have come up on four separate trips to visit the area that was sprayed, and see what little I could of the community.

Congressman McCarthy. How long did you spend on these trips?

Dr. Martin. These were 1-day visits.

Congressman McCarthy. How many did you make?

Dr. Martin. Four. As a result of seeing the area, and talking to some of the people in the area, I was curious to see if just what degree the community might have been affected by this. I wasn't prepared to believe that people, or animals could be affected by herbicide sprays because the little I heard indicated that those who work with herbicides stand underneath the spray plane and are occasionally drenched by the chemicals, and don't suffer ill effects.

So it seems incredible that people in this community could be complaining of such an effect, but they were.

Indeed as a result it seemed to me that it was important to listen to them and try to understand what they were saying, and try to come to terms with the only observers who witnessed an event that wasn't supposed to have happened.

It also seemed to me that some of the people involved in the work with herbicides were unprepared for this sort of experience, they weren't even listening to the complaints. So I presumed to do that.

Congressman McCarthy. And what did you find in the course of your four trips?

Dr. Martin. There is one other person that's involved in what I'm going to say next, I don't know if she's here or not.

Within the last month a student from Massachusetts by the name of Miss Adelaide Frick and she was willing to go on a door-to-door basis, and interview people in the community apart from the ones that I talked to.

Congressman McCarthy. Excuse me, is Miss Frick present?

Dr. Martin. I have the results, a summary of her door-to-door investigation in the area, the purpose was to see if there complaints comi...rom any other source other than the individuals that I talked to. The trips ...at I'd made up here and the design was to on a door-to-door basis talk to approximately 50 people in the canyons close to the sprayed area, and to another 50 over in Crestwood, which I believe is east of Globe at a further—at a point further remote from the area that was sprayed.

So what Miss Frick did was then conduct a door-to-door interview with people close to the sprayed area, and another group of 50 further away from it.

Congressman McCarthy. What did she find, do you have the report? We would like to have that for the record.

Dr. Martin. I'd be glad to give you a copy.

Congressman McCarthy. Would you care to summarize it?

Dr. Martin. I'll simply read about a paragraph from the report that summarized it, and of course, the individuals are not identified in this report, and the complete questionnaire is not represented here, simply the highlights of it.

There are three key questions, two that have to do with personal health, and one that has to do with livestock. It turned out that few people do have livestock in either—neither the spray area, or in Crestwood, but quite a number have pets. This is what she found.

Regarding pets, 13 cases in which animals were effected, and one must presume some relationship to spraying although in no individual case perhaps could this be directly proved.

This is the experiences of people living in this community who know the nature of the community, and then feel that something has happened that's a little bit out of the ordinary.

Thirteen cases in which animals acted, three kittens lost; two dogs lost; infertile eggs, one; rabbits not breeding, two; chickens not laying, one; burro lost, one; sick dogs, three reports.

Now, as far as people are concerned near the spray area, 23 of 56 indicated illness over the past 2 years which may be spray associated. Some people had absolutely nothing wrong with them, or were not concerned. They thought that those that were complaining were imagining it happened, an event that had no bearing in the real world, that it was in the minds of the people reporting.

Other reported, and we're quite convinced that their experiences were related to the events of last June, or earlier when herbicide spraying had haped.

Of the 23 reporting illness, 21 were reporting breathing difficulties. Many of these are attributed to the times of spraying. Some are attributed to smelter smoke, there's no avoiding the fact that this area that experiences a good deal of smelter smoke. Some of these people may be reporting an effect that is indeed caused by smoke, I don't know.

There were five reports of serious diarrhea, including one entire family. Four reports of chest pains, including one false heart attack, one report of coughing of blood, one report of subnormal temperature. Two reports of numb pain in arms; two reports of hemorrhaging; two reports of irregular periods; one report of miscarriage; two others by hearsay.

Fifty-six people interviewed, 42 mentioned some damage to plants, although the purpose of this questionnaire was not to consider plant damage.

Now, in Crestwood at a great distance from the——

Congressman McCarthy. Was the interviewer able to determine if such complaints were prevalent before the spraying began?

Dr. Martin. I don't know how one would do that. In fairness to the people in the Forest Service who have worked with this project, one simply can't conduct a scientific experiment at this point in time. All we can do is talk to the people who were the observers, or ones—or residents in the area, and while their memories are still hopefully fresh, recover some information, just having to take them at their word.

Congressman McCarthy. Let me just clarify. Is the interviewer ascribing these conditions to the spraying based on the interviews with the people? Do

they say that these phenomena results were the results of the spraying, or don't they know?

Dr. MARTIN. Yes, some of them would rather not say. The question is in effect, "Have you experienced any sickness which might be related to herbicide spraying of this area."

It's a leading question in part. It's not a question that denies any ignorance of the fact that herbicide spraying had taken place in the area.

I am sure there are many faults of a questionnaire of this sort that a professional psychologist would recognize.

Congressman MCCARTHY. Let me say as a point of information, we will shortly have put into the record a scientific data of the results on human beings of 2,4,5-T, which I think you will find bear a similarity to phenomena you've just described.

I wonder if you would go beyond Miss Frick's survey to give us benefit of your own observations of what you saw, and if you were able to reach any conclusions about the effects of the spraying on either humans, vegetation, or animals?

Dr. MARTIN. Well, the effects on vegetation impressed me as ones that have to be watched over a period of time. Again, this problem of who's to make the investigation, and how it's to be conducted are important. The incident is over, and in the minds of some local people, hopefully will never occur again.

The problem is, what really happened? I was up on four separate trips, or 4 separate days, I saw some things that I have not seen in Arizona vegetation before. Such as the presence on Century plants of flowering way out of season, and immature new plants going on the old stocks of old ones without normal seed being set.

I understand that this particular species of Century plant is known to do that, and other botanists have seen such a feature.

The area that was sprayed, not all plants are dead in it. Some species like Manzanita are remarkably resistant up to this point.

The effectiveness of the treatment is doubtful. The areas of spray aren't dead. The effects of spray on the outside areas on different plants have to be watched over a period of time to fully appreciate the change in phenology, the changes of flowering time, the change of time when the leaves appear, and when they fall, the way the tradition of plants may be as far as overall growth is concerned, and if one wants to demonstrate the herbicide-caused effect on vegetation. It's also necessary to take into consideration all the other environmental variations that aren't under control either, such as rainfall and temperature.

Congressman MCCARTHY. But, you did find evidences of drift outside the project area?

Dr. MARTIN. Yes.

Congressman MCCARTHY. Did you find evidences of 2,4,5-T in any of the adjacent streams, or did you seek to find it?

Dr. MARTIN. No, I collected samples only from within the project area, soil samples and water samples.

Congressman MCCARTHY. You found evidences of 2,4,5-T in the water you've collected within the project area?

Dr. MARTIN. The samples that I collected and submitted to a laboratory in California came back with a report of the presence of 2,4-D, and smaller amounts of 2,4,5-T.

Congressman MCCARTHY. In the water?

Dr. MARTIN. There was a trace in the water, there was up to one part per million in the soil of 2,4-D.

Congressman MCCARTHY. Is there anything that you or your colleague could add which would be pertinent to our inquiry?

Dr. MARTIN. I would make one recommendation, and then if Steve Russell has anything he would care to add.

The recommendation would simply be that hospital records, doctors' records, the veterinary records of those doctors and veterinarians in the Globe area be gone over very carefully by proper professional people.

Congressman MCCARTHY. At that point I think we should put into the record a memo of conversation with Mr. Peter Riddleberger of my staff, and

Dr. Grantville Knight, M.D., 2901 Wilshire Boulevard, Suite 345, Santa Monica, Calif.

This conversation took place on February 6, 1970.

Dr. Knight informed Mr. Riddleberger that he has two patients under his care from Globe, Ariz. While his examination is not complete, he is of the opinion that their malady is associated with the recent spraying of Silvex containing 2,4,5-T by the U.S. Forest Service. Dr. Knight is of the opinion that an investigation is warranted, and offered to submit a statement of his findings upon completion of his examination subject to the approval of his patients.

Miss Frick is here now, and I wonder if she could sit next to Dr. Martin and Doctor, if you would be good enough to reread that portion alluded to?

Dr. MARTIN. This simply summarizes the interviews that Miss Frick conducted in the canyons that is Kellner Canyon, Russell, Sixshooter, and Icehouse. Fifty-six interviews in that particular area, and some people who had serious complaints to make were not considered in this interview.

What I found just in tabulating what her questionnaire revealed was that 23 of 56 individuals indicated illness over the past 2 years, which may be spray associated, 21 individuals reported breathing difficulties, many of these are attributed to the times of spraying, but not all. Some were attributed to smelter smoke.

There were five reports of serious diarrhea, including one entire family.

Miss FRICK. Yes.

Dr. MARTIN. Four reports of chest pain, including one false heart attack; one report of coughing of blood; one report of subnormal temperature.

Two reports of pains, or numbness in arms; three reports of uterine hemorrhaging; one report of a miscarriage.

There were two others that I thought were hearsay, but I wasn't sure had really occurred in family that you interviewed, and then finally all the questionnaires wasn't directed to plant damage, there were 42 people interviewed who mentioned at least some damage to their plants in that area.

Now, the Crestwood account shows much less effect, and this is what one might expect because of the distance further away from the area of spray.

Congressman MCCARTHY. Doctor Russell, is there anything that you would add to the record here that would be helpful?

Dr. RUSSELL. I don't think I would add to the record, but I'm in agreement with Dr. Martin's statement.

Congressman MCCARTHY. You are, you've studied the information he has available?

Dr. RUSSELL. I have seen much of the general information, but I've conducted no investigation of my own into it.

Congressman MCCARTHY. Thank you, Gentlemen, and Miss Frick, very much. I'd like to now recall Prof. Galston.

Doctor, as we discussed here I understand you have some scientific data on the effects on human beings of 2,4,5-T. I wonder if you would cite the source of this information, and the findings?

Dr. GALSTON. Mr. Congressman, I'm very happy to present this information because in the course of my wanderings around on this day I have found that certain individuals tend instinctively to disapprove any allegations of direct damage to human beings or animals.

Now, as I hoped I made clear yesterday, very small doses of 2,4,5-T can cause birth abnormalities in laboratory animals, and that is now actively under investigation, and we've discussed to see whether it might be due to this impurity called dioxin, or whether it was due in fact to the chemical.

But now, the question is, can we actually produce an effect on mature individuals, let us say male individuals, totally apart from pregnant females bearing embryos in uteri, and I should say that there is a fairly sizable respectable scientific literature on this, and if one looks in a variety of sources, including the sort of encyclopedia of clinical toxicology by Gleason and Coughlin, and can find citations to many articles, and I have reference to a few here.

Now, 2,4-D can produce, if it's administered in very massive quantities, it can produce death in the small animals, and there are even a few cases

recorded of its having produced very severe symptoms in man. The latest data however, comes from 2,4,5-T, and I would like to read to you a brief account of an article published in 1959 by T. Flint entitled "Dermatitis and Kidney Damage Ascribed to Weed Killer 2,4,5-T."

Flint relates an episode involving two sisters, age 4 and 6 years, who had played for several hours in a yard which had been sprayed heavily a short time before with the Ortho brand of 2,4,5-T, brush killer. This was used for the control of poison oak.

This spray contained 16.4 percent of the isopro ester of 2,4,5-T in an oil base.

Now, I should mention parenthetically, I don't have the exact data at hand but Kuron contains much more than that, I believe in excess of 60 percent of this same ester.

The next day both girls exhibited generalized erythema—reddening of the skin—and edematus swelling of the oral and vaginal mucous membranes.

The pulse rate and body temperature were not elevated, but both children were described as appearing slightly toxic. The limbs and eyelids were slightly swollen as the mucous membranes of the mouth were inflamed. On the 8d day there were signs of kidney damage. Albumen was noticed in the urine. There was no evidence of liver injury, the urinary abnormalities persisted for about 2 weeks, but 2 months later the urine specimens for both patients were normal.

Now, there are other reports in which 2,4-D, and 2,4,5-T are alleged to have caused toxic effects on the nervous system as measured by the electroencephalogram. That is after ingestion, there was a desynchronization of the electrical activities of the nervous system, I bring these points up only to reinforce the fact that no chemical is completely innocuous. Some individuals are more sensitive than others, and some may require a big dose, and some a small dose to have these abnormal effects produced, but I share with Dr. Martin the view that when people appear and say that they have been adversely affected by these chemicals, immediate and adequate attention should be given to the possibility that these reports will furnish yet additional data to supplement the rather large amount of scientific data already existing.

Congressman McCarthy. Thank you, Dr. Galston. I wonder if you could—give us your observations after your inspection of the sprayed area, and the area where it drifted.

Is there anything that you at this point care to have in the record?

Dr. Galston. Well, I'll say a few words. I want to make it perfectly clear that after 24 hours in Globe, Ariz., I don't want to pose as an expert either on the program, or the effects on vegetation, or on people, but as a biologist working in this area, there is some conclusions I think I can make which point out the need for still further investigation, and everything I say should be held in that light.

What did I see on my brief trip yesterday? Well, I would classify them in several categories.

Number 1, at the helispot, overlooking the picnic area, I observed and smelled residues, there was no doubt that you could smell residual diesel oil which was primarily the carrier for the herbicide which had been splashed during the loading operation onto the helicopter.

Now, if you could smell it, there was a good deal around, and that would indicate that there are definitely residues in certain selected areas, how much there was I can't say, how much there might be in the soil, or in the water, I cannot say, but it seems to me that I could smell evidences at various points in my trip. So that there probably are residues here and there, and those could serve as a continuous supply of leaching, I suppose, into the waters of the area, one should not discount that possibility.

The second category was definite plant damage, and the plant damage was both the desired plant damage in the canyon, and undesired plant damage in the vicinity of homes, which was due to the drifting, I assume, the herbicide.

In the canyons we could see, and these were pointed out to me by some of our Forestry friends who were with me, the desired killing of such plants as

Manzanita and Oak, and the desired persistence of what they considered more desirable plants such as gerardia.

Now, I suppose a question could be raised as some of the local residents have been raising undesirable, and desirable, according to whose criteria, and by what judgmental values. Manzanita and Oak do live on these hillsides, they do transpire to water, and I suppose their killing is desirable in the contention of wanting to avoid the evaporation of water. Whether after you are all through with the operation and plant to grass, which is the stated objective of this clearing observation, you are going to save very much water, I'm not sure, and whether, in fact, the esthetics of the environment will be improved another stated objective of this operation is also I'd say open to question, I would think it would be a very useful operation for those groups charged with making policy to hold some public hearings at which citizens could come with their points of view. I think a lot of this fracas is due to poor interchange of information between official agencies, and the citizens. If there had been open hearings, and announcements, this is what we intend to do, this is why we are doing it, and this is how we are going to do it, and have objections recorded at the time, a lot of the acrimony that's built up here might have been avoided.

Now, so far as the damage of plants around homes, there is no doubt about it, it has occurred. I have seen it, and as a plant physiologist, I could testify that this is typical damage due to herbicide drift. I think that this points up a lesson when you discharge herbicides from the nozzles of spray on a helicopter, you are getting an assortment of droplet sizes, the big drops are going to fall quickly, the small drops are going to be carried for longer distances. I think until the technology is improved, the so-called invert sprays is one possibility here, and new types of booms for spraying are another, it seems to me that it's very unwise to spray in areas where homes are so intimately associated with the forest and woodland, that you are trying to control. You cannot pinpoint the spray, you cannot keep it out of the water, and you cannot prevent inadvertent spray damage to the nearby residences, and I would say that there are certainly many sprays in the country where the application of aerosol sprays is a highly beneficial practice.

From my cursory look here that I would say the intervening of house and the canyons in which spraying is desired, is so intricate that the slightest miscalculation, the slightest air movement, the slightest malfunctions of the spray equipment would lead to damage to the property, and I don't know how that could be worked out technically, and I would want assurance that those problems are looked into.

I think the people whose plants have been damaged ought to be compensated in some way because the damage has been considerable around some homes, and I think it's unfair to expect these people to bear the brunt of this kind of inadvertent drift operation.

Now, I did see damaged animals, and I talked with humans who alleged that they were adversely affected.

All I can say here is the damage is there, and spray operations did occur, but I know of absolutely no scientific evidence which would link the spray operation to the damage, and I think the people who showed me the damaged animals showed it to me in the spirit that this could be a consequence of spray operations, but they weren't sure, and certainly I'm not sure, but unlike some people I would not immediately offhand say this is ridiculous. It could be as I have shown from my previous reading from this scientific compendium, and I could document further a lot of the symptoms that people are reporting here have been reported for massive doses of 2,4-D. So we should not leave the possibility that this did occur, but a much more scientific information is required.

My overall view after one day of looking around is one of puzzlement. I wonder why it's desired to initiate this kind of an operation in this kind of an environment. The stated objective is to improve water runoff, and water runoff will benefit, I presume, the citizens of a nearby urban area, Phoenix, which is growing rapidly, and which has a lot of water requirements, and their water

requirements will grow as the years go by. We know this is an arid a[illegible]-by the way, not being an Arizona resident, and not being a politician, I perhaps could say some things here which a lot of people were thinking, but haven't brought forth.

Truly, water is going to be wilting in this area for others. So far as I can see unless nuclear technicology makes it available on a massive scale, which I don't foresee, if you take water from this area to give to another area, you are, in fact, robbing Peter to pay Paul. If you are robbing water from here, you are going to partially change the kind of vegetation, perhaps you are going to denude some of the areas in order to increase the runoff, this involves a comparative set of rules. Whose object is going to be gored here, whose interests are paramount? Well, clearly cities are not going to be able to grow indefinitely, we are going to have to put some limit on them, we know, for example, that the city of Los Angeles got into a lot of trouble with smoke because there are just too many people there. In the same way cities in the Southwest may have to limit their size ultimately based on the number of people they can support on the amount of water resources there are. The trying to take every amount of water out of the Country brings a possibility of a very serious question.

Now that President Nixon among others is calling for a campaign to restore the environment, it might be that we would want to look at this whole project in the context of what we are doing to the entire State, and to the entire countryside.

Finally, I would like to merely renew my suggestions that the people who formulated this policy, who set up this whole spray program should identify themselves, and should request the contributions of the citizenry as an input to this whole program.

I think that policy should not be made without question. This is a democratic society in which citizens have responsibility to interest themselves in the making of policy, and—my faith in the American people, and in their desire to run their own country has been to a certain extent reinforced by seeing a group of aroused citizens here out to protect their rights.

Thank you very much.

Congressman McCarthy. Thank you, Doctor Galston. I think the points you make are valid. One that I would just enlarge on a bit is that I am presently working on legislation to be established to support a National Growth Policy, I think growth has to be commensurate with the resources and of course, in this case, water is a critical resource.

I would conclude these hearings now with a couple of observations. I think it's important to know that 2,4,5-T was developed at the Army's chief Germ Warfare Research Center at Fort Detrick, Md. My experiences in investigating the Army's chemical and biological warfare programs, and policies, has not encouraged me about some of the actions that have been taken, without taking into consideration some of the unforeseen consequences. For instance, when they wanted to dispose of waste from nerve gas production at the Rocky Mountain arsenal near Denver, they first dumped this material into ponds on the arsenal's property. They didn't expect that it would find its way out. They thought it would be just absorped in the water on the pond. It wasn't, it was carried out into adjacent streams, and the neighboring countryside, and killed among other things livestock and 6 square miles of sugar beets.

They then dug a deep well and figured the best way to dispose of it was by dumping it deep into the earth. That set off 1,500 earthquakes in the Denver area, some of them up to six on the Richter scale, and caused great alarm in the community. They finally had to pull out this material, and of course the earthquakes stopped.

Then, they thought they should ship it across the entire United States. They thought this would be safe. Scientists later said it would risk the lives of thousands of people, the plans also called for dumping this large quantity of nerve gas and other materials into the Atlantic Ocean. They thought that would be safe.

Scientists later said it could destroy all marine life in 600 cubic miles of the Atlantic Ocean, with a cataclysmic effect on ocean's production cycle.

Now, I cite these instances not in reproaching the Army [illegible] the C.B.W. establishment, but I think that this particular program ha[illegible] questionable record.

We find 2,4,5-T developed by the Army's Germ and Gas Warfare establishment, 25 years ago to this date. We do not know for sure whether it will produce birth defects in human beings, I find it unwise to say the least to use such a substance without being sure that it is safe. For some reason the burden of proof seems to be on me and my colleagues in the sense that the attitude is, "we'll keep using it until you can prove it unsafe." Well, I quarrel with the basic assumption, I think that it should be just the reverse, I don't think that any toxic substance whether herbicide, pesticide, drug, whatever, should be used, sold in the United States until it can be shown that it is not harmful to human beings, that it doesn't produce cancer, or birth defects, or genetic effects.

One would think that we have learned from the Thalidomide experience, but apparently we haven't.

I also find it incredible that the Dow Chemical Corp. could have succeeded in helping reverse an order from The White House.

Now, I read this section from the statement of October 29 wherein the President's science adviser said that certain agencies of Government, the Department of Defense, the Department of Interior, the Department of Agriculture would do certain things, will inaugurate a new policy. Now we have the letter received today from The White House addressed to me, advising me that The White House is backing off from this directive, and is saying that the statutory responsibility resides with the individual agencies.

I find it personally unconscionable that in light of the Bionetics findings, and the scientific data cited by Doctor Galston this morning about the proven effects of 2,4,5-T on females, that this substance would be continued to be used on wide scale in the United States, and for that matter in Vietnam where even larger quantities are used.

I welcome the U.S. Senate Subcommittee on Investigation into this. I will prepare a full report which will appear in the public documents that will be developed as a consequence of our trip will be made available to not only the Senate Commerce Committee, but appropriate other committees of the Congress, as well as to the study of the American Association for the Advancement of Science under the directorship of Professor Messelson of Harvard.

We finally conclude by thanking the officials who have been most helpful, and to the residents of Globe who have been most hospitable, and I would hope that this experience here might have effects far more reaching than the small area of Globe, Ariz., and that perhaps as a result at least in part of what we have discovered here, that we will stop using 2,4,5-T around the world until we can run a series of tests that show that it is not harmful to this generation, and to the next generation.

Thank you very much.

Appendix 6

Albuquerque, N. Mex., *February 26, 1970.*

Hon. Richard D. McCarthy,
House of Representatives,
Washington, D.C.

Dear Mr. McCarthy: Thank you for your letter of February 16 and for the opportunity to furnish additional documents or statements for the record of your hearing in Globe.

For the Record Regarding Winds

In my testimony I promised to furnish you with additional data on windspeeds during the 1969 spray project. While windspeed was measured by the Project Air Officer who used a pocket anemometer, no record of observations was made. He did, however, maintain a record of application flight times

which shows when the work was shut down due to winds exceeding 10 miles per hour. The following table summarizes these important times from this record:

Date	Time	Remarks
June 8, 1969	1505	Shutdown (wind exceeds 10 m.p.h.).
June 8, 1969	1703	Resume operations (wind below maximum).
June 8, 1969	1935	End operation for day.
June 9, 1969	1018	Shutdown (wind exceeds 10 m.p.h.). End operation for day.
June 10, 1969	1115	Do.
June 11, 1969	1250	Do.

Because allegations of "gale winds" during application have been made, it is of interest to compare the above shut-down times with winds recorded at the Globe Fire Weather Station. The Globe Station records are for observations made only once daily at 1300 hours, but do not indicate the presence of "gale winds" on any day of the project. These 1300 hours observations are as follows:

Date	Direction	Speed (m.p.h.)
June 8	SW	5
June 9	SW	16
June 10	W	14
June 11	SW	16

As can be seen from the two tables, the only day on which applications extended beyond 1300 hours was June 8, when the 1300 hours observation was only 5 miles per hour. The June 11 shut-down time of 1250 hours would tend to infer that winds did possibly exceed 10 miles per hour when compared with the 1300 hours observation of 16 miles per hour. Ranger Moehn has stated that winds did not exceed 10 miles per hour in the area of the spray application, and this is quite possible since spray work was high up in Russell Gulch, in the lee of sheltering mountains to the Southwest, on that date.

OTHER ITEMS FOR THE RECORD

Additional copies of the Forest Service Interim Position Statement and of the map showing the limit of infrared detection of dead and distressed vegetation (as of October 1969) are enclosed for the record.

As I recall, Professor Galston asked for additional information on the 3-Bar research studies related to water yield. Since the Interim Position Statement digests these, I suggest that the Statement will serve for the record, but would be glad to arrange for you or for Dr. Galston to receive a copy of the rough draft of the manuscript referenced in the Statement.

Since the herbicide container converted to a trash barrel, and found in Kellner Canyon during your field tour, became a matter of importance to the press, the following additional information may serve as a useful insertion for the record: (1) The Dow Chemical Company label does not specify that the container be destroyed (copy of specimen label enclosed); (2) As a matter of good practice, we prefer that all pesticide containers not be reused, and when it was found that trash barrels were being made of the containers by the Globe District, the Regional Forester directed by memorandum on January 29 that all Southwestern Region Ranger Districts discontinue such uses; (3) Ranger Moehn, in response to the Regional Forester's direction, had all such trash barrels picked up earlier in the week of your visit; (4) presence of the container in the creek at the Kellner recreation area cannot be explained by District personnel who were in the area and had not seen it prior to your field tour; (5) the container had been washed with water and detergent prior to painting for use as a trash barrel.

Also on your field tour, there seemed to be some misunderstanding regarding application of herbicide to the live stream in Kellner Canyon. While the stream was flowing when you were in the area, it was not a live stream at the point visited at the time of application. We do not deny that some herbicide may have drifted to live streams, as evidenced by some tip damage to trees in the Kellner Recreation Area where there was a live stream, but that drift actually reached the water has not been established.

While the Interdepartmental Panel of Scientists headed by Dr. Fred H. Tschirley arrived following your hearing, their findings are of sufficient importance to the matter under consideration, that we desire to have the enclosed press release issued by them inserted in the record.

It was a pleasure working with you and Mr. Riddleberger during your visit. If the Forest Service can be of any further assistance, please let us know. We will appreciate receiving three copies of the hearing record when available.

Sincerely,

JOHN M. PIEROVICH,
Assistant Regional Forester.

45-362 O - 70 - 11

FOREST SERVICE INTERIM POSITION: KELLNER CANYON-RUSSELL GULCH HERBICIDE SPRAY PROJECT AND THE SOUTHWESTERN REGION CHAPARRAL PROGRAM, February 9, 1970

INTRODUCTION

Background on Kellner Canyon-Russell Gulch Project

The Kellner Canyon-Russell Gulch Project is a part of the Chaparral Management Program of the Tonto National Forest. The primary objective of this project is to improve water yield, but other program objectives and resulting benefits are intended to be met as well. Improved water yield and other Chaparral Program objectives are discussed below.

This project was initiated in 1965 following extensive local discussions and a press release which appeared in the local paper. Rather than the usual practice of applying prescribed fire as the initial treatment, herbicides were used. This was because of the known tendency for streams in this area to produce flash floods; herbicide treatment was considered to be unlikely to contribute to flooding, whereas large areas treated by fire could.

Chemicals used in this project are listed by year of use in Table 1, which is appended. These are all Federally Registered Compounds and were applied in keeping with the laws and label instructions governing their safe use.

Following the 1969 Application of Herbicide, Tonto Forest Supervisor Robert Courtney received a complaint in the form of a petition bearing 154 signatures of people in and near Globe, Arizona. Following the initial complaint, Courtney requested a team of qualified individuals to visit the area for a general assessment of alleged herbicide damage. This team reported some limited damage to vegetation on certain private properties.

Chaparral Management Objectives

Objectives of managing chaparral on the Southwestern National Forests are to:

1. Improve water quality and yield through reductions of the potential for sedimentation following wildfire and through reductions in evapo-transpiration losses where modification of existing vegetation is proper.

2. To enhance the scenic value of the Chaparral zone through development of varied patterns resembling the natural variety sometimes found in unprotected chaparral; these patterns range from savanah-like grass and forb areas to newly regrowing chaparral, to relic stands of mature chaparral.

3. To improve wildlife habitat through creation of additional edge effect and through maintenance of vigor and new growth in desirable species.

4. To reduce the high costs of protecting chaparral from wildfires through the establishment of breaks in heavy fuel continuity, making it more possible to avert fires of conflagration proportions.

5. To increase forage production for wildlife and livestock through the release of native grasses and the establishment of new grass stands.

6. To improve access for both the observer of wildlife and the hunter through a system of near-primitive roads to strategic fire control locations and through the openings that will result in treated areas.

It is intended that each of the above objectives will be met through Multiple Use Coordination Procedures. These require that regardless of the primary purpose of any project, proper consideration be given to other forest uses and values. Because of the intense interest in improving Southwestern water quality and yield, both Federal watershed management and cooperator funds have been made available for this work as a primary purpose. Each of the objectives of chaparral management is fairly well understood by the interested public except for this one of improvement in water yield. Even some experts have, until recently, discounted the potential for augmenting water supplies through alteration of shrub cover in the chaparral type.

Much of the research leading to improved understanding of the potential for additional water has been done on the 3-Bar Experimental Watersheds near Roosevelt Dam on the Tonto National Forest, Work there was begun in 1956. Two reports from this work are of particular interest.

> Pase, C.P., and P.A. Ingebo, 1965, "Burned chaparral to grass: early effects on water and sediment yields from two granitic soil watersheds in Arizona," Proceedings Ninth Annual Arizona Watershed Symposium, 4 pp illus.
>
> Hibbert, Alden R., Unpublished 1970 Manuscript on file with Rocky Mountain Forest and Range Experiment Station: "Increases in streamflow vary with rainfall after converting brush to grass."

The latter report is cited because it contains data not previously available which are regarded as more reliable (due to additional years of streamflow measurement) and which indicate greater promise of improved water yields than previously expected. Increases due to watershed treatment have varied from 1.5 area inches to 14.0 area inches. The two test watersheds averaged an increase in water yield, for the period 1959 through 1969, of from 4 to 6 area inches.

Progress and Direction of Studies--The Kellner Canyon-Russell Gulch Project

Task Forces No. 1 and No. 2 (Completed Work)

The first two teams to examine the area were concerned with visually detectable effects of the 1969 herbicide application. Due to the similarity of some insect and desease symptoms to symptoms of herbicide effects, the second team included specialists in entomology and plant pathology. It was on the basis of this team's findings that many plants alleged to be damaged from herbicide drift were determined to be affected by other causes.

It should be noted that while all complainants have been advised of Forest Service claim-for-damage procedures, only one formal claim has been filed. This claim was not for properties identified as damaged in the Task Force No. 2 Report, and has thus been disallowed.

Infrared Photography and Interpretation for Distressed Vegetation (Work In Progress)

While the second Task Force reported that some visually detectable herbicide drift had occurred from the 1969 spray project, extending approximately one-fourth mile north of the project, their assessment did not include previous years' effects, nor was it concerned with delineation of the sprayed area as a whole.

In order to more accurately define the limits of herbicide effect on plants from all years of spraying, aerial infrared photography has been employed. Interpretation of these aerial photographs has made possible a preliminary delineation of the exterior boundary of destressed and dead vegetation. Both the visually detected drift line reported by Task Force No. 2 and the External Limit of Infrared-detected distressed and dead vegetation are shown on the appended PRELIMINARY map. It is important to note that internal exclusions have not been delineated and that field verifications are not yet completed for the infrared interpretation.

Environmental Effects (Work in Progress)

Work is underway in this study to assess the total effect of the Kellner Canyon-Russell Gulch Project on the environment. Some of the key considerations included in this study are listed below.

1. Possible further evidence of drift of herbicide sprays through such herbicide residues as are detected in soil samples north of the project area. Initial soil sampling was within the project and on two transects toward the northeast corner of the project. This corner was selected as the best to test the hypothesis that soil residues from drift might be found, since prevailing winds are from the Southwest.

   Initial laboratory analysis reports have indicated low concentrations of Silvex and 2, 4-D at some locations (maximum detected concentration off the project to date is 0.16 p.p.m. Silvex). Especially at these low levels of concentration, it is possible that other sources of contamination may induce "background" which could lead to erroneous conclusions. For this reason, we are proceeding to cross-check analysis procedures while, at the same time, widespread sampling north of the project is scheduled.

   It would be premature to reach any conclusion regarding drift at this time.

2. Herbicide levels in water samples. Water sampling and analyses have been underway for some time. Project methods called for interruption of application at all stream channel crossings, and as far as we have been able to determine, no herbicide was applied directly to water. Some soil-leaching and runoff is to be expected. All samples we have taken, or taken by private individuals and brought to our attention, are less than the Federal water quality criterion of 0.1 p.p.m. 1/

3. Effect of Treatment on Esthetics. While it is evident the dead vegetation over this area is not pleasing, our concern here is with the next needed steps to actually provide enhancement of the scenic resource. It is sometimes necessary to tolerate temporary degradation of the appearance of an area as a cost of ultimate improvement. This study is intended to better define tolerable limits, explore alternatives, and recommend treatments to completion. Concurrently, we are assessing the past, present, and projected fire hazard in order to build conflagration control concepts into the landscape design.

4. Effects on Animals and Plants. Initial observations by wildlife experts have shown no marked effect upon wildlife.

1/ Surface water criteria for public water supplies table appearing in: Water Quality Criteria issued as a report to the Secretary of Interior, April 1, 1968, and published by the Federal Water Pollution Control Administration.

On the other hand, repeated claims have attributed varied maladies of humans and animals to the project's herbicide sprays. Lacking private medical histories or other solid bases for evaluation, we believe it more sound to rely on published results of laboratory tests. These are to be used in determining expected effects on animals for rates of application used. Yet to be published laboratory results are needed to complete this study as it relates to animals.

A further consideration of this study is that for proper perspective, all of the environmental influences on the area must be weighed. Two examples help to bring this need to focus. One is the frequent presence of smoke from nearby smeltering operations, especially when an inversion and northwest winds combine to produce a thick accumulation in the basin north of the Pinal Mountains. The other is household and industrial uses of herbicides which may have induced additional residues into the affected area.

While neither the effect of possible air pollution in the area nor the possible contamination by other herbicide uses are known, their importance as suspect environmental effects cannot be discounted.

FOREST SERVICE POSITION

We share deeply the concern of the people in this area with their environment. The Forest Service has no intention of persuing a course which will adversely affect the health and safety of its National Forest neighbors, nor which will permanently detract from the scenic or other qualities of the Forests.

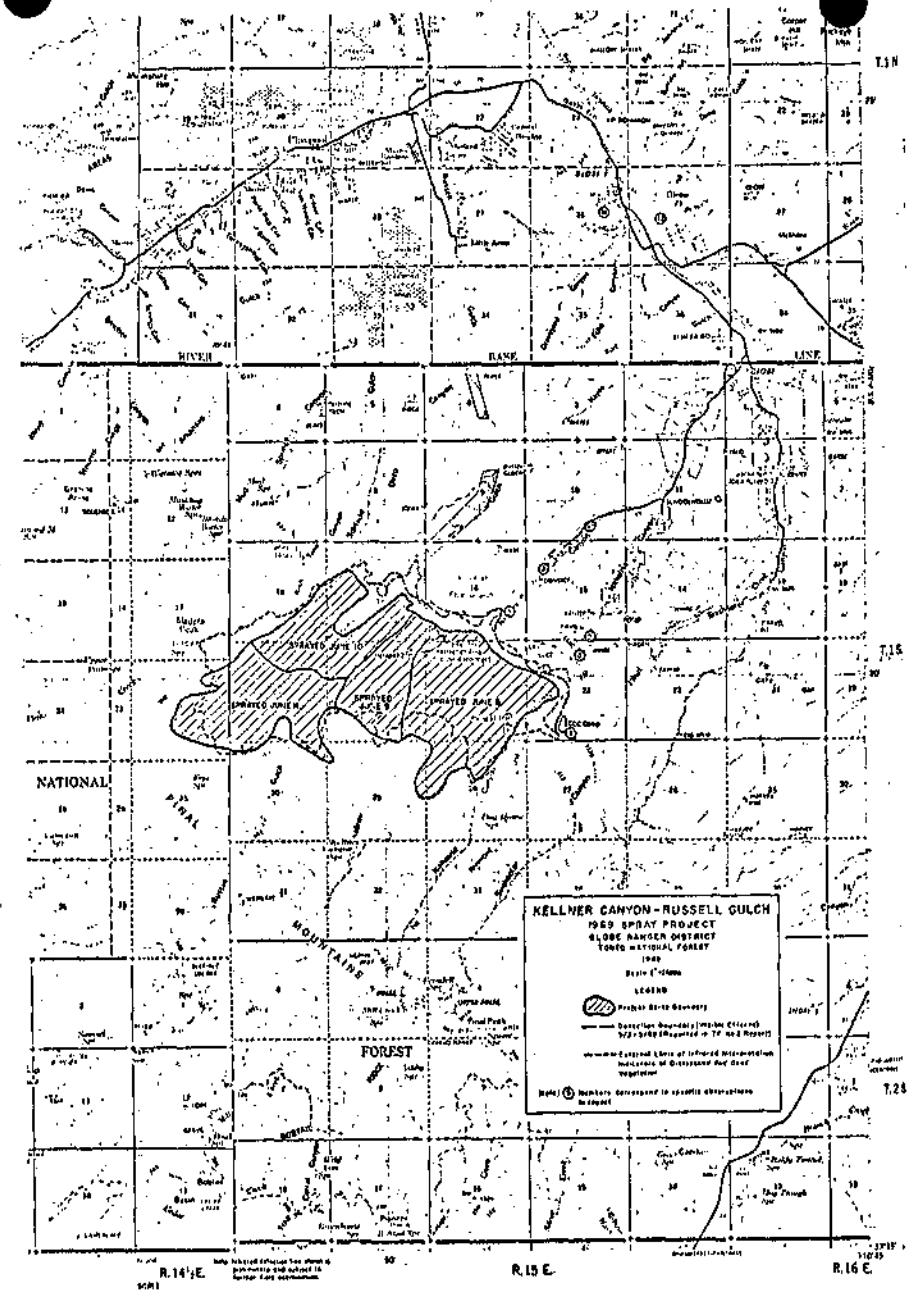
It is our position that the studies we have underway, as well as the outcome of public meetings concerned with herbicides and with the overall conduct of the chaparral program, must determine the ultimate decision on deferment. For this reason, we believe it would be premature to state at this time either when the deferment may be lifted, or what new guidelines will be followed.

It is our further position that it would be unwise to base decisions on the future use of the herbicides employed, solely upon alleged or suspected effects in the vicinity of the Kellner Canyon-Russell Gulch Project. There are many environmental influences operating in this area which must be better understood. Also, many of the questions raised about these chemical compounds can be resolved only through carefully controlled laboratory experiments.

Year	Chem. Name	Trade Name and/or Mfr.	USDA Reg.	Lbs. Acid Equivalent per Gal. of Mixture	Application Rates/Acre (Acid Equivalent)
1965	2 4 D	Mansanto	524-115	6	1 lb.
	2 4 5 T	Thompson-Hayward	148-431	6	1 lb.
1966	2 4 D	Mansanto	524-115	6	1 lb.
	2 4 5 T	Thompson-Hayward	148-431	6	1 lb.
1968	Silvex	Kuron (Dow)	464-162	4	2 lbs.
1969*	Silvex	Kuron (Dow)	464-162	4	2 lbs.
	2 4 D	Mansanto	524-115	6	1 lb.
	2 4 5 T	Thompson-Hayward	148-431	6	1 lb.
	2 4 5 T	Hercules	891-46	4	2 lbs.
	2 4 5 T	Hercules	891-45	4	2 lbs.

*In recent discussions with project personnel, we have learned that in addition to the Silvex reported on the project accomplishment report and used in our earlier correspondence, a small amount of Mansanto 2,4 D and Thompson-Hayward 2,4,5-T arrived mixed with the mixing equipment and was applied in 1969. Registration numbers, mixtures and rates were the same as reported above for 1965 and 1966. There was also a 30-gallon supply of Hercules 2,4,5-T on hand from earlier field trials which was used.

TABLE 1 - Record of Herbicides Applied to the Kellner Canyon-Russell Gulch Project



PRESS RELEASE - February 20, 1970

Government Interdepartmental Panel of Scientists

The panel is carefully examining the evidence collected during its visit. The study will continue and will include analyses of the numerous samples of blood, soil, water, fruit and plants for the herbicides, a possible contaminant (dioxin), as well as various agents producing disease in man, animals and plants. However, to date, we can summarize a few of our findings as follows:

1. The application of herbicides in the Pinal Mountains near Globe, Arizona was made by the Tonto National Forest starting in 1965. The most recent application of the herbicide was made by helicopter on June 8, 9, 10 and 11, 1969.

2. The materials used in the treatments in 1965, 1966, 1968 and 1969 included 2,4-D, 2,4,5-T, and silvex. These chemicals came from different sources. In 1969, 30 gallons of 2,4,5-T produced by the Hercules Chemical Company and 935 gallons of silvex produced by the Dow Chemical Company were used. The silvex is reported by Dow Chemical Company to contain less than 1 ppm of the dioxin. Analyses will be made of silvex and the other herbicides for dioxin and the active herbicide ingredients.

3. There are reports of the aircraft flying over private properties but not spraying; and other reports of the herbicide being applied just outside the project area. There is clear evidence of drift of the herbicides on a number of plants on some of the nearby properties.

4. Human illnesses have been reported by several residents in the Globe region. Many of the residents with complaints were interviewed by a medical member of the panel. These are complaints that commonly occur in the normal population; the eye irritation in one individual may be related to the spraying. Nine doctors serving the area of Globe were interviewed and there was general agreement that there had been no significant increase in human illness related to the spraying. However, blood samples were obtained and additional studies are planned to verify or rule out this possibility.

5. Reports from the wildlife specialists indicate no significant effects on birds, deer, and other wildlife. There are reports of reductions of birds on a few properties but there are other reports that bird and other wildlife populations in and near the project area are normal.

6. Information obtained from owners of livestock and observations of animals did not indicate any illnesses that do not commonly occur in other regions. It is doubtful that the spraying of the herbicides or dioxin caused the afflictions in the goat and duck because the goat was born before the treatment and the duck was hatched about 4 miles away from the treated area.

7. There was evidence of woody plant mortality from root rot, and also visible damage to certain yard trees from several kinds of insects and woodpeckers or sapsuckers. Other plant injuries were observed that appeared to be caused by low soil moisture, air pollution and unusual soil properties.

8. The phenoxy herbicides following normal use do not usually persist for more than 8 months in soil and water. Additional analyses are in progress to determine the presence or absence of herbicides.

Senator HART. We are adjourned to resume on the 15th of this month in this room.

(Whereupon, at 5:15 p.m., the Subcommittee was adjourned, to resume on April 15, 1970.)

EFFECTS OF 2,4,5-T ON MAN AND THE ENVIRONMENT

WEDNESDAY, APRIL 15, 1970

U.S. SENATE,
COMMITTEE ON COMMERCE,
SUBCOMMITTEE ON ENERGY, NATURAL RESOURCES AND THE ENVIRONMENT,
Washington, D.C.

The Subcommittee met, pursuant to adjournment, at 10 a.m., in room 1318, New Senate Office Building, Hon. Philip A. Hart, presiding.

Present: Senators Hart and Baker.

Senator HART. The Committee will be in order.

Our first and distinguished witness is the Surgon General, Dr. Jesse Steinfeld.

STATEMENT OF DR. JESSE STEINFELD, SURGEON GENERAL, DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE: ACCOMPANIED BY DR. DAVID GAYLOR, DR. DIANE COURTNEY, AND DR. DALE LINDSAY

Dr. STEINFELD. Thank you, Senator Hart.

Accompanying me are Dr. Diane Courtney, on my right, of the Pharmacology and Toxicology Branch of the National Institute of Environmental Health Sciences, Dr. Dale Lindsay, associate commissioner for science (FDA) and Dr. David Gaylor, chief of the Biometry Branch of the National Institute of Environmental Health Sciences.

I have a prepared statement.

Senator HART. Yes. I suggest you read it and if there is any footnoting or extension that you want to make as you go along, feel free to do it.

Dr. STEINFELD. Thank you, sir.

I am pleased to appear before you today to discuss the herbicide known as 2,4,5-T, our efforts to determine its hazard to health, and subsequent action to protect human health.

The production of 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) in the United States increased from 8 to 40 million pounds per year in the last decade. In the United States, 2,4,5-T is principally used as a weedkiller in clearing range and pasturelands, roadsides and rights-of-way, in suppressing aquatic weeds, and in eliminating weeds in croplands. It is also used to reduce weeds in turf. The use of 2,4,5-T and its salts and esters on food crops has been registered by the

U.S. Department of Agriculture on the basis of no residues in the marketed food.

To insure that the foods reaching markets are free of residues, the FDA has monitored the food supply in selected cities. About 5,300 food samples were analyzed for 2,4,5-T and other pesticides in the last 4 years. Residues of 2,4,5-T, at trace levels (less than 0.1 part per million), were found in 25 of these samples. In 1965, one sample contained 0.19 parts per million; in 1966, another sample contained 0.29 parts per million. It is my opinion that the results of the monitoring program justified the registered use of 2,4,5-T on selected food crops, in the absence of any known toxicity of 2,4,5-T.

The development of a balanced public policy which considers benefits and risks associated with the use of a compound such as 2,4,5-T is an exceptionally difficult matter. Great public fear of the possible implications for man has followed reports of harm in laboratory animal tests. And yet frequently it is not known with certainty what laboratory animal tests may mean for man. We are obligated to make decisions of great health and economic importance on the basis of very limited evidence of potential hazard; prudence allows no other course. We are aware that both good and bad consequences may result from our actions.

The enormous strides taken in achieving the prosperous and healthy life we now enjoy in an industrial age has created problems and uncertainties which are not easily overcome. The resolution of these uncertainties and solution of these problems will require national commitment and broad public education and understanding.

At this point, I would now like to read the joint announcement of Secretaries Hardin, Finch, and Hickel, prepared in accord with the Interagency Agreement for Protection of the Public Health and the Quality of the Environment in Relation to Pesticides. This is the first public release of this announcement.

Agriculture Secretary Clifford M. Hardin, Interior Secretary Walter J. Hickel, and HEW Secretary Robert H. Finch today announced the immediate suspension by Agriculture of the registrations of liquid formulations of the weed killer, 2,4,5-T for use around the home and for registered uses on lakes, ponds, and ditch banks.

These actions are being taken pursuant to the Interagency Agreement for Protection of the Public Health and the Quality of the Environment in Relation to Pesticides among the three Departments.

The three Cabinet Officers also announced that the Department of Agriculture intends to cancel registered uses of non-liquid formulations of 2,4,5-T around the home and on all food crops for human consumption (apples, blueberries, barley, corn, oats, rye, rice and sugar cane) for which it is presently registered.

The suspension actions were based on the opinion of the Department of Health, Education and Welfare that contamination resulting from uses of 2,4,5-T around the home and in water areas could constitute a hazard to human health.

New information reported to HEW on Monday, April 13, 1970, indicates that 2,4,5-T as well as its contaminant dioxins, may produce abnormal development in unborn animals. Nearly pure 2,4,5-T was reported to cause birth defects when injected at high doses into experimental pregnant mice, but not in rats. No data on humans are available.

These actions do not eliminate registered use of 2,4,5-T for control of weeds and brush on range, pasture, forest, rights of way and other non-agricultural land.

Users are cautioned that 2,4,5-T should not be used near homes or recreation areas. Registered uses are being reviewed by the three Departments to make

certain that they include adequate precautions against grazing treated areas until long enough after treatment by 2,4,5-T so that no contaminated meat or milk results from animals grazing the treated area.

While residues of 2,4,5-T in meat and milk are very rare, such residues are illegal and render contaminated products subject to seizure. There is no tolerance for 2,4,5-T on meat, milk or any other feed or food.

USDA will issue guidelines for disposal of household products containing 2,4,5-T. The chemical is biologically decomposed in a moist environment.

Background Information

Secretary Finch's Commission on Pesticides, which reported its findings in November and December 1969, extressed concern that research conducted at Bionetics Research Laboratories, under the direction of the National Cancer Institute, indicated that 2,4,5-T had produced a number of birth defects when fed or injected into certain strains of mice and rats. Because the test material contained substantial concentrations of chemical impurities (dioxins), the birth abnormalities could not be attributed with certainty either to 2,4,5-T, or to the impurities known to be present.

Representatives of the chemical industry pointed to evidence of extreme potency of the impurities as toxic agents. They demonstrated that 2,4,5-T now being marketed is of a greater purity than that which had been tested in the Bionetics experiments and urged that further testing be undertaken to clarify the questions raised.

Responding to this suggestion and utilizing materials supplied by one of the major producers of 2,4,5-T, scientists at the National Institute of Environmental Health Sciences promptly initiated studies to determine whether 2,4,5-T itself, its impurities or a combination of both had caused the earlier findings, and whether the 2,4,5-T now being marketed produces birth abnormalities in mice and rats.

The experiments were completed last week and the statistical analyses performed over the weekend. On Monday and Tuesday of this week the analyses of the data were presented to the regulatory agencies of the Federal Government and to the members of the Cabinet.

The dioxin impurities and the 2,4,5-T as it is now manufactured, separately produced birth abnormalities in the experimental mice.

Because absolutely pure 2,4,5-T was not available for testing, it is possible only to infer from certain of the observations that the pure 2,4,5-T probably would be found to be teratogenic if it were tested. But, since pure 2,4,5-T is not marketed and could not be produced in commercial quantities, this is not a practical issue for consideration.

In exercising its responsibility to safeguard public health and safety, the regulatory agencies of the Federal Government will move immediately to minimize human exposure to 2,4,5-T and its impurities. The measures being taken are designed to provide maximum protection to women in the childbearing years by eliminating liquid formulation of 2,4,5-T use in household, aquatic and recreational areas. Its use on food crops will be cancelled, and its use on range and pastureland will be controlled. Maximum surveillance of water supplies and marketed foods will be maintained as a measure of the effectiveness of these controls. These measures will be announced more specifically in the Federal Register shortly.

While the restriction to be imposed upon the use of this herbicide may cause some economic hardship, we must all cooperate to protect human health from potential hazards of 2,4,5-T, other pesticides and the dioxins.

The three Secretaries commended the chemical industry for its prompt and willing cooperation with the National Institute of Environmental Health Sciences in the studies to clarify questions raised by the initial studies of this herbicide and for working closely with the FDA in the other studies still underway. They urged the full support of industry, agriculture and the home gardner in insuring the safe use of 2,4,5-T and other pesticides which contribute in important ways to the welfare of the Nation.

That is the end of the press release and I would add that it is my understanding that Secretary Packard of the Department of Defense sent a memorandum to the Joint Chiefs of Staff saying the Department will suspend the use of 2,4,5-T in all operations pending evaluation of the data.

I will return to the prepared testimony.

At this point, we would like to provide for the record a summary description of the results of these latest studies of the National Institute of Environmental Health Sciences,[1] completed this past week. I shall be pleased to respond to questions about these data but suggest that the Committee not be burdened by a detailed oral presentation of the findings which have been stated briefly in the foregoing announcement.

This leads me to brief mention of the studies which will be presented next by Dr. Verrett. Commencing in the fall of 1969, Dr. Verrett reinstituted tests of the embryotoxicity and teratogenicity of 2,4,5-T, its contaminating dioxins, and related chemicals.

Dr. Verrett is to be commended for promptly attacking these problems and for going to the very considerable trouble of purifying the 2,4,5-T by repeated recrystallization. However, I must express concern about the degree of reliance which has been placed upon chick embryo studies. While the studies in chick embryos are in general agreement with those in studies of rodents at the NIEHS, it is to be emphasized that they do not clarify the uncertainties as to significance for man.

I believe that it is imperative that everyone involved in the development of a national policy for dealing with the many questions posed by 2,4,5-T and other pesticides be aware of the complexity as well as the importance of the issues, together with the limitations of our ability to estimate potential hazards to human health posed by these substances.

It is essential that we strive to respond wisely to the discoveries which have been made in this field, and resist the temptation to resort to measures which may be more extreme than the evidence warrants. For example, 2,4,5-T is probably the most effective means of controling poison ivy, poison oak, and other noxious weeds to which a substantial portion of the population react badly. It has been estimated that 60 percent of the American population is sensitive to either poison ivy or poison oak, and that from 5 to 10 percent of Americans suffer a reaction to the poisons from these weeds each year. Some of these individuals become quite ill and incapacitated by their reaction to these poisons.

By contrast, we are not aware of any reliable evidence that 2,4,5-T, indeed any of the pesticidal chemicals, has resulted in human birth abnormalities. These remarks should not be interpreted as evidence of indifference to what may be a potential hazard to health. The record clearly reveals a series of responsible actions by the Administration to the results of recent laboratory tests. Prudence has characterized these decisions and actions and will continue to guide the Department in these matters.

[1] See p. 98.

In keeping with the pattern established with the naming of the Secretary's Commission on Pesticides, the thorough study of pesticide problems by the Commission, and the Administration's prompt action to implement the recommendations of the Commission, we now commit ourselves to the following actions:

We shall strive to develop better means for predicting in laboratory animal systems the potential hazard posed for man by chemical pesticides.

We are aware of a great need for a centralized clearinghouse for information of all types on pesticides. We plan to have such a clearinghouse established jointly by the National Library of Medicine and the FDA in the very near future. Other agencies having similar interests and needs will be invited to participate in this undertaking.

The need to continue certain closely restricted uses of 2,4,5-T will require a high level of surveillance activity to insure protection of the human population from exposure through water sources. This will be done.

The Food and Drug Administration will continue to examine a variety of foods for the possible presence of residues of pesticides, and will take appropriate action through the interdepartmental agreement to protect the public health.

This completes my prepared statement, Senator Hart.

My colleagues and I will be pleased to answer any questions.

Senator HART. Thank you Doctor.

Just as you began, we were joined by the able Senator from Tennessee, Senator Baker.

I understand that the announcement you just read us relates to both powdered and liquid forms of 2,4,5-T shipped in interstate commerce.

But what about the 2,4,5-T which is now on the shelf? What do we do about that?

Dr. STEINFELD. You mean on the shelves in the homes and the shelves in the stores?

Senator HART. Yes, the places for retail sale.

Dr. STEINFELD. I think there is a distinction between the suspension of the registration and the cancelling for registration and I would like to call on Dr. Lindsay to describe in more detail, the procedures involved.

Dr. LINDSAY. The suspension is a little more drastic than the cancellation, because it is a final action until some other action is taken, whereas the cancellation permits hearings and has the statutory procedure for appeal during which time the pesticide may be used while it is being reviewed.

Senator HART. Well, the suspension, the more drastic remedy, was directed at the liquid form.

Do I read that correctly?

Dr. STEINFELD. Yes.

The suspension by Agriculture of the registrations of liquid formulations of the weed killer for use around the home and for registered use on lakes, ponds, and ditch banks.

We reviewed the concentration of 2,4,5-T in a number of formulations and found the concentrated form is present in liquids and could present a hazard.

The amount of 2,4,5-T in some of the solid fertilizer-type materials was much less and therefore, the more drastic action was taken regarding those compounds.

Senator HART. As you read that suspension sentence I did not hear a suspension extended to the use of 2,4,5-T on food crops.

Dr. STEINFELD. The three Cabinet officers announced they intended to cancel the registered use of nonliquid formulation around the home and on all food crops for human consumption, so that all of these registered uses will be cancelled.

Senator HART. But the use of liquid formulations on food crops, as I understand the announcement, was not.

Dr. LINDSAY. As far as I know all of the use on food crops is from the liquid application.

Senator HART. So there would be no application to food crops under this order, as you understand it?

Dr. LINDSAY. As I understand it.

I am not aware of any dry material used on food crops.

Senator HART. Well, let me get back to my point of departure. You have suspended for certain applications 2,4,5-T in liquid form. As Dr. Lindsay said, that is the more drastic sanction.

Now, with respect to that 2,4,5-T in liquid form, the order today has what effect on the marketing and use on shelves or in homes?

Dr. STEINFELD. Well, I don't know exactly what the Department of Agriculture will do. This is not an FDA activity. I am certain they will move quickly and appropriately. I think a significant statement is on page 2 of the release, which says the "U.S. Department of Agriculture will issue guidelines for disposal of household products containing 2,4,5-T. The chemical is biologically decomposed in a moist environment".

The intent is to get rid of all 2,4,5-T around the household. I assume it would not be available for use in households where pregnant women would have access to it. I don't have the details of those actions.

Senator HART. I see we don't have anybody on the witness list this morning for the Department of Agriculture, but would you agree it would be very inappropriate for the Department of Agriculture to permit continued vending of liquid 2,4,5-T for any of the purposes for which you have suspended it, even though it is now in retail distribution?

Dr. STEINFELD. I think this announcement will have dramatic impact. Our meetings with the Department of Agriculture on Monday and Tuesday would lead me to believe they are going to take appropriate and vigorous action.

Senator HART. Would you describe as appropriate, walking into a store and seeing the thing on the shelf and saying, take it off? That seems appropriate to me.

Dr. STEINFELD. Idon't know the mechanisms which they have to insure compliance.

Senator HART. If they have it and don't do it, don't you think it would be inappropriate and if they don't have it, don't you think Congress should give it to them?

Dr. STEINFELD. Certainly they should have the authority to do what is required to protect the public health, and I think they do have this.

Senator HART. Well, we will find out.

Dr. STEINFELD. I am sorry, I don't know.

Senator HART. You are talking to another nonexpert, so don't feel bad.

Mr. Bickwit has greater expertise than I, so we will let him deal further with the problem.

But there is one passage in your announcement that particularly interests me. In the press statement which you read, there is a paragraph which states: "The regulatory agencies of the Federal Government will move immediately to minimize human exposure to 2,4,5-T and its impurities. The measures being taken are designed to provide maximum protection to women in childbearing years by eliminating formulation of 2,4,5-T use in household, aquatic and recreational areas. Its use on food crops will be canceled and its use on range and pasture land will be controlled."

You say on food crops its use will be "canceled."

But is it not a very technical definition only of that term that permits you to say it will be canceled on food crops, because in liquid form I take it, it may still be used, or am I wrong about that?

Dr. STEINFELD. When the use is canceled, such a notice is published in the Federal Register, I believe.

And then there is a 30-day period for comments, is that not correct, Dr. Lindsay?

Dr. LINDSAY. Yes.

Dr. STEINFELD. After which appropriate action is taken.

Senator HART. I think what I am more concerned about is my desire to understand precisely what may or may not be done with this formulation in application to food crops.

In liquid form may it continue to be used?

Dr. STEINFELD. You mean during the 30-day period while the—I am afraid I don't understand.

Senator HART. It has been suggested to me that there would continue to be no restrictions with respect to the use in liquid form on food products.

Now, is my information correct on that?

Dr. STEINFELD. No, sir, the use on all food crops will be eliminated as promptly as the law permits through cancelation of the registration, whether in dry or liquid form or any form. There will be no use on food crops, Senator Hart.

Senator HART. All right. I think this is a desirable clarification, since there were some who had felt otherwise.

You say it will be eliminated as promptly as is possible under the law. It could be eliminated more promptly by a suspension than a cancelation?

Dr. STEINFELD. Yes.

Dr. LINDSAY. Yes, I am not aware of what the Department of Agriculture's intent is with regard to carrying this on.

The main idea was to get it into effect at the earliest possible time where it would be likely to come in contact with women of childbearing age.

Senator HART. I am trying to ask why the different treatm
Why with respect to certain forms and use is it merely canceled?

Although that sounds very dramatic, it means if you want to use it, go ahead and use it until somebody resolves differences which may arise over the action. Why handle some uses on a cancelation basis and some by suspension?

Is it because those uses and forms that you suspended more intimately or directly come in contact with women of childbearing age?

Dr. STEINFELD. Yes, I believe that is the reason.

Right now there is a zero tolerance on foods, and any foods that had any measurable toxicity would be subject to seizure. I believe the intent was to move as quickly as possible, but we wanted to alert women who may have liquid formulations around the home, who may be spraying it, that it may present a hazard. We will take appropriate steps to try to warn the female population, particularly of childbearing age.

That is the reason for the more dramatic action in the one instance, and the less dramatic but, I believe nonetheless complete, action, however, nonetheless in others.

I guess I have here a legal phrase; I think for suspension one must show an imminent hazard to health, and this, perhaps, is the reason.

Senator HART. I don't envy you that business of interbalancing.

You describe the judgement that you seek to arrive at as a product of weighing the imminence of danger against the values that are identified as following from the use of the pesticide. As a layman, probably we would tend to oversimplify it.

Now, having admitted this may be an oversimplified impression, why isn't it a more prudent balancing act to say, well, there is danger here because we can't establish that there is no danger and we are not going to get hung up on the degree of imminence of the danger. We are just going to say, to be sure there isn't any danger, we are going to suspend this.

Why aren't you tempted to resolve this balancing operation in that manner?

Dr. STEINFELD. I am not sure I am the one who makes all these decisions of balancing, Senator Hart. My role of course, is concerned with public health and safety. But we are always balancing things.

Certainly in medicine, in picking drugs to use for diseases, sometimes the treatment is worse than the disease. If it should turn out that these materials can be safely used on range and pastureland, that there is a period in which there is biodegradability during which the materials will effectively disappear, and yet permit the person who raises his cattle or dairy cows to have a better—I don't really know the name, I am a city boy, a small-town boy, not a farmer—but better able to have better cows, more milk, better meat, then there are appropriate reasons for using this chemical.

I think the real problem, Senator Hart, is that we do not have an effective, adequate substitute for certain uses. I think this is the key issue.

The other good chemical which kills poison ivy and poison oak is a carcinogen in some animals and not proven for many, but it is a very potent chemical that will destroy poison ivy and poison oak. So there is another balance that one must weigh.

Senator HART. But into that formula you have to th w the sort of economic possibility that if this were suspended, if it just wasn't permitted to be marketed for this purpose, and if there is a need for a cure for the ill that this thing treats, maybe there would be a renewed effort to find a third alternative.

Dr. STEINFELD. I believe the action which has been taken today will lead to more intensive research to find an alternative to 2,4,5-T to destroy the particular kind of herbs it is capable of destroying.

Senator HART. Mr. Bickwit.

Mr. BICKWIT. I am sorry to go over the matter of use on food crops again, but I do want to clear this up so that we know precisely what the situation is. It says in the first paragraph of your press release that liquid formulations of the weed killer 2,4,5-T for use around the home, for registered use on lakes, ponds and ditch banks will be suspended. Do you intend to include within that list of uses, the use on food crops?

Dr. STEINFIELD. I think that the wording for food crops is otherwise. It would be canceled rather than suspended.

Mr. BICKWIT. I am talking about liquid formulation.

Dr. STEINFELD. As I read the actions taken, there will be a cancelation of registered use of nonliquid formulations around the home and on all food crops.

Mr. BICKWIT. That is clear, but what I want to know is what action is proposed with respect to the use of liquid formulations on food crops.

Dr. STEINFELD. My interpretation of this would be—I am not a lawyer but I now see what you are driving at. I think this should have been worded, and we will have to check into it, "liquid and nonliquid formulations around food crops." The intent is not to use the formulation on food crops.

Mr. BICKWIT. So the use of liquid and nonliquid formulations on food crops will be canceled?

Dr. STEINFELD. I cannot speak for the three Cabinet officers. It is my understanding that the intent is not to permit use on any food crop for human consumption.

Mr. BICKWIT. Well, you will permit use on it pending appeals?

Dr. STEINFELD. Pending the legal activities.

Dr. LINDSAY. But there is no permitted residue of 2,4,5-T on any food. It would be subject to seizure.

Mr. BICKWIT. Now, I would like to deal with your statement that an imminent hazard needs to be present before suspension can take place. Is that to say that there is no imminent hazard from the use of 2,4,5-T on food crops?

Dr. STEINFELD. In the studies which have been done, the market basket sampling and the measurement of foods for 2,4,5-T, as I mentioned, it is a very rare instance where these things are found, and in sugar cane the herbicide is probably destroyed in the processing by heat. We do not really know. The action we are taking is based on teratogenicity in mice and the fact that dioxins also cause teratogenicity in rats and perhaps in hamsters. It is a possible hazard.

Mr. BICKWIT. Is that what you need to cancel as opposed to suspend-a possible hazard?

Dr. STEINFELD. I do not know the law that well. I really do not know the exact wording of the law, do you, Dr. Lindsay?

Dr. LINDSAY. No. I am sorry. This is Agriculture's bag, and I do not know it.

Senator HART. Let us order printed in the record at the conclusion of your testimony the appropriate sections of the Federal Insecticide Fungicide and Rodenticide Act.

Dr. STEINFELD. Fine.

Mr. BICKWIT. Have you any information derived from your tests on the degradability of dioxin?

Dr. STEINFELD. Dr. Courtney is a pharmacologist.

Dr. COURTNEY. We have no information on that.

Mr. BICKWIT. In other words, then, it is possible that dioxin is both persistent and accumulative in human beings?

Dr. COURTNEY. That is possible. It is also possible that it can be metabolized.

Dr. STEINFELD. I would like to volunteer something, that is, that the dioxin which produced the results that we will submit for the record is a very potent teratogen for mice in 10,000 to 30,000 times smaller a dosage than 2,4,5-T as we could obtain to pinpoint which chemicals were the villains. And I think it raises another issue, that is, where else in man's environment could these chemicals be found?

We have not shown that these chemicals are teratogenic for man, but we may want to take action. The Food and Drug Administration and Agriculture are presently studying a number of other pesticides in the manufacture of which poly-chlorinated phenols are subjected to heavy temperatures and may produce dioxin. So I think we are having an important study carried out there.

Mr. BICKWIT. Are you looking outside the herbicide area as well?

Dr. STEINFELD. We must look wherever polychlorinated phenols are subjected to high temperatures. We must look for the presence of dioxin and if we find them we shall have to take appropriate action.

Mr. BICKWIT. But the appropriate action is not to find that an imminent hazard exists?

Dr. STEINFELD. I do not know what the appropriate action is. I know we are going ahead with this activity.

Mr. BICKWIT. I take it you do know what the data are with respect to 2,4,5-T and you do know dioxin is present and you do know it is very potent and yet you have concluded it is not an imminent hazard. If it were you would have suspended rather than canceled use.

Dr. STEINFELD. You mean suspended all use everywhere? Is this what you mean?

Mr. BICKWIT. Yes.

Dr. STEINFELD. I think the question of imminent hazard would relate to pregnant women, but we do not know it is teratogenic for man. Use out in rangelands and forests and so forth, I do not see as a hazard to pregnant women.

Mr. BICKWIT. Clearly you have no evidence that it is not.

Dr. STEINFELD. No, I have no evidence that it is not, nor that it is, actually. It is a potential.

Mr. BICKWIT. And when you have no evidence either way you conclude that it is not an imminent hazard?

Dr. STEINFELD. I am tempted to make an analogy, but I probably should not. It is difficult to state that there is no evidence that a number of things are not a hazard to health. I think we are in a never-never land, and where we can, we should try to get as much good hard data as we can and act accordingly.

Mr. BICKWIT. Is there any evidence either way on the accumulativeness of dioxin?

Dr. STEINFELD. I do not think there is any evidence on dioxin. This is a new area which has opened up which we will have to study intensively.

Mr. BICKWIT. Thank you.

Senator HART. I am not sure this will come out as an effective analogy, but think for the moment of the general attitude on pot—marijuana the prevailing view appears to be that since we cannot be sure it is not harmful, it ought not to be used. Is it not correct now that there is at least disagreement as to whether it is harmful or not?

Dr. STEINFELD. I think most physicians, and I am the father of teenagers, feel that pot is harmful.

Senator HART. You cannot be sure it is not harmful. Is not that your parental attitude?

Dr. STEINFELD. I feel it is harmful because it represents an attempt to escape from reality at a time when children must adjust to the outside world and become independent. So I find it harmful as a crutch which particularly the teenagers and those growing up must not use.

Senator HART. Well, you have destroyed my analogy. I was going to pursue it on the assumption that you would agree you cannot be sure it is not harmful. You say you are darn sure it is harmful?

Dr. STEINFELD. Yes, as far as teenage use, I think psychologically it is harmful. I do not think we can be sure of enzyme changes or long-term liver effects, this sort of thing. I do not think is is possible to be sure, but I would say it is harmful.

Senator HART. What if you were unsure, then would you say let us go ahead, although I am not sure? Or would you say do not use it? You say with respect to the pesticides, you balance it and say since we are not sure it is harmful, go ahead?

Dr. STEINFELD. I think we have some evidence in animals that 2,4,5-T is a teratogen and dioxins are present, and while we cannot be certain that women, mankind, behave similary to the mouse, yet pregnant women should not be exposed to this. This is a prudent action.

Mr. BICKWIT. Do you know the date on which the National Cancer Institute received the first progress report raising the possible teratogenic nature of 2,4,5-T in mice?

Dr. STEINFELD. I have with me a chronology regarding 2,4,5-T. It is a few pages, but it is triple spaced. If you would like I could read it to you.

Senator HART. Was that a part of the insert that you presented?

Dr. STEINFELD. We can provide it to you, and if you would like I can read it into the record.

Mr. BICKWIT. We would like it for the record.

Dr. STEINFELD. Maybe it would be useful to go through the chronology. With your permission, I will.

Senator HART. Please.

Dr. STEINFELD. In presenting the following chronology I should take a moment of the Committee's time to commend Dr. Kotin and Dr. Falk for their foresight and initiative in undertaking the studies which were conducted under their guidance by Bionetics Research Laboratories. This commendation extends also to the scientists in the National Cancer Institute who assumed responsibility for successful completion of the study after Drs. Kotin and Falk transferred to the National Institute of Environmental Health Sciences. It consumed large amounts of their time and energy without assurance that the investment would be rewarded. The total cost of this study approximated $3.5 million, and approximately 20,000 animals were studied.

Summer 1963: The National Cancer Institute (National Institutes of Health) awarded a contract to the Bionetics Research Laboratories (Falls Church, Va.) to perform studies of the toxicology, carcinogenicity, teratogenicity, and mutagenicity of pesticides and industrial chemicals which were to be selected by scientists of the National Cancer Institute, according to protocols to be devised by the scientists of the Institute.

During the fall, 1963, the chemistry and toxicology of the chemical compounds to be studied were examined and planning of the large-scale carcinogenicity screening operations was initiated.

Fall and winter 1964: Large-scale screening activities in carcinogenicity were initiated and plans for teratology studies were drawn up.

June 1966: First indication of possible teratogenicity of 2,4,5-T. At a dose of 113 mg/kg of body weight, 2,4,5-T, now recognized as containing substantial concentrations of dioxin impurities, produced an elevated incidence of cystic kidneys in one strain of mice. The 2,4,5-T had been administered by injection.

At that point we did not know whether the results produced by injection were significant. The 2,4,5-T had not been fed.

November of 1966: 2,4,5-T of a similar grade of purity administered by injection at a dose of 133v./kg. body weight was found to be teratogenic in another strain of mice.

The results obtained in June and November 1966, in the absence of information about rates of clearance of injected 2,4,5-T from the blood stream, were regarded as of uncertain significance. This route differs from human exposure and possible differences in metabolism could be very important.

January 1968: Oral administration of 2,4,5-T of similar purity was initiated in mice. The data produced in this study indicated teratogenicity (cystic kidneys and cleft palate).

May 1968: Oral administration of 2,4,5-T of similar purity at a dose of 113 mg./kg. of body weight produced cleft palate in another strain of mice.

September 1968: First draft of the final report of the data on carcinogenicity and teratogenicity was delivered to the National Cancer Institute by the Bionetics Research Laboratories. It should be emphasized that these carcinogenicity data were in an incompletely analyzed state and required scrutiny for possible errors, plus numerous statistical analyses. The first evidence of teratogenicity obtained in rats fed 2,4,5-T was reported.

October 24, 1968: The draft report of the "raw" data mentioned immediately above was provided to Dr. Fitzhugh in the Food and Drug Administration.

October-November-December 1968: Scrutiny of the carcinogenicity data was undertaken by the National Cancer Institute scientists and report writing begun.

January 30, 1969: At a meeting of scientists from the National Institutes of Health with representatives of the regulatory agencies, Consumer Protection and Environmental Health Services, the National Academy of Sciences, and the chemical industry, attended also by Drs. Philippe Shubik and Samuel Epstein, the first two volumes of the final report of data on carcinogenicity, submitted by Bionetics Research Laboratories were made available. In addition a special preliminary report on the teratogenicity of 2,4,5-T, exclusive of data pertaining to the other teratogenicity studies, was provided to all participants in the meeting.

The analyses of the carcinogenicity data had been given priority because of its volume and the apparent potential significance, based upon the indications of the raw data. It had been intended to completely analyze the teratogenicity data immediately following completion of the analysis of the carcinogenicity data.

At the meeting of January 30 a number of uncertainties in the analyses of the carcinogenesis data were pointed up by Drs. Epstein and Shubik and one of the senior scientists in the National Cancer Institute. On this basis, it was decided to withhold publication of the data and findings until additional animal specimens had been examined and certain features of the study design had been reanalyzed. For the same reason, it was decided that a presentation planned for the March 1969 meeting of the Society of Toxicology would be withdrawn from the program.

January–September 1969: Extensive statistical analyses of the teratology data were performed by the National Institute of Environmental Health Sciences.

March 1969: In the course of the appropriations hearings, Dr. Endicott promised to provide the results of the carcinogenicity studies to the Congressional Record just as soon as the analyses could be completed. This was accomplished in the last week of April or the first week of May 1969.

June 1969: The preliminary report of the carcinogenicity findings was made in the Journal of the National Cancer Institute.

June 1969: The Technical Panel on Carcinogenicity for the Secretary's Commission on Pesticides was appointed and included scientists from the National Cancer Institute and the National Institute of Environmental Health Sciences.

June 1969: The intent to name a teratology panel to the Secretary's Commission on Pesticides was made known to the National

ancer Institute liaison member of the Commission. The spo[illegible]neous offer by the Institute's liaison member of the commission to supply the Bionetics data on teratology was declined by a member of the staff of the Commission.

July–September, 1969: Members of the staff of the National Cancer Institute and the National Institute of Environmental Health Sciences actively engaged in the work of the technical panels on carcinogenicity and terabology. Further analyses of the teratogenicity data were performed.

August 15, 1969: Request made by the Teratology Panel for the Bionetics data on teratogenicity.

September 11, 1969: Data on teratogenicity provided to the Teratology Panel. Delay in part related to procedure involved in clearing permission for the data and in part related to putting the data into a condition suitable for examination by those who had not participated in their development.

Fall 1969: FDA studies on embryotoxicity, and teratogenicity of 2,4,5-T and dioxins reinstituted, as described in Dr. Verrett's testi-

November 25, 1969: Meeting of National Institutes of Health scientists with those from FDA and Dow Chemical Co. to plan further studies to clarify roles of 2,4,5-T and dioxin impurities in the production of teratological abnormalities.

November and December 1969: Secretary's Commission reports published.

January 1970: New teratological studies initiated at National Institute of Environmental Health Sciences using materials provided especially for the purpose by Dow Chemical Co.

April 10, 1970: Above teratological studies completed.

April 12, 1970: Analysis of the above data completed.

April 13 and 14, 1970: Interpretations of the above-mentioned findings by representatives of the regulatory agencies and parties to the interagency agreement for protection of the public health and the quality of the environment in relation to pesticides, and presentation of conclusions and proposed actions to members of the Cabinet.

That is a long chronology. I am sorry. I thought it would be shorter.

Senator HART. You have taken the words from me, it is a "long" time after that first bell was sounded before we got this morning's action. I am sure it is always easier to play it from the 20–20 vision of the grandstand up here than from the vantage point of the summer of 1966 when the first bell rang. But that is still a long time.

Dr. STEINFELD. The studies were initiated at a time when this sort of thing was not ordinarily done. As we have more and more chemicals and materials put into our environment we must be more and more careful about the effects they produce.

Senator HART. How can we compress the period between June of 1966 and April 15, 1970, in the future? What mechanism do you now visualize which will avoid this sort of lag from recurring?

Dr. STEINFELD. If the procedures for registration of materials for use on food crops required teratogenicity studies as well as other

long term chronic toxicity studies, as it is my under[illegible]ding that they now do, we may be able to avoid this in the future.

The idea would be to prevent the introduction rather than react some years later, after the material was used, not only ubiquitously but in large quantities. I think this is the direction we must go, to prevent the introduction of materials rather than to react after they are used.

Senator HART. Wouldn't this require the burden of proof to be on those who want a market?

Dr. STEINFELD. Yes.

Senator HART. To make the affirmative case that it is not dangerous. That is correct, isn't it?

Dr. STEINFELD. Yes, I think the thing we really need are good predicting systems for man. I think it would be ideal if we had some in vitro systems which would tell whether a compound is going to be toxic. This is what we need, a lot more research and correlation of animal data with human epidemiologic data. I hope we never do experiments on man but we can collect data in retrospect epidemiologically in individuals who may have been exposed to chemicals or certain diseases and so forth.

Senator HART. Mr. Bickwit?

Mr. BICKWIT. You obviously have done some thinking about how to patch up the system and I don't want to cry unduly over spilt milk, but do you have any idea why, when NCI received this first progress report, that it did not immediately pressure Bionetics to go into an all out effort to acquire further data quickly instead of allowing them approximately 2½ years to complete their tests?

Dr. COURTNEY. The first statement NCI made was "Repeat the study and make sure it is right," and that is just what we did. We went to a different strain of mouse, then we went to a rat. By the time we did all of these studies, it took a bit of time.

Dr. STEINFELD. We were also studying similar chemical pesticide structures, so we could see if it was a larger problem than just this one. This was all going on at the same time.

Mr. BICKWIT. Did the other pesticides that you were studying exhibit the same kind of alarming data?

Dr. COURTNEY. I don't know how you describe it as alarming.

Mr. BICKWIT. Would you not describe it as alarming?

Dr. COURTNEY. Yes. We had some other pesticides that we were concerned with at the time and, of course, without repeated studies we could not make a judgment. So some pesticides were not as alarming and some were more and as we repeated the tests we got our results. This pesticide seemed to give us a positive response every time we studied it.

Dr. STEINFELD. I would say we are not particularly pleased with the fact that it took so long to get all the data out. The first time around in one of these situations always takes longer and hopefully in the future we will be able to move much more rapidly.

Senator HART. I was just thinking of all the things that have happened since that first alarm bell. We have elected two-thirds of the Senate, a new President, gotten further into Vietnam.

Mr. BICKWIT. According to your chronology, if I read it correctly, the data from Bionetics were first made available to FDA on October 24, 1968?

Dr. STEINFELD. Yes, the draft of the raw data was provided to Dr. Fitzhugh on October 24, 1968.

Mr. BICKWIT. Do you believe FDA, one of the government agencies responsible for the regulation of pesticides, should have known about these preliminary indications prior to a time more than 2 years after the data first became available?

Dr. STEINFELD. I think in retrospect we could look at this and speed everything up and inform everyone very quickly. I can't give you the reasons why, (a), the information was not rapidly disseminated as soon as it was confirmed and, (b) why things didn't move much more rapidly and on a larger scale. But I would point out that the material used was heavily contaminated with dioxins. In this interval we have identified the dioxins, and we are moving, I think, on a broad scale to try to find out where else dioxins may be found. I am not trying to look for a silver lining in a dark cloud but I do think we have a lot better data and a lot more information as to just what did the job; it probably was the concentration of the dioxins used in the Bionetics experiments which was responsible for the teratogenicity.

Mr. BICKWIT. Then you do regard this as a dark cloud?

Dr. STEINFELD. I would say the darkest part is that, whatever the rules were, we permitted the utilization of the material without testing for what may be a significant hazard to man, teratogenicity.

Senator HART. Doctor, I commented earlier on the fact that no witnesses are scheduled today from the Department of Agriculture. My interest at that time bore on the action, if any, that would be taken to remove from retail channels and from shelves at home, perhaps, this product as a result of the announcement that you gave us today.

The Secretary of Agriculture participated with Secretary Finch and Secretary Hickel in this announcement suspending or canceling 2,4,5-T. I am reminded and I must confess my own memory of this testimony is not clear, but it has been suggested to me that when witnesses speaking for the Deparment of Agriculture testified before this subcommittee last week, they took the position that the evidence did not warrant an action such as is taken today.

I won't say that they promoted or advocated its use, but—Mr. Bickwit, have you found any passage that bears on this?

Mr. BICKWIT. Yes.

Senator HART. From the transcript this sentence is cited. This is from a Department of Agriculture witness who addressed us on the seventh of this month.

> In view of all the information now available, we have not found that registered use of 2,4,5-T without a finite tolerance on food crops warrants a suspension or cancellation of such registered use.

Now, that testimony is April 7. You say that on April 13 the analysis which had been completed 2 days before were presented for proposed action. Whatever else you can say about it, it points up again the fact that on April 7, notwithstanding the patterns beginning in June of 1966, indicating possible serious danger, this one Department was still telling us, on the record, what I just read you.

Dr. STEINFELD. I would have agreed with that position last week. I was surprised to see the data that developed over the weekend. It

appeared to me it was the dioxin that was the likely villain in this piece, not the 2,4,5-T; the particular batch of the 2,4,5-T used in the experiments was heavily contaminated with dioxin. Our goal was to pin down the fact that it was dioxin and probably not 2,4,5-T which was the teratogen and get rid of dioxins wherever they are found.

So I think last week I would have said the same thing, Senator Hart. The data over the weekend have changed the picture completely

Senator HART. Yes; that will be made part of the record.

Well, then we all wind up saying it is a darn shame this past weekend had to be the first time when you got the solid information, which information was a result of an alarm bell that rang in June of 1966.

We all agree on that.

Do you anticipate that the centralized clearinghouse which you made reference to in your prepared testimony can assure that this kind of timelag no longer will occur?

Dr. STEINFELD. I hope that that will help. Our other attempts at coordinating activities with regard to pesticides will also help. The Secretary has a special commission: we have an interagency group of Agriculture, Interior, and HEW; we have Dr. Russell Train, Environmental Quality Council; I hope all of these will help us avoid problems such as we are facing today.

Senator HART. I would ask our staff to obtain for the record the announcement which you anticipate the Department of Defense is about to make. You did indicate that they were——

Dr. STEINFELD. Idon't know if they will make an announcement If is my understanding that this is an action that Deputy Defense Secretary Packard has initiated this morning.

Senator HART. If there is any announcement in connection with this, let it be a part of the record. I understand there is a big departmental request outstanding for a major purchase order for 2,4,5-T. I would like to find out whether that contract request now will be withdrawn in light of Deputy Defense Secretary Packard's position. I would assume it would. But let us make it a matter of record.

Is there anything any of you would care to add, given the exchange we have had this morning?

Dr. STEINFELD. I would add one final statement. We used inbred strains of animals and large doses of compounds in order to try to find a particular phenomenon. The problem is that man is not inbred; we don't breed brothers and sisters and so we can't predict. We have a tremendous variation among people in this country; some people may have missing enzymes of a particular type that may make a chemical extremely hazardous at a very low dose.

We have taken actions because we muct act prudently. We don't want to alarm the public, but we do want to react prudently and protect the public health.

Senator HART. Amen.

Thank you very much, gentlemen.

Dr. STEINFELD. Thank you.

(The information referred to earlier follows:)

REGISTRATION

Sec. 4.a. Every economic poison which is distributed, sold, or offered for sale in any Territory or the District of Columbia, or which is shipped or delivered for shipment from any State, Territory, or the District of Columbia to any other State, Territory, or the District of Columbia, or which is received from any foreign country shall be registered with the Secretary: Provided, That products which have the same formula, are manufactured by the same person, the labeling of which contains the same claims, and the labels of which bear a designation identifying the product as the same economic poison may be registered as a single economic poison; and additional names and labels shall be added by supplement statements; the applicant for registration shall file with the Secretary a statement including--

(1) the name and address of the registrant and the name and address of the person whose name will appear on the label, if other than the registrant;
(2) the name of the economic poison;
(3) a complete copy of the labeling accompanying the economic poison and a statement of all claims to be made for it, including the directions for use; and
(4) if requested by the Secretary, a full description of the tests made and the results thereof upon which the claims are based

b. The Secretary, whenever he deems it necessary for the effective administration of this Act, may require the submission of the complete formula of the economic poison. If it appears to the Secretary that the composition of the article is such as to warrant the proposed claims for it and if the article and its labeling and other material required to be submitted comply with the requirements of section 3 of this Act, he shall register it.

c. If it does not appear to the Secretary that the article is such as to warrant the proposed claims for it or if the article and its labeling and other material required to be submitted do not comply with the provisions of this Act, he shall notify the applicant for registration of the manner in which the article, labeling or other material required to be submitted fail to comply with the Act so as to afford the applicant for registration an opportunity to make the corrections necessary. If, upon receipt of such notice, the applicant for registration does not make the corrections, the Secretary shall refuse to register the article. The Secretary, in accordance with the procedures specified herein, may suspend or cancel the registration of an economic poison whenever it does not appear that the article or its labeling or other material required to be submitted complies with the provisions of this Act. Whenever, the Secretary refuses registration of an economic poison or determines that registration of an economic poison should be cancelled, he shall notify the applicant for registration or the registrant of his action and the reasons therefor. Whenever an application for registration is refused, the applicant, within thirty days after service of notice of such refusal, may file a petition requesting that the matter be referred to an advisory committee or file objections and request a public hearing in accordance with this section. A cancellation of registration shall be effective thirty days after service of the foregoing notice unless within such time the registrant (1) makes the necessary corrections; (2) files a petition requesting that the matter be referred to an advisory committee; or (3)

files objections and requests a public hearing. Each advisory committee shall be composed of experts, qualified in the subject matter and of adequately diversified professional background selected by the National Academy of Sciences and shall include one or more representatives from land-grant colleges. The size of the committee shall be determined by the Secretary. Members of an advisory committee shall receive as compensation for their services a reasonable per diem, which the Secretary shall by rules and regulations prescribe, for time actually spent in the work of the committee, and shall in addition be reimbursed for their necessary traveling and subsistence expenses while so serving away from their places of residence, all of which costs may be assessed against the petitioner, unless the committee shall recommend in favor of the petitioner or unless the matter was referred to the advisory committee by the Secretary. The members shall not be subject to any other provisions of law regarding the appointment and compensation of employees of the United States. The Secretary shall furnish the committee with adequate clerical and other assistance, and shall by rules and regulations prescribe the procedures to be followed by the committee. The Secretary shall forthwith submit to such committee the application for registration of the article and all relevant data before him. The petitioner, as well as representatives of the United States Department of Agriculture, shall have the right to consult with the advisory committee. As soon as practicable after any such submission, but not later than sixty days thereafter, unless extended by the Secretary for an additional sixty days, the committee shall, after independent study of the data submitted by the Secretary and all other pertinent information available to it, submit a report and recommendation to the Secretary as to the registration of the article, together with all underlying data and a statement of the reasons or basis for the recommendations. After due consideration of the views of the committee and all other data before him, the Secretary shall, within ninety days after receipt of the report and recommendations of the advisory committee, make his determination and issue an order, with findings of fact, with respect

to registration of the article and notify the applicant for registration or registrant. The applicant for registration, or registrant, may, within sixty days from the date of the order of the Secretary, file objections thereto and request a public hearing thereon. In the event a hearing is requested, the Secretary shall, after due notice, hold such public hearing for the purpose of receiving evidence relevant and material to the issues raised by such objections. Any report, recommendations, underlying data, and reasons certified to the Secretary by an advisory committee shall be made a part of the record of the hearing, if relevant and material, subject to the provisions of section 7(c) of the Administrative Procedure Act (5 U.S.C. 1006(c)). The National Academy of Sciences shall designate a member of the advisory committee to appear and testify at any such hearing with respect to the report and recommendations of such committee upon request of the Secretary, the petitioner, or the officer conducting the hearing: Provided, That this shall not preclude any other member of the advisory committee from appearing and testifying at such hearing. As soon as practicable after completion of the hearing, but not later than ninety days, the Secretary shall evaluate the data and reports before him, act upon such objections and issue an order granting, denying, or cancelling the registration or requiring modification of the claims or the labeling. Such order shall be based only on substantial evidence of record of such hearing, including any report, recommendations, underlying data, and reason certified to the Secretary by an advisory committee, and shall set forth detailed findings of fact upon which the order is based. In connection with consideration of any registration or application for registration under this section, the Secretary may consult with any other Federal agency or with an advisory committee appointed as herein provided. Notwithstanding the provisions of section 3.c. (4), information relative to formulas of products acquired by authority of this section may be revealed, when necessary under this section, to an advisory committee, or to any Federal agency consulted, or at a public hearing, or in findings of fact issued by the Secretary. All data submitted to an advisory committee

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In support of a petition under this section shall be considered confidential by such advisory committee: Provided, That this provision shall not be construed as prohibiting the use of such data by the committee in connection with its consultation with the petitioner or representatives of the United States Department of Agriculture, as provided for herein, and in connection with its report and recommendations to the Secretary. Notwithstanding any other provision of this section, the Secretary may, when he finds that such action is necessary to prevent an imminent hazard to the public, by order, suspend the registration of an economic poison immediately. In such case, he shall give the registrant prompt notice of such action and afford the registrant the opportunity to have the matter submitted to an advisory committee and for an expedited hearing under this section. Final orders of the Secretary under this section shall be subject to judicial review, in accordance with the provisions of subsection d. In no event shall registration of an article be construed as a defense for the commission of any offense prohibited under section 3 of this Act.

d. In a case of actual controversy as to the validity of any order under this section, any person who will be adversely affected by such order may obtain judicial review by filing in the United States court of appeals for the circuit wherein such person resides or has his principal place of business, or in the United States Court of Appeals for the District of Columbia Circuit, within sixty days after the entry of such order, a petition praying that the order be set aside in whole or in part. A copy of the petition shall be forthwith transmitted by the clerk of the court to the Secretary, or any officer designated by him for that purpose, and thereupon the Secretary shall file in the court the record of the proceedings on which he based his order, as provided in section 2112 of title 28, United States Code. Upon the filing of such petition the court shall have exclusive jurisdiction to affirm or set aside the order complained of in whole or in part. The findings of the Secretary with respect to questions of fact shall be sustained if supported by substantial evidence when

considered on the record as a whole, including any report and recommendation of an advisory committee. If application is made to the court for leave to adduce additional evidence, the court may order such additional evidence to be taken before the Secretary, and to be adduced upon the hearing in such manner and upon such terms and conditions as to the court may seem proper, if such evidence is material and there were reasonable grounds for failure to adduce such evidence in the proceedings below. The Secretary may modify his findings as to the facts and order by reason of the additional evidence so taken, and shall file with the court such modified findings and order. The judgment of the court affirming or setting aside, in whole or in part, any order under this section shall be final, subject to review by the Supreme Court of the United States upon certiorari or certification as provided in section 1254 of title 18 of the United States Code. The commencement of proceedings under this section shall not, unless specifically ordered by the court to the contrary, operate as a stay of an order. The court shall advance on the docket and expedite the disposition of all causes filed therein pursuant to this section.

e. Notwithstanding any other provision of this Act, registration is not required in the case of an economic poison shipped from one plant to another plant operated by the same person and used solely at such plant as a constituent part to make an economic poison which is registered under this Act.

f. The Secretary is authorized to cancel the registration of any economic poison at the end of a period of five years following the registration of such economic poison or at the end of any five-year period thereafter, unless the registrant, prior to the expiration of each such five-year period, requests in accordance with regulations issued by the Secretary that such registration be continued in effect.

Senator HART. Our next witness, as the Surgeon Gen[illegible] has already indicated, is Dr. Jacqueline Verrett.

STATEMENT OF DR. JACQUELINE VERRETT, FOOD AND DRUG ADMINISTRATION, DEPARTMENT OF HEW

Dr. VERRETT. Thank you, Mr. Chairman, for this opportunity to discuss our investigations of the relationships between chlorinated phenoxy herbicides, chlorinated dibenzo-p-dioxins, and the chick edema factors.

Chick edema disease was first recognized in 1957, when large numbers of broiler flocks in the United States suffered what appeared to be an epidemic disease. The affected birds appeared droopy, with ruffled feathers, and had difficulty breathing. In many flocks, more than 50 percent of the birds died as a result of the disease. Of the millions of birds affected, those autopsied consistently displayed hydropericardium (accumulation of fluid in the pericardial sac), accumulation of fluid in the abdominal cavity, subcutaneous edema, and additionally liver and kidney damage.

In 1958 the investigations of a number of laboratories indicated that the causal agent was contained in fats, and specifically in the unsaponifiable fraction of fats in the commercial poultry rations. In laying hens the toxic fat caused a rapid drop in egg production. Pullets receiving toxic fat during the full growing period did not come into production, and mortality was very high. Hydropericardium, the most common lesion found in young birds, was not found in birds of laying age.

The chick edema factor found in the toxic fat in the 1957 outbreak was presumed to have arisen as a byproduct of industrial production of stearic and oleic acids, since the unsaponifiable materials from this process were the components of fat in the poultry ration. Subsequently, the toxic substance was found to be present in several different types of fats. It was demonstrated to be present in samples of commercially produced oleic acids and triolein, in acidulated vegetable oils, and in inedible animal tallows. The demonstration of the presence of the chick edema factor in commercial fats led to the ruling by the Food and Drug Administration in 1961 that higher fatty acids intended for food additive use must be free of the chick edema factors. The presence of the factor was to be ascertained by a chick bioassay based on the volume of pericardial fluid in birds fed the fat under investigation.

Beginning in 1958, fat that had first been proved to be toxic to chicks was used by various investigators in experiments with other species, and was demonstrated also to produce deleterious effects in rats, mice, turkeys, pigeons, guinea pigs, swine, dogs, and monkeys.

Early investigations of feeding toxic fats to rats indicated that they are more resistant than chicks in short term feedings, but when fed in sufficient dosage, extracts of the toxic fat produced definite deleterious effects as shown by growth depression, enlarged and fatty livers, marked involution of the thymus, and enlarged adrenals.

Guinea pigs fed 2½ percent toxic fat stopped growing at 6 weeks, and death losses occurred at 8 weeks. At a level of 4½ percent the

weight losses occurred after 3 weeks, and death [illegible]4 weeks. The pathology observed was congestion of the lungs and mottled livers.

Dogs fed 10 percent toxic fat in their rations lost hair on their backs and shoulders (alopecia), and there was poor reproduction and lactation performance. Whelped pups were either dead or weak, and the mothers seemed to have an insufficient milk supply. Pups removed before weaning and fed a normal ration showed an immediate and dramatic increase in growth. Other litters maintained on the toxic fat ration postweaning demonstrated inferior growth performance.

Monkeys have demonstrated considerable sensitivity to toxic fat materials. In one study nine monkeys received a toxic triolein at a level of 25 percent in their diets. One monkey died at 1 month, and four died at 3 months. At the 3-month period, corn oil was substituted for the toxic triolein, but the other four monkeys died from 3 weeks to 5 weeks later, in spite of this substitution. Of the nine monkeys fed the toxic triolein, eight were autopsied and showed signs of jaundice, pancreatic atrophy, and fibrosis, hemosiderosis, fatty liver with necrosis, bile duct proliferation, and gross hemorrhage in the intestinal tract. No such pathology was seen in the control monkeys in this study.

A second study with 36 monkeys given a toxic fat at levels from 0.125 to 10 percent of their diet, demonstrated an inverse relationship between the concentration of the toxic fat in the diet and their mean survival time. Those given the highest level (10 percent) had a mean survival time of only 91 days, while those given the lowest level (0.125 percent) had a mean survival time of 445 days. It has been estimated that the highest level provided approximately 728 micrograms total chick edema factors, while the lowest level diet provided approximately 100 micrograms total intake. The toxic fat was lethal at all levels studied, and the animals were sacrificed when possible just before death. During the last 30 days of life, all monkeys developed alopecia, generalized subcutaneous edema, accumulation of fluid in the abdominal and thoracic cavities, and hydropericardium. There were decreases in red and white blood cell counts, total serum protein values, and altered serum-protein ratios. There was also cardiac dilatation and myocardial hypertrophy and edema. Finally, the experimental monkeys had reduced hematopoiesis and spermatogenesis, degeneration of the blood vessels, focal necrosis of the liver and gastric ulcers.

Limited experimentation with mice, pigeons, and turkeys, indicated that toxic fat in the diet led to reduction in growth without hydropericardium or accumulation of abdominal fluid. Similarly, swine, fed toxic fat at a level of 9 percent of their ration, showed poor weight gain, but one pig sacrificed 6 weeks after the start of the study showed no gross or microscopic lesions attributable to the ration.

One important finding in the studies with chickens, is the apparent storage of the chick edema factors in chick tissues. The unsaponifiable fraction of carcasses (exclusive of intestines, head, and feet) of chickens fed the toxic fat was very potent in producing hydropericardium in other birds when incorporated in their rations. Other investigations of the distribution of the chick edema factors in the

chick tissues indicated significant levels in bone, heart, intestine, kidney, liver, and skin. The liver contained more than 80 percent of the total detected. A similar determination of the distribution in rats indicated the presence of chick edema factors only in liver and in the feces.

During the years that the previously described toxicity investigations were taking place, the toxic fats were undergoing intensive chemical analyses to concentrate, purify, and finally determine the nature of the compounds responsible for chick edema disease. At all steps of these procedures, the path of the toxic material was confirmed by assay in young chicks. This proved to be a time-consuming and difficult job because of the complexity of the fatty materials. A major breakthrough in this effort came when it was found that a highly purified crystalline material possessing the properties of chick edema factor contained chlorine. This indicated that it was not a natural component of the fat in which it occurred.

Work in several laboratories obtained similar results, and examination of the purified material by a variety of analytical techniques suggested that chick edema factors could be highly substituted (chlorinated) derivatives of naphthalene, biphenyl, anthracene, or even structures common to the chlorinated pesticides of the DDT family. These latter compounds were ruled out when tested in the chick feeding assay, but some derivatives of the former classes of compounds were tested and found in some instances to be toxic, and indeed produce similar lesions to those observed with authentic toxic fat. However, none of these compounds demonstrated the high order of toxicity, or the complete chick edema syndrome when so tested. Finally, by means of single crystal X-ray crystallography, it was demonstrated that a pure compound isolated from a toxic fat was a hexachloro-dibenzo-p-dioxin. This structure was verified by infrared, ultraviolet, and mass spectrometry data. Final confirmation came when this particular compound was synthesized and found to produce the same lesions in chicks as the compound isolated from the toxic fat.

The finding that a chlorinated dibenzo-p-dioxin was a chick edema factor explained why different investigators had isolated materials similar in their capacity to elicit chick edema disease, but yet in their purest forms, had slightly different chemical properties. The large number of isomers possible (more than 60) in this family ranging from mono- to octa-chloro-dibenzo-p-dioxins illustrates the complexity of the problem. It then became a problem of determining whether some or all of these compounds are in fact chick edema factors, and what their relative capacities in this regard might be.

The chlorinated dibenzo-p-dioxin structures have been known in organic chemistry many years, and became particularly noteworthy, when in manufacturing processes with chlorophenols, their formation as byproducts posed serious occupational hazards. The most potent in this regard seems to have been the symmetrical tetrachloro-p-dioxin which was formed in the manufacture of 2,4,5-trichlorophenol. These chlorinated compounds were found to cause a serious and persistent disease referred to as chloracne. This disease was first described in 1899. Associations of this disease with chlorinated dibenzo-p-dioxins were made by the Germans, who had several outbreaks of this disease in their factories. There have also been similar occurrences in the Netherlands and in this country, in factories manufacturing chlorophenol compounds. It should also be pointed out, that other compounds, such as the chlorinated naphthalenes, anthracenes, biphenyls and dibenzofurans, are known to be acnegenic, but as in the case of the toxic response in chicks, these materials are less potent than the chlorinated dibenzo-p-dioxins. In the case of the chloracne associated with dioxin, the human symptomatology extends to other mucous membrane irritation, porphyria cutanea tarda, hirsutism, hyperpigmentation, increased skin fragility, severe damage to the internal organs, particularly hepatotoxicity, and central nervous system disorders, as indicated by neuromuscular symptoms and psychologic alterations, and other systemic symptoms. Most of these occupational exposures in Germany occurred in the 1950's, and followup examination of these affected workers in recent years indicate that the recovery period is lengthy, with many workers still having demonstrable adverse effects from prior exposure. Similar observations have been made on exposed workers in the United States of America. The tetrachlorodibenzo-p-dioxin was demonstrated to produce the chloracne in humans after the application of only 20 micrograms. The rabbit ear is especially sensitive, with concentration of 0.001 percent to 0.005 percent producing severe reactions after local application. This assay using the rabbit ear is apparently used as an indicator in some plants of the content of this particular dioxin in the manufacturing process. Hence, the serious health significance of these compounds for humans has, inadvertently, been clearly documented.

Research in Germany and Japan indicated that the magnitude of this problem was indeed large, since the formation of the chlorinated di-benzo-p-dioxins would be facilitated in the saponification procedures used in various processes involving chlorophenols. A further complication is that a given chlorophenol preparation is generally contaminated with other isomers, increasing the possibility of formation of a wide spectrum of chlorinated di-benzo-p-dioxins beyond those to be expected from the predominant component. Evidence that this does occur will be discussed shortly in connection with the chicken embryo studies of these materials.

During the time the previously described investigations of the chick edema factors were underway, many of which were carried out by FDA investigators, methodology was developed for detecting the chick edema factors using sensitive gas-liquid-chromatographic (GLC) techniques. It became apparent that authentic toxic fats consistently gave peaks with specific retention times, and these peaks were used as an indication of chick edema factor in a suspect sample. Confirmation of this was obtained using the chick feeding assay. In the light of recent knowledge of the chlorodioxins as chick edema factors, it has been possible to establish that the materials being detected were hexa-, hepta-, and octa-chlorodibenzo-p-dioxins. Although toxic fat samples did indeed contain peaks corresponding to dioxins of lesser chlorine content, i.e., di-, tri-, tetra-, penta-, these are not detectable with this particular analytical procedure because their particular peaks are obscured by other components,

including pesticide residues present in the samples. Other GLC procedures are being developed to detect these latter dioxins.

In the early 1960's the chicken embryo was being used in toxicological evaluations of a wide variety of materials. It was hoped to develop a rapid and sensitive screening system to pinpoint compounds with significant toxic and teratogenic effects for further study. In view of the demonstrated sensitivity of the chicken to chick edema factors, the chicken embryo was used to assay toxic fat samples, and found to present the same syndrome as observed in the chick feeding assay. A high mortality was observed with toxic fat extracts, and additionally, hydropericardium, generalized and massive edema, eye, beak, and leg defects, and necrotic livers were apparent on gross observation. No microscopic studies have been conducted on embryos or hatched chicks in these investigations.

In parallel with other investigations, the chicken embryo was used to test the toxicity of the chick edema factors isolated from toxic fats. It was also found that the chlorinated biphenyls, naphthalenes, anthracenes, and other compounds did indeed elicit a toxic response, and in some instances, the chick edema syndrome was present. But in no case were any of these materials as potent as the toxic components isolated from toxic fats, and were generally less potent by a few orders of magnitude.

After the identification in 1966 of a hexachloro dibenzo-p-dioxin as a chick edema factor, studies were initiated in which various isomers of chlorinated dibenzo-p-dioxin were prepared and tested in the embryos. Although the investigation was not extensive or complete, it illustrated that isomers prepared by pyrolyzing selected chlorophenols did give chloro-dibenzo-p-dioxins with GLC retention times duplicating those in the authentic toxic fats, and likewise, produced the chick edema syndrome in the treated embryos. It was also apparent from this study that the various isomers (that is, those with different chlorine content, and those with identical chlorine content, but with chlorine atoms positioned differently on the molecule) varied in their toxicity, although in all cases only microgram or less quantities were required to elicit the toxic response. It is not possible to give exact figures for the toxicities obtained in this study, since most of the individual dioxins were contaminated with traces of others. Nevertheless, it was apparent that the symmetrical chlorodioxin prepared from 2,4,5-trichlorophenol (2,3,6,7-tetrachloro dibenzo-p-dioxin) was more potent than any of the others tested, even recognizing its lack of purity.

During this investigation, samples of the chlorophenols, both technical and reagent grades, were examined by GLC to determine if preformed chloro-dioxins were present. The presence of chlorodioxins was demonstrated by GLC, and these materials, which can be removed by appropriate techniques, were then tested in the chicken embryo system and did indeed produce chick edema. A current study of similarly contaminated chlorophenols, containing from 18 ppb to 95 ppm of chloro-dioxins with six or more chlorine atoms are currently under test.

This investigation was not pursued further in view of the fact that there had been no known occurrences of chick edema disease since the late 1950s and, hence, such research had a low order of priority.

However, in early 1969 there was another large outbreak of the disease in North Carolina and the toxic factor was traced to the fat component of the feed. The toxicity was confirmed by the chicken embryo test with fractions of the hexa-, hepta-, and octa-chlorodibenzo-p-dioxins from the crude fat.

An investigation of the processing plant in which the toxic vegetable oil products were produced revealed a proximate operation for the manufacture of chlorophenol formulations. However, it is still not possible to conclude that this accounted solely for the presence of dioxins in the fat, or whether they were at least to some extent in crude oil from a prior contamination.

Since that time FDA has initiated an investigation of the oils of other manufacturers and processors and in a few cases GLC analysis has indicated the presence of chloro-dioxins. These have not as yet been confirmed by chicken embryo bioassay; however, it is noteworthy that in the case of these subject samples there is no known adjacent manufacturing operation that would give rise to direct chloro-dioxin or chlorophenol contamination, so that entry of the chloro-dioxins from other sources must be considered.

This 1969 outbreak of chick edema disease, coupled with the question of contamination of the herbicide 2,4,5-T by chlorinated dioxins led us to renew this investigation. In the past 6 months the herbicides 2,4-D and 2,4,5-T, as well as the particular tetrachlorodioxin purported to be the teratogenic agent responsible for the effects in the Bionetics study of 2,4,5-T have been under study.

In an effort to assess the edema-producing capacity, the teratogenic activity, and the acute toxicity of these materials, samples of the original Bionetics 2,4,5-T, from Diamond-Alkali, were obtained for comparison with a sample representative of the current manufacture of Dow Chemical Co.

The Bionetics 2,4,5-T is reported to contain 27 plus or minus 8 parts per million of the 2,3,6,7-tetrachloro dibenzo-p-dioxin, with the content of other dioxins unknown. The current production of Dow 2,4,5-T has 0.5 parts per million of this dioxin, with no analysis for higher chloro-dioxins reported, but does contain almost 5 percent of other impurities, mostly isomers of 2,4,-D 2,4,5-T, and chlorophenols and chlorophenoxy compounds of undetermined structure.

All investigations using the chicken embryo involved administration of the compounds by injection through the air cell of the egg, either preincubation or at the 4th day of incubation.

A comparison of the Bionetics 2,4,5-T with the Dow 2,4,5-T indicates that with respect to the ability of the materials to produce embryonic mortality, the Bionetics 2,4,5-T is more potent. The Bionetics 2,4,5-T has an LD_{50}—that is, kills 50 percent of the treated embryos—of approximately 25 micrograms per egg, 0.5 parts per million, while the Dow 2,4,5-T LD_{50} is approximately 100 micrograms per egg, 2 parts per million.

With respect to teratogenic effects, both samples produce chick edema syndrome in the nonviable embryos, and hatched chicks, including eye defects, beak defects—predominantly cleft palate—short and twisted feet—the result of tendon slippage—and diffuse and localized edema in various parts of the body.

With both of these samples of 2,4,5-T these teratogenic effects are observed at levels inducing no significant embryonic mortality.

The Dow 2,4,5-T still produces the chick edema syndrome at 50 micrograms, one part per million, a level where only 12 percent mortality is observed, while the Bionetics sample has similar effects as low as 6.25 micrograms per egg, 0.125 parts per million, a level inducing only 16 percent mortality. Both of these mortalities are close to that induced by the solvent alone.

It should also be emphasized that the chick edema syndrome is not observed in the embryos treated with the solvents only, at any level, or in the control flock.

A sample of 2,4,5-T from a chemical supply company was subjected to three recrystallizations before test. With the present GLC techniques no chlorodioxins are detectable in this purified sample. When tested in embryos it produced chick edema syndrome at 5, 10, and 25 parts per million, all levels which induced no more than 15 percent mortality in the embryos.

This same sample was subjected to an additional purification by seven extractions to remove dioxins that might have been present, but were below the current detection levels.

This repurified sample is still clearly teratogenic in the embryos since when tested at a level of 2.5 parts per million it produced 20 percent incidence of the malformations previously described, though no significant edema was seen grossly. The mortality induced was 24 percent, which is higher than that of the sample prior to the extensive extraction procedure.

It is also noteworthy that the embryonic mortality occurred soon after treatment, and the hatched chicks had bleached down, indicative of an aberration in the normal pigment formation.

With respect to the 2,3,6,7-tetrachlorodibenzo-p-dioxin, early investigations of this compound in a preparation containing some 2,3,7-trichlorodibenzo-p-dioxin indicate a high order of toxicity and teratogenicity.

Whether prepared by pyrolysis of 2,4,5-trichlorophenol, or direct chlorination of dibenzo-p-dioxin, the test preparations, which contained approximately 50 to 55 percent of the tetrachlorodioxin and 20 to 25 percent of the trichlorodioxin, produced significant mortality, that is greater than 20 percent, and chick edema syndrome in more than 40 percent of the treated embryos at levels of five ten-millionths of a milligram per egg, or 10 parts per trillion.

More recent investigations, with two samples of the tetrachlorodioxin, both of purity greater than 95 percent, indicate edema and terata at 20 parts per trillion. These samples have only become available within the past month and additional testing is underway at lower and higher levels.

It should also be mentioned that the herbicide 2,4-D as a commercially available sample, and a purified sample, a mixture of the N-butyl esters of 2,4-D and 2,4,5-T, and a sample of silvex, a related herbicide, have been tested.

Terata and chick edema syndrome have been observed with all of these materials at levels of 10 parts per million and above. Lower levels are under investigation and the levels of dioxins in these samples are also being determined.

Studies have recently been initiated in the FDA using pregnant golden hamsters, intubated on day 6 through 10 of organogenesis with the test compounds.

The Dow 2,4,5-T, 0.5 parts per million tetrachlorodioxin, tested at 100 m./k. yielded about 80 percent fetal deaths and those pups born alive had gastrointestinal hemorrhages.

The thrice-recrystallized 2,4,5-T sample referred to earlier, with no detectable chlorodioxins, when tested at a level of 100 m./k. produced an average fetal mortality of 55 percent.

Among 38 live pups, three abnormals were found: One with a deformed hind limb and two with inadequate fusion of the skull.

At lower doses the fetal mortality was less, but still higher than that observed in control hamsters.

When the extensively repurified sample of 2,4,5-T was tested in hamsters no gross terata were observed at 100 m./k., but the number of early fetal deaths was 70 percent, indicating a definite embryotoxic effect and corroborating the observation of increased mortality in the chick embryo studies. Additional tests with this compound are underway.

A dioxin preparation containing approximately 51 percent 2,3,6,7-tetrachloro- and 21 percent 2,3,6 trichlorodibenzo-p-dioxin yielded 98 percent fetal deaths at 9.1 micrograms per kilogram. Gastrointestinal hemorrhages and eye anomalies—absence of lid—were present in many of the pups.

Tests with the purer tetrachlorodibenzo-p-dioxins are underway.

The numbers of animals in the hamster tests are too small to be considered statistically valid, but there are definite indications that alterations in fetal viability and gastrointestinal hemorrhages do occur at the levels tested.

In summary, the chick embryo studies and additionally the preliminary hamster data indicate that the current production 2,4,5-T containing 0.5 parts per million of the 2,3,6,7-tetrachlorodibenzo-p-dioxin is teratogenic and embryotoxic in these test systems.

Further, an extensively purified 2,4,5-T sample, with no chlorodioxins detectable with the present techniques, has indicated significant embryotoxicity in the hamster and chick embryo, and additionally produced gross terata in the chicken embryos, making it impossible at this point in time to exonerate it of teratogenic or other adverse effects on the embryos that may have some health significance.

The data for 2,4-D in chick embryos likewise demonstrate these effects in current production materials.

These studies have in no way assessed another and perhaps more complicated aspect of this problem, and that is the interactions of the various chlorodioxin isomers with each other in the many combinations in which they are likely to occur, or the possible interactions, including potentiation or synergism, between the chlorodioxins and the chlorophenols, herbicides and other materials in which they are found.

At this point, with your permission, Senator Hart, I would like to insert in the record the documents containing the data from which this testimony was derived.

Thank you. That is the end of my prepared statement.

Senator HART. Yes, they will be received and placed in the record after your oral testimony

Dr. VERRETT. I realize I presented a rather lengthy and complicated piece. I will be happy to answer questions and elaborate if you wish.

I also have some samples of embryos, if you would care to see them, or chicks.

Senator HART. Yes, I heard about them.

The statement is not an easy one for one not trained in your discipline.

I take it, Doctor, these are some of the chicks that have the deformities that you are talking about?

Dr. VERRETT. That is right. These are chicks that have been treated with the tetrachlorodibenzo-dioxin. I would like to put them out on the table but it is not safe.

As you can see, they cannot stand up. These chicks hatched early this week. They have noticeable edema. This is really the ankle, if you wish, of the chicken and this is really a result of the malformation.

These are normal chicks; as you can see, they have no difficulty standing and walking.

These are chicks that have been treated with the ethanol, the solvent alone or have been untreated. We always run some at the same time that have had no treatment and I think the comparison between that one and this one, if you wish, is quite obvious.

Senator HART. Your last statement referred to a series of compounds.

Dr. VERRETT. Yes.

Senator HART. Exactly what are these?

Dr. VERRETT. It is a family of compounds which I may say very briefly is a series of phenols having a varying chlorine content. They go from dichlorophenol, which is the precursor or the compound from which 2,4-D is made, all the way up to pentachlorophenol. All of these materials are very widely used as herbicides. They also have a very broad use in industry and, in other words, are capable of being in the environment and if they are contaminated with dioxins, of also putting dioxins in the environment.

What I was trying to say is that we have looked at these chlorophenols, apart from just the herbicides which are derived from them, and we know from past study, and there is a paper in the material that I have included in the record which demonstrates, that these materials are already contaminated with dioxin. The amount of the contamination at this point is——

Senator HART. It is very difficult to hear the witness. I do not know what the distraction is, but please be patient.

Dr. VERRETT. We do know, at least by GLC (gas-liquid-chromatography) just chemical techniques—I will distinguish between chemical and biological techniques—by chemical analysis, we know these materials are contaminated with chlorodioxins.

In addition to that, we know there are many of these chlorinated dioxins that are involved and not simply the single one which has been the subject of the 2,4,5-T. There are approximately 60 or more.

We are aware from our previous work that these materials are already in chlorophenols as they are being used. This is the point I was trying to make.

Senator HART. The point I was trying to make by raising it is the necessity that there be no more delay with respect to establishing the dangers, the hazards, the potentials for harm in this whole variety, this whole family of products, given the kind of damage that you have so dramatically demonstrated here from one single element of the environment.

Dr. VERRETT. Yes. I might say—of course I could not bring very many—these are chicks treated with the tetradioxin in question, which is the most pertinent to the hearing. There are some here which have been treated with 2,4-D. Thes are 2,4,5-T treated chicks. We do have underway an investigation of all the other dioxins. It is a question of having to synthesize these materials and test them in pure form. But by inference at least, in our experience with the chick edema problem in the past, we know all of these dioxins are in fact toxic. We do know that. We have that information.

As I said, at least in chicks, we know there is a storage and possible transmission from chickens that have been fed these materials to the human. The possibility does exist.

Senator HART. By handling, as you just did?

Dr. VERRETT. No. I hope not. The tetradioxin is potent. I do not know whether handling this bird will harm you, but chloracne is a very serious disease and persistent disease and there is no known cure.

We are not aware that the other dioxins are as potent as that in this regard, but they have yet to be evaluated.

To our knowledge the herbicides we have been discussing have not actually been tested to see if any other of the dioxins are also present.

The concern, apparently, has revolved around the particular dioxin because it is extremely potent; even if the others are less potent, of course, they may still be important.

Senator HART. I understood your explanation with the rats and the monkeys to indicate that this—that the dioxin in 2,4,5-T has an accumulative effect.

Dr. VERRETT. The effects that are seen would indicate that. I would be unable to say that has been proven, but the fact that, for example, in the one monkey study, when they were fed for 3 months and then the animals that did not die during that course were put on a ration free of it, still died in subsequent months, and that would indicate that either the damage had been done by the initial exposure or there was storage such that it finally did have its effect.

But it was fatal in all cases. So the animal data would indicate—again we cannot apply that with certainty about the human—storage or persistence, if you wish to use that word.

Senator HART. Then your attitude, which you say is not conclusive and certainly does not relate directly to humans, points to the persistence, the cumulative character of the dioxin, rather than in the other direction, that it is not cumulative?

Dr. VERRETT. I would say it indicates it is probably cumulative.

Senator HART. Do you know any experiments that point in the direction that it is probably not cumulative?

Dr. VERRETT. I am not aware of any, no. The human data I cited with respect to occupational exposure would also indicate that it is probably cumulative in the human, of course.

Senator HART. You used a word here which I take it means burning.

Dr. VERRETT. Pyrolysis. Not exactly burning. In the sense I used it, it means reacting under conditions of elevated temperature, but not the actual burning of the material itself. It indicates a high temperature reaction, perhaps, heat applied to the material in order to make the reaction take place, but not in the sense of actually igniting it.

But it does indicate that heat, in other words, facilitates the formation of these compounds (dioxins), if that is what you are arriving at.

Senator HART. What if some of this material is just put in the city dump and burned? Could that burning inadvertently produce dioxin?

Dr. VERRETT. I could only say that the likelihood is there; yes.

Again, lack of actual experimentation does not give us any evidence or proof of this, but the fact is that these materials are formed when chlorophenols are subjected to heat and that would indicate to me that that is definitely a possibility.

Senator HART. What common products, or what common articles contain chlorophenol?

Dr. VERRETT. There are so many that I wouldn't be able to name all. But, every piece of newspaper or paper of any kind probably has chlorophenol used in the manufacture of paper. I am sorry I am not in an area which would enable me to give you total usage figures, but they are considerable.

This is washed out to some extent, but there probably are some chlorophenol residues, and paper would be one item and one which we can say is very widely used.

Another example—well, leather is cured by using chlorophenols in the tanning process. So leather materials contain it. There would be any number of other everyday items that would possibly have it.

Senator HART. Well, just as I am reluctant to have pictures taken, I am reluctant to make these contrary statement and yet this one is not inappropriate.

If the materials that we customarily—and for generations, centuries I guess—have been throwing into a fire contain chlorophenol, when you burn them, it is your opinion that dioxins can result——

Dr. VERRETT. Could possibly result; yes.

Senator HART. Is it possible that some of the birth defects for which there has been no medical explanation to date are the result of this kind of thing, where we have always done it and it has never seemed to hurt us?

Dr. VERRETT. I would say it is a possibility. It would be for others to assess this situation, but I think it is a distinct possibility because of the fact that these materials (chlorophenols) are ubiquitous, and if, in fact, chlorodioxins are formed in the environment, this is a possibility.

I should point out the studies done in mammals have been done by feeding, while the largest exposure may come from inhalation or dermal contact. That was the source of exposure occupationally to the tetrachlorodioxin. So here we have to be concerned not only about eating, but inhalation and perhaps contact exposure.

This brings up the subject of other materials. For example, we are also investigating——

Senator HART. I was just going to say that it is hard to visualize a substitute for paper or leather, but can one have leather and paper without this material?

Dr. VERRETT. I would think so. I should point out some of the materials (chlorophenols) are washed out in the processing. The total amount used in the processing does not always remain in the products. I did not mean to imply that. That brings up another question: Are they washed out into the rivers et cetera?

Nevertheless, I am not really sufficiently knowledgeable of the technology to say whether something else could be substituted or not.

Senator HART. Mr. Bickwit?

Mr. BICKWIT. I have heard several people criticize these chick embryo studies as being overly sensitive. I am not sure of this, but I believe the Secretary of HEW has criticized them.

Can you respond to that criticism?

Dr. VERRETT. Well, I have not as yet. As I mentioned in the testimony very briefly, this technique was started with just that idea, of finding a sensitive technique, if at all possible, for assessing toxic and also teratogenic response.

One of the great difficulties in all of the toxicology animal work, using mammals and primates, even, is the difficulty of relating, of course, to the human. There is perhaps another generation gap, if you can use that word, between the chicken or an avian species.

I would say without hesitation that these studies, for example, proved that this material is not for the birds, if I may phrase it that way. I would certainly not say that you can conclusively state that there is a human hazard from the results with the chick embryo.

However, inferentially we have evidence of that, and we have the inadvertent tie-in because of the chlorodioxin toxicity already known in primates and humans. I would certainly hope the human is less sensitive than the chick, but I feel what we really need, and I think that is pointed out very much by these hearings, is a very sensitive test, and then it remains to show this is not the case in the species more closely related to man.

I would rather demonstrate that something has an adverse effect in a sensitive system and then, by appropriate study, find out whether it will be relevant to man, than miss it altogether in an insensitive test. Although we do not try to make direct correlations, we feel certain anything seen in this system is worthy of further study, and I should also like to add we are trying to keep the study in the proper perspective.

That is, we use levels (doses) when possible that are relevant to the human exposure or other animal exposure and not simply try to produce effects with excessive amounts of material.

Mr. BICKWIT. You state on page 4 that the most potent dioxin in the production of serious occupational hazards, seems to have been the tetradioxin.

What actual experimental evidence is there that suggests or confirms that tetra is the most potent of the dioxins?

Dr. VERRETT. You mean in animal work?

Mr. BICKWIT. Yes.

Dr. VERRETT. Of course the tests referred to earlier by Dr. Steinfeld show in that system that the tetradioxin is extremely potent in rats. I am not aware that the other dioxins have ever been studied.

Mr. BICKWIT. Have the other dioxins ever been compared in potency in chick analysis?

Dr. VERRETT. Yes. As far as I know only in the chick embryos.

Mr. BICKWIT. So, unless we can rely on the chick studies, we may be in very serious trouble, even more serious than it now appears on the basis of available evidence.

Dr. VERRETT. That is right.

Mr. BICKWIT. Are there any data available, either in mammalian studies or chick studies on the relative toxicity of dioxins compared to thalidomide?

Dr. VERRETT. If you used the chick as an exhibit or even the mammalian studies with the tetradioxine, which is the only one being studied at the moment, you would have to say this material is some 100,000 to a million times more potent in these particular species.

Now, I should add that the abnormal effects (terata) that we are seeing are not the same as we see with thalidomide, but the potential for producing abnormalties that we do find, it is of that order.

Senator HART. Doctor, thank you very much.

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THE CHICK EDEMA FACTOR

A toxic factor in some feed grade fats and fatty acids produces hydropericardium and ascites in young chicks when 9 µg. per kilogram body weight are fed per day. There are a number of these toxic compounds, containing large amounts of chlorine. One has been characterized and synthesized.

In 1957, large numbers of chickens suffered from what appeared to be an epidemic disease. Losses attributable to this epidemic have been estimated in the millions of dollars. The affected birds appeared droopy, showed ruffled feathers, and had difficulty breathing (L. Friedman, *Feedstuffs, March 17, 1962*).

In some flocks, over 50 per cent of the birds died with typical symptoms of the disease (V. L. Sanger *et al., J. Am. Vet. Med. Assn.* **133**, *172 (1958)*). When autopsied, the birds had pale hearts with hydropericardium, and livers that were pale, mottled, and had an irregular granular surface. In the advanced stages, the abdomens were distended, and contained 100 to 500 ml. of clear, straw-colored fluid. The pericardial cavity is most susceptible to fluid accumulation, with the abdominal cavity next, and then subcutaneous tissue (D. F. Flick, C. D. Douglass, and L. Gallo, *Poultry Sci.* **42**, *855 (1963)*). The kidneys were pale and swollen; the fatty tissue of the gizzard was edematous; and the duodenum was swollen and soft (Sanger *et al., loc. cit.*). Hydropericardium was not as prominent in the older birds as in the broilers (Friedman, *loc. cit.*).

No acceptable explanation is available for the abnormalities associated with feeding the chick edema factor. Despite accumulation of fluid in the pericardial and abdominal cavities of the affected birds, the hematocrit, blood volume, and moisture content of the heart, skeletal muscle, skin, and kidney were normal. However, the total body water content of the birds was significantly increased. On the basis of these observations, it was suggested that the chick edema factor increased permeability of the cardiac vascular bed (Flick, Douglass, and Gallo, *loc. cit.*). Additional support for this hypothesis came from the finding that the toxic factor produced no appreciable change in the proportion or level of plasma proteins (Flick, D. Firestone, and J. P. Marliac, *Poultry Sci.* **44**, *1214 (1965)*).

The type of diet had a marked effect on the rate at which the chicks' bodies accumulated water. Chicks fed a natural grain ration containing 4 per cent toxic fat showed an increased body moisture content (78 versus 72 per cent for the controls) only at the end of the third week. Birds fed the same level of toxic fat in a semipurified diet showed a marked increase in body water content after seven days (79 versus 73 per cent) (Flick, Douglass, and Gallo, *loc. cit.*).

During 1958, a number of laboratories traced the disorder to the presence of a toxic substance in the unsaponifiable fraction of fats added to commercial poultry rations. This fraction contained a toxic substance(s) which presumably was present in a by-product of industrial production of oleic and oleic acids.

The toxic substance was present in a variety of types of fats. These included a sample of triolein, which on the basis of chemical analyses was found to be of "exceptional quality" (A. Yartzoff *et al., J. Am. Oil Chem. Soc.* **38**, *60 (1961)*). When this triolein was incorporated into a chick ration at a level of 15 per cent, the birds showed severe symptoms of hydropericardium. Similar symptoms were produced when chicks were fed rations containing distillates or residues secured in commercial production of fatty acids. The toxic substance(s) was also found in inedible animal tallows, acid-refined vegetable oils, and several commercially produced oleic acids and triolein (D. Firestone, W. Horwitz, Friedman, and G. M. Shue, *J. Am. Oil Chem. Soc.* **38**, *418 (1961)*). A report from another laboratory confirmed the presence of the chick edema factor in various industrial fats (J. C. Alexander, R. J. Young, C. M. Burnett, and H. D. Hathaway, *Poultry Sci.* **41**, *22 (1962)*).

The presence of the chick edema factor in commercial fats led to the ruling by the Food and Drug Administration that methyl esters of higher fatty acids intended for use as a food additive must be free of the chick edema factor (*Federal Register Dec. 9, 1961, 26 F.R. 11838; 121.224*). The presence of the factor was to be ascertained by a chick bioassay based on the volume of pericardial fluid in birds fed the fat under investigation.

That salt was necessary in the chicks' ration for the development of hydropericardium when toxic fats were fed was suggested by Alexander and co-workers. They observed typical symptoms of the disturbance only when the ration contained sodium chloride; extra salt accentuated the condition. Although this syndrome had many of the earmarks of a vitamin E deficiency, neither antioxidants (including vitamin E) nor selenium had any curative effect.

Sensitivity of different species to the toxic factor in these fats varies considerably. When monkeys were fed a sample of triolein that produced hydropericardium in chicks, the five monkeys died within three months (Yartzoff *et al., loc. cit.*). The ration contained 25 per cent triolein. The monkeys showed signs of jaundice, pancreatic atrophy and fibrosis, hemosiderosis, fatty liver with necrosis, and gross hemorrhage in the intestinal tract.

Pigs fed a ration composed of equal parts of a highly toxic chick ration and a normal swine ration gained weight and appeared to show no abnormalities (Sanger *et al., loc. cit.*). However, when a toxic fat was incorporated into a swine ration at a level of 9 per cent, the five shoats gained 0.72 pounds per day while the controls gained 2 pounds (L. C. Scott, *J. Am. Vet. Med. Assn.* **137**, *258 (1960)*). Despite the poor weight gains, the one pig sacrificed about six weeks after the start of the study showed no gross or microscopic lesions attributable to the ration.

The fat from the latter pig was rendered and incorporated into a chick ration at a level of 4 per cent. None of the chicks fed the ration containing the rendered lard developed toxicity signs or symptoms. This finding suggested that the pig did not store appreciable amounts of the toxic substance in its adipose tissue.

The apparent absence of the toxic factor in pork fat is in contrast to chicks' fat, where storage appears to occur. The unsaponifiable fraction of carcasses of chickens fed the toxic fat was very potent in producing hydropericardium in other birds (Friedman *et al., J. Assn. Official Agr. Chem.* **42**, *129 (1959)*).

A more recent study of the response of different species to the chick edema factor utilized the unsaponifiable fraction of a toxic fat. This was fed to young chicks and rats (T. C. Campbell and Friedman, *Proc.*

Soc. Exp. Biol. Med. **121**, *1283 (1966)*). The weanling rats fed the unsaponifiable fraction at a level of 9 μg. per kilogram body weight per day of the chick edema factor showed a 3.7 per cent loss in weight over the six day feeding trial. No gross tissue alterations were apparent on autopsy. The livers, however, were 21 per cent heavier than those of the controls when expressed on a body weight basis, while the adrenals were 50 per cent heavier. The heart, kidney, and spleen were of normal size.

Chicks fed a comparable amount of concentrate developed hydropericardium in six days. The livers of these birds were 15 per cent heavier than those of the controls. The statement was made that "the increase in liver weight in the chicks was not due to moisture or fat." Such an observation should have been documented and checked by histological studies.

A few years ago, R. E. Harman and collaborators (*J. Am. Chem. Soc.* **82**, *2078 (1960)*) reported isolation of a crystalline substance from a feed-grade tallow. This compound produced hydropericardium in chicks when incorporated into a commercial ration at a level of 0.1 p.p.m. A report (Yartzoff *et al., loc. cit.*) indicated that the crystalline substance contained 47 per cent chlorine.

Apparently, there are a number of compounds that behave like the chick edema factor. These compounds move closely with the toxic factor during molecular distillation, on thin layer chromatography, and show similar peaks on gas chromatography. Not all these compounds are toxic, and toxicity appears to vary in those that are.

Part of these difficulties may be resolved with characterization and synthesis of one of the toxic substances. By means of single crystal x-ray crystallography, J. S. Cantrell, N. C. Webb, and A. J. Mabis reported (*Abstracts, Am. Crystallographic Assn. Meeting, p. 27 (1967)*) that the compound isolated in their laboratories was 1,2,3,7,8,9-hexachloro-dibenzo-p-dioxin. This work was supported by infra-red, ultra-violet, and mass spectrometry principally by J. C. Wooton and W. R. Courchene (*J. Agr. Food Chem.* **12**, *94 (1964)*). This structure was verified by synthesizing the compound. The latter produced the same lesions in chicks as the compound isolated from the toxic fat.

Significant progress has been made in identifying one of the toxic substances present in some batches of fats intended for animal foods. There are still a number of unanswered questions, the most important of these being: What is the possible significance of these compounds to human health? Since substances with physiological properties similar to the chick edema factor can be stored in the adipose tissue of chicks, can they also accumulate in human tissue when minimal amounts are inadvertently ingested?

Another important question is: What is the source of these compounds? Apparently, the chick edema factor has been associated only with fats and fatty acids subjected to a considerable amount of heat during their processing. If the source of the chlorine in the toxic compound could be identified, it might be possible to devise methods for its removal or elimination before marketing the fat.

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Studies of the Chicken Edema Disease Factor*

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Introduction

When the problem of "X" or "Edema" in poultry was brought to our attention in December of 1957, a considerable amount of work had already been done. It was established that drugs added to the feed, as well as contamination by drugs or heavy metals such as lead or arsenic, was not responsible. The disease was not caused by bacterial, viral, or parasitic infection, and evidence was accumulating that the only feed ingredient associated with all the various outbreaks was the fat. The symptoms have been described in detail in the preliminary report of Schmittle, *et al.* (*J. Am. Vet. Med. Assoc.*, 132, 216 (1958)), which also summarized some of the work leading to the incrimination of the fat added to the ration.

The characteristic symptoms of this disease are the presence of excessive fluid in the pericardial sac, in the abdominal cavity (water belly), and less often subcutaneously, accompanied by high mortality beginning approximately in the third week. The striking resemblance of the "edema disease" symptoms to those recorded for the exudative diathesis syndrome of vitamin E deficiency in chicks made attractive the hypothesis that the disease outbreaks were the result of an induced vitamin E deficiency caused by the use of poor quality fats.

It was convenient, because of other work in progress in our laboratory at the time, to use the A.O.A.C. vitamin D_3 chick test ration and single comb white Leghorn chicks in an attempt to produce the condition.

Our first feeding trial with a sample of feed collected from one of the "disease areas" was a false start, since after six weeks on the ration the chicks were normal and showed no unusual symptoms upon postmortem examination. The first experiment in which we successfully produced the characteristic symptoms of the "chick edema disease" involved a sample of fat that had been collected by one of our Food and Drug inspectors at a feed manufacturing plant and which had been known to have produced the disease, and also a sample of a "tarry" by-product from the manufacture of oleic and stearic acid, which presumably had been mixed into this fat sample to the extent of about 40%. In this experiment the vitamin E hypothesis was tested. Also, the suitability of the A.O.A.C. ration for this investigation was checked against a feed resembling more closely a practical commercial ration.

Basal Ration and the Vitamin E Hypothesis.—Table 1 shows the composition of the A.O.A.C. basal ration and the special basal ration. The major differences are the substitution of Drackett (an isolated soybean protein) for the casein of the A.O.A.C. diet, and the inclusion of alfalfa leaf meal and linseed oil meals. From the results shown in Table 2, it is clear that: (*1*) the fatty acid (F.A.) by-product is much more effective in producing the disease than the sample of fat (INV. Fat) that had actually been used by feed manufacturers; (*2*) the special basal ration is to be preferred to the A.O.A.C. diet, since the severity of the edema symptoms is increased, growth performance is improved, and gizzard erosion is eliminated; (*3*) the feeding of vitamin E in large

Table 1. Basal chick rations

	AOAC	Special
Whole yellow corn, ground	58	45
Wheat flour middlings	25	19
Crude precipitated casein	12	—
Calcium phosphate (precipitated)	2	2.14
Iodized salt	2	2.04
Non-irradiated yeast	2	3.2
$MnSO_4 \cdot H_2O$	0.02	0.02
Cod liver oil	1	1
Dehydrated alfalfa leaf meal	—	1
Linseed oil meal	—	10.6
Drackett protein	—	16
	102.02	100.00

* Presented at the Seventy-second Annual Meeting of the Association of Official Agricultural Chemists, Oct. 13-15, 1958, at Washington, D.C.

Table 2. Effect of suspect fat and by-product fat on chicks

Group	Diet	Added Fat	Av. Wt. 20-Day (Grams)	Death	No. of Chicks Showing Edema Symptoms Marked	Mild	No.	Normal
1	A.O.A.C.	6% lard	147	0	—	—		20
2	A.O.A.C.	6% INV. fat	135	0	—	7		12
3	Special	6% INV. fat	153	0	10	2		4
4	Special	3.6% lard + 2.4% F.A. by-product	147	(4) [b]	7	9		—
5	Special	3.6% lard + 2.4% F.A. by-product + vitamin E [a]	143	0	5	5		9

[a] 10 mg *dl*-alpha tocopherol per chick per day by mouth.
[b] Av. survival time, 20.2 days.

doses does not prevent the occurrence of the disease, but does seem to decrease the severity of the symptoms. However, in an independent study by P. C. Underwood and C. G. Durbin at the Beltsville Laboratories of our Veterinary Medical Branch, with Rhode Island Reds, the oral administration of 1 mg per day per chick of *dl*-alpha tocopherol acetate had no effect, the symptoms being slightly more pronounced in the supplemented group. The conclusion that some material associated with the "fatty acid by-product" is responsible for the disease, and not a simple vitamin E deficiency, was confirmed in a subsequent test, when the fatty by-product was fed at 7% of the diet and all chicks showed marked to severe symptoms at autopsy; only 2 of 19 chicks survived the twenty-third day, and the average survival of 17 chicks was 19.4 days. In a similar group fed 10 mg of *dl*-alpha tocopherol per chick per day, the average survival was 20.5 days for 15 chicks; 4 chicks survived to the twenty-third day. All showed marked to severe symptoms.

Chemical Findings.—Part of the chemical data on these samples is shown in Table 3. Findings of particular significance were that these "fats" (F.A. by-product) were actually free fatty acids containing an unusually large amount of unsaponifiable material. Furthermore, the unsaponifiable fraction is qualitatively different from that found in fresh animal fat such as lard, by virtue of the large percentage that reacts in the Liebermann-Burchard test, compared to the much smaller proportion that reacts with digitonin. In the lard the proportion is almost one to one.

Table 3. Comparison of suspect fats with lard

	% Unsap.	Liebermann-Burchard Positives % of Unsap.	Digitonin Precipitable % of Unsap.
Lard	0.35	23.0	21.7
Inv. fat	3.8	70.0	7.6
F. A. by-product	10.7	92.5	2.3

[a] A-5 Unsaturated sterol.
[b] 3-β-OH Δ-5-Unsaturated sterol.

Urea Fractionation.—The fatty acids from the fatty by-product, after saponification, were fractionated (Fig. 1) with urea into two portions: the normally occurring fatty acids that form urea adducts, and modified or abnormal fatty acids that do not form urea adducts. About 76% of the fatty acids *form* urea adducts; the *remainder (24%)* comprise the urea filtrate.

Effect of Urea Fractions on Rats and Chicks.—The urea filtrate fatty acid fraction was fatal to weanling rats (40–60 g) upon oral administration. Two successive daily doses of 0.4 ml resulted in marked loss of weight and death by the fourth day. Two doses of 0.2 ml caused a marked weight loss from which recovery began at the fourth day. This effect is similar to that observed in our laboratory with the fatty acids that do not form urea adducts which were derived from heated cottonseed oil, and to the observations recorded by Crampton, *et al.* (*J. Nutrition*, 44, 177 (1951)) with heated linseed oil. Chicks react similarly, although they seem to be more resistant to the lethal effects of direct feeding than the rats. However, not enough observations were made to establish the relative susceptibility of the two species. All polymers and hydrogenation products (see Fig. 1) were toxic.

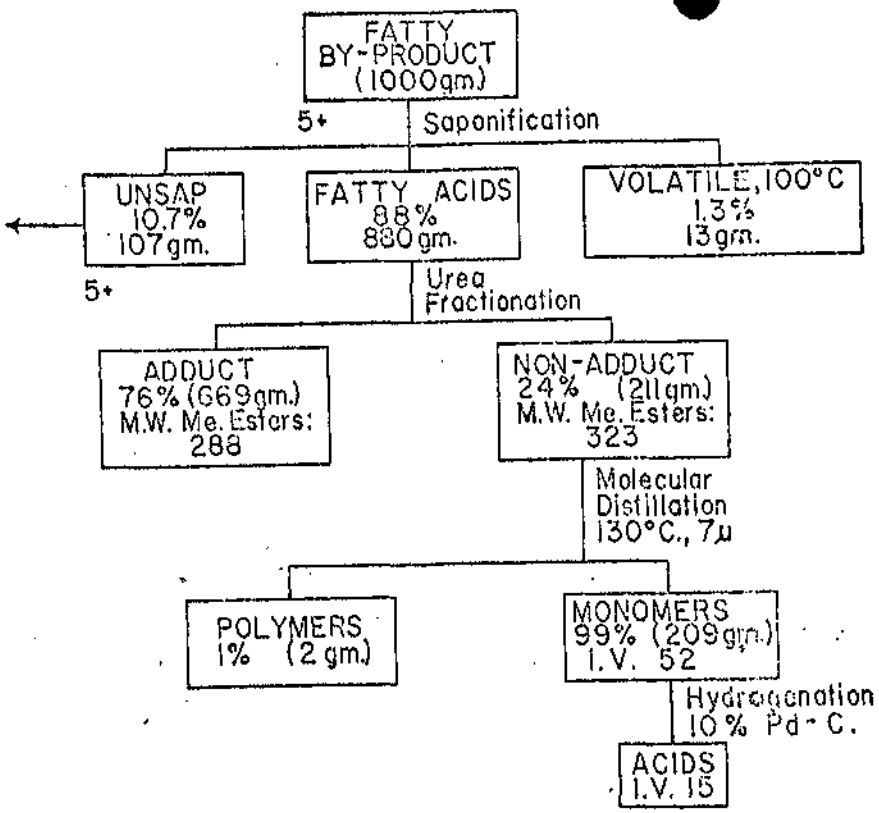

Fig. 1.—Fractionation of fatty acid by-product. (Numbers followed by + sign are toxicity scores; see footnote c, Table 5.)

The urea adduct fraction of the fatty acids produced no unusual effects either in rats or in chicks.

Effect of Unsaponifiable Fraction on Rats.—The significant findings of feeding the unsaponifiable fraction of the fatty acid by-product to rats are seen in Table 4. One ml of the unsaponifiable fraction was fed to each of four rats every day for 22 days. Control animals received no supplement to the basal ration. The experimental group contained two males from different litters, with litter mates in the control group. The females of both the test and control groups were all from one litter. The growth rate was significantly depressed—females were more drastically affected than males; the size of the thymus decreased; and the size of the liver markedly increased, which was accounted for only in part by an increase in liver fat. No other significant differences in the relative weights of organs (kidneys, thyroids, hearts, adrenals, etc.) were noted, although small differences cannot be demonstrated by such small groups.

Although deleterious effects were produced in rats both by the urea filtrate and by the unsaponifiable portion, symptoms similar to those of the "edema disease" syndrome were not observed in this species.

Effect on Chicks.—When added to the diet of chicks at a level equivalent to 7% of the original fatty by-product, the urea filtrate produced a significant growth depression but, as Table 5 shows, very few edema disease symptoms. The small amount of activity

Table 4. Results of feeding unsaponifiables from "fatty acid by-product" to weanling rats by stomach tube (1.0 ml/rat/day)

	Males[a]		Females[b]	
	Control	Fed	Control	Fed
22-day wt gain, grams	138	91	114	40
	112	94	115	48
Liver wt, g/100 g body-wt	6.00	9.65	5.66	10.00
	5.77	10.03	6.31	9.08
% Fat in dry liver	12.6	14.8	10.9	14.9
Thymus, g/100 g body wt	0.220	0.095	0.234	0.085
	0.258	0.162	0.169	0.073

[a] Males paired by litter; 2 litters.
[b] Females all from one litter.

indicated in the urea filtrate was shown in later experiments to reside in the unsaponifiable residue that had not been completely extracted. It is clear that the edema disease-producing material is associated with the unsaponifiable portion of the fatty by-product. In Table 5 are also shown the variations in severity of the disease produced by a series of graded doses. Although such a tabulation as shown here cannot give a complete picture of the range of observations encountered, it indicates how the pattern of symptoms seen in any one group depends upon the dose of active material fed. In these studies the dose was usually high enough to produce severe symptoms, and as we became more familiar with the disease we became more confident of the results obtainable with smaller groups. Whenever possible we now use five chicks per group;

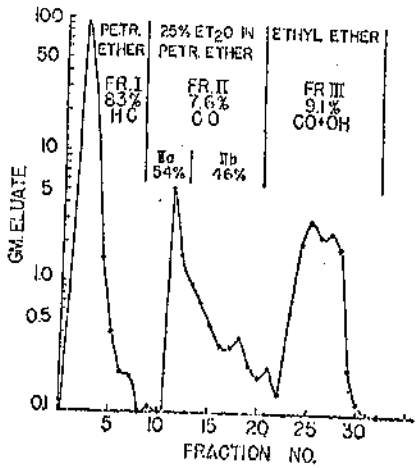

Fig. 2.—Alumina chromatography of unsaponifiable fraction from toxic fatty by-product.

occasionally we have used as few as two, when the amount of sample was limited.

Concentration of Toxic Factor

Alumina Chromatography.—The first step in our attempts to isolate the factor that caused the "edema disease" from the unsaponifiable portion of the fatty by-product was chromatography on alumina (1:6). A chromatogram (Fig. 2) was obtained by plotting the weight of material eluted by increments of solvent. Petroleum ether was used until the elution curve seemed to have reached a minimum, then was followed by 25% ethyl ether in petroleum ether. The elution was completed with 100% ethyl ether to give three distinct fractions of increasing polarity. In general, Fraction I could be characterized as hydrocarbon (HC), Fraction II as containing carbonyl compounds, and Fraction III as containing sterols. The ultraviolet absorption spectra show that Fraction I has characteristic absorption peaks at 223, 227, 234, and 243 mμ, suggesting the presence of significant amounts of cholestadiene, either the 2,4 or 3,5 isomer or both.

In anticipation of the possibility that the toxicity would reside in Fraction I (83% of the unsaponifiable material) a sample of 3,5-cholestadiene was prepared by W. L. Hall in time to be included in the third feeding trial. The results of this experiment indicated clearly that there was no response to cholestadiene but that all the activity responsible for the chick edema disease was in Fraction II, which constituted approximately 8% of the unsaponifiable or about 0.85% of the original product.

Purification of Fraction II.—Three different approaches were made simultaneously to the problem of further purifying Fraction II: (*1*) counter current distribution (iso-octane: methanol solvent system with 500 transfers[1]), (*2*) chemical separation of carbonyl containing compounds by the Girard Reagent T, and (*3*) re-chromatography on alumina with a higher ratio of adsorbent to sample and more gradual elution.

Table 5. Effect of by-product fat and its fractions on c

Expt.	Sample	% in Diet	Deaths	No. Chicks Showing Edema Symptoms: Severe	Moderate	Mild	No. Normal	Tox. Score[c]
2	F.A. by-product	7	17 (19.4)	2	—	—	—	4.9
2	F.A. by-product + vitamin E[b]	7	15 (20.5)	2	2	—	—	4.7
2	Urea adduct	4.8	—	—	—	—	20	—
2	Urea filtrate	1.5	—	—	—	4	14	0.2
2	Unsap.	0.70	15 (20.2)	6	—	—	—	4.7
4	Unsap.	0.50	3 (20.3)	6	—	1	—	4.0
4	Unsap.	0.25	—	—	7	3	—	2.4
4	Unsap.	0.125	—	1	5	2	3	2.0
4	Unsap.	0.062	—	—	—	—	10	—

[a] Figures in parentheses indicate average survival time in days.
[b] 10 mg *dl*-alpha tocopherol per chick per day by mouth.
[c] The toxicity score is computed by assigning a score of 5 for each chick that died of the edema disease, 4 for each chick that showed one or more of the characteristic symptoms, i.e., hydropericardium, hydroperitoneum, or subcutaneous edema, in severe degree, a score of 3 for moderate symptoms, and a score of 1 or 2 for mild symptoms. The average of the individual scores is tabulated as the toxicity score.

For the counter-current distribution experiment 8.4 grams of Fraction II were loaded into the first 10 tubes of a 500 tube Craig all-glass counter-current apparatus. After 500 transfers, the contents of every 10 consecutive tubes were combined, and after evaporation of the solvents the weight of solids was determined in each of the 50 fractions obtained. The resulting distribution curve, i.e., solute *vs.* tube numbers, is shown in Fig. 3.

The 50 fractions were combined according to the peaks indicated by the distribution curve into 10 fractions for biological testing (Fig. 3). Each fraction was added to the test diet at a level equivalent to 7% of the original fatty by-product. Figure 4 shows the separation procedure diagrammatically, and indicates the amount of material recovered in each fraction. The activity seemed to be equally distributed between Fraction 5 (tubes 240-320 containing 2.239 grams) and Fraction 6 (tubes 321-343 containing 0.600 grams), as indicated by the toxicity scores. Fraction 6 was the most potent, showing as much activity at a level of 4.2 mg per 100 grams of feed as had been observed with 5 grams of original product. It is of interest that the toxic fractions 5 and 6 did not react with digitonin and did not show the L.B. reaction.

In preparing material for the chemical separation (Fig. 5) Fraction II was cut into two approximately equal portions; a characteristic yellow band was used as the landmark for the separation. Each of the fractions (IIA and IIB) was treated identically. First, after evaporation of the ether the material was heated with methanol, then cooled. When the hot methanol solution cooled, white crystals were obtained. The infrared spectrum for these crystals indicated a carbonyl compound with a long aliphatic chain. A synthetic compound, dipalmitone (prepared by Jonas Carol), had a very similar infrared spectrum.

Approximately 16% of Fraction IIA was insoluble in methanol. To the methanol-soluble portion was added twice the weight of Girard Reagent T and to the mixture was added 10% its volume of glacial acetic acid. The mixture was allowed to stand for two hours at room temperature before the water-soluble derivatives were separated from the unreacted material ("non-ketonic"). The "non-ketonic" portion was dissolved in hot acetic acid and heated with two times its weight of Girard Reagent T on the steam bath for 10 minutes, and the unreacted material was separated from the water-soluble derivatives. Approximately 23% of sample

[1] We gratefully acknowledge our debt to Procter & Gamble workers for suggesting the solvent system.

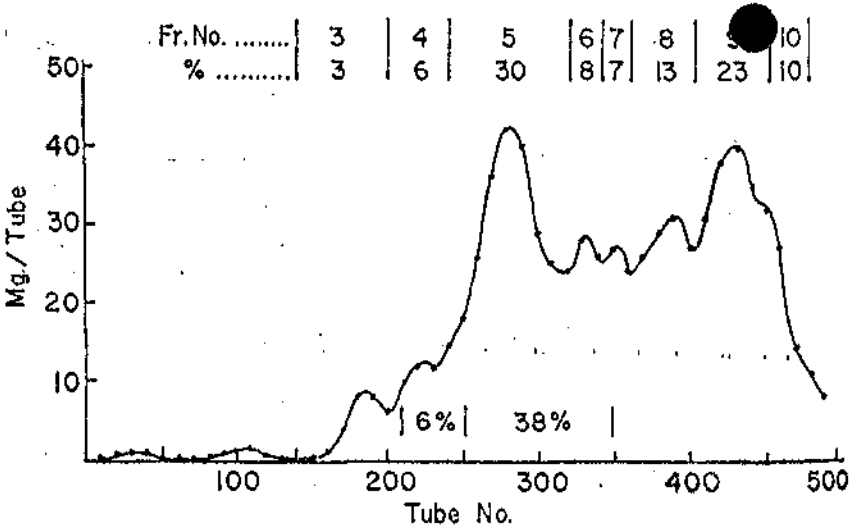

Fig. 3—Counter-current partition of Fraction II. Methyl alcohol:iso-octane system, 500 tubes, 28 hours.

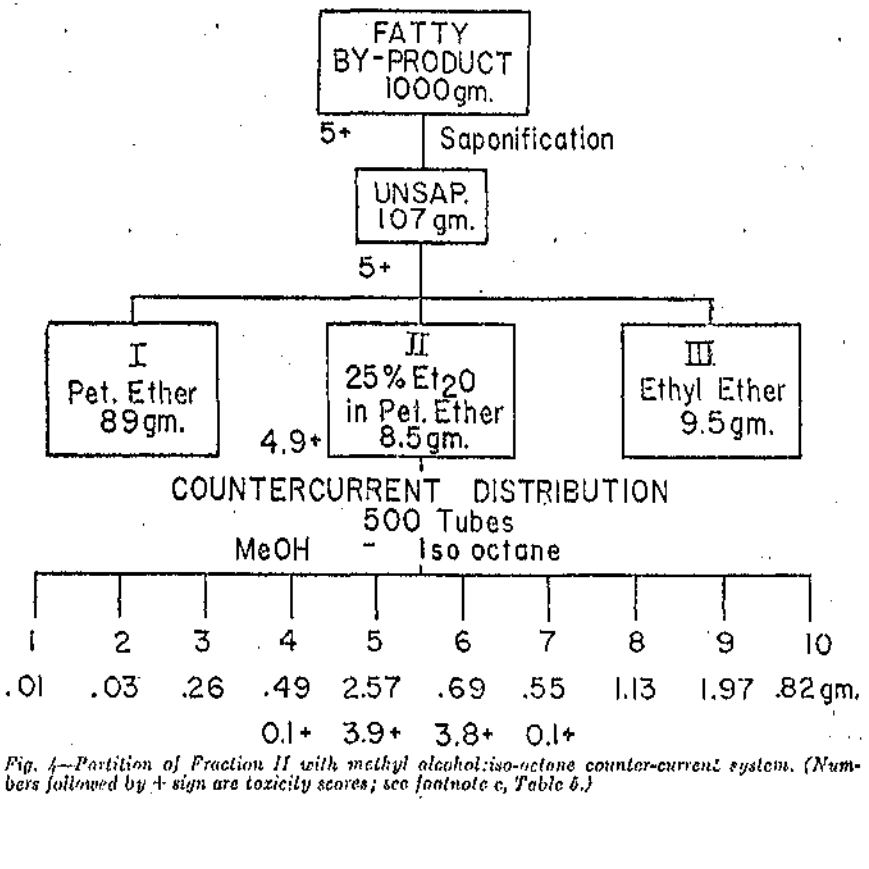

Fig. 4—Partition of Fraction II with methyl alcohol:iso-octane counter-current system. (Numbers followed by + sign are toxicity scores; see footnote c, Table 6.)

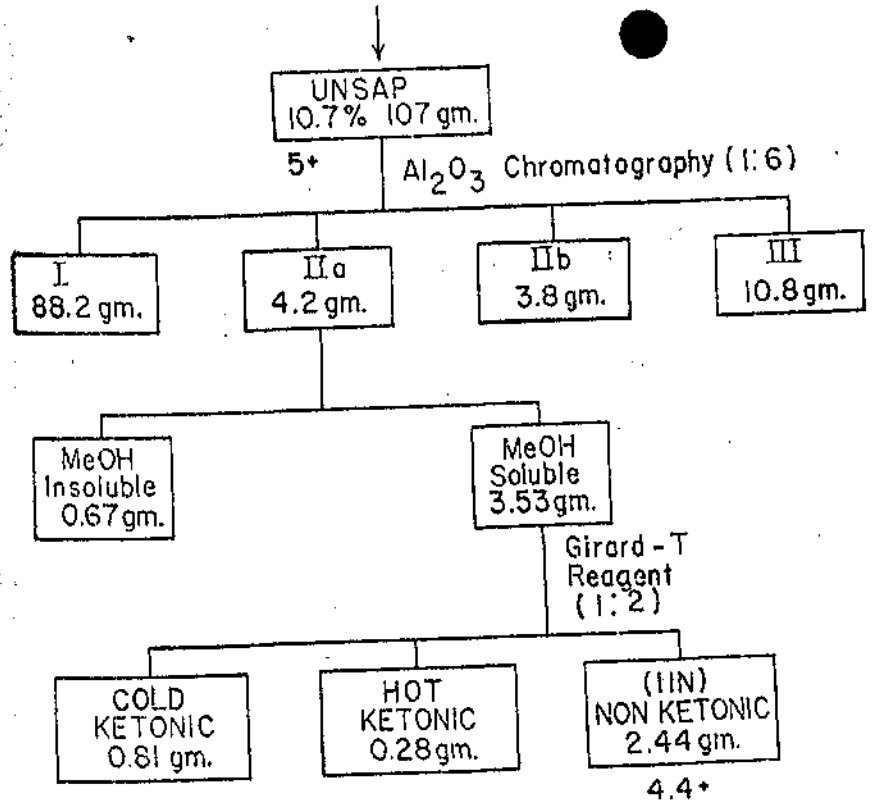

Fig. 6—Further fractionation of Fraction II.

had reacted in the cold and 8% more with heat; 69% did not react under these conditions and was classified as "non-ketonic" (11N). The various fractions derived from Fraction IIA in Fig. 5, as well as a similar set derived from Fraction IIB, were also fed at a 7% equivalent level in the diet. The synthetic dipalmitone was fed at a level of 10 mg per 100 grams. As indicated, there was no disease-producing activity in any of the fractions obtained from IIB and only the "non-ketonic" Fraction 11N was very active. Surprisingly, however, the infrared spectrum of Fraction 11N definitely indicated the presence of carbonyl groups. This so-called "non-ketonic" material was again treated with Girard Reagent T, this time with five times the weight of reagent instead of two times, and was heated at 80° C for 15 minutes. After the water-soluble derivatives were separated from the unreacted non-ketonic material, two-thirds of the 11N fraction proved to have reacted. The non-ketonic residue was again treated with excess Girard reagent and heat, and this time 50% of the "non-ketonic" material reacted. When the original material from the Girard derivatives was regenerated and the reaction was repeated with excess Girard reagent, again only 67% reacted to form ketone-Girard derivatives. It was clear that our active "non-ketonic" Fraction 11N consisted largely of compounds containing "hindered" carbonyl groups that would react with Girard reagent with great reluctance. Of additional interest is the fact that these "hindered ketone" fractions had characteristic ultraviolet spectra with a major absorption max-

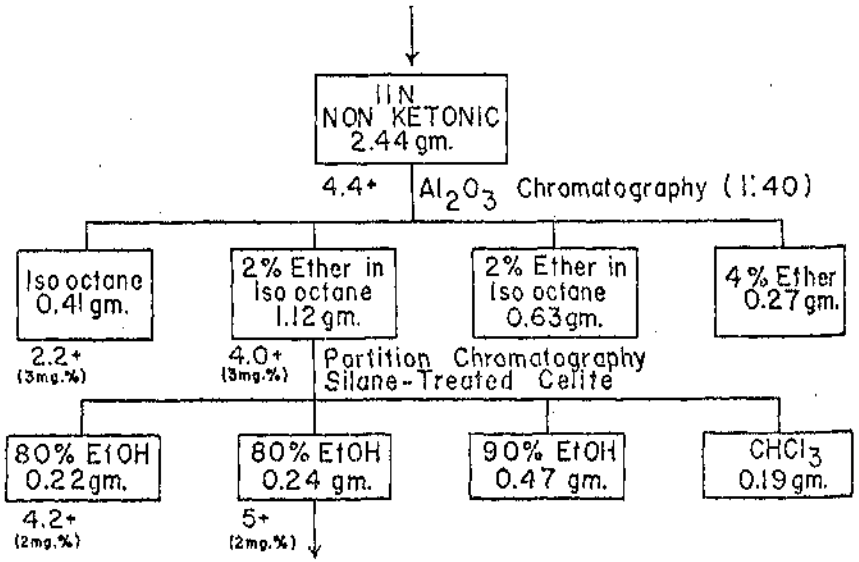

Fig. 6—Chromatography of Fraction 11N. (Toxicity scores at the levels fed appear below boxes.)

imum at 233 mμ and a secondary maximum at 200 mμ.

The disease-producing activity of Fraction 11N was further concentrated (Fig. 6) by chromatography, first on alumina. A ratio of one part sample to 40 parts alumina was used, and the column was eluted successively with iso-octane, 2% ethyl ether in iso-octane, and 4% ethyl ether in iso-octane. The fractions were monitored by ultraviolet spectrophotometry as they were eluted and then were combined for feeding on the basis of spectral characteristics. The first part of the material eluted with 2% ethyl ether in iso-octane had a definite phenanthrene type of ultraviolet spectrum with characteristic maxima at 259 mμ and 300 mμ, and with no peak at 234 mμ. The fraction immediately preceding, eluted with 100% iso-octane, did not show a phenanthrene type of spectrum but did have a major absorption peak at 234 mμ and a smaller one at 260 mμ. The phenanthrene spectrum disappeared upon further elution with 2% ethyl ether in iso-octane. Only the iso-octane and the first 2% ether fractions showed toxicity at a level of 3 mg per 100 grams of feed. The fraction characterized by the phenanthrene ultraviolet spectrum produced the more severe edema symptoms.

Next, this "phenanthrene" material was further concentrated by partition chromatography on a column consisting of 5 grams of silane-treated Celite plus 4 ml of iso-octane. Mobile solvents were 80% ethyl alcohol saturated with iso-octane, followed by 90% ethyl alcohol saturated with iso-octane. The first and second 80% alcohol fractions were both very toxic at a level of 2 mg per 100 grams of feed. These two steps (Fig. 6) represent approximately an 8-fold concentration of the toxic material of Fraction 11N. The first 80% alcohol cut had a typical phenanthrene ultraviolet spectrum and no peak at 234 mμ, whereas the second 80% alcohol fraction shows a major absorption maximum at 234 mμ, in addition to the characteristic phenanthrene peaks at 259 and 300 mμ.

Simultaneously with the work just discussed, another study of chromatography on alumina was made with a higher ratio of adsorbent to sample (100:1) and more gradual elution (Fig. 7). We had learned that Fraction II obtained from the unsaponifiable fraction could be cut directly from the first alumina column into two parts (using the characteristic yellow band as a landmark)

and that only the first cut contained edema disease-producing material (Fig. 2). This Fraction IIA was eluted from the second column with large volumes of petroleum ether (three arbitrary cuts), followed in succession by large volumes of 0.2%, 1%, 2%, and 5% and 25% ethyl ether in petroleum ether. The seven fractions indicated in Fig. 7 were tested at a level of 3 mg per 100 grams of feed. Only Fractions 2 and 3 showed activity. The methyl alcohol-insoluble portion from Fraction 3 showed activity. This finding did not agree with earlier experience, but may be explained by the larger quantities of material involved in these particular operations as compared to the earlier experiment. Apparently the active material has a more limited solubility in methanol than first supposed. In one of our most recent experiments, the methanol-soluble portion of Fraction 3 from the second alumina column was subjected to partition chromatography on silane-treated Celite as previously described (Fig. 8). This time only 80% ethanol was used as the mobile phase and the eluate was partitioned into forerun, tailings, and two major fractions, 82A and 82B, according to their ultraviolet spectra. Fraction 82A consisted mainly of material with a phenanthrene spectrum, and 82B showed the major peak at 234 mμ. Fraction 82A was rechromatographed on the silane column, using 75% ethanol as the mobile solvent, and again cut according to the ultraviolet spectra into a forerun, a fraction with a phenanthrene spectrum, an overlap fraction showing approximately equal absorption peaks at 259 and 234 mμ, and a fraction with a major peak at 234 mμ, approximately 6 times as intense as at 259 mμ. All of the fractions were fed, and each of the major fractions was found capable of producing the chick edema disease when fed at a level of 0.5 mg per 100 grams of diet with a severity approximately equal to that observed in chicks fed 5% of original fatty by-product. This is the first instance where fractions with distinctly different absorption characteristics had approximately the same biological activity.

Another recent experiment involves the reduction of one of our potent fractions with sodium borohydride. After the borohydride was separated, the treated material was

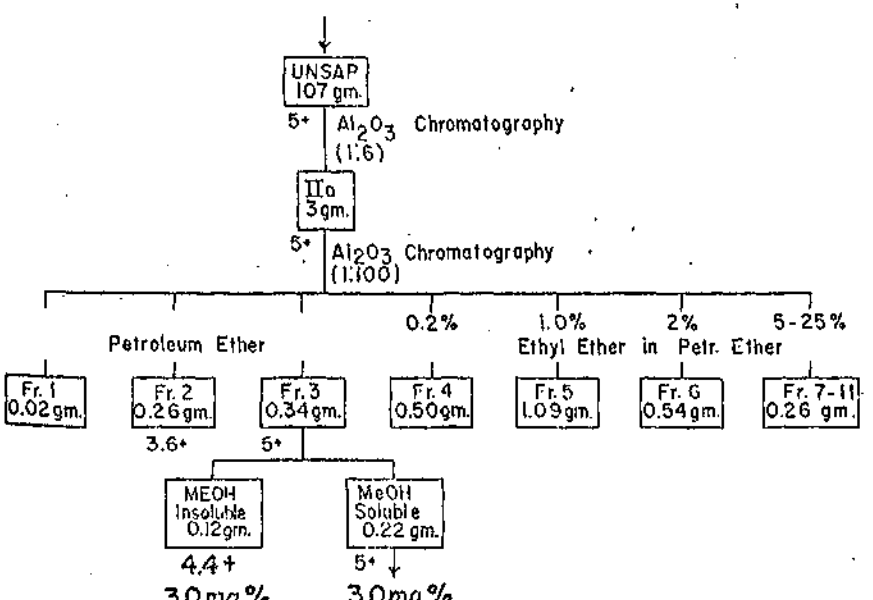

Fig. 7—Chromatography and elution of Fraction IIA (1:100 column).

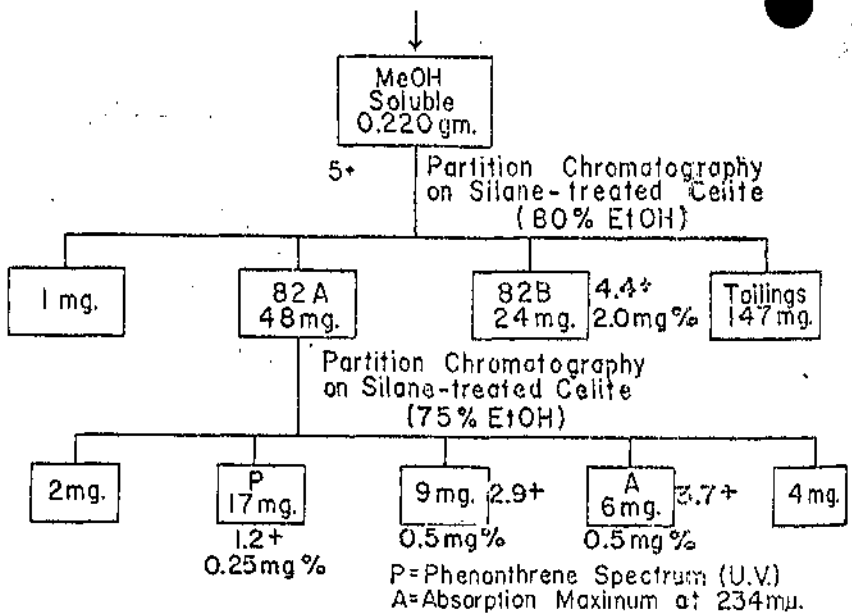

Fig. 8—Reverse phase partition chromatography of MeOH-soluble portion of Fraction 3.

chromatographed on alumina into three approximately equal (30:30:40) fractions of increasing polarity. The infrared spectra indicated that the first fraction was largely hydrocarbon, the second fraction had the carbonyl group present, indicating that borohydride also reacts reluctantly and incompletely with the hindered ketones, and the third fraction contained hydroxyl groups. There was very little disease-producing activity left, all of it limited to the first fraction. This may mean that either the activity is lost upon reduction but that not all of the material reacts under the conditions used, or that the reduced compound retains only part of the original activity. In either case, these data are additional evidence that the active material contains a carbonyl compound.

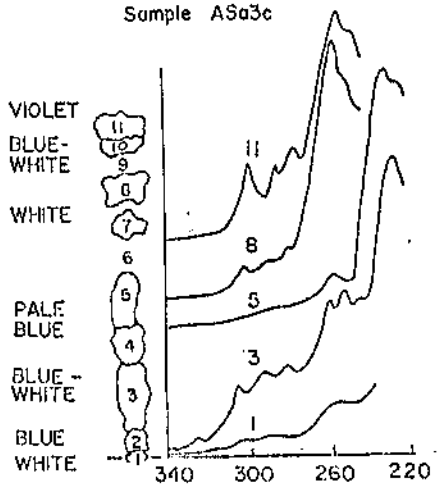

Fig. 9—Paper chromatography, and ultraviolet curves of fluorescent spots obtained from a highly toxic fraction.

Each of the fractions discussed here, and many others have been studied by ultraviolet and infrared spectrophotometry and the pertinent observations have been mentioned.

E. O. Haenni has been studying the fluorescence spectra, both activation and emission, of each fraction, and will report the results when the data have been analyzed.

In addition, as the potency of the disease producing factors has been concentrated, we have tested the purity of each fraction by paper chromatography. We are using 8×

9½ Whatman No. 31 paper, washed with methanol and then coated with mineral oil that has been pre-washed with sulfuric acid and methanol. The papers are developed with methanol in an ascending system for periods varying from 2 to 20 hours. For the longer periods the solvent is permitted to evaporate from the edge of the paper through the slotted glass cover. After the papers are dried, they are examined under ultraviolet light and the fluorescent spots are traced. Figure 9 shows a typical paper chromatogram. The spots are cut out and eluted with methanol, and the ultraviolet spectra of these solutions are determined. Figure 9 represents a fraction of high purity that has been tested by a chick feeding experiment; it is of considerable interest to note that the ultraviolet spectra of these spots are of both types discussed earlier, namely, the phenanthrene type with an absorption maximum at 259 mμ and the type with a maximum at 234 mμ. The ultraviolet curve of the original solution was of the phenanthrene type.

If we were to speculate about the chemical nature of the disease-producing material from the evidence available at this point, we would visualize a pair or a series of closely related compounds, with a 2-ring naphthalene structure, or more likely a 3-ring nucleus, similar to phenanthrene; a carbonyl group in a long aliphatic side chain; and molecular weights of 500–600.

Appearance of Poison in Flesh of Chickens

Once it had been established that the chicken edema disease was caused by a toxic principle, the possibility that edible chicken flesh might contain this toxic material had to be considered. As soon as we discovered the unusual characteristics of the unsaponifiable material from the [illegible] fat and fatty by-product, we compared the unsaponifiable fraction of the carcasses of chickens that had been fed these products with the unsaponifiable fraction from the carcasses of normal control chickens. About 25% more unsaponifiable material per unit of fresh chicken weight was obtained from the test chickens than from the control group. When the unsaponifiable material was chromatographed on alumina, about 18 times more hydrocarbon material was obtained from the test group. The test group hydrocarbon fraction gave a strong positive reaction in the Liebermann-Burchard Test whereas comparable material from the control group was negative. Furthermore, upon comparing the hydrocarbon fractions by differential ultraviolet spectrophotometry, definite absorption peaks appeared which were similar to those observed from Fraction I of the unsaponifiable portion of the fatty by-product.

This evidence indicated beyond doubt that some of the material from the disease-producing fat was deposited in the flesh of chickens when they received it in their diet. This, of course, did not prove that the chicken meat contained the toxic material, especially after it was learned that the disease-producing activity was in Fraction II and not in Fraction I. In order to settle this point, the unsaponifiable portion obtained from freshly ground chicken carcasses, not including intestines, head, or feet, was fed to chicks at levels of 1.0, 0.5, 0.25, 0.125, 0.05, and 0.025% in the test ration. As can be seen in Table 6, symptoms of edema-disease were observed at all levels fed. The unsaponifiable material from normal control chickens fed at 0.5% of the diet produced no abnormal symptoms of any kind.

Table 6. Results of feeding unsaponifiable extract of chickens that had been fed fat or fatty acid by-product

Expt. No.	% in Diet	Deaths	No. Chicks Showing Edema Symptoms: Severe	Moderate	Mild	No. Normal
3	1.0	2 (15.5)	—	—	—	—
4	0.5	5 (16.6)	—	—	—	—
5	0.25	5 (19.0)	1	—	—	—
	0.125	4 (21.0)				
6	0.050	—	—	3	2	1
	0.025	—	—	1	1	3

* Figures in parentheses show average survival time in days

We have examined samples of various types of fats and fatty acid products in a survey that is still continuing. The edema disease-producing product is a characteristic not confined to the production of a single manufacturer.

Summary

In summary, from the studies so far, it may be concluded that:

(1) The chicken edema disease is caused by a toxic factor in a fatty by-product of stearic and oleic acid manufacturing operations.

(2) The disease-producing factor has been concentrated approximately 10,000 times by saponification, chromatography of the unsaponifiable portion on alumina, and reverse phase partition chromatography on silane-treated Celite.

(3) The evidence presented indicates (a) the presence of compounds with a two-ring nucleus similar to naphthalene or a three-ring nucleus similar to phenanthrene, (b) the presence of a "hindered carbonyl" group, probably in a side chain. Based on the relatively low intensity of the carbonyl peak at 5.8 μ a compound of molecular weight 500–600 is suggested. However, the evidence on the presence of carbonyl in the toxic compounds is not conclusive and does not completely rule out the possibility of a toxic hydrocarbon.

(4) The fatty acid fraction from this fatty by-product contains significant quantities of non-urea adduct-forming compounds that are toxic, although they do not produce edema disease.

(5) The edema disease-producing compounds are present in significant amounts in the flesh of chickens on rations which contain toxic material.

(6) The edema disease-producing material is not confined to the production of a single manufacturer.

Note

Since this paper was presented, repeated chromatographic purifications of the toxic materials on alumina and on silane-treated Celite have now permitted the isolation of several distinctive fractions. One of these fractions, possessing the ultraviolet absorption spectrum of substituted naphthalenes ($\lambda_{max.}$ at 236, 280, 296 mμ; shoulder at 323 mμ; $\lambda_{min.}$ at 253 mμ; $A_{253} \sim 14 \times A_{296}$), proved highly toxic. The infrared spectrum of this material showed little absorption in the carbonyl region. It is concluded that the "naphthalene" fraction comprises a mixture of hydrocarbons. Assuming that alkyl-substituted naphthalene is the only chromophore present, the average molecular weight, calculated from the absorptivity at 236 mμ (E, 1%, 1 cm $\cong$ 3200) is in the vicinity of 250.

Acknowledgment

The extensive work outlined in this paper was made possible by the active cooperation of many other individuals, both within and outside the Food and Drug Administration. We are indebted to W. C. Ault and D. H. Saunders of the Eastern Utilization Research and Development Division of the U.S. Department of Agriculture, who prepared 1,330 grams of unsaponifiable material from the fatty by-product, and to these members of the Food and Drug Administration: Stanley Nesheim, Division of Food, who prepared an equal amount of the unsaponifiable material; Jerome Eisner, Division of Food, who helped prepare the charts; J. H. Jones, John Winniger, and Meyer Dolinsky, Division of Cosmetics, who assisted in the countercurrent distribution study; Jonas Carol, Division of Pharmaceutical Chemistry, who helped to interpret the infrared curves and to synthesize a sample of dipalmitone; P. C. Underwood and C. G. Durbin, Bureau of Medicine, who provided market-size chickens that had been fed the toxic material, for use in the carcass residue studies; W. L. Hall, Division of Nutrition, who synthesized a sample of 3,5-cholestadiene; and the numerous inspectors of the Food and Drug Administration who provided essential information without which this work would not have progressed.

We are also indebted to the other individuals and laboratories, who are simultaneously investigating this problem, for discussions that provided information helpful in guiding us in our studies. Among these are Ralston Purina Company, the Procter & Gamble Company, and Hales and Hunter.

The Occurrence of the Chick Pericardial Edema Factor in Some Oleic Acids and Products Derived Therefrom [1]

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Division of Eastman Kodak Company, Rochester, New York

A material causing the chick edema syndrome has been reported (1, 2, 3, 4) to occur in specific lots of feed-grade animal fats. These fats were reported to contain the edema-producing factor as a trace impurity produced during certain fat-processing operations (2, 3). Birds fed diets containing this unidentified factor develop an edematous condition, characterized by pericardial edema (distention of the pericardial sac with fluid, also termed "hydropericardium") and in more severe cases ascites and gross liver and kidney damage. In the young chicks the initial gross symptoms are characterized by abdominal distention and labored breathing. Manifestations of the pericardial edema factor appear to be limited to poultry (1).

The active pericardial edema-producing factor has not yet been isolated, but certain characteristics of the pericardial edema factor have been reported by Brew *et al.* (2) as follows: a) presumably a hydrocarbon derivative, possibly of cholesterol, b) a molecular weight of about 360, c) associated with fractions which give the Liebermann-Burchard test for sterol residues, d) it can be concentrated by molecular distillation of fats containing the factor. The edema syndrome is not produced by fat *per se* but appears to be related to impurities in certain lots of fats subjected to special fat-processing operations.

F. W. Hill of Cornell University reported to us that high-level feeding to chicks of a sample of a glycerol ester of oleic acid resulted in some mortality with gross symptoms resembling pericardial edema. Our studies have confirmed his observations and furthermore have shown that the pericardial edema-producing factor is present in many samples of commercially-available oleic acid as well as in glycerol esters made therefrom. Molecular distillation of an active oleic acid or of a glycerol mono-ester of an active oleic acid was found to concentrate the pericardial edema-producing factor.

TECHNIQUE

In this investigation a rapid, sensitive bioassay procedure, developed in our laboratories, was employed (5). The diet used is a modification of that described by Brew *et al.* (2) and consists principally of purified casein, gelatin, glucose, and 16% test fat. Day-old chicks are fed this diet *ad lib.* immediately on arrival. Subgroups are sacrificed at 10, 14, and 21 days. In general, a higher incidence of pericardial edema is observed in the subgroups sacrificed at 10 and 14 days than in the group sacrificed at 21 days. At autopsy, chicks are examined for the appearance of pericardial edema, ascites, and the appearance of complications, such as liver and kidney damage or labored breathing. Bioassay groups consist of 10 or 15 chicks for each sample of fat. Using a weighting procedure described in Table I, a pericardial edema-activity score is calculated. This makes possible a semi-quantitative comparison between the relative activities of various fat samples.

OCCURRENCE

Oleic acid samples (U.S.P.) from four manufacturers were assayed biologically and chemically (acid value and unsaponifiable content). As indicated in Table I, several of the oleic acid samples were strongly active, but others showed little or no activity. The pericardial edema activity scores showed that no correspondence existed between the presence of the pericardial edema-producing factor and the acid value or the content of unsaponifiables.

Tests for the pericardial edema-producing factor in certain commercial samples of glycerol esters (monoglycerides and monodiglycerides) of oleic acid are summarized in Table II. Seven different manufacturers of glycerol esters are represented. The majority of these ester samples were active. Again, there is no correlation between unsaponifiable content and pericardial edema activity.

In four instances, samples of both the oleic acid and the glycerol ester pro-

[1] Communication No. 265 from the Research Laboratories of Distillation Products Industries, Division of Eastman Kodak Company, Rochester, N. Y.

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Sample No.	Cut, percent	Analysis		Bioassay Results						Activity ratio[c] pos./total	Activity score,[d] percent
				Pericardial edema				Other deaths[b]	Negative		
		Acid value	Unsap. percent	Plus death	Plus complications[a]	Plus ascites	Uncomplicated				
TABLE I.—OLEIC ACIDS AND THEIR PE (PERICARDIAL EDEMA) ACTIVITY											
1		201.0	0.45						21	0/21	0
2		202.5	0.68		2	1			7	3/21	110
3		201.2	0.77			1			8	1/10	33
4		202.8	0.52			2			8	2/10	60
5		203.0	0.18	1	1	1			6	3/10	133
6		202.5	0.28		4		3		4	7/11	200
7		201.3	0.51						10	0/10	0
8		204.9	0.50				1		8	1/9	22
9		204.4	0.77		3	4	3			10/10	300
10		203.9	0.52						10	0/10	0
11		202.0	0.20						10	0/10	0
TABLE II.—OLEIC ACID ESTERS AND THEIR PE (PERICARDIAL EDEMA) ACTIVITY											
1		2.2			3	1	2		0	6/6	320
2		1.6	0.12			1	5		4	6/10	130
3		1.7	0.13	3	4	4	13		6	24/30	230
4		2.2	0.26						11	0/11	0
5							1		13	1/14	14
6		0.5	0.32			2			9	2/11	55
7		0.3	0.63						9	0/9	0
8		2.8	0.5	1			4	3	2	5/10	160
9		4.7	0.49	2	1		3		4	6/10	200
10		7.1	0.35			1			8	1/9	33
11		1.2	0.46					3	6	0/9	33
12		5.5	1.2				2	3	9	2/14	50

TABLE IV.—MOLECULAR DISTILLATION OF AN OLEIC ACID (SAMPLE 6, TABLE I)

Charge:				4		3	4	7/11	200
F-1	11.9						10	0/10	0
F-2	10.3								
F-3	29.1			1			8	1/9	45
F-4	29.6								
F-5	9.7								
F-6	7.2	2	1	5			2	8/10	290
r	1.2								

TABLE V.—MOLECULAR DISTILLATION OF A GLYCEROL MONO-ESTER OF OLEIC ACID (SAMPLE 2, TABLE II)

Charge				1	5		4	6/10	130
F-1	10.4	3	2	4			1	9/10	350
F-2	9.4								
F-3	30.6			1	1	1	6	2/9	67
F-4	31.5								
F-5	11.6								
F-6	2.8		1	2	1		6	4/10	120
r	1.0								

[a] Complications include gross liver and kidney damage or labored breathing.
[b] Early deaths in excess of control groups, PE not observed.
[c] Chicks with positive pericardial edema/total chicks in test.
[d] The "PE activity score" is a numerical index of the severity of the activity. The products of the number of chicks in each category times its weighting factor are summated and divided by the total number of chicks as follows:

$$\text{PE activity score} = 100 \times \frac{5\times(\text{PE}+\text{death})+4\times(\text{PE}+\text{compl.})+3\times(\text{PE}+\text{ascites})+2\times(\text{PE})+1\times(\text{other deaths})}{\text{Total chicks in test group}}$$

2,647

2,221

duced therefrom were bioassayed as summarized in Table III. The glycerol esters showed activity only when the corresponding oleic acid was active. With inactive oleic acids or those with low activity the corresponding glycerol ester did not have increased activity. Thus the edema factor was not formed during the manufacture of the glycerol esters.

CONCENTRATION STUDIES

Molecular Distillation of an Oleic Acid. A sample of oleic acid known to contain a moderate amount of the pericardial edema-producing factor was fractionated in a 14-in. molecular still into six fractions as indicated in Table IV. The first and last fractions and a composite of the middle fractions were bioassayed as described above. The first 12% strip cut was entirely free of the pericardial edema-producing factor. The middle cuts, representing approximately 60% of the input fat, were only slightly active. The last 7% fraction showed a concentration of the edema factor with an increased activity over the input oleic acid. Thus the pericardial edema-producing factor was concentrated in the last fraction after the bulk of the oleic acid was removed by molecular distillation.

Distillation of a Glycerol Mono-ester of an Oleic Acid. A sample of a glycerol mono-ester of oleic acid, prepared from an oleic acid known to contain the pericardial edema-producing factor was fractionated into six fractions in a 14-in. molecular still, as described in Table V. The first 10% cut, the last 3% fraction, and an intermediate fraction representing approximately 60% of the input material were bioassayed as described above. An increased concentration of the pericardial edema-producing factor was observed in the first 10% cut. Some pericardial edema-producing factor was present in the other fractions examined.

DISCUSSION

The material which produces pericardial edema in chicks has been reported previously only in certain lots of fats subjected to special fat-processing operations (1, 2, 3). Therefore the finding that some U.S.P. oleic acids possessed a high degree of activity was unexpected. Feed-grade fats that contained the edema factor characteristically had high unsaponifiable levels ranging above 6% (2). However, in the present study, samples of the more active oleic acids had unsaponifiable levels ranging from 0.2 to 0.7%. This indicates a 10- to 30-fold concentration of the edema factor in the unsaponifiable fraction and suggests that the unsaponifiable fraction of an active oleic acid might be a starting material for isolation of the factor.

Molecular distillation of a fat which contains the factor concentrated this factor in the more volatile fractions (2). Molecular distillation of an active glycerol mono-ester of oleic acid also concentrated the edema factor in the more volatile fractions, but separation from the monoglyceride fraction was not as complete as it was from the higher-distilling triglycerides. Molecular

TABLE III.—COMPARISON OF PE (PERICARDIAL EDEMA) ACTIVITY OF OLEIC ACIDS AND THEIR CORRESPONDING GLYCEROL ESTERS

Oleic Acid			Glycerol Ester		
Sample No. (from Table I)	PE activity ratio	PE activity score	Sample No. (from Table II)	PE activity ratio	PE activity score
	pos./total	*percent*		*pos./total*	*percent*
5 (75%)	3/10	133			
11 (25%)	0/10	0	2	6/10	130
5	3/10	133			
1	0/21	0	3	25/31	230
3	1/10	33	4	0/11	0
			5	1/14	14

distillation of an active oleic acid concentrated the edema factor in the less volatile fraction. Since fatty acids, such as oleic acid, distill at temperatures lower than the corresponding monoglycerides, the distillation range of the edema factor appears to lie above that of oleic acid and below that of the glycerol mono-ester of oleic acid.

SUMMARY

A number of samples of commercially-prepared (U.S.P.) oleic acids and glycerol esters of oleic acids were shown to contain a material which produces pericardial edema in chicks. The active material was concentrated from either the oleic acid or the glycerol mono-ester by molecular distillation.

Four of the 11 samples of oleic acid were inactive; this indicates that the pericardial edema is not caused by the fatty acid itself but by an incidental material in the fatty acid.

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STUDIES OF THE CHICK EDEMA FACTOR. II. ISOLATION OF A TOXIC SUBSTANCE[1]

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A crystalline halogen containing material producing chick edema symptoms at 0.1 part per million in the diet has been isolated from a sample of triolein which was toxic to monkeys. This material is similar to that reported by Harman *et al.* (4) but differs somewhat in ultraviolet spectral properties.

In a symposium on the chick edema disease in October, 1958, several laboratories (1,2,3) presented reports on their progress toward the isolation and elucidation of the toxic factor responsible for the occurrence of this unusual syndrome. It was established that the disease is caused by a toxic factor in the unsaponifiable fraction of a fatty by-product of industrial stearic and oleic acid manufacturing operations, and it was further suggested that the factor might posses a polynuclear or steroidal structure. A note, later published as an addendum to the contribution from this laboratory (2), reported that the toxic factor was associated with eluates from alumina and "silane-treated Celite" chromatographic columns which exhibited the ultraviolet absorption spectra of polysubstituted naphthalenes (λ_{Max} at 236 mμ, secondary λ_{Max} at 286 and 296 mμ). Neighboring cuts from these chromatograms showed the characteristic spectra of phenanthrene derivatives (λ_{Max} at 259, 282, 292, 300 mμ) and of simpler naphthalene derivatives (λ_{Max} 228–233 mμ, secondary λ_{Max} 270–280 mμ).

Subsequent purification of substances that had an absorption peak at 236 mμ demonstrated that they were not the most toxic fractions in our materials. Furthermore our computations showed that the toxic factor must be potent when present in the diet at levels of a fraction of one part per million, Recently Harman *et al.* (4) have reported the isolation of the chick edema factor in crystalline form from a feed-grade tallow. Their substance was toxic to chickens at 0.1 p.p.m. in the diet and had an ultraviolet absorption spectrum with a major peak at 244 mμ, a lesser peak at 312 mμ, and a shoulder at 238 mμ. A private communication from Tishler of the same laboratory (5) disclosed that the crystalline substance contains chlorine to the extent of about 47%.

Ames *et al.* (6) have observed the presence of the toxic factor in some commercial oleic acids. We have studied a sample of triolein which had been an

ingredient in a series of dietary treatments involving changes in the level and types of fats to which a group of Cebus monkeys had been subjected. The following summary of experimental results relative to these monkeys was received from O. W. Portman and S. B. Andrus of the Department of Nutrition, Harvard School of Public Health.

Of a group of nine monkeys that received this triolein in their diets at a level of 25% by weight, one died at one month and four at three months. After three months on the triolein diet corn oil was substituted for the triolein. The other four monkeys died from three weeks to five months later even though triolein had been discontinued and replaced by corn oil. Of 14 monkeys in the colony that did not receive triolein but were supplied other fats and oils at 25% of the diet by weight, there was only one spontaneous death. Eight of the nine monkeys fed triolein were autopsied and showed the following findings: jaundice (4.8?); pancreatic atrophy and fibrosis (6); hemosiderosis (6); fatty liver (5); bile duct proliferation (3); extramedullary erythropoiesis (3); necrosis of liver (2); gross hemorrhage in gastrointestinal tract (2); and erythrocytophagocytosis (1). Several features including marked anemia in several instances suggested the possibility of a hemolytic process. Pancreatic changes were most pronounced in the two monkeys that survived longest (seven to nine months from the beginning of triolein feeding). The severity of the lesions in the pancreas was unrelated to that of the hepatic changes. With the exception of fatty changes in the liver, the above findings have not been reproduced in rats. These observations are from an experiment not designed to study a toxic principle, and it would be unwise to draw firm conclusions with respect to the toxicity of the triolein from these limited data.

The fact that, in our laboratory, marked symptoms of chick edema disease were produced by this sample of triolein suggests the possibility that the chick edema factor may have been responsible for the toxic effects noted in the triolein-fed monkeys.

We now wish to report the isolation of a highly toxic crystalline substance from this triolein and to describe its properties.

EXPERIMENTAL

The triolein sample was of excellent quality with an unsaponifiable content of 0.87% and a steroidal hydrocarbon (1) absorbance of 0.07. Only 0.01% of oxirane oxygen (epoxide) was detected. Examination of the fatty acids as the ethyl esters by gas chromatography showed that oleic acid constituted 70.9% of the total acids with 3.6% linoleic, 0.3% linolenic, 13% palmitoleic, 9.4% palmitic and shorter-chain fatty acids, and 1.8% of a C_{17} fatty acid containing one double bond (by inference from relative retention time). Urea filtrate fatty acids were present to the extent of 4.73% (6a).

When fed to chicks at a level of 15% in the diet, the triolein produced the symptoms of chick edema disease with a severity approximately equivalent to that observed with a diet containing the toxic fatty product used in our original studies (2) at the 5% level. The unsaponifiable fraction of the triolein was proportionately richer in the toxic factor than any other materials available to us.

The toxic factor in 17.6 kg. of triolein was concentrated by molecular distillation under pressures of 10–20 microns at temperatures up to 200°C. The distillate (289 g.), which contained all of the toxic factor, was saponified, and 166 g. of unsaponifiable material were recovered. The portion soluble in petroleum ether (155 g.), was chromatographed on 3 kg. of Fisher Alumina A 540 in a 4-ft. x 3½-in. column, using petroleum ether as eluent. Two-liter fractions were collected, and substances with the absorption spectra of naphthalene and phenanthrene derivatives were eluted in fractions 6–15. The bulk of the material containing cholestadiene and related hydrocarbons (129 g.) was eluted in the first three days, and the remainder was recovered by the use of more polar solvents. These foreruns and tailings were devoid of potency in the chick edema test.

Fractions 6 to 15 were combined (668 mg.) and chromatographed on 2,500 g. of the more retentive Merck Alumina No. 71707. No material was eluted with petroleum ether. Foreruns were eluted with 1–2% ethyl ether in petroleum ether, and fractions exhibiting the spectra of naphthalene and phenanthrene derivatives (239 mg.) were obtained when 5% ethyl ether in petroleum ether was employed as eluent. This material was again chromatographed on Merck

alumina, using 250 g. in a 42 x 4.5-cm. tube. Several fractions which eluted with 5% ethyl ether in petroleum ether exhibited an absorption maximum near 245 mμ as well as the peaks previously observed at 234–236 mμ and 255–260 mμ. These fractions were combined, and the eluted material was partitioned on 5 g. of silane-treated Celite, by reverse-phase chromatography, using 4 ml. of iso-octane in the immobile phase, and 80% alcohol saturated with iso-octane as the mobile solvent.

Fractions exhibiting maximum absorption at 245 mμ, but not at 235 mμ or 255μ, were combined for two further chromatographic purifications on alumina. Merck alumnia was deactivated with 3% its weight of water, and the ratios of alumina to sample and column size 2,000:1 in a 25 x 1-cm tube and 25,000:1 in a 25 x 2-cm. tube, respecively. Iso-octane, redistilled and chromatographed over silica gel, was employed as eluent, and fractions were again monitored by ultraviolet spectral absorbance fractions devoid of inflection and 255 mμ were combined and evaporated to dryness. The solid residue was dissolved in a small volume of boiling iso-octane and stored over-night in the refrigerator. White crystals weighing 2.64 mg. were obtained.

The ultraviolet absorption spectrum of the crystals dissolved in iso-octane was characterized by maxima at 245 mμ ($E^{1\%}_{1cm.}=718$) and 300 mμ ($E^{1\%}_{1cm.}=50$) and inflections at 240 mμ ($E^{1\%}_{1cm.}=655$) and 310 mμ ($E^{1\%}_{1cm.}=76$). The substance sublimed on the hot stage about 239°C. The infrared absorption spectrum of the substance, incorporated into a potassium bromide disc, showed evidence of aliphatic and aromatic linkages but no bands characteristic of oxygen or nitrogen functions.

The reported presence of chlorine in the toxic substance isolated by the Merck group (5) prompted a Beilstein test, which showed the presence of halogen. The presence of halogen in the crystalline material being reported here was confirmed by examination in a microcoulometric gas chromatograph.[1] (In this instrument the sample is fractionated by gas-liquid chromatography, and, as they are eluted from the column, the compounds are pyrolyzed at 800°C. under oxidizing conditions. The halogen acid formed from halogenated materials is titrated in a microcoulometer.)

In the chick biossay the substance, when fed at *ca.* 1 p.p.m. in the diet, produced severe hydropericardium, hydroperitoneum, and liver damage, with death occurring within 12 days. At 0.1 p.p.m. in the diet marked hydropericardium was evident at autopsy after a three-week feeding period.

The isolation of the toxic substance had been complicated by the presence of another material with an absorption maximum at 248 mμ. This second substance was isolated by the same techniques of chromatography, monitored by ultraviolet spectrophotometry, and a yield of white crystals was obtained. The ultraviolet spectrum of this material was identical with that of the toxic substance except that it was shifted 3 mμ so that the major absorption peak was at 218 mμ. It behaved similarly to the toxic substance in the microcoulometric gas chromatograph, showing a similar retention time and a similar halogen content. However, it was completely inactive in the chick edema test when fed at 1 p.p.m. in the diet.

In order to obtain additional crystalline material the portion remaining from nondestructive testing, fortified with the adjacent fractions from the final chromatography (of high potency, judging from the ultraviolet spectrum), was further chromatographed according to the previous isolation scheme. Small quantities of phenanthrene derivatives were separated with a consequent diminution in the ultraviolet absorption in the 250–300 mμ region. Furthermore, although there was no indication of nonhomogeneity by paper chromatography, the ratio of absorbances of the shoulder at 240 mμ to the peak at 245 varied from fraction to fraction, suggestion the presence of an additional component.

The extinction values reported above may be in error because of inaccuracies in weighing on account of the difficulties in handling the small amount of material involved. There is no doubt however of the validity of the relative absorbances at various wave-lengths. The spectrum of the substance reported here differs somewhat from that reported by Harman *et al.* (4), particularly in the ratio of the absorbance at the maximum (244 or 245 mμ) to the absorbance at the inflection at 238 or 240 mμ.

[1] Dohrmann Manufacturing Company, Palo Alto, Calif.

DISCUSSION

The toxic substance which we have isolated from triolein resembles that recovered by Harman *et al.* (4) from animal feed tallows. However the divergences in their properties suggest either that we are dealing with two different but closely related compounds, or that one or both of the preparations is still a mixture of related compounds despite the fact that only a single spot could be obtained on paper chromatography in a number of solvent systems. In view of the manifest difficulties involved in isolating a pure compound in minute quantities from a myriad of substances with similar properties it would be hazardous, as Harman warns, to infer chemical structures from spectral data. Nevertheless it should be pointed out that the spectra obtained by us and by Harman *et al.* are strongly reminiscent of those exhibited by highly substituted naphthalenes (7). Furthermore the toxic factor occurs in association with a bewildering array of aromatic naphthalene and phenanthrene derivatives, as we have previously noted (2). The detection of chlorine in large proportions in a toxic preparation, and the ultraviolet spectrum observed, suggest a possible relationship with chlorinated naphthalenes. Pentachloronaphthalene possesses an an absorption maximum at 248 mμ and a secondary maximum at 312 mμ (8), and it has been shown to cause hyperkeratosis in cattle (9) and several other species of animals including chickens (10). Other chlorinated naphthalenes also are toxic (11).

The possibility that the chick edema factor is a chlorinated napthalene derivative cannot be ignored. Samples of tetrachloronaphthalene and hexachloronaphthalene, kindly provided by Engel and Bell of the Virginia Polytechnic Institute, who had demonstrated that these compounds could produce hyperkeratosis in cattle, were without effect in the chick edema test. Furthermore these compounds, despite the similarity of their ultraviolet spectra and their chromatographic behavior to the toxic substance, showed considerable difference in the microcoulometric gas chromatograph. For instance, chlorinated pesticides, aldrin and heptachlor, showed retention times of 10 min., tetrachloronaphthalene 9 min., and hexachloronaphthalene 14 min., whereas the toxic substance, as well as its inactive analogue with the absorption maximum at 248 mμ, had retention times of 37–38 min. It is tempting to speculate that the greater retention-time of the toxic material is related to a greater molecular weight or to a substituent conferring different solubility and polarity properties.

We are continuing our studies toward the isolation of the toxic factor. It is necessary that the chemical nature of this substance be elucidated to make possible a rapid chemical test for its detection, to clarify its origin, to verify the suggestion of its severe toxicity to primates, and to study its action in other species.

ACKNOWLEDGMENT

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Collaborative Bioassay for Chick Edema Factor*

By CARL D. DOUGLASS and DONALD F. FLICK (Division of Nutrition, Food and Drug Administration, Washington 25, D.C.)

A substance contained in certain processed fats and fatty products used in poultry feeds has been implicated in the outbreak of the condition called "chick edema disease" which occurred in 1957 (1). A number of laboratories are actively working to isolate and identify the agent responsible for the disease (2–5) and each has developed its own bioassay, which in each case has admirably served its specific purpose.

Ames and co-workers (6), early in 1960, reported this factor in oleic acid samples destined for human consumption. The pres-

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ence of this highly poisonous substance in human food products immediately resulted in a regulation from the Food and Drug Administration specifying that all such products must be free of "chick edema factor" in order to be incorporated in food. From the point of view of the regulated industry and the regulatory agency, the need to develop an assay method of adequate specificity and sensitivity, the results of which are comparable from laboratory to laboratory, can hardly be overemphasized. Since the chemical characterization of the substance has not proceeded far enough to provide the basis for a chemical or physical assay, it is necessary to use the bioassay. This method has successfully proved itself capable of providing for the detection and semiquantitative estimation of small amounts of this factor.

When the need for the standardization of a method became acute, investigators in eight laboratories that are currently engaged in some phase of work on the chick edema factor were invited to participate with us in a collaborative study and to submit suggested procedures. All graciously responded, and from the procedures which they forwarded, the authors selected what they considered the most desirable features of each and combined them into the procedure presented here.

Three collaborative test samples were prepared by thoroughly mixing a fat, known to be toxic, with cottonseed oil in such proportions that the amount of toxic fat in the samples was in the ratio of 1:2:4.

Each of the collaborators was sent the procedure given below as well as four collaborative test samples identified by number. Sample 1 was USP cottonseed oil; Samples 2, 3, and 4 contained, respectively, 1, 2, and 4 grams of toxic fat in 16 grams of sample. Report sheets suitable for recording the following specific information were also sent: chick number, initial body weight, final body weight, weight gain, heart fluid volume, presence or absence of peritoneal and subcutaneous fluid, and dates of early deaths.

Noteworthy features of the assay procedure are the use of white leghorn cockerels as the assay animal; a semi-synthetic ration in which the test samples are contained at 16%; and a 21 day feeding period. We included a request that groups be assigned to provide for observations to be made at the 14th day as well. The collaborators were asked to score the results visually, by use of a suggested scale of values, as well as by measurement of the pericardial fluid volume.

METHOD

Reagents

(a) *Salt mixture.*—

	G/60 G MIXT.
$CaCO_3$	15
$Ca_3(PO_4)_2$	14
K_2HPO_4	9
NaCl	8.5
Na_2HPO_4	7.3
$MgSO_4.7H_2O$	4.86
$MnSO_4.4H_2O$	0.42
Fe citrate	0.4
$ZnCO_3$	0.2
$CuSO_4.5H_2O$	0.02
$Na_2SeO_3.5H_2O$	1.2 mg

(b) *Vitamin mixture.*—

	AMOUNT, G
Folic acid (1.0% triturated in powd. glucose)	10
Biotin (0.1% triturated in powd. glucose)	7.5
Vitamin B_{12} (0.1% triturated in powd. glucose)	2.5
Niacin	2.0
Ca pantothenate	0.50
Thiamine	0.50
Riboflavin	0.375
Pyridoxine·HCl	0.200
Menadione	0.025
Celluflour (Alphacel), to make	500

(c) *Fat-soluble vitamin mixture.*—

	AMOUNT/KG MIXT.	AMOUNT/KG DIET
Vitamin A acetate, cryst.	900,000 USP Units	9,000 USP Units
Vitamin D_3, cryst.	100,000 IC Units	1,000 IC Units
D-α-Tocopheryl acetate	2.00 g	20 mg
Corn oil, to make	1.00 kg	10 g

Table 1. Summary data of the collaborative assay for chick edema factor

Coll.	Diet	Assay Period (Days)	No. of Chicks	No. of Early Deaths	Average Weight Gain, Total (g)	Average Hydropericardium, Visual Score	Average Hydropericardium, Fluid Volume (ml)	Average Hydropericardium, Score From Volume	Edema Incidence, Ascites	Edema Incidence, Subcutaneous	Edema Incidence, Hydropericardium
1	1	14	12	0	39.5 ± 3.2	0	0.054 ± .004	0	0/12	0/12	0/12
	1	21	12	2	73.0 ± 4.3	1+	0.12 ± .025	1+	1/12	0/12	1/12
	2	14	12	1	52.8 ± 4.3	0	0.11 ± .009	0	0/12	0/12	0/12
	2	21	12	1	78.2 ± 4.9	12+	0.26 ± .039	9+	0/12	0/12	7/12
	3	14	12	0	47.0 ± 3.2	7+	0.23 ± .09	3+	1/12	1/12	1/12
	3	21	12	2	69.4 ± 4.6	20+	0.92 ± .29	21+	3/12	2/12	10/12
	4	14	12	3	20.2 ± 3.3	20+	0.71 ± .25	15+	1/12	2/12	6/12
	4	21	12	5	39.4 ± 5.1	27+	1.58 ± .72	22+	6/12	3/12	10/12
2	1	14	6	1	26.2 ± 6.7	1+	0.028 ± .013	0	0/5	0/5	0/5
	1	21	5	0	59.6 ± 12.6	0	0.072 ± .013	0	0/5	0/5	0/5
	2	14	7	1	32.7 ± 9.2	9+	0.095 ± .021	0	0/6	0/6	0/6
	2	21	5	0	70.0 ± 4.3	10+	0.59 ± .24	7+	0/5	0/5	4/5
	3	14	6	0	39.8 ± 2.9	7+	0.155 ± .079	2+	1/6	1/6	1/6
	3	21	5	1	63.2 ± 4.7	15+	1.43 ± .49	14+	2/5	1/5	5/5
	4	14	7	3	39.3 ± 7.6	20+	2.05 ± .78	16+	5/7	6/7	5/7
	4	21	5	4	57.0 ± 9.6	15+	1.80 ± .66	15+	4/5	3/5	5/5
3	1	14	12	0	46.1 ± 2.0	0	0.023 ± .001	0	0/12	0/12	0/12
	1	21	12	0	104.1 ± 3.3	0	0.085 ± .016	0	0/12	0/12	0/12
	2	14	12	0	51.4 ± 2.5	4+	0.101 ± .018	1+	0/12	0/12	1/12
	2	21	12	0	118.2 ± 6.3	3+	0.145 ± .036	4+	0/12	0/12	3/12
	3	14	12	0	48.7 ± 2.6	12+	0.39 ± .13	9+	2/12	6/12	4/12
	3	21	12	1	89.5 ± 4.0	7+	0.28 ± .12	7+	1/12	8/12	5/12
	4	14	12	0	45.4 ± 3.3	14+	0.54 ± .21	14+	3/12	4/12	6/12
	4	21	12	1	86.1 ± 4.4	23+	1.46 ± .45	25+	8/12	9/12	9/12
5	1	14	12	0	42.3 ± 1.6	0	0.059 ± .008	0	0/12	0/12	0/12
	1	21	12	0	91.3 ± 8.9	0	0.081 ± .034	0	0/12	0/12	0/12
	2	14	12	0	43.5 ± 2.9	0	0.127 ± .006	3+	0/12	0/12	3/12
	2	21	12	2	41.8 ± 5.1	10+	0.306 ± .052	10+	1/12	1/12	8/12
	3	14	12	0	28.1 ± 2.1	1+	0.102 ± .021	2+	0/12	2/12	2/12
	3	21	12	4	35.0 ± 1.4	16+	0.66 ± .29	17+	10/12	8/12	11/12
	4	14	12	0	22.3 ± 1.8	11+	0.37 ± .17	9+	1/12	5/12	6/12
	4	21	12	8	32.3 ± 5.1	19+	0.80 ± .25	20+	8/12	9/12	9/12
7	1	14	12	0	22.6 ± 5.9	0	0.05 ± 0	0	0/12	0/12	0/12
	1	21	12	0	33.4 ± 10.0	0	0.054 ± .004	0	0/12	0/12	0/12
	2	14	12	0	11.2 ± 6.3	0	0.058 ± .005	0	0/12	0/12	0/12
	2	21	12	1	16.2 ± 7.2	8+	0.196 ± .093	3+	1/12	4/12	1/12
	3	14	12	2	25.4 ± 5.2	4+	0.166 ± .078	2+	1/12	2/12	1/12
	3	21	12	2	35.9 ± 10.3	10+	0.427 ± .203	9+	1/12	2/12	4/12
	4	14	12	1	12.4 ± 7.2	10+	0.391 ± .14	9+	3/12	5/12	5/12
	4	21	12	9	22.0 ± 15.6	6+	0.204 ± .078	6+	3/12	6/12	5/12
8	1	14	12	0	42.9 ± 3.8	0	0.061 ± .015	0	0/12	0/12	0/12
	1	21	12	0	100.8 ± 7.3	3+	0.093 ± .013	0	0/12	0/12	0/12
	2	14	12	0	54.5 ± 3.2	9+	0.125 ± .019	2+	0/12	0/12	2/12
	2	21	12	1	78.1 ± 4.7	15+	0.183 ± .023	4+	0/12	2/12	4/12

(*Continued*)

Assay Ration.—

	%(w/w)
Sucrose, *commercial*	20.3
Corn starch, commercial	20.3
Casein (vitamin-free)	20
Fat (USP cottonseed oil or assay fat)	16
Gelatin	13
Salt mixt., (a)	6
Vitamin mixt., (b)	2
Salt, iodized	1.2
Fat-sol. vitamin mixt., (c)	1
Choline chloride, 25% aq. soln	0.2

Treatment of Experimental Animals

Use day-old white leghorn, single comb cockerels. On day of receipt of chicks, tag individually, record body weights, and place in brooder cages equipped with heater. Use room with controlled temp. and humidity.

Offer control ration contg 16% cottonseed oil as fat and H_2O *ad libitum*. After 48 hr, weigh chicks and do not use any chick which is outside limit of mean body wt by ±5 g. Place 12 chicks in each brooder cage and record body wt and date of beginning feeding regimen.

Assay Period

Continue 1 group on control ration contg 16% cottonseed oil and substitute test fats for all or part of the 16% cottonseed oil in assay rations. Check chicks daily for fatalities and for presence of adequate food and H_2O. Record all deaths and cause of deaths. For each death due to other than accidental cause, autopsy and record presence of hydropericardium, hydroperitoneum, subcutaneous edema, and amount of heart fluid to 0.01 ml. Autopsy remaining chicks on 21st day and record findings as above.

Postmortem Examination for Quantity of Heart Fluid

(a) *Sacrifice of chicks.*—Sacrifice by means of cervical dislocation and proceed as follows:

(b) *Exposure of heart.*—With dissecting scissors make small transverse cut in skin over full diam. of abdomen. Peel skin toward head and lay skin fold over head. This cutaneous incision permits wide field exposure of subcutaneous area over thoracic and abdominal cavities for examination for subcutaneous edema. Skin may be reflected caudally to afford wider field of vision. Record (+ or −) evidence of subcutaneous edema.

Insert blunt tip of scissors thru body wall, and make transverse incision of musculature to lower rim of rib cage, avoiding cutting into organs of peritoneal cavity.

Lift breastbone with fingers, insert blunt tip of scissors, and carefully enlarge incision by cutting on each side of chest cavity thru rib joints up to clavicles. (Do not cut into subclavian vessels.) With sufficiently wide cut, fingers may be used to protract incised chest cavity and thus permit clear observation of substernal attachment of pericardium. Firmly clasp pericardial attachment with fingers and reflect flap of sternum so as to expose heart with pericardium intact.

Estimate visually and record severity of pericardial edema according to following table:

Pericardial Edema	*Score*
Absent	0
Slight	+
Moderate	++
Severe	+++
Very severe	++++

(c) *Withdrawal of heart fluid.*—*(1) For vols estimated as <1.0 ml.*—Firmly clasp apex of pericardium with small forceps and make small incision in heart sac. Insert small spatula into incision of pericardium and push heart to one side. Carefully aspirate fluid into 1.0 ml tuberculin syringe. Blunt-end 18 gauge needle permits more complete aspiration of small vols. To reduce formation of air bubbles during aspiration, prerinse needle and syringe with *n*-butyl alcohol. Do not include vol. of *n*-butyl alcohol in measurement of fluid.

(2) For vols estimated as >1.0 ml.—Insert sharp hypodermic needle on 10 ml syringe into intact pericardium. Aspirate as much as possible of the heart fluid (*n*-butyl alcohol rinse is unnecessary). Collect and measure remainder with blunt-end tuberculin syringe. Record total vol. heart fluid.

(d) *Other observations.*—Observe and record other obvious changes such as peritoneal edema (ascites), liver changes, kidney changes, etc.

Results

Seven of the nine cooperating laboratories submitted results in time for inclusion in this report. Their observations are compiled in Table I.

It is seen that mortality due to the toxic factor does not become appreciable until the third week on the diet containing the highest level of the toxic fat. Note that three of the

this value falls at the high end of the normal range of the volumes of pericardial fluid for chicks of the age and weight used in the bioassay.

From the observations reported by the collaborators it may be generalized that of the three anatomical sites in which fluid may accumulate, hydropericardium occurs with the greatest frequency, followed in order by peritoneal and subcutaneous accumulation. This is in agreement with earlier observations.

The appended charts (Fig. 1) show graphically the response of the volume of peri-

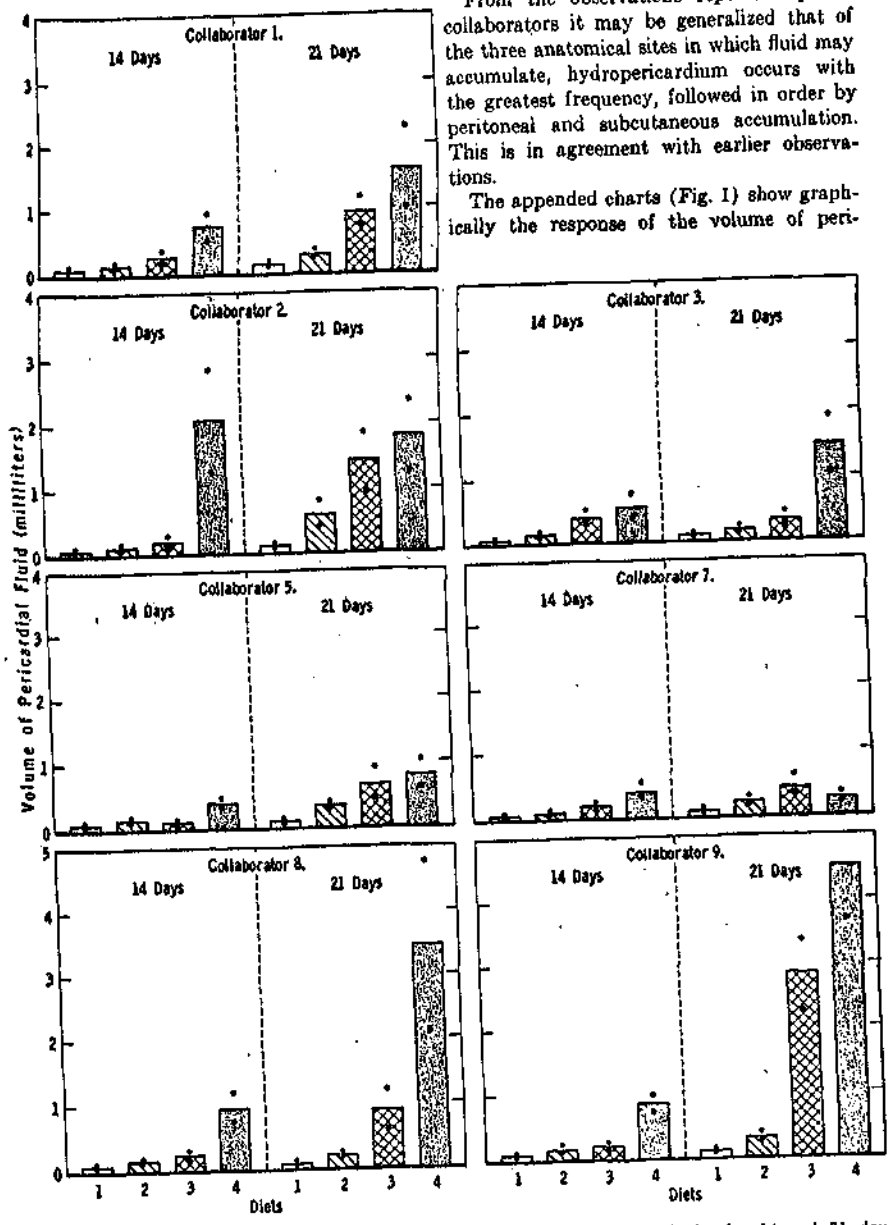

Fig. 1—Volume of pericardial fluid (± S.E. of the mean) versus level of toxic fat for 14 and 21 day feeding periods.

Table 1. (*Continued*)

Coll.	Diet	Assay Period (Days)	No. of Chicks	No. of Early Deaths	Average Weight Gain Total (g)	Average Hydropericardium Visual Score	Fluid Volume (ml)	Score From Volume	Edema Incidence Ascites	Subcutaneous	Hydropericardium[a]
	3	14	12	0	45.1 ± 2.8	13+	0.203 ± .066	5+	1/12	1/12	4/12
	3	21	12	2	72.5 ± 8.3	28+	0.878 ± .339	17+	4/12	7/12	8/12
	4	14	12	1	48.3 ± 5.4	28+	0.903 ± .303	20+	5/12	8/12	8/12
	4	21	12	5	75.1 ± 6.4	26+	3.44 ± 1.40	19+	6/7	6/7	7/7
9	1	14	12	0	54.6 ± 3.3	0	0.09 ± .01	0	0/12	0/12	0/12
	1	21	12	1	104.4 ± 9.6	0	0.10 ± .015	0	0/12	0/12	0/12
	2	14	12	0	57.3 ± 5.6	1+	0.15 ± .07	2+	0/12	0/12	1/12
	2	21	12	0	108.0 ± 7.1	10+	0.29 ± .05	10+	0/12	0/12	9/12
	3	14	12	0	63.3 ± 3.6	4+	0.19 ± .045	5+	0/12	0/12	4/12
	3	21	12	1	104.0 ± 11.8	36+	2.86 ± .55	42+	9/12	5/12	12/12
	4	14	12	0	60.4 ± 4.8	21+	0.84 ± .24	22+	6/12	2/12	11/12
	4	21	12	5	89.6 ± 6.7	35+	4.52 ± .86	34+	11/11	9/11	11/11

[a] Hydropericardium incidence: based on measurement of pericardial fluid.

collaborators reported deaths of four birds on the control diet. Since these deaths could not have been due to the factor, this and the foregoing observation indicate that mortality measurement alone is not a reliable index of the presence of the chick edema factor. It has been found that signs of chick edema disease are the development of hydropericardium, hydroperitoneum, and subcutaneous edema, in that order, as the dose of toxic material is increased. At higher dosage levels, death will occur from the 10th day. Birds dying of chick edema disease invariably exhibit these signs. Deaths unaccompanied by any of these signs cannot be attributed to the toxic agent.

Weight gains of the chicks were somewhat depressed at the higher levels of toxic fat. In the control groups, the average weight gains at the end of the assay periods were quite variable among the laboratories. This variability may be due to borderline nutritional inadequacies of the basal diet that possibly were aggravated by hereditary or environmental factors or a combination of both, as may have happened in the case of Collaborator 7's group. Perhaps it would have been wise to follow the suggestion of certain of the collaborators and to have an antibiotic incorporated in the test ration.

Visual subjective scoring of the degree of hydropericardium according to the instructions given in the collaborative procedure is seen to agree fairly well with a score calculated from the actual measurement of pericardial fluid as shown in Table 2. The figures given in Table 1 represent the sum of the plus scores for individual chicks within the group. It appears that there was greater accuracy in visual scoring of large toxicity responses than in the smaller responses. While visual scoring may be adequate in judging the presence of a large amount of toxic material, it is inadequate in those borderline cases where controversy is most likely to develop.

We have arbitrarily adopted 0.2 ml as the upper limit of normal heart-sac fluid volume. This choice is justified on the basis of the work of Shue and Gallo (7), who report that

Table 2. Scoring by Referee of pericardial edema

Volume of Pericardial Fluid	Toxicity Score
<0.20	0
0.21–0.40	+
0.41–1.00	++
1.01–2.00	+++
>2.01	++++

cardial fluid to the level of toxic fat in the diet. It is seen to vary directly with this level. It is likewise seen that an appreciably greater response to the dose is obtained after a three-week feeding period than after a two-week period. Although there is a high incidence of negative responses in a group of chicks on a low level of toxic fat, the average response of the group as measured by the volume of pericardial fluid is proportional to the dose level within the dose limits of this experiment.

Comments of Collaborators

Most of the comments were directed at the nutritional adequacy of the basal ration. One investigator has suggested that dry, stabilized vitamins A, D, and E be used rather than the crystalline products used in the procedure. This suggestion appears to be meritorious and has been incorporated in the recommended procedure as optional. Another objected to the mode of addition of choline to the diet and suggested a 25% dry, free-flowing preparation which is commercially available. For the sake of convenience, this has likewise been incorporated as optional. Objection was made by another collaborator to adding the water-soluble vitamins on Celluflour as the carrier. Since many laboratories routinely use other of the dietary ingredients as carriers for the vitamin mixture, we see no reason why any of the major components cannot be used for this purpose.

Weight gains obtained on the diet are not optimal. This, however, is not critical from the standpoint of the assay except, as one collaborator points out, that it is easier to carry out the procedure of withdrawing the heart-sac fluid from a larger bird than from a smaller one. Since the diet has given excellent pericardial fluid responses, major changes cannot be justified at this time.

Other collaborators have suggested that an antibiotic be incorporated in the ration, that crude casein be substituted for the vitamin-free casein, and that commercially available salt and vitamin mixtures be used. These appear to be desirable from the standpoints of economy and convenience and will form the basis of a modification of the diet to be used in further collaborative work. The level of sodium chloride was likewise the subject of question on the basis that 2% of salt is too high and subjects the chick to extra "stress." It has been found in our laboratory that this level favors the development of the edema. Selye and Stone (8) have found that extra sodium chloride in the diet of chicks accentuates the development of edema produced by certain steroids. Biester and Schwartz (9) state that 6–8 grams of sodium chloride per day is not harmful to 9 week-old chicks. We cannot see that a change here is justified.

One collaborator reported that the ration was unpalatable to his chicks. The weight gains reported by him were much lower than in any other laboratory. We have no explanation for this effect. It is suggested that this laboratory may have some peculiar problem and that the performance of these chicks is not typical.

Several of the collaborators preferred to use heavy breeds of chicks rather than leghorns. We have retained leghorns for the reasons of uniformity of response, widespread availability, convenience, and the demonstrated sensitivity of this breed.

Summary and Recommendations

A successful collaborative study of a procedure for the detection and assay of the chick edema factor in fats and fatty materials has been carried out. The results indicate that the method as studied is satisfactory for the intended purpose. Although it is demonstrated here that the method studied is capable of differentiating contaminated from uncontaminated fats, it would be desirable to carry out additional collaborative studies on samples of lower potencies than those used here.

It is recommended[1]—

(1) That the method for bioassay of chick edema factor, presented in this report, be adopted as first action.

(2) That collaborative studies be continued.

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(1) Editor's Note, *This Journal*, 42, 120 (1959).

[1] These recommendations were approved by the General Referee and by Subcommittee C, and were adopted by the Association. See *This Journal*, 44, 70 (1961).

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Progress in the Chick Edema Problem

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It has been almost four years since I first became aware of the problem that we know today as "chick edema disease." Because of our activity on this problem, my colleagues and I have had the opportunity to become acquainted with many scientific groups and individuals working in the same area with whom we have enjoyed a fruitful cooperation and pleasurable association. We hope that they feel as kindly toward us as we do to them, but sometimes, I known, they wish as we do that they had never heard of chick edema disease.

This problem has been most difficult and progress frustratingly slow. Despite the meager amount of new information that can be added at this time, especially since the most recent advances were reported by Dr. Artman recently, it is nevertheless worthwhile to review the several aspects of this problem and see its present status in full perspective.

As you recall, during 1957 an epidemic disease caused millions of dollars in losses among broiler flocks throughout a large part of the U.S. After elimination in succession of all other possibilities, attention was focused on the fat ingredient of the feed as the etiologic agent. A series of reports in 1958 from several laboratories described the manifestations of the disease and definitely implicated a toxic fat or a toxic substance in fat as the cause. The characteristic symptoms were droopiness, ruffled feathers, labored breathing and high morbidity and mortality. Autopsy findings revealed hydropericardium, abdominal ascites (water belly), subcutaneous edema, swollen liver, swollen and pale kidneys, etc. In laying hens the toxic fat caused a rapid drop in egg production. Pullets receiving toxic fat during the full growing period did not come into production, and mortality was very high. Hydropericardium, the most common lesion found in young birds, was not found in birds of laying age.

SYMPTOMS DIFFERENT

These differences in susceptibility and symptoms in different age groups of the same species should be noted. The feeding of toxic fat to other species has not produced such striking results as with young chicks, with the exception possibly of monkeys. However, every species that has been tested has shown evidence of deleterious effects. Very little work has been done with rats. Our very limited experience indicates that they are much more resistant than chicks in short-term feedings, but that when fed in sufficient dosage, extracts of the toxic fat produce definite deleterious effects as shown by growth depression, enlarged and fatty livers, and marked involution of the thymus.

I recall few reports of the effects of toxic fat on swine, but again, in our own limited experience we have seen depressed growth . . . and have demonstrated the presence of toxic factors in the meat of hogs that had been fed toxic fat.

I am indebted to Dr. Wilcke of Ralston Purina for reports of studies on guinea pigs and dogs. Guinea pigs fed 2½% toxic fat stopped growing after six weeks, and death losses occurred after eight weeks. At a level of 4½% toxic fat weight losses occurred after three weeks and deaths after four weeks. Control groups receiving non-toxic fats did not show weight loss or deaths. The only observed pathology at the conclusion of the experiment was congestion of the lungs and mottled livers.

In experiments with three different breeds of dogs, using Purina Dog Chow in which 10% of toxic fat was substituted for the usual normal fat, there was poor reproduction and lactation performance. The females on the toxic fat ration whelped pups that were dead or weak, and, furthermore, the mothers seemed to have an insufficient milk supply. When the pups were removed before weaning and fed a normal ration the increase in growth was immediate and dramatic. Also, the females on the toxic fat ration tended to lose hair on their backs and shoulders. With the ration containing toxic fat, post-weaning growth tests (6–18 weeks) with five litters of pups demonstrated inferior growth performance using either weight gain or increases in body length as the criterion.

WORK WITH OTHER SPECIES

In these experiments with other species, fat that had first been proved to be toxic to chicks had been used. In the case of monkeys, a sample of triolein

had produced irreversible toxic symptoms for no apparent reason [illegible] was proved toxic to chicks and was the source from which we isolated a highly purified crystalline "chick edema" factor. At the present time, then, "toxic fat" that produces "chick edema disease" has been demonstrated also to produce deleterious effects in rats, guinea pigs, swine, dogs and monkeys. It should be emphasized that the toxic fat undoubtedly contains many other substances that may have effects in these other species. Definitive information as to the effect of "chick edema factor" (CEF) in other species must await tests with purified CEF. Triolein-fed monkeys probably received the "purest" source of CEF. However, purified CEF should be administered to monkeys to verify the implication that the severe toxic symptoms observed with triolein were due to CEF.

Relatively little has been done to throw light on the mechanism of the toxic effect. Flick and Gallo in our laboratories have reported that in young chicks showing symptoms of the disease, the intra-cellular water was not changed. Neither were the total blood and plasma volumes altered so that the observed edema was primarily interstitial. Hemoglobin and hematocrit levels were low and blood glucose levels were decreased in advanced stages of the disease. Plasma sodium, potassium and chloride were not affected. In the liver, neutral fat was decreased and phospholipid increased. Preliminary experiments by Flick give some indication of increased membrane permeability, but these must be repeated under much more rigorous conditions.

DISSERTATION ABSTRACT

A most interesting study in chicks has been reported by J. R. Allen, Jr. Part of the results were presented at the federation meetings in March, 1961, and the complete study is available as the dissertation of J. R. Allen, Jr., University of Wisconsin, 1961. I will quote portions from the dissertation abstract [Dissertation Abstracts 22. [2], 545 (Aug., 1961)]:

"Experiments conducted to determine the effects of 'toxic fat' on mice, pigeons and turkeys demonstrated a reduction in growth without hydropericardium or ascites."

In chicks, "Microscopic examination of the tissues of the test animals revealed lymphocytic foci in the epicardium and myocardium. Edematous fluid separated the myocardial fibers. Edema of the lungs was a frequent observation in the experimental birds.

". . . Blood pressure indicated the test birds had an elevated average mean pressure in the right ventricle of 6 cm. water and 2 cm. water in the vena cava. Electron micrographs of the myocardium revealed shrunken, vacuolated mitochondria in the test animals.

"'Toxic fat' produces a reduction in growth rate of experimental animals. This reduction depends on the age of the animal and the level of 'toxic fat' added to the diet. Hydropericardium and ascites are a frequent lesion in the animals receiving from 1.0 to 5.0% 'toxic fat.' When this level was reduced to 0.25% in the diet, reduced testicular development was a more sensitive criterion than hydropericardium, ascites or weight gain for evaluating chronic toxicity.

"The mechanism by which 'toxic fat' induces hydropericardium and ascites appears to be associated with degeneration and edema of the myocardial fibers. These data would tend to eliminate the kidneys, liver and endocrines as the primary cause of edema. The early development of hydropericardium, increased venous pressure, enlarged hearts, mitochondrial changes in the myocardium and generalized edema suggest that the myocardium may be directly inhibited; however, altered capillary permeability has not been excluded. It is believed that cardiac decompensation and increased capillary permeability act together in producing the excessive extravascular fluid collection and the demise of the animal."

The wide range of susceptibility within and among species and the variety of toxic effects that have already been noted would make it appear logical that some primary unit of structure and function such as the mitochondrion, may be the target of the toxic factor and that the observed differences may be explained by factors such as absorption, specific binding, transport, detoxication, etc., that determine the local concentration of any substance in a specific site.

Another possible mechanism that had suggested itself quite early is that the toxic factor interferes with the normal regulation of electrolyte and water balance. Selye and Stone, back in 1943, described the production of edema symptoms in chicks by certain steroids and the accentuation of these symptoms by increasing the salt intake of the chicks. Alexander has shown that it is possible to produce hydropericardium in chicks by increasing the NaCl in the ration and to prevent its occurrence even with CEF by eliminating NaCl from the diet. Work in our own and other laboratories has illustrated beautifully the interaction of nutritional factors on the susceptibility of the chicks to a toxic agent. For example, the present AOAC bioassay diet for "chick edema factor" is probably four times more sensitive than the assay diet we used originally, although both have approximately the same NaCl content.

A point to remember is that hydropericardium as a symptom of toxicity in chicks is not new. In addition to NaCl and certain steroid hormones, chapter 40 in Biester and Schwarte on Poisons and Toxins indicates that:

(1) Zinc phosphide, used as a rodenticide produces "various degrees of congestion with the accumulation of some serous fluid in the pericardial sac as well as in the abdominal cavity in some cases."

(2) Alpha naphthyl thiourea, the rodenticide ANTU, shows in poisoned chicks evidence of lung edema and excessive quantity of fluid in the pericardial sac.

(3) Sodium monofluoroacetate, compound 1080, another very effective rodenticide, produces in chicks distention of the pericardial sac with clear straw-colored fluid, in addition to other marked pathological changes on the heart and lungs.

(4) Chlordane. "The primary lesions found in all fatal cases were in the heart. Excessive quantities of fluid were found in the pericardial sacs. . . ."

The weed "corn cockle" and several species of Crotalaria produce seeds which are toxic, and in chicks the toxic symptoms include hydropericardium.

However, in each case other characteristic pathology is usually present, and in no case is the purified toxic principle of the same high order of activity as the toxic substances that have been isolated from toxic fats.

It is well known now that chicks can efficiently utilize large amounts of fat in properly balanced rations. The use of fat as a standard ingredient of poultry feeds grew as the price of fat calories dropped and became competitive with calories derived from corn. The chick edema disease epidemic of 1957 was a totally unexpected consequence of this growing practice. Many of you are familiar with the story of how the toxicity was associated with a fatty by-product of stearic and oleic acid manufacture that had been blended with feed grade fats.

Very large quantities of fatty acids are used industrially in the manufacture of lubricants, rubber, paints, asphalts, roofing, chemicals, and to a much smaller extent in foods. Relatively low grades of fat are split into fatty acids and glycerol at high temperatures and pressures, sometimes with the aid of catalysts. The glycerol is recovered and the fatty acids are distilled under vacuum. The first distillate may be used directly as the highest grade of mixed fatty acids or it may be separated by a low temperature crystallization process into stearic (saturated) and oleic (unsaturated) fractions. The residue from this distillation is resplit and redistilled. The second distillate yields a lower grade of fatty acids. The residue from the second distillation is usually suitable for use on highways or in rubber manufacturing, but occasionally it is again recycled to obtain a third distillate and another residue. It was this third residue that had been blended with feed grade fat for use in feeds.

Every sample of residue of this type from several manufacturers of fatty acids proved to be rich in chick edema toxicity. Our first impression, therefore, was that the toxic factor was produced during the splitting and distillation steps and that it was concentrated in the residue along with other non-volatile unsaponifiable substances. Closer study of the various stages of fatty acid production soon revealed that the toxic factor was distillable and was present to some extent in the first distillates which were used for the production of the best grades of fatty acids, and those intended for food purposes. This discovery was made independently and reported by Ames, et al., who had found several samples of oleic acid and a monoglyceride made from such an oleic acid to be contaminated.

All this happened just after the passage of the Food Additives Amendment. The fatty acid manufacturing firms were very cooperative in providing us

[redacted] information and samples. However, they sincerely believed that they were not part of the food business, that the bulk of their production went into non-food industrial purposes. In our visits and talks with their technical people we advised them to study the application of the new legislation to their industry. They realized that a substantial proportion of their highest grade of materials did enter food channels when their customers started asking for guarantees that the fatty acids met the requirements of the Federal Food and Drug Law. At that point the technical committee of the Fatty Acid Producers Council became actively engaged in a study of this problem, and we have enjoyed the whole-hearted cooperation of the fatty acid industry. While they made studies to determine what part of their processes were responsible for the production of the toxic material, studies were continuing on the isolation and chemical characterization of the active substances. Three years ago at the AOAC meeting a symposium on chick edema was held at which reports from the Purina laboratories, the Quaker Oats laboratory, the Procter & Gamble research laboratories and the Food and Drug laboratories described the progress made up to that time. The Merck group had recently entered the problem, and, although they did not report, they also had traveled a similar road.

Every step and experiment had to be followed by the chick bioassay. However, during the first year all the groups had made steady progress at about the same rate. First it was demonstrated that the "chick edema" toxicity was entirely in the unsaponifiable portion of the fat. Then, in our work, the unsaponifiable was separated by chromatography on alumina into three fractions of increasing polarity by elution with petroleum ether alone, then with a mixture of ethyl and petroleum ether and finally with 100% ethyl ether. The first, or hydrocarbon, fraction contained 83% of the unsaponifiable, and its ultra-violet absorption spectrum indicated the presence of cholestadiene. The second fraction was characterized as ketonic and infrared absorption spectra suggested the presence of dipalmitone. The third fraction consisted of sterols and oxidized materials. Simultaneous bioassay of these three fractions and synthetically prepared samples of cholestadiene and dipalmitone showed that only fraction 2, but not the dipalmitone, was toxic.

Several approaches were tried simultaneously to purify fraction 2. I will not take the time to describe our experiments with a 500 tube counter-current distribution between iso-octane and methanol, or the separation of carbonyls with Girard-T reagent or the consecutive rechromatography on alumina and silane treated Celite. Suffice it to say that as our fractions became more potent we relied more and more on ultra-violet spectrophotometry as an indication of concentration and purity. Two distinct types of fractions were obtained, one with the ultra-violet spectra characteristic of naphthalene compounds and the other of phenanthrene compounds. Both fractions contained the toxic factor. However, the sum of their toxicities was not comparable to that of the starting material in the final purification steps despite the fact that practically all of it was accounted for by weight in the eluates. Since only the fractions that had a significant weight had been tested biologically, the insignificant, hardly visible residue of less than 0.5 mg. in the practically "empty" beaker that represented the cut between the two major fractions was rinsed into a chick diet and to our surprise was very active at approximately 0.1 ppm., representing the most potent material we had obtained. At this stage we had practically exhausted our raw material, and we had to start from the beginning once again.

During the course of these studies we had learned that Drs. Portman and Andrus in the department of nutrition at the Harvard School of Public Health had lost a number of monkeys in a nutritional study. They had used a synthetic triolein as the source of fat and had to abandon the experiment. At a Gordon Research Conference I had the opportunity to discuss this experience with Dr. Portman. Fortunately about 40 lb. of the triolein was still available. It proved to be toxic in the chick edema assay. The triolein was of excellent quality, containing only 0.0% of unsaponifiable material. This unsaponifiable proved to be the richest source of the toxic material we had ever examined.

The toxic fraction was separated from the triglyceride by molecular distillation. The distillate from 17.6 Kg. of triolein was saponified, and the unsaponifiable was chromatographed on alumina to remove the cholestadiene fraction. The naphthalene and phenanthrene-containing cuts were collected, yielding 670 mg. of material. This fraction was rechromatographed on more retentive alumina with 5% ether in petroleum ether, and 240 mg. of naphthalene-phenanthrene material was separated. This was rechromatographed in the same system and three distinct types of U.V. spectra began to emerge in the fractions: Naphthalene (235 mμ), phenanthrene (260 mμ) and a new peak at 245 mμ. These materials were combined and chromatographed on a silane treated Celite-iso-octane column, with 80% alcohol saturated with iso-octane as the mobile phase. The cuts with maximum absorbence at 245 mμ were further purified by two more chromatographic treatments on alumina at a very high sample: adsorbent ratio (1:2,000 and 1:25,000). The final fraction was completely free of absorption peaks near 235 and 260 mμ. This fraction was evaporated to dryness, dissolved in a small volume of boiling iso-octane and stored overnight in the refrigerator. White crystals were obtained, weighing 2.6 mg. and representing a concentration over the original triolein of three million-fold. This material at 1 ppm. in the diet killed the chicks in 12 days and produced typical hydropericardium when fed at .05 ppm. in a 21-day test.

During the last stages of this work the Merck group [Harmon, et al., JACS 82, 2078 (1960)], announced the isolation of a crystalline chick edema factor from a toxic fat. On July 19, 1960, the Merck group informed us that they had found 47% chlorine in their crystalline material. The presence of chlorine in our material was quickly confirmed by the use of the Dohrmann microcoulometric gas chromatograph.

In this test, a few micrograms of sample is injected into a gas chromatograph and the components as they emerge are pyrolyzed at 800° under oxidizing conditions. The halogen acid formed from halogenated materials is titrated automatically in a microcoulometer.

Of interest also is the isolation of a crystalline material that had an ultraviolet absorption spectrum identical with that of the toxic material but shifted 3 mμ so that the major peak was at 248 mμ instead of 245 mμ. It behaved like the toxic substance in the microcoulometric gas chromatograph, showing a similar retention time and halogen content. However, it was completely inactive in the chick edema test when fed at 1 ppm. in the diet.

The finding of chlorine in the toxic substance was a major breakthrough. It was no longer necessary to think in terms of a naturally occurring material that had changed chemically under the conditions of industrial fatty acid production. The possibility of contamination with one of many familiar chlorinated hydrocarbons was obvious. Samples of tetrachloronaphthalene and hexachloronaphthalene, kindly provided by Engel and Bell of Virginia Polytechnic Institute, who had demonstrated that these compounds produced hyperkeratosis in cattle, were without effect in the chick edema test. We have tested a long list of chlorinated compounds including aldrin, dieldrin, lindane, DDT, DDE, BHC, chlordane, toxaphene, methoxychlor, and a series of Halowaxes, without any definitely positive indication. Furthermore these have all been heated with oleic acid at 250° C for long periods and then fed to chicks, with negative results. Also, on the theory that tallow may sometimes be bleached with active chlorine materials which may chlorinate a sterol nucleus, we have chlorinated cholesterol, squalene, estone and equilenin with negative results. To test the theory that the toxic substance is a metabolite of a chlorinated insecticide we have fed large doses of chlordane, methoxychlor, heptachlor, aldrin and dieldrin to rats for a month and are feeding the unsaponifiable extract of the rat carcass in the chick test. The results to date show that chlordane and methoxychlor produce no response.

INDUSTRIAL EXPERIMENTS

Similar experiments have been carried out and are still in progress by at least two industrial laboratories under conditions of fatty acid production. The results so far have been largely negative or at best only suggestive but not clear cut. Dr. Artman has reported that chlorination of naphthalene and phenanthrene by substitution reactions has produced CEF active products. The chlorinated phenanthrene is particularly promising, since preliminary fractionation experiments indicated the possibility that a highly toxic compound was produced.

Dr. Boyd O'Dell and colleagues at the University of Missouri observed hydropericardium and other symptoms of chick edema disease in chicks housed in freshly painted cages. They traced the responsible agent down to one of the paint ingredients, a chlorinated biphenyl sold under the name

[illegible]lors of different chlorine content. Some were not toxic; others produ[illegible] the disease but only at relatively high feeding levels, e.g., 200 ppm. It may b[illegible] that an impurity in these compounds is the toxic agent, or that they are innately toxic at the high levels fed. Of interest is the use of one of these Arochlors in some insecticide formulations. At the present time there has been no real evidence developed to implicate any product or compound. However, the circumstantial evidence is stimulating considerable speculation and activity.

The feed industry has managed by careful control of ingredients to avoid a recurrence of the 1957 epidemic. The color test developed by Brew, et al., at Purina has been very useful in screening out toxic fats. This test is not specific but is useful since the presence of large amount of steroidal compounds that give this test usually indicates still residue that may be toxic. It is useless, however, for fatty acids and other products such as triolein, monoglycerides, etc., that give no response in this test, but which sometimes are quite toxic.

GEORGIA OUTBREAK

Last year, at this time, an outbreak of the chick edema disease occurred in Georgia. Although considerable chlordane residues were found in the feed, we do not believe they were responsible for the symptoms observed. Furthermore, the best information we have indicates that only rendered fat was used in this feed and no product of the fatty acid industry was involved. With each new development the scope of the problem increases. First we were concerned only with still residues, then also with fatty acid distillates and their derivatives. For a long time it was felt that only the fat derived from animals was involved. Recent evidence from sources in the fatty acid industry and our own studies indicates that some vegetable fat sources may yield fatty acids contaminated with CEF.

Still another development has occurred to further complicate the picture. Early this year in the course of our regulatory activities, we examined a sample of oleic acid. All the test chicks died by the end of the second week, with symptoms of severely stunted growth, ascites, jaundice and pathology of the liver and other organs, but with no hydropericardium. This sample had been tested by the manufacturer last year before the adoption of the present AOAC test procedure. The testing laboratory had used the procedure we ourselves had used in our earlier work, and had found the sample negative for chick edema. Repeat of the test in both laboratories by both procedures confirmed both findings. The sample is free of chick edema disease factor when tested on a diet of natural ingredients, and the chicks survive in apparent good health. On the casein-sucrose diet of the AOAC chick edema test, the chicks fail to grow and die early with the described symptoms but no hydropericardium. Furthermore, the dose response curve for this effect is very steep, since the ratio of the dose that gives a maximum effect to the dose that produces a minimum effect is less than two, as compared to a ratio of four to five for chick edema factor. Evidence from preliminary fractionation studies also indicates that this is an entirely different substance. There is no other information as to its characteristics at this time. There is evidence that this toxic contamination has occurred in different places from time to time and in a variety of fatty acid samples and may occasionally occur together with CEF. Here again we must anticipate that this material may occur elsewhere independently of the fatty acid industry.

For whatever comfort we may derive, it should be noted that chick edema disease has been observed in England. In a letter to the editor of the Veterinary Record of June 10, 1961, C. C. Wannop of the Houghton Poultry Research Station draws attention to a condition apparently identical with that reported by Sanger, et al., and Schmittle, et al., in 1958, that has appeared in several broiler flocks. In a personal note dated Sept. 29, he says that the condition has disappeared for the time in his country.

At the present time fatty acids can be used in the manufacture of foods or food ingredients only if they are free from CEF. This requirement made necessary the development of a bioassay which has been accomplished by collaborative work and is now being adopted as official by the AOAC.

I have tried to review the history of this troublesome problem, what little is known of its toxicology and its physiological aspects, the speculations as to the origin of the contamination, and attempts to track down its sources. I have sketched quickly our own attempts at isolation and identification of the toxic factor and have alluded to the most recent developments along this line that were reported at the AOAC Section of Fats and Oils by Dr. Artman.

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Occupational Intoxication Occurring in the Production of Chlorophenol Compounds by H. Bauer, K. H. Shulz and U. Spiegelberg, Hamburg

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INTRODUCTION, GENERAL

In the last few years, professional work in the production of chlorinated phenols has been the cause of group outbreaks of acne in at least three West

German chemical works, the acne persisting over a long period and being associated with other effects on the health.

Diseases in a group of 17 workers from a company in North Rhine/Westphalia were reported by BAADER and BAUER, as well as by BRINKMANN in 1950/51. The workers in that company were engaged in the production of pentachlorophenol. Apart from comedone acne with various degrees of secondary pustular infection and boils, most of the workers, whilst still in the first stages of the skin diseases, also experienced pain and weakness in the lower limbs, mild paraesthesia, heart complaints and indeterminate psycho-vegetative disturbances. Subsequent examination of the records of 17 cases[1] revealed the following findings:

All 17 were suffering from an acne, 4 of these being very severe, 8 fairly severe, and 5 moderately severe to mild. In almost all cases there were extensive pustular infections and boils, 4 with bursitis on the elbow.

Other disturbances amongst the workers included 11 cases of bronchitis, 6 of myocardiac damage, 2 of cirrhosis of the liver (one of which proved fatal), 9 of neuritis symptoms (severe pains in the lower extremities in 7 patients, sensibility disturbances in 4 cases, mild paresis without atrophy in 2 patients, and 2 cases of weakening of the Achilles' reflex). Seven workers complained of physical conditions such as continuous fatigue, depression, lack of vitality, nervousness, slight headaches, disturbed sleep, and decrease in libido and potency.

A larger number (about 60 cases, Prof. Hergt) of similar conditions occurred in two Mid-Rhenish companies amongst workers who had been engaged for long periods, generally several years, in the production of trichlorophenol (saponification of 1,2,4,5,-tetrachlorobenzene to 2,4,5-trichlorophenol by treatment with methanolic caustic soda solution). These trichlorophenol workers, like those in a third group of affected persons from the Hamburg region who will subsequently be dealt with in more detail, suffered from further disturbances to health, these often not occurring until a fairly long time after occupational exposure had ceased. In the course of a discussion on a paper by SPIEGELBERG, who referred briefly to our Hamburg cases in a lecture at the 1960 North-West German Neurologists' and Psychiatrists' Congress in Lüneberg on psychopathological delayed and chronic damage following occupational intoxication. Janzarik described largely identical disturbances amongst workers from the Mid-Rhenish companies.

The third group comprised 31 workers in a Hamburg company. Those of this group who were affected were engaged in the trichlorophenol department of the company, in which the herbicide 2,4,5,-trichlorophenoxyacetic acid was manufactured from technical 2,4,5-trichlorophenol by heating trichlorophenol together with caustic soda solution and monochloroacetic acid in autoclaves. After completion of this esterification process, the end product was purified by double recrystallization. The task of the workers consisted first of all in charging the autoclaves, for which purpose the trichlorophenol in flake form had to be removed by shovel from open barrels. In this operation, a fine dust formed and dispersed throughout the room.

Other operations were concerned with filling and controlling centrifuges and regulating feed and outlet pipes. Since it was the workers most exposed to contact with trichlorophenol who suffered from the severest skin conditions, it was logical from the outset to suspect the causal noxa to be present in the trichlorophenol. The extent to which this assumption was valid is discussed later in this paper in connexion with etilogy.

SOME CLINICAL OBSERVATIONS

Of the 31 workers of this Hamburg company, 9 are still receiving medical attention 5 years after the termination of occupational exposure, this being due to residues of their acne, chronic neuromuscular weakness of the leg musculation, vaso-vegetative lability and, most especially, marked psychopathological disturbances. Details of the established complaints and damage to health are given in the table. The development of the skin conditions in the patients followed, by and large, a uniform pattern. Numerous comedones formed, first on the face, especially on the cheeks above the malar bones, forehead, temples, chin and ears, after which folliculitis, pustules, boils and retention cysts occurred as a result of secondary infections. As the disease progressed, these symptoms spread in the majority of patients, especially to the sides of the neck, back of the neck, upper half of the back, chest, forearms, genitals and thighs. Numerous boils formed, particularly on the back of the neck and on the back. The efflorescences were generally located so closely together that scarcely any follicles remained unchanged.

In certain workers who had apparently been more strongly exposed, the development of these acne-like symptoms preceded a dermatitis associated with erythema and swelling, this extending to the region of the eyes, the cheeks and the forehead. At about the same time, blepharoconjunctivitis occurred in several patients, this, like the skin symptoms, becoming chronic in some cases.

As the table of findings shows, spots or, in certain cases, patches of pigmentation occurred in the faces of some patients, these giving the skin a dirty, greyish-brown appearance.

The overall clinical picture was identical to the symptoms occurring after working with chlorinated naphthalenes, diphenylenes and other aromatics as first described by HERXHEIMER (1899) and subsequently by several other authors (BETTMANN, HOLTZMANN, TELEKY, HERZBERG, BRAUN, GRIMMER, etc.). (For further details, see W. BRAUN and A. RISSE-SUNDERMANN (1959)). Although not a completely exact description, the designations "chloracne" and "perna disease" have become the most popular for these forms of occupational intoxication.

TABLE OF FINDINGS

Skin and mucous membranes: Dermatitis of the face in initial stage; comedones, retention cysts, nodules, pustules, boils; patches of pigmentation; blepharoconjunctivitis.

Internal organs: Loss of appetite; abdominal complaints; loss of weight; reduction in general condition; altered acidity of the gastric juice; gastritis; damage to liver; pulmonary emphysema, dyspnea; myocardiac damage; blood pressure; edema; pathological urine finding (renal damage).

Nervous system.—Neurological: Muscular pains; weakness in legs; (general) fatigue; increased sleep requirements; paresthesia; headaches; attacks of giddiness; orthostatic collapse tendency; paresis (implicit); coordination disturbances; hypaesthesia; reflex irregularities; vegetative hyperexcitability; EEG finding[1]; EMG finding.[2]

Nervous system.—Psychopathological: Decrease in initiative and interests; hyperaesthetic-emotional traits; pronounced fluctuations in intensity; disturbances in memory and concentration; disturbances in libido and potency; alcohol intolerance; depressions; decrease in impulsion; affective disturbances in the restricted sense of the term; experimental weakness in mental capacity; organic Rohrschach psychogram; individual neurotic traits.

n. = normal, abn. = abnormal, p. = pathological, n.s.p. = not definitely pathological, v.mU. = premature fatigue in the electromyographic series stimulus test.

The course of the dermatological manifestations proved to be extremely obstinate in our cases. The therapeutic measures employed (drainage of the comedones, external keratolytic and antibacterial measures, as well as the internal administration of antibiotics in severe cases) could not prevent the reformation of comedones, retention cysts and boils in the first year or two, although there was no further contact with the causal noxae. Only after a long time did the tendency to relapses cease. A residual condition now to be found, particularly amongst the serious cases, is closely arranged pitted scars which have a disfiguring effect, especially where localization occurs in the face (pseudo-atrophodermia vermiculata).

All affected workers reported pronounced fatigue and weakness in the legs, often with pain, especially in the region of the proximal leg musculation. These conditions were marked, even in the early stage of the disease, and in some cases even before the development of skin changes. Paraesthesia was reported in the records or in spontaneous information in only 2 of 9 cases.

[1] Our thanks are due to the Berufsgenossenschaft der Chemischen Industrie for placing their records and other documents at our disposal, and also for their understanding in the sometimes lengthy clinical examinations.

[2] Our thanks are due to Doz. Dr. BOCHNIK and Dr. BUSCHART for conducting the electro-encephalographic investigations, and to Dr. PUFF and Dr. RUEDAS for the electro-myographic investigations.

Implicit paresis or atrophy, weakening of the reflexes, or absence of [illegible] expansion reflexes as a sign of toxic polyneuropathy was not establi[illegible] in any of the cases. Two of the patients examined indicated a decrease in sensibility with isolated epicritical disturbances in the lower limbs. No definite signs of neurogenic damage that could have been expected with peripheral nerve lesions were established electro-myographically; premature fatigue, which was recorded in the series stimulus test, requires further confirmation regarding both the method and the raised findings. As these findings show, the neuromuscular disturbances do not fit in with the typical picture of toxic polyneuritis or polyneuropathy.

The electroencephalographic examination produced an abnormal electroencephalogram in 6 cases, with frequency lability and dysrhythmic groups of a partly asymmetrical character. In one of the patients examined, accentuated dysrhythmia and raised cerebral excitability were revealed after photostimulation. The electroencephalographic changes found were uncharacteristic and afforded no diagnostic viewpoints of any real consequence.

Some of the workers examined complained of headaches, attacks of giddiness and orthostatic collapse tendency. In 5 of the 9 patients examined, there were distinct signs of vegetative hyperexcitability, fine tremor of the hands, increased perspiration on the hands and legs, axillary perspiration, raised dermographism and suggestions of Chvostek's sign. The blood pressure value measured during out-patient check-ups were all in the normal region, though at the lower limit of the norm in 5 of the 9 patients examined. Orthostatic collapse tendency was not established either during out-patient visits or during in-patient observation by an internist. In 2 cases, myocardiac damage was suspected.

Abdominal complaints such as a feeling of fullness, pressure in the stomach and liver region, and slight pain, were reported by 5 of the 9 patients. There were 4 reports of disturbances in the gastric secretions, 3 of subacidity, one of hyperexcitability and one radiographic finding of gastritis.

Very thorough investigations were conducted as part of repeated out-patient examinations and in-patient observation to ascertain any liver damage.[3] Whilst the liability reactions were uncharacteristic in all cases, the bromphthalein test indicated slight delay in the dyestuff excretion in 2 instances. In 3 cases, the liver biopsy produced pathological findings, these comprising 2 cases of slight perihepatitic changes and in one case a fatty liver with inflammatory symptoms and slight fibrosis of the liver. Owing to the clinical and histological findings, it was suspected that a condition following virus hepatitis existed in this instance. Deposits of ferrous and non-ferrous yellow-brown pigment were established in this case, although these did not correspond to the grey, non-ferrous pigment discovered by KALK and WILDHIRT in chlorophenol intoxication. The excretion of erythrocytes in the urine of one worker whose renal findings were otherwise normal remained unaccounted for.

The psychopathological changes in the chlorophenol workers who were all psychiatrically and psychopathologically examined were especially remarkable. In 6 cases the course could be observed over a 2-year period and there was an opportunity for objective anamnesis investigation and experimental psychological examinations.[4] With a very large degree of agreement, a subjective syndrome of complaints was reported by the patients under investigation, this syndrome extending from the psychoneuropathic complaints in the region of the extremities, cardiovascular and abdominal symptoms to the mental/spiritual sphere, especially in the modes of behaviour associated with the vital forces (BÜRGER-PRINZ). Considered in detail, there were reports of disturbances in the vital senses such as general sense of weakness, feeling of fatigue, indisposition, sense of insecurity, inner restlessness and a feeling of illness. The basic mental mood was reported to be deteriorated and lowered towards behaviour characterized by dissatisfaction or sullenness and irritation. Not infrequently, a mood component of fear and unease was present. Changes in affectivity in the restricted sense of the term were reported by the patients in the form of increased emotional reactions, irritability, tendency to fits of temper and also a certain hebetude.

[3] Prof. Dr. HORNBOSTEL and Dr. SCHONFELDER, I. Med. Univ.-Klinik, Hamburg-Eppendorf.

[4] Our thanks are due at this point to Dipl. Psychologist W. von SCHUBERT, for conducting the tests (Rorschach psychogram and Hamburg-Wechsler intelligence test for adults).

General loss of strength and reduced inner vitality and impulsion were symptoms noted in each of the cases observed. The probands described reduction in initiative and interests, weak willpower, reduced efficie[illegible] and more rapid exhaustion in physical and mental/spiritual matters.

The subjective and objective anamnestic psychopathological picture is further complicated by a number of additional symptoms that are present with a greater or lesser degree of regularity. Disturbances of the instincts occurred in practically all cases. Thus, probands suffered from a sharp reduction in potency, and most also from decreased libido. The appetite had deteriorated and occasionally there were substantial fluctuations in weight. The sleep and sleep requirements of most probands were distributed in that there was an increase in disturbances to sleep itself and concurrently, but less often, to the process of falling asleep.

In certain cases, a decrease in mental capacity, especially disturbances of the memory and perception, were mentioned. In the majority of instances, alcohol intolerance was noted. Finally, mention should be made of hyperaesthetic traits with hypersensitivity to light and noise. Marked fluctuations of the psychopathological syndromes described were reported in nearly all cases.

Changes were described in the intensity of the symptoms, daily patterns of fluctuation occurring in favour of early morning, late morning or evening hours. In many probands, there were intervals of some days or weeks in which they were practically free from complaints.

For completeness' sake, a further two less common phenomena are described. Two probands stated that the general symptoms and the polyneuropathic symptoms were relieved for a varying length of time by the use of cold media (cold showers and washing with cold water). One proband reported abnormal, constantly changing eating habits, such as a wish for nothing but black bread, milk soup, or three litres of milk daily, there being no desire for food of other types at these times.

Compared with the multifarious polysymptomatic subjective pictures, the objective psychopathological signs can be recorded at less length. In exploratory conversations, the majority of the probands displayed a distinct, slightly depressed and subdued mood, which could be brightened only slightly or not at all. So far as impulsion was concerned, the patients examined gave an impression of lifelessness; their psychomotivity was feeble and fatigued. The impression was rather one of slight cerebral organic impulsion reduction than of inhibition. The affective modes of behaviour were occasionally notable for their reduced reactivity and oscillation capacity, though also because of lability and decompensability. In 2 cases, pronounced hypochondria and, in one case, slight but distinct alienation of the total personality were recorded.

The psychological tests are significant for the discovery of finer intellectual performance shortcomings and psycho-organic disturbances in affectivity. In the majority of the probands tested by the Hamburg-Wechsler intelligence test for adults (HAWIE), there was a significantly raised percentage of degeneration, this providing a certain indication of an acquired decrease in mental capacity. In the Rorschach psychogram, coartation of the experiential type, signs of weakened emotional reactivity, poor concentration, reduction in tempo, sluggishness of the mental processes and a tendency to perservation point to cerebro-organically governed changes.

DISCUSSION

On the basis of the findings in three independent groups of chlorophenol workers, which together included more than 100 affected by diseases, a characteristic clinical picture is provided, the most important features of this being the following disturbances:

1. Following initial dermatitis of the face and symptoms of irritability on the part of the conjunctiva; often together with gradually developing acne primarily in the region of the face, then the back of the neck, shoulders and upper trunk, and in severe cases on the entire body, with comedones, pustules, boils and patches of pigmentation. In several cases with severe irritation of the mucous membranes of the face and the upper respiratory tract; sometimes with continuing blepharoconjunctivitis.

2. In several cases, disturbances connected with the internal organs, especially damage to the liver, with deposits of a nonferrous pigment as a

characteristic biopsy finding. In some cases, chronic bronchitis and [illegible] instances of myocardiac damage.

3. In all cases, general fatigue and weakness principally affecting the proximal muscles of the lower limbs, often with pain in the musculation and in some cases paraesthesia and slight hypaesthesia. In isolated cases only, more pronounced disturbances of sensitivity, slight paresis (implicit) and weakening of reflexes.

4. A psychovegetative syndrome with the following disturbances: Subjective: Disturbances of the vital senses, disturbances in the basic mental mood and affectivity, disturbance in impulsion, weakness of memory and concentration, hyperaesthetic traits, vegetative dysregulation, tendency to orthostasis, sleep disturbances, much increased sleep requirements, disturbances of the instinct sphere, reduction in libido and potency, and alcohol intolerance.

Objective.—psychopathological: Reduction in impulsion, subdepressive traits of a type characterized by genuine vital moments of depression, disturbances in affectivity in the sense of a certain levelling-out, increased excitability; occasionally hebetude, hypochondria and personality alienation.

Experimental psychological. HAWIE: Increased degeneration percentage; Rorschach psychogram: Coartation of the experiential type, signs of weakened emotional reactivity, weakness of concentration, reduction in tempo, sluggishness of mental processes, tendency to perseveration.

The dermatological picture of the chlorophenol intoxication described shows extensive agreement with the disease caused by chlorinated aromatic hydrocarbons as first described by HERXHEIMER and later by several authors (see BRAUN, RISSE-SUNDERMANN). On the basis of the observations that chlorinated naphthalenes were principally responsible, WAUER, and later TELEKY, suggested the designation "perna disease" (*PER*chlorinated *NA*phthalene). TELEKY pointed out that the chloracne already described by HERXHEIMER in 1899 was produced not by pure chlorine but by chlorinated hydrocarbons or the simultaneous action of chlorine and tar. Further observations on perna disease made by MITTELSTADT, FLINN and JARWIK, DRINKER and collaborators, and GREENBURG and collaborators, indicated that not only the skin symptoms but also fatigue, loss of appetite, giddiness, and severe liver damage with acute yellow atrophy of the liver leading to death can result from work with chlorinated naphthalenes. BAADER mentions epidemics at American shipyards during the Second World War. In his description of the cases occurring in America and Great Britain, sometimes with a fatal outcome, TELEKY refers to the report of BROWN, President of the Halowax Co., New York (1937), that only the manufacture of the higher stages of chlorination and the combination with chlorinated diphenyls and other substances led to severe damage to the health and in some cases to fatal acute yellow atrophy of the liver. TELEKY also refers to the animal experiments by C. K. DRINKER and collaborators to support the view that only the higher chlorinated diphenylamines produce serious damage.

The general symptoms in occupational chlorophenol intoxications are apparently more pronounced than those occurring with the lower chlorinated naphthalenes employed earlier. This fact was also observed by TRUHAUT and collaborators amongst workers who had been using pentachlorophenol for wood preservation, as well as KUBOTA in Japan, who mentions multifarious disturbances of the autonomous nervous system and who observed several fatal cases. In all three German groups of chlorophenol intoxication, liver damage was established, the damage that was most pronounced and studied most intensely being that found amongst the cases of disease occurring in two Mid-Rhenish companies (HERGT, KALK and WILDHIRT). In all the groups, several cases of chronic emphysema bronchitis and myocardiac damage were found, although these disturbances did not occur nearly so regularly as the pronounced fatigue and neuromuscular weakness, which we observed in all our patients. The psychosyndrome described was equally regular, this being found not only by us but also by JANZARIK and RICHERT to a completely identical degree amongst the Mid-Rhenish workers.

The psychopathological syndrome could be distinguished with a sufficient degree of certainty by differential diagnostics from endogenic psychosis, especially mild cyclothymic diseases, neurotic personality developments and organic psychosyndromes of different etiology, and somewhat presenile or

cerebrosclerotic processes of degeneration. Phenomenologically [illegible] relationships to the pseudoneurasthenic syndrome, which is described in connexion with a large number of occupational intoxications such as those caused by lead, carbon monoxide, manganese, thallium, arsenic, carbon disulphide, trichloroethylene, etc. (for relevant articles, see BORBELY, von HATTINGSBERG MEGGENDORFER, MOESCHLIN, PENTSCHEW, TELEKY)—and especially the relationships to particular endogenic mood conditions—are obvious. Lowering of the vital level, moments of depression, vegetative symptoms, and, not least, fluctuations in intensity can be observed predominantly in endogenic-depressive conditions. On the other hand, alcohol intolerance, hyperaesthetically excitable and polyneuropathic traits influence the differential diagnostic aspect more in the direction of an apparently exogenic condition. The somewhat older psychiatric literature should be borne in mind in this connexion (MEGGENDORFER, STERTZ), this placing the neurasthenic syndrome quite definitely in the pattern of exogenic symptom complexes. Not least, reference should be made to the phenomenological relationship of our observations to the (exogenic) hyperaesthetic-emotional conditions of weakness of BONHOFFER, which, from the psychopathological aspect, have significantly been designated by EWALD as no longer heteronomous but homonomous in the sense propounded by KLEIST.

Despite the phenomenological relationships discussed, the psychopathological delayed syndrome of the chlorophenol workers scarcely corresponds completely with any of the known clinical pictures. In any case, the question of a special psychic-vegetative delayed intoxication syndrome, which was discussed by SPIEGELBERG in connexion with observations on persons suffering chronic occupational damage from military poison gas, also demands consideration in view of the observations mentioned in this paper.

It has been possible to rule out psychogenic-neurotic moments so far as our subjects are concerned, provided that individual neurotic conditions, i.e. characterogenic and experiential situative data are involved. Two of the nine probands exhibited considerable psychopathic or neurotic structural elements. However, it was easily possible to separate these two probands from the other completely or largely non-neurotic cases. Certain "collective-neurotic" factors have, in our opinion, to be taken into account as an unfortunate but practically unavoidable fact in all group investigations but especially those involvidg etiological evidence (SPIEGELBERG). Reactions of this type have also been observed in our cases in the sense of a superimposed psychogenic accessory with, as it were, "physiological" but not inadequate, individual-neurotic (complex-determined) idemnification wishes. The psychopathological analysis of the individual case and the comparison of the findings in each instance with such independent collectives of the same etiology afford sufficient protection from authoritative and scientific false assessments. Despite the long course, the prognosis of the psychopathological intoxication results appears favourable. Although technical aspects of the pension situation have not yet been finally clarified, there was, on the whole, a certain subjective improvement in the symptoms, or else they remained static. We have not observed any objective deteriorations, except for the momentary intensity fluctuations. The experience of the Mainz Nerve Clinic (RICHER) and the impressions of works medical staff (KNECHT) suggest a benign course of acute and chronic intoxications, provided no toxic parenchyma damage, as such, influences the prognosis unfavourably.

It seemed appropriate to attribute the toxic action to the high-chlorinated chlorophenols, this view being supported by animal experiments conducted by MACHLE and THOMAS, H. KITZMILLER, a series of other investigators (KEHOE, DEICHMANN, GRUEBLER, BOYD, McGAVACK, TERRANOVA, PICCIONE cited acc. to von OETTINGEN) and also our own animal experiments. KIMMIG and SCHULZ were, however, able to show that the use of non-industrial, analytically pure, high-chlorinated chlorophenols (trichlorophenols, pentachlorophenols) does not lead to the characteristic symptoms of chlorophenol intoxication.

Animal experiments were carried out with a view to discovering the noxne causing the symptoms. The rabbit's ear proved to be a suitable test object since it is possible to produce the changes on this with the substances causing chloracne, these changes closely resembling those of human chloracne (HOFMANN and NEUMANN, BRAUN, LANDES, etc.). Brushing with a

substance which is active in this respect leads at first to patches of [illegible] in conjunction with reddening, swelling and flaking; then, some days later, hyperkeratosis linked with the follicles and also small cysts occur, these being easy to record histologically, as well. In addition to the brushing experiments, tests were carried out on rabbits to determine the general toxicity, whilst cats, too, were used for testing a number of substances. In these tests it was found, in corroboration of findings derived by OETTEL and also HOFMANN from similar cases of intoxication in a large chemical works in southern Germany, that the substances producing chloracne possess marked liver toxicity in rabbits. It was possible to trace effectively the damage to the parenchyma of the liver intra vitam with the micro-modification of the bromsulphthalein test given by HOFMANN and OETTEL. In autopsies, diffuse steatoses and extensive necrosis of the parenchyma of the liver were found. The investigations, which have already been reported (SCHULZ 1956; KIMMIG and SCHULZ 1957) led to the following results:

The effective substances must have occurred in the alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene to 2,4,5-trichlorophenol, this having been carried out technically under pressure at about 180°C in the presence of methanol and caustic soda solution. However, it was not the trichlorophenol itself but the by-products that formed in small quantities in the course of the pressurized phenol process that were regarded as the causal noxae; for it was not possible to produce any of the above-named changes on the rabbit's ear with pure, repeatedly distilled 2,4,5-trichlorophenol or with 1,2,4,5-tetrachlorobenzene, although they did occur with the trichlorophenol used technically.

Since the isolation of defined compounds from the residue occurring in the distillation of technical trichlorophenol was not possible at first, compounds were synthesized by chemical means and given to us for testing on animals where there was a certain likelihood that these substances may occur as by-products in the saponification of tetrachlorobenzene to trichlorophenol. The substances initially available were various chlorination products of the diphenyl ether and the dibenzofuran (diphenylene oxide).

Although the diphenyl ether and its 1× to 4× chlorinated derivatives, and also dibenzofuran and monochlorodibenzofuran were ineffective in experiments on animals, 3× and 4× chlorinated dibenzofurans, even in concentrations as low as 0.05%, produced the symptoms mentioned on the rabbit's ear. Single doses of 0.5 to 1 mg/kg administered orally produced severe liver damage in rabbits, this leading to the death of the animals in most instances.

The clinical observation of a laboratory assistant engaged elsewhere, who fell ill with severe chloracne after exposure to tetrachlorodibenzodioxine, indicated the chlorine derivatives of the dibenzodioxine.

Tetrachlorinated dibenzodioxines, especially 2,3,6,7-tetrachlorodibenzodioxine, were highly effective on the rabbit's ear, even in low concentrations. Three brushed applications with 0.01–0.005% solutions (in polyglycol) were sufficient to cause severe areas of inflammation and follicularly arranged hyperkeratosis. When administered orally, single doses of 0.05–0.1 mg/kg body weight led to severe liver damage and generally the death of the animals.

The assumption that 2,3,6,7-tetrachlorodibenzodioxine is actually of considerable importance in causing the chloracne diseases occurring in the chemical works received further substantial support from the chemical angle. It was possible to prove that this compound is formed from two molecules of sodium trichlorophenolate in association with the cleavage of NaCl under the pressure and temperature conditions prevailing in the autoclave.

It was, moreover, possible to isolate the named tetrachlorodibenzodioxine from the by-product occurring in the technical pressurized phenol process (alkaline saponification from tetrachlorobenzene to trichlorophenol). To prove that 2,3,6,7-tetrachlorodibenzodioxine is capable of producing alterations in the form of chloracne not only on the rabbit's ear but also on human skin, one of us (SCHULZ) carried out a test on his own body. Two brushed applications of a 0.01% solution on a circumscribed skin area of the forearm led within two days to a mild dermatitis, then some days later to a follicular hyperkeratosis and comedones, these also being easy to record histologically. The etiological significance of this substance for the diseases described here seems to us to be sufficiently evidenced by this experiment. However, it is not impossible that other chlorinated aromatic compounds with highly toxic characteristics may occur in this technical process, these possibly not having been so far identified or tested in experiments on animals.

The experimental and clinical findings are an impressive example of the fact that, in works pathology, substances occurring in small quantities as by-products in chemical processes can be of importance. In experiments to discover the origin of frequency occurring occupational intoxications, this viewpoint should not be ignored. If it is possible to prove that impurities attached to the main product should be regarded as the causal noxae of an occupational disease, this constitutes an important prerequisite for successful prophylaxy.

In our own special case, it was possible, by changing the plant chemistry aspect of the manufacturing process, to prevent the formation of the highly toxic, multi-chlorinated dibenzodioxines and dibenzofurans. Since than, 2,4,5-trichlorophenol is again being manufactured in this works and processed into the herbicide 2,4,5-trichlorophenoxyacetic acid, without symptoms of intoxication of any kind occurring amongst the workers.

A Technic for Testing Acnegenic Potency in Rabbits, Applied to the Potent Acnegen, 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin*

E. Linn Jones, M.D. and Helen Krizek, Ph.D.

Follicular hyperkeratosis is an important feature of the occupational disease known as chloracne, which is characterized by the appearance of papules, comedones and cysts after exposure to industrial materials containing highly chlorinated diphenyls, highly chlorinated naphthalenes, and other chlorinated aromatic compounds. A characteristic epithelial hyperplasia and hyperkeratosis can be produced on the inner surface of the rabbit ear by such compounds (1, 2), and a difference in intensity of response has been noted and suggested as a basis for comparative tests (2). Experimental studies heretofore (1–7) have been, however, directed chiefly to the ability or the failure of various materials to produce this effect in experimental animals and in man, and in delineating its gross and its histological features; these studies have used either material of unstated origin, or else mixtures (*e.g.* Halowax, 1014). We have attempted to study the phenomenon of acnegen-induced hyperkeratinization on the rabbit ear in a quantitative fashion by recovering and weighing the keratin formed after applying known amounts of a single, well-characterized chemical compound under controlled conditions. To recover keratin a new technic was developed based on the resistance of this material to digestion by pepsin. As test compound we have chosen 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, which has been reported to be so potent that painting the rabbit ear three times with a 0.05%-0.001% solution was sufficient to produce the acneform response (7). With a compound of such potency, the expected effects could be produced without the necessity of applying the material in an ointment or as a crust, circumstances which would have made very uncertain the quanity actually in contact with the skin.

Experimental

Preliminary gross and histologic observation indicated that 0.3 micrograms applied to the rabbit's ear gave, by gross observation, a minimal follicular plugging whereas 0.02 micrograms caused no observable effect. Accordingly, 0.3 micrograms was chosen as the lowest dose; in addition dose levels of 1.0, 3.0 and 10.0 micrograms were studied.

Seven mature, white, male rabbits were used for each dose level, except in the first studied (0.3 micrograms) for which only six were used. Seven days after wax epilation †, of the inner surface of the ears, 1 ml. acetone solution of the compound was applied to one ear of each of the rabbits, and 1 ml. acetone to the other. Special effort was made to distribute the liquid uniformly over

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† With "Improved Zip."

the entire inner surface, and to aid in securing uniformity the dose was divided into three applications, made on successive days. The right ear was used as control for some rabbits in each group, and the left for others. Fourteen days after the first application, three biopsy samples, extending through the cartilage, were taken under procaine anesthesia, with a 9 mm. punch. One sample was taken from the middle, one from the posterior and one from the anterior area of the ear, at a level about 15 mm. distal to the notch of the ear. (Fig. 1) The discs were washed free of blood without delay. Under a dissecting microscope at 9× magnification, the moist samples were plucked free of any adhering hairs and clots; and the cartilage was removed with sharp #10 Bard-Parker scalpel. A thin coating of white petrolatum was applied to the epidermis. Each disc was floated in a 50-mm. petri dish containing 10 ml. 0.1% pepsin (Worthington Biochemicals "2× Crystallized" product) in 0.24 *N* HCl and incubated 4 hours at 37°C., at the end of which period the keratin disc was gently lifted out, free of any undigested dermis by inverting and gently irrigating it, and resuspended in 10 ml. 1:1 V/V ethanol: diethyl ether mixture. A light aluminum-foil cup 9 mm. in diameter, with perforated bottom, previously washed with ether and weighed to 0.02 mg., was brought close to the disc, which was then gently transferred, with follicular projections up, with the aid of a scalpel handle, into the cup. After four hours at room temperature in the covered petri dish, the solvent was aspirated. A similar leaching with 10 ml. ether was made, after which the cup in the covered petri dish was dried in vacuo overnight. The cup and sample were weighed to the nearest 0.02 mg.

Additional biopsy samples were taken on about half the animals and examined histologically after routine hematoxylin and eosin staining.

The following method was found convenient for preparing a small quantity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.[1] The sodium salt of 2,4,5-trichlorophenol was prepared by dissolving 1.6 g. metallic sodium in 25 ml. absolute ethanol in a 100-ml. round-bottomed flask, adding 20 g. of 2,4,5-trichlorophenol (Eastman "Practical Grade," recrystallized from petroleum ether) and distilling off the ethanol. The salt was cautiously heated until the copious evolution of acid fumes which took place at 200–250° C. subsided, after which the flask was kept at 350–400° C. for 30 hours. Two zones were found in the distilling head. The lower of these, a dense, compact mass, had a melting point of 230–300° C. and was only slightly soluble in chloroform. This was the crude acnegen. The upper zone of coarse crystals soluble in chloroform was probably a tetrachlorobenzene formed in a competing reaction. Two batches of the crude acnegen were combined and recrystallized twice from anisole to yield 0.25 g. product. Its synthesis is represented in Fig. 2.

Analysis. Calculated for $C_{12}H_4O_2Cl_4$: C, 44.76%; H, 1.25%; Cl, 44.05%. Found: C, 44.31%; H, 1.40%; Cl, 44.18%.[2] The melting point was 295–300° C.; literature values for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin prepared by chlorination of dibenzo-*p*-dioxin: 295° C. (8), 320–325° C. (9). The infrared spectrum showed a doublet at 1310–1322 cm.$^{-1}$ in the range reported for a series of dibenzo-*p*-dioxin derivatives (10). The ultraviolet absorption spectrum of the compound in absolute ethanol had a maximum at 233 mμ and one at 307 mμ; the respective molecular extinction coefficients were 46,500 and 4,250.

RESULTS

Histological sections showed a characteristic hyperkeratosis of the follicular epithelium and a marked hyperplasia of the surface epidermis. At low dose level some sebaceous cells were present; at high dose level, only a few were seen, and the follicle was filled with a keratinous mass. (Figs. 3 and 4). There was marked thickening of the epidermal keratin, although this was only rarely observed on the slides, presumably because it was lost in the cutting and processing.

[1] Following the practice of *Chemical Abstracts* we describe our command as 2,3,7,8-tetrachlorodibeno-*p*-dioxin. From a comparison of its preparation with the mode of origin in the Industrial process discussed by Kimmig and Schulz (7) we conclude that it is the same as their 2,3,6,7-tetra-chlorodibenzo-*p*-dioxin.

[2] Analyses by Micro-Tech Laboratories, Skokie, Ill.

The complete removal of all non-keratinized tissue from the biopsy by pepsin digestion was confirmed by histological section of a keratin disc. (Fig. 10).

In general, the keratin discs recovered from the treated animals were less fragile and thicker than the controls. At lower dosages the follicular keratin was usually observed as tree-like forms representing casts of the multilobated sebaceous glands. (Figs. 5A, 6, and 7). These follicular projections were not present in the controls. (Fig. 5B). At higher doses the keratin in the follicles appeared as a larger structure of smooth, oval shape typical of comedones. (Fig. 8).

Weights of keratin recovered from the biopsy samples are given in Tables 1 and 2. In Table 1 are listed average weights for the three biopsy specimens taken from each ear. In subsequent experiments biopsy specimens taken from the anterior, the middle and the posterior portion were distinguished, in an effort to assess the importance of the site of biopsy removal with respect to the weight of keratin recovered.

DISCUSSION

For each rabbit there was calculated a value for the relative increase in weight, $(T - C)/C$, where T = average weight for the three biopsy samples from the treated ear and C = the corresponding average for the control. These values and their average for each dose level are plotted (Fig. 9) against the dose. The averages vary approximately linearly with the logarithm of the dose, but individual $(T - C)/C$ values at each dose deviate widely. The deviation may be due, in part, at least, to failure to secure uniform spreading of the acnegen, in spite of the precautions taken. Loss of keratin during manipulation of the samples may be another source of deviation; however, such an error would be expected to be most important for smallest amounts of keratin handled (*i.e.* the controls). An inspection of the control weights does not reveal a corresponding spread of values. Further, the probability of losing keratin was greater for the rabbits showing greatest response, because these tended to have some loosely adhering scales and large comedones which might be expressed and lost in the course of tissue removal and subsequent processing; actually, therefore, the spread of response may be larger than our results indicate. In addition, gross observation indicated that some rabbits gave a weaker response than others at the same dose level. We feel, therefore, that the values reflect a real individual variation. Inspection of Tables 1 and 2

TABLE I.—RECOVERY OF KERATIN FROM EARS OF RABBITS AFTER APPLICATION OF 0.3 MICROGRAMS 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

Rabbit	Milligrams of keratin per 9-mm biopsy sample [1]	
	Treated ear	Control
31	0.98	0.97
49	1.07	0.70
47	1.91	1.31
66	1.88	1.18
84	1.58	1.20
57	1.13	1.31

[1] Average of three samples from, respectively, anterior, middle, posterior.

reveals some tendency for greater response to occur in animals for which control values were high, but this tendency is by no means clear-cut. We found no correlation between intensity of response and weight of the animal.

There appears to be no consistent difference in the three positions, anterior, middle and posterior, along the line of section, either for the control or for the treated ear, with respect to weight of keratin recovered. Average value for keratin recovered from the controls was remarkably constant: 1.11 ± 0.19, 0.85 ± 0.18, 1.02 ± 0.26 and 1.02 ± 0.16 mg. for the rabbits treated with, respectively, 0.3, 1.0, 3.0 and 10.0 micrograms.

The technic developed in the present study might be used for a c[illegible] of acnegenicity of various materials if the test substance is applied [illegible] and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to the other. Such a procedure might avoid the complications introduced by individual differences, and reduce the number of animals necessary. Control values could be secured independently from untreated animals. Three biopsy samples may not be necessary; an analysis of the data showed that values calculated from only one (the middle) biopsy sample were not significantly different from those based on the average for three.

A fairly good correlation was found between gross observations and intensity of response as assessed by weight of keratin, but there were some deviations. Thus all the animals at 0.3 micrograms dose level were assessed grossly as showing follicular dilatations. However only four showed increase in weight of keratin. At 3.0 micrograms large comedones were observed on four rabbits, and these showed the greatest relative increase in weight of keratin; on one other, comedones were observed; but the response in terms of weight recovered was less than for two which gave the gross impression of follicular papules.

TABLE 2.—RECOVERY OF KERATIN FROM EARS OF RABBITS AFTER APPLICATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN

Rabbit	Milligrams of keratin per 9-mm. biopsy sample							
	Treated ear				Control			
	Anterior	Middle	Posterior	Average	Anterior	Middle	Posterior	Average
Dose: 1 microgram								
16	1.24	0.54	0.90	0.89	0.65	0.54	0.46	0.55
15	2.14	1.82	1.70	1.89	0.94	0.70	0.64	0.76
30	1.60	1.14	1.26	1.33	0.88	0.76	0.72	0.79
17	2.16	1.52	2.52	2.07	0.80	0.82	0.58	0.73
11	2.04	1.36	1.26	1.55	1.14	1.08	1.14	1.12
7	3.82	2.08	2.52	2.81	1.08	1.08	1.04	1.07
27	3.44	2.86	2.06	2.79	0.88	1.16	0.86	0.97
Dose: 3.0 micrograms								
18	0.86	1.22	0.96	1.01	0.90	0.64	0.92	0.82
19	1.76	0.94	0.84	1.18	0.78	0.90	1.32	1.00
20	1.80	1.08	1.58	1.49	1.08	0.54	0.92	0.85
21	7.00	8.54	5.64	7.06	1.80	0.88	1.26	1.31
22	2.82	2.04	1.38	2.08	0.88	0.64	0.74	0.75
23	3.00	2.88	5.86	3.91	1.24	0.98	0.78	1.00
24	5.86	6.14	5.56	5.85	1.94	1.30	0.90	1.38
Dose: 10.0 micrograms								
32	3.28	3.30	2.02	2.87	0.88	0.96	0.80	0.88
33	2.68	2.48	2.60	2.59	1.20	1.08	1.20	1.16
34	1.26	1.62	1.18	1.35	0.78	1.16	0.78	0.91
35	6.56	7.12	5.84	6.51	1.32	0.80	1.08	1.07
36	5.14	2.90	3.84	3.96	0.98	1.08	1.16	1.07
37	1.64	1.34	2.18	1.72	1.18	1.28	0.52	0.99
38	6.28	4.02	4.90	5.07	1.08	1.06	1.08	1.07

Attention should be called to the great toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Kimmig and Schulz (7) reported that 0.5–1.0 mg. per kg. orally was lethal to most of their rabbits. In our preliminary experiments a rabbit receiving topically 0.3 micrograms in acetone, died at the end of [illegible] days, and two others receiving, respectively, 30 and 2 micrograms died within a week. However, there were undoubtedly other contributing factors, because in all animals treated subsequently no toxic symptoms were observed.

SUMMARY

1. Hyperkeratinazation induced on the rabbit ear by the acnegen 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is studied by a new technic based on weighing keratin recovered after careful pepsin digestion.

2. When applied in acetone solution to the rabbit ear, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin is effective at microgram levels. Effect of dose and individual differences in response are discussed.

3. The new technic, using 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, is suggested for comparing acnegenicity of various substances.

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DISCUSSION

Dr. Peter Flesch, Philadelphia, Pa.: Since the criterion of acnegenic activity appears to be the conversion of the sebaceous cells into keratin-forming cells, I would like to ask, what did you see in the histologic sections?

Dr. E. Linn Jones, (in closing): In the paper we will have histologic sections of treated glands and the digested keratin disc.

In regard to histology there is varying response, depending on the amount applied. With lower doses there is conversion of the cells in the follicle to keratinizing squamous cells, with occasional remnants of sebaceous cells in pockets here and there. With larger doses no sebaceous cells can be found. The entire follicle is converted into a large keratin-filled papule.

STUDIES OF THE CHICK EDEMA DISEASE

2. PREPARATION AND BIOLOGICAL EFFECTS OF A CRYSTALLINE CHICK EDEMA FACTOR CONCENTRATE

BY

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Studies of the Chick Edema Disease

2. PREPARATION AND BIOLOGICAL EFFECTS OF A CRYSTALLINE CHICK EDEMA FACTOR CONCENTRATE

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INTRODUCTION

Following the early reports of the chick edema disease (CED), Sanger *et al.* (1958), Potter *et al.* (1959), Allen (1961) and Allen and Lalich (1962) reported toxicological studies which established that the disease differed from known poultry diseases. A number of investigators reported on the purification, isolation and partial chemical characterization of toxic factor (Brew *et al.*, 1959; Friedman *et al.*, 1959; Wootton and Alexander, 1959; Ames *et al.*, 1960; Harman *et al.*, 1960; Yartzoff *et al.*, 1961; Wootton *et al.*, 1962; and Wootton and Courchene, 1964).

Brew *et al.* (1959) reported that broiler chicks developed the disease when fed a fraction purified 3200-fold from the original starting material. A fraction purified 10,000-fold which elicited CED was reported by Friedman *et al.* (1959). Yartzoff *et al.* (1961) reported that a fraction purified 1,000,000-fold from a low potency commercial triolein produced hydropericardium (HP) when fed to day-old chicks at 50 p.p.b. in the diet, and resulted in death at 1 p.p.m. Wootton and Courchene (1964) found one fraction (designated α 3.02) which was highly toxic for the chick. These workers estimated that ingestion of 5 µg. of the α 3.02 fraction was enough to kill one chick. Firestone *et al.* (1963) reported that signs of the disease were elicited by one fraction fed at 0.1 p.p.m., which substantiated the report by Yartzoff *et al.* (1961).

The purpose of this paper is to report our recent studies on the purification and biological effects of a concentrate of the chick edema factor (CEF) isolated from a crude toxic fatty material (TFM) known to produce the chick edema disease (Flick *et al.*, 1962, 1963). Preliminary data are included on the effects of CEF-containing material on egg hatchability and development of the chick embryo.

MATERIALS AND METHODS

Preparation of CEF Concentrate. Eighteen pounds of unsaponifiable material was isolated from 180 pounds of toxic fatty material (TFM) by saponification and extraction of the unsaponifiable fraction with petroleum ether:diethyl ether (1:1, v./v.). The petroleum ether was redistilled and the solvent boiling between 40-60°C. was collected and used. The unsaponifiable fraction was chromatographed on Fisher Alumina (Cat. No. A 540) essentially as described by Yartzoff *et al.* (1961). Fractions with ultraviolet absorption spectra that matched those of naphthalene and penanthrene derivatives were combined and chromatographed on Merck Alumina (Cat. No. 71707). Foreruns were eluted with petroleum ether. Additional fractions that eluted with 5% diethyl ether and that exhibited the spectra of naphthalene and phenanthrene derivatives were collected, combined and concentrated. The concentrate was chromatographed on columns of Celite:H_2SO_4:fuming H_2SO_4 (1:1:1) and eluted with CCl_4. The foregoing procedure was a modification of AOAC method 24.111 (a) (Horwitz, 1960). The CCl_4 eluates were re-chromatographed on Merck Alumina, and individual fractions were collected and checked by ultraviolet spectrophotometry. Fractions eluting with 10% diethyl ether with absorption spectra of naphthalenes (absorption maxima in the range of 240–250 mµ.) were combined and re-chromatographed on Merck Alumina with isooctane as the eluant. Individual fractions were collected and bioassayed, and a highly toxic crystalline fraction (CEF concentrate) weighing 79 mg. was obtained.

The CEF concentrate was examined by microcoulometric and electron capture gas chromatography. A microcoulometric gas chromatograph (Dohrmann Manufacturing Company, Palo Alto, California) was used at a column temperature of 250°C. with a 6-foot × ¼ inch (i.d.) aluminum column packed with 20% Dow-Corning High Vacuum Grease on acid-washed Chromosorb W. Details of this technique were described previously by Firestone *et al.* (1963). For electron capture detection, an Aerograph Hy Fi (Model 600B) gas chromatograph (Wilkins Instrument and Research Inc., Walnut Creek, California) was used. This instrument was equipped with a tritium source electron capture detector. A stainless steel column, 5 feet × ⅛ inch, packed with 5% SE-52 silicone gum rubber on 60-80 mesh Chromosorb W, was used at a column temperature of 202°C. and a nitrogen (carrier gas) flow rate of 50 ml./minute.

Feeding Procedure and Biochemical Methods. Day-old Single Comb White Leghorn

TABLE 1.—*Composition of basal ration*

Component	Level
	% (W/W)
Corn meal, yellow	36
Wheat flour middlings	15
Alfalfa meal	1
Linseed meal	8
Protein, soya (Drackett)	16
Salts, A.O.A.C.[1]	3
Yeast	3
NaCl, iodized[2]	1
Cod liver oil	1
Cottonseed oil	16
	100

[1] For composition of A.O.A.C. salt mixture, see report by Flick *et al.* (1963).
[2] This addition gave a total of 1.99% NaCl in the diet.

cockerels were fed *ad libitum* and provided with fresh water at all times. They were handled and maintained as reported previously (Flick *et al.*, 1963).

The composition of the basal diet used is shown in Table 1. The crystalline CEF concentrate, dissolved in chloroform:ether, was added to the cottonseed oil, and the oil was warmed with stirring to remove solvent. The CEF concentrate was fed at levels of 50 and 200 p.p.b. for three weeks.

Body weights and feed intake were determined and at weekly intervals chicks were anesthetized with diethyl ether, volume of hydropericardium (HP) was measured according to the procedure outlined by Douglass and Flick (1961), and postmortem observations were recorded. Blood samples were withdrawn from the right ventricle with needles and syringes moistened with heparin, and the following tests were performed: microhematocrit (Natelson, 1961), whole blood glucose (Somogyi, 1952) and total plasma proteins and plasma protein fractions (Gornall *et al.*, 1949). Other blood samples were allowed to clot, and copper determinations[3] were made on the sera.

[3] Samples of serum were collected in acid-rinsed tubes, frozen under carbon dioxide and sent to Dr. E. W. Rice, Presbyterian Hospital, Pittsburgh, Pennsylvania for determination of copper.

Hatchability Study. For this pre... study, commercially available White Leghorn fertile eggs were injected with the test materials into the yolk sac prior to incubation according to the technique described by McLaughlin *et al.* (1963).[4] Control eggs were injected with 0.1 ml. oil. Experimental eggs were injected with 10, 20 or 50 µl. of the unsaponifiable fraction of crude TFM from which the crystalline CEF concentrate was prepared. Eggs were candled each day after the 5th day of incubation. Dead embryos were removed and examined for gross malformations. After incubation, unhatched eggs were opened for gross observation. Chicks which hatched were observed for 3 days.

RESULTS AND DISCUSSION

GLC Analysis of CEF Concentrate. Gas chromatographic separations of crystalline CEF concentrate were obtained by using both the microcoulometric and electron capture techniques. A typical electron capture chromatogram of our purified preparation is shown in Figure 1(a). The chromatogram revealed that within a 70 minute run at least eight components were present in the preparation. Wootton *et al.* (1962) isolated two components, which were designated α 3.02 and α 3.17 (gas chromatographic retention time relative to methyl arachidate). Each of these two components contained a high-melting fraction that produced edema and a low melting fraction that did not produce edema. Our toxic concentrate was chromatographically compared to the low melting fractions of Wootton *et al.*[5] The peaks of the latter two compounds, obtained with the electron capture technique, are shown in Figure 1(b) and 1(c). The chromatogram of our CEF concentrate shows a peak (retention time of 10.5 relative to aldrin) having the same retention time as the α3.02 inactive isomer.

The relative peak area (% of total area of the chromatographic peaks) of the 10.5 component was estimated by using the retention × peak height method of Carroll (1961). By this method the 10.5 peak obtained in the microcoulometric chromatogram represented about 20% of total components.

Chick Response. Results obtained on weekly weight gain, food consumption, feed/gain ratio and calculated consumption of the CEF are tabulated in Table 2. The weight gains among control birds increased at a fairly constant rate each week. Weight

[4] Injection directly into the yolk has been studied in over 70,000 eggs (personal communication from Dr. McLaughlin) with approximately 300 chemicals. India ink and various dyes were injected in conjunction with several different studies. Injected materials were found to consistently remain in the yolk and no great difficulties were encountered which were not amenable to correction.

The unsaponifiable fraction is a liquid residue of the toxic fatty material which is a still bottom residue resulting in the commercial production of fats. The unsaponifiable fraction comprised 10.1% of the crude starting material and contained hydrocarbon residues and fatty acid degradation products in addition to the chick edema factor. When centrifuged 1000 × g for 15 minutes at 24°C., the unsaponifiable material contained <0.5% (v./v) of precipitable material. See Rrew *et al.* (1959), Friedman *et al.* (1959), and Wootton and Alexander (1959).

[5] Kindly supplied by Dr. N. R. Artman, Procter & Gamble Company, Cincinnati, Ohio.

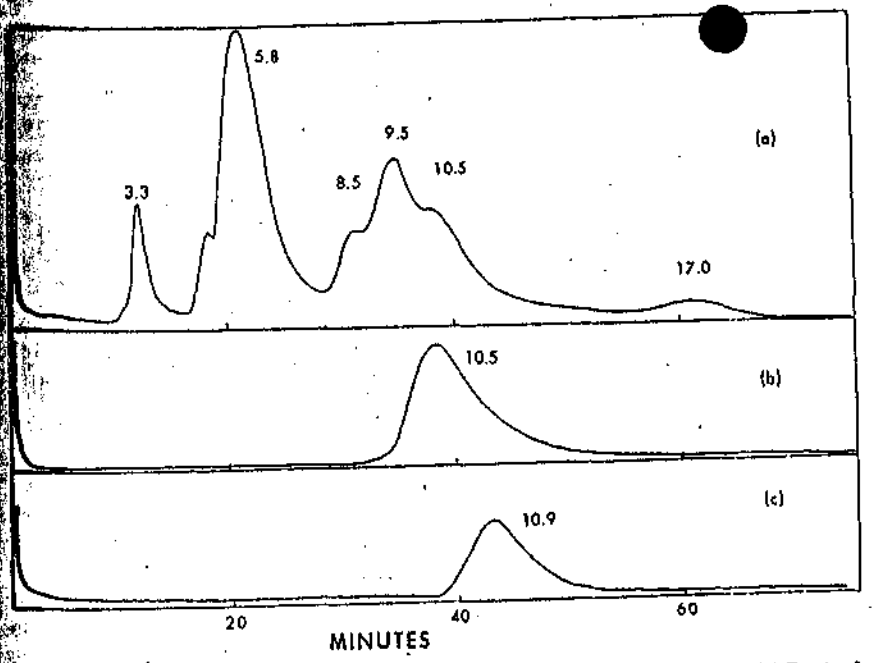

FIG. 1. Gas chromatograms of (a) toxic concentrate, (b) Procter & Gamble α 3.02, and (c) Procter & Gamble α 3.17. See text for details on instrumentation. The numbers adjacent to chromatographic peaks are retention times relative to aldrin.

TABLE 2.—*Weekly weight gain, feed consumption, feed/gain ratio and ingestion of crystalline CEF concentrate*

CEF level	No. of chicks	Mean weight gain[1]	Diet consumed[1]	No. of deaths	Feed/gain ratio	CEF concentrate consumed	
p.p.b.		g.	g.			Total μg.	μg./100 g. B.W.
			Week 1				
0	52	27.4±1.1[2]	38.5	0	1.41	0	0
50	47	29.8±1.4	42.0	0	1.41	2.1	3.2
200	47	28.0±1.4	42.7	0	1.53	8.5	13.1
			Week 2				
0	39	36.7±1.6	82.6	0	2.25	0	0
50	36	29.3±2.0	80.5	0	2.75	4.0	4.1
200	38	39.0±2.2	72.1	1[3]	1.85	14.4	14.4
			Week 3				
0	23	58.4±2.1	139.3	0	2.39	0	0
50	20	27.5±3.0	112.0	0	4.07	5.6	4.4
200	22	40.6±2.7	133.7	3[4]	3.29	26.7	18.3

[1] Mean values/cage (8-10 chicks/cage in 5-6 cages/treatment).
[2] S.E. of the mean.
[3] This chick died on 11th day with edema.
[4] These chicks were severely edematous.

gains among birds fed 200 p.p.b. CEF were similar to those of controls for the first two weeks but were less than those of controls for the third week. The birds fed 50 p.p.b. CEF grew less during the second and third weeks than the controls or the birds fed 200 p.p.b. Weight gains were equivalent among all groups during the first week.

There were a few deaths, all of which were associated with feeding CEF concentrate (Table 2). The chicks that died had edema. Birds with severe edema always had gross organ changes. These pathological changes were not so severe as those observed among chicks fed the crude TFM (unpublished observations). This observation may be an indication that the purified CEF preparation has been at least partially separated from substances in the crude fat that not only enhance edema formation but also may lead to extensive and more severe pathological changes.

From the data in Table 2 on diet consumption, it is apparent that the CEF concentrate did not alter the amount of diet ingested during the first week. CEF concentrate at 200 p.p.b. caused a moderate decrease in diet consumption during the second week, and at 50 p.p.b. led to a decrease in diet consumption during the third week. The feed/gain ratio was elevated during the second week among birds fed 50 p.p.b. CEF and was further increased during the third week. The feed/gain ratio for the birds fed 200 p.p.b. CEF concentrate was elevated during the third week but it was lower than the ratio for the birds fed 50 p.p.b. From the data, however, it is apparent that feeding of the concentrate was accompanied by a moderate decrease in feed utilization.

From determination of feed intake and knowledge of the amount of crystalline CEF concentrate incorporated into each diet, the amount of purified material ingested was calculated. These calculations are given in Table 2. The total μg. CEF concentrate consumed was 11.7 μg./chick for those fed p.p.b. and 49.6 μg./chick for those fed 200 p.p.b. The amount of CEF concentrate ingested in μg./100 g. of body weight/chick is shown also in Table 2. The total amount of purified concentrate ingested (49.6 μg.) was about ninefold greater than the amount (5 μg.) that Wootton *et al.* (1962) calculated could kill one

TABLE 3.—*Weekly levels of plasma proteins of S. C. White Leghorn cockerels fed CEF concentrate*

CEF level	Albumin	Globulin	A/G ratio	Total protein
p.p.b.	g. %	g. %		g. %
		Day-old		
—	2.19±.05[1]	1.76±.13	1.27±.12	3.95±.10
		Week 1		
0	2.03±.07	1.28±.09	1.66±.19	3.31±.09
50	1.98±.09	1.38±.12	1.47±.10	3.37±.07
200	1.96±.13	1.23±.09	1.59±.19	3.23±.08
		Week 2		
0	2.21±.11	1.07±.04	2.08±.11	3.31±.11
50	2.51±.05	0.98±.06	2.63±.16	3.50±.10
200	2.11±.17	0.95±.08	2.29±.21	3.08±.22
		Week 3		
0	2.29±.11	1.39±.11	1.76±.20	3.68±.11
50	1.99±.20	1.87±.31	1.17±.18	3.88±.37
200	1.96±.16	1.19±.12	1.77±.26	3.15±.20

[1] S.E. of the mean.

chick. The difference between these values may be due to a number of reasons. First, Wootton *et al.* (1962) did not report the amount of food ingested by their birds fed the α 3.02 fraction. Second, they used a heavy breed of chick, while we have used a light breed. Third, though both diets were synthetic, they were not identical. Fourth, their α 3.02 fraction was essentially homogeneous, while our preparation contained perhaps as many as eight components (compare a, b and c, Figure 1). Nevertheless, the prediction of Brew *et al.* (1959) that the chick edema factor may be detectable when fed to chicks at levels of 2.0 p.p.m. or less has been confirmed by our studies and by those of Wootton *et al.* (1962).

The results obtained for plasma albumin, globulins, A/G ratio and total proteins are presented in Table 3. In general, there were no appreciable differences in either the plasma protein fractions or the total plasma proteins when considered on the group basis. Some chicks with severe edema had marked hypoalbuminemia and hypoproteinemia, in confirmation of the observations of Alexander *et al.* (1962). From the data it seems unlikely that the massive edema formed was the result of such modest alterations in circulating proteins. These data support the hypothesis of Flick *et al.* (1963) that the mechanism of CEF toxicity resulting in edema formation may be the effect of CEF on capillary permeability, and perhaps more specifically, on causing chemical alterations in endothelial intercellular cement substance, as postulated by Allen (1961). Even though there was no appreciable depression of circulating albumin, at least sufficient to result in hypoalbuminemic edema (Best and Taylor, 1955; and Smith and Jones, 1961), it may be that even slight decreases in albumin could enhance fluid transfer across endothelial membranes damaged by the chick edema factor.

The degree of development of HP in response to the ingestion of the CEF concentrate is shown in Table 4. The concentrate fed at the 200 p.p.b. level produced a marked increase in fluid which accumulated within the pericardial sac. The magnitude of this response is of the same order as reported previously by Flick *et al.* (1963), using 4% crude TFM in a similar diet. The HP group scores show that the 50 p.p.b. level of CEF did not result in abnormal volumes of pericardial fluid according to the ranges of volumes reported by Shue and Gallo (1961). The HP incidence shows that all the birds fed 200 p.p.b. CEF had abnormally high levels of fluid in the pericardial sac. Also shown in Table 4 are values for hematocrit, serum copper and whole blood glucose. The packed cell volumes were decreased by the two levels of CEF concentrate fed. These decreased hematocrits were not so low as those reported previously by Flick *et al.* (1963) from feeding 5% crude TFM with a practical type of ration.

Serum copper levels (Table 4) were elevated at the 50 p.p.b. level but no statistically significant change from normal occurred at the 200 p.p.b. level of CEF concentrate. Since it had been observed that the liver, heart and kidney were adversely in-

TABLE 4.—*Hydropericardium (HP), hematocrit, serum copper and whole blood glucose values of S. C. White Leghorn cockerels fed CEF concentrate for 3 weeks*

CEF level	Hydropericardium			Hematocrit	Serum Copper	Glucose[3]
	Mean volume	Score[1]	Incidence			
p.p.b.	ml.			% cells	μg. %	mg. %
0	0.060±0.012 (8)[2]	0	0/8	34.2±1.5 (5)	51± 6 (7)	226±11 (8)
50	0.138±0.018 (4)[4]	0	0/5	28.0±0.8 (8)	95±11 (6)	240± 7 (6)
200	4.68 ±1.64 (6)	30+	6/6	27.1±1.4 (8)	41± 8 (5)	217±10 (8)

[1] HP score determined from HP volumes according to the following scale: <0.20, 0; 0.21-0.40 1+; 0.41-1.00, 2+; 1.01-2.00, 3+; 2.01-3.00, 4+; 3.01-4.00, 5+; 4.01-5.00, 6+; 5.01-6.00, 7+; and >6.00, 8+.

[2] These levels are considerably higher than glucose levels among birds fed crude TFM in a purified ration (Flick *et al.*, 1963) in another experiment which resulted in rather marked hypoglycemia (controls: 146±5 mg. %; vs. severe disease: 110±8 mg. %).

[3] S.E. of the mean (number of observations).

[4] One sample of HP fluid lost from this group.

volved in the chick edema disease (Allen, 1961; Alexander *et al.*, 1962; and Flick *et al.*, 1963), and that changes in these organs had been involved in the mechanism of edema formation (Smith and Jones, 1961), it was thought that determination of the levels of serum copper in the birds with the disease might be of some diagnostic value. Scheinberg and Sternlieb (1963) reported that human patients with Wilson's disease had severe liver disease, and many patients had serum copper dyscrasias caused by excessive urinary excretion, malabsorption from the intestine, decreased protein synthesis (particularly in severe malnutrition) and severe hepatic dysfunction. It may be that the elevated serum copper among the chicks fed 50 p.p.b. CEF concentrate was associated with either decreased liver utilization of copper or decreased renal excretion. The adverse effect on serum copper of CEF concentrate fed at 50 p.p.b. is not clear, but may be indicative that CEF toxic activity is oligodynamic and more specifically oligotoxic (more toxic at low levels than at higher levels).

Whole blood glucose levels were not appreciably altered by feeding the concentrate (Table 4). Crude TFM, however, fed in a purified ration, frequently elicited a marked hypoglycemia among chicks in advanced stages of the disease (see footnote 2, Table 4).

From what is known to date, the chick edema factor elicits a number of signs of intoxication which not only accompany the feeding of crude TFM but are more severe when the crude material is fed. The finding that our CEF concentrate contained 8 peaks by electron capture gas chromatography (Figure 1) indicates that at least 8 compounds were present in the purified preparation fed in these studies. It may be that only one, or a few, of these compounds possess the necessary molecular configuration to produce signs of the disease equivalent to the estimated potency of the α 3.02 fraction.

Hatchability Study. The preliminary results obtained from injection of White Leghorn eggs with the unsaponifiable fraction of TFM, which contained CEF, are shown in Table 5. The percent hatch of control eggs was within the expected range reported by McLaughlin *et al.* (1963). Chicks which hatched appeared to be normal in size and development by gross observation. When fertile eggs were yolk-injected with 10 μl. of the undiluted unsaponifiable fraction containing CEF, the hatch was 40%; injection of 20 μl. resulted in

TABLE 5.—*Effect of CEF-containing unsaponifiable fraction of TFM on embryonic development and egg hatchability*

Unsap. injected	No. eggs injected	Hatch	Observations	
			Embryo	Chick
μl.		%		
0[1]	20	93[2]	—	Normal
10	20	40	Malformations of right cerebral hemispheres, legs and beaks; small embryos	Weight retardation, sparse and deformed feathers.
20	20	20		
50	5	0	—	—

[1] Controls were injected with 100 μl. of corn oil.

[2] Normal expected % hatch (McLaughlin *et al.*, 1963).

20% hatch and 50 μl. completely inhibited hatching. Embryos which failed to hatch exhibited one or more of the following developmental anomalies: malformed beak, lack of development of the right mesencephalon, eye defects, growth retardation or leg deformities. The deformities observed were not studied further. The deformities found were common to the embryopathies which occurred and were not related to level of CEF-containing fraction injected. Embryos which hatched after injection with 10 or 20 μl. of unsaponifiables exhibited sparse and defective feathering (down) and were small compared to the controls. This study revealed that CEF-containing unsaponifiables are capable of interfering with normal embryonic development and hatchability.

SUMMARY

The following studies were performed: gas-liquid chromatographic (GLC) separation of a purified crystalline concentrate containing the chick edema factor (CEF); feeding of the concentrate at 50 or 200 parts per billion (p.p.b.) in a semi-synthetic diet to day-old S. C. White Leghorn cockerels for three weeks and determination of growth, feed intake, feed/gain ratio, mortality, total intake of CEF concentrate, plasma proteins, hydropericardium (HP), hematocrit, serum copper, whole blood glucose; injection of CEF-containing unsaponifiable fraction and determination of its effects on embryonic development and on egg hatchability.

The following observations were made: 1) presence of approximately eight components (GLC separation) in crystalline material, 2) moderate growth depression at 50 p.p.b. level of CEF concentrate, 3) essentially normal feed consumption, 4) moderately increased feed/gain ratio, 5) increased HP at 50 p.p.b. level of CEF concentrate, 6) severe HP at 200 p.p.b. level of CEF concentrate, 7) essentially no group changes in plasma proteins, 8) moderate decrease in hematocrit, 9) no change or moderate elevation of serum copper (50 p.p.b. CEF concentrate), and 10) normal blood glucose levels.

A preliminary hatchability study revealed that a CEF-containing material led to a decreased hatch of injected eggs and to development of embryonic deformities.

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NUTRITIONAL ADJUNCTS

Chick Edema Factor. III. Application of Microcoulometric Gas Chromatography to Detection of Chick Edema Factor in Fats or Fatty Acids

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A rapid screening test for detecting chick edema factor in fats consists of adsorption chromatography of extracted unsaponifiables on alumina, followed by analysis of specific fractions by a microcoulometric gas chromatograph which is sensitive only to halogens. This chromatographic method appears to be more sensitive than the chick bioassay.

The widespread occurrence of chick edema disease in 1957 resulted in the deaths of millions of young chickens. The toxic materials causing this disease have been found to be chlorinated aromatic hydrocarbons, occurring in toxic fats in association with a large number of relatively nontoxic aromatic materials with similar chemical and physical properties. This paper describes a screening procedure for detection of such toxic fats. Specific fractions of unsaponifiable matter isolated from the fats are examined by using a microcoulometric gas chromatograph, an instrument which can detect submicrogram amounts of halogen. The presence of slow-eluting substances is an indication of the chick edema factor in the fat.

The precise structure of the substances causing chick edema disease have yet to be determined. Preliminary work on the detection, isolation, and characterization of the toxic agents was reported in 1959 by several laboratories (1-3). Subsequently,

Harmon and co-workers (4) isolated a toxic substance in crystalline form from a feed grade tallow. A private communication from Tishler of the same laboratory (5) disclosed that the crystalline substance contained about 47% chlorine.

Yartzoff and co-workers (6) isolated a crystalline halogen containing material that produced chick edema symptoms at 0.1 ppm in the diet from a sample of triolein. This triolein was toxic to monkeys, producing changes in the liver, kidney, pancreas, and other organs. More recently, Wootton and co-workers (7) isolated three compounds from a toxic fat which produced chick edema disease. Mass spectra of two of the compounds indicated a molecule which has a molecular weight of 391 and contains six chlorine atoms. Ultraviolet spectra were consistent with the concept that these materials are highly chlorinated aromatic compounds.

Ames and co-workers (8) and Firestone and co-workers (9) reported the occurrence of chick edema disease factor in oleic acid samples destined for human consumption. A food additive regulation[1] of the Food and Drug Administration now requires that food grade fatty acids be "free of chick edema or other toxic factor." At present, the detection and assay of chick edema factor in fats is carried out by a bioassay procedure (10–12) that requires 21 days to complete.

We observed that unsaponifiable matter from toxic fats contained a number of chlorinated components which had greater retention times than chlorinated pesticides when examined in a microcoulometric gas chromatograph;[2] our observation prompted this investigation of the use of microcoulometric gas chromatography for detecting the presence of chick edema factor in fats. Chick edema factor is presumed to be present if one or more gas chromatographic peaks with retention times relative to aldrin of 5 to 20 are found; its absence is presumed if analysis of the equivalent of 100 g of a fat or fatty acid fails to reveal the presence of these gas chromatographic peaks.

METHOD

Extraction of unsaponifiable matter (modification of AOAC method 26.064(5).—Reflux 111 g sample with 270 ml alcohol and 55 ml 50% (w/w) KOH for 1 hour. Transfer mixture to 2 L separator, rinsing flask with 325 ml H_2O, and add rinsings to separator. Add 300 ml petroleum ether, A.C.S. (redistilled, retaining cut with b.p. 40–60°C), and shake vigorously. Let layers separate, breaking emulsions that may have formed by adding 10 ml alcohol and swirling gently. Draw off lower layer, and transfer upper layer to another separator. Repeat extraction 3 times with 300 ml portions of petroleum ether and combine extracts. Wash extracts twice with 60 ml portions of H_2O by swirling gently. Wash petroleum ether extracts first with 60 ml H_2O and then with 60 ml of an alkaline dilute alcohol soln (dissolve 28 g anhydrous K_2CO_3 in 600 ml H_2O and then add 400 ml alcohol), and repeat washings in same order. Wash extracts with 60 ml portions of H_2O until neutral to phenolphthalein. Transfer extract to a 2 L erlenmeyer and dry by adding 20 g anhydrous Na_2SO_4, swirling vigorously, and letting the solution stand a half hour. Decant solution through a glass funnel, containing a pledget of cotton in the neck and holding 20 g anhydrous Na_2SO_4, into another 2 L erlenmeyer. Wash first erlenmeyer and funnel with three 10 ml portions of petroleum ether, transferring washings from the erlenmeyer through the funnel and into the filtered solution. Evaporate most of solvent on steam bath, and transfer extract to 100 ml tared fat flask containing several boiling chips. Evaporate solvent on steam bath and complete drying under a gentle current of air, or by evacuating flask to 0.5 cm of mercury while swirling on steam bath. Determine weight of unsaponifiable matter.

Fractionation of unsaponifiable matter by alumina chromatography.—To a chromatographic column, 25 mm o.d. × 300 mm long, fitted at the bottom with a coarse porosity fritted glass disk and Teflon stopcock, add redistilled petroleum ether, dried prior to use with anhydrous Na_2SO_4, until column is ¾ full. Weigh 50 g aluminum oxide (Merck reagent, No. 71707), and transfer to column. Store the alumina in tightly closed bottle, and close bottle as soon as possible after weighing out portions for chromatography. Let alumina settle, and when air bubbles stop rising to the surface of the solvent, place a disk of coarse filter paper on top of the alumina. Cover the disk with 20 g anhydrous Na_2SO_4. Drain the excess petroleum ether so that it is level with the upper surface of the Na_2SO_4.

Transfer unsaponifiable matter to the chromatographic column, using a total of 20 ml petroleum ether. Allow liquid level to fall so that it is just below the top of the Na_2SO_4. Elute sample with 400 ml portions of each of the following solvents (dried prior to use by shaking with anhydrous Na_2SO_4): Petroleum ether (fraction 1), 5% ethyl ether in petroleum ether (fraction 2), and 25% ethyl ether in petroleum ether (fraction 3). Collect eluates in 500 ml erlenmeyer flasks, add several boiling chips, and evaporate to small volume on steam bath. Transfer residues to tared fat extraction flasks, evaporate solvent, and weigh. Transfer to 2 g short style vials having screw cap with tin liner, and evaporate solvent.

Microcoulometric gas chromatography.—Dissolve 4 g silicone grease (Dow Corning High Vacuum Grease) or Dow Corning DC 200 silicone fluid (12,500 centistokes) in 200 ml chloroform on steam bath. Add 16 g acid-washed Chromosorb W (Johns-Manville Co.), and stir continuously until most of solvent evaporates (about half an hour). Let stand on steam bath 1 hour, and place in vacuum oven at 50°C overnight to remove residual solvent.

Pack the coated Chromosorb W into a 3' length of 0.25" o.d. aluminum tubing plugged at one end with glass wool, using a Burgess Vibratool. (Two 3' columns may be prepared from 20 g coated Chromosorb W.) Add a plug of glass wool to the open end of the column and bend it into a tight spiral, using a 3" diameter mandril. Condition the column at 275°C for 48–72 hours, passing nitrogen through at 20 ml per minute.

Prepare a 1.00×10^{-3}% solution (10 mg/L) of aldrin in hexane or benzene and chromatograph 100 µl portions in a Dohrmann microcoulometric gas chromatograph at 246–248°C, using a nitrogen flow rate of 50–100 ml per minute so that aldrin elutes in 2.3–3 minutes. Use the 128 ohm range setting.[3] Determine area of aldrin peak by triangulation, or with a disc chart or electronic integrator installed on the strip chart recorder, and calculate recovery of aldrin using the following equation (applicable to chlorinated compounds):

$$\mu g\ \text{Aldrin} = (\text{peak area, in.}^2) \times [\text{recorder sensitivity (min./in.)(mv/in.)}] \times (35.5\ \text{g/eq.}) \times (60\ \text{sec./min.}) \times (10^6\ \mu g/g)(10^3\ \text{v/mv})(10)/(\text{sensitivity range, ohms}) \times (\%\ \text{chlorine in compound}) \times (96{,}500\ \text{coulombs/eq.}).$$

For a 0.1 mv/in. recorder sensitivity, 2 min/in. chart speed, and 12.8 ohms sensitivity range resistance, the equation above reduces to:

$$\mu g\ \text{Aldrin} = (\text{area} \times 34.5)/\%\ \text{chlorine}.$$

The number of strokes of a disc chart integrator coupled to a chromatography recorder[4] equivalent to each square inch of area can be determined as follows: (*a*) Remove the fuse from the strip chart amplifier; (*b*) move the pen upscale on the strip chart a known distance from the baseline; (*c*) run the chart a known distance; and (*d*) divide the calculated area (height × distance traversed by the pen) by the number of strokes obtained.

By using the formula calculated as described above, a recovery of at least 70% of the aldrin injected should be obtained.

Dissolve fractions 2 and 3 from alumina chromatography separately in benzene to give 100 µl solution, and chromatograph each solution in the Dohrmann instrument. (For analysis of more than about 60 mg of each fraction, approximately 50% benzene solutions of up to 250 µl volume should be prepared and injected. Do not inject more than 125 mg material into column). First chromatograph 1/10 of the fraction, and if no chromatographic peaks with $R_A = 5$ or greater are observed, chromatograph the remaining 9/10 of the fraction (equivalent to 100 g starting sample). Chromatograph a portion of aldrin before each sample, and calculate R_A value (retention time relative to aldrin) of each peak in the sample chromatogram, using a millimeter rule to measure retention times. Record R_A of gas chromatographic peaks in the range $R_A = 5$–20. Peaks in this range are indicative of the presence of chick edema factor. The presence of broad bands with no definite peaks is not indicative of the presence of chick edema factor.

(*Note:* Types of samples which are found from experience to be generally free of components characteristic of toxic fats may be examined as described above in 100 g portions, the sample saponified by refluxing with 240 ml alcohol and 50 ml 50% (w/w) KOH, and all of each of the polar alumina fractions gas chromatographed.)

[1] *Code of Federal Regulations, Title 21, Section 121.1070.*

[2] Dohrmann Manufacturing Company, Palo Alto, Calif.

[3] This setting will have a resistance of 12.8 ohms when the chromatograph is used with a 1 mv strip chart recorder.

[4] Minneapolis-Honeywell Model Y 153X (Minneapolis-Honeywell Regulator Co., Philadelphia, Pa.), or equivalent.

Results and Discussions

Relative Retention Times of Chlorinated Pesticides and Chlorinated Materials from Toxic Fats.—A number of chlorinated pesticides and several chlorinated materials isolated from toxic fats were chromatographed in the Dohrmann instrument at 248°C with the 3' column. Retention times relative to aldrin (R_A) are shown in Table 1. The pesticides are representative of the whole range of retention times displayed by chlorinated pesticides. A toxic factor isolated from triolein (6), an inactive analogue, and a concentrate prepared from a toxic fat, all yielded chromatograms with peaks of $R_A = 5$ or greater whereas the pesticide peaks were all less than $R_A = 4$. The toxic factor from triolein as well as the toxic fat concentrate produced chick edema when fed to young chicks at a level of 0.1 ppm in the diet.

Table 1. Relative retention times of chlorinated pesticides and materials isolated from toxic fats

(3 foot, ¼ in. diameter column, 20% silicone grease, 80% Chromosorb W; carrier gas flow rate, about 60 ml/min.; column temperature, 248°C; injection block temperature, 270°C)

Sample	Retention Time vs. Aldrin (R_A)
Chlordane	1.0
Heptachlor	0.9
Kepone	2.2
Mirex	3.6
Strobane	0.5–3.5
Tedion	3.4
Toxaphene	0.6–3.8
Toxic factor from triolein	5.0
Inactive analogue from triolein	9.0
Concentrate from a toxic fat	2.3, 3.6, 5.4

The chick edema-producing factors isolated by Wootton and co-workers (7) had retention times relative to methyl arachidate of 1.17, 3.02, and 3.17 when chromatographed at 250°C on a 20% silicone column. Since aldrin elutes twice as fast as methyl arachidate under these conditions, it would be expected that these toxic factors would have R_A values of about 2.4, 6.0, and 6.3 at 250°C on silicone columns. When a low-melting inactive isomer[3] having the same retention time ($R_A = 6.0$) as one of the toxic factors was chromatographed in the Dohrmann instrument, the following R_A values were obtained at 246°, 248°, and 250° respectively: 6.6, 6.4, and 6.2.

Preliminary Analysis of a Group of Toxic and Nontoxic Fats. Microcoulometric Analysis of Unsaponifiable Matter Without Prior Fractionation on Alumina.—A group of 7 toxic and 7 nontoxic fats were examined initially. The fats are described in Tables 2

Table 2. Data on toxic fats

Component	Manufacturer	% Unsaponifiable Matter	Organic Cl in Unsaponifiable Matter, ppm
1. Tallow acids, still distillate	1	18.3	10
2. Tallow acids, still distillate	1	14.8	47
3. Tallow acids, still distillate	2	10.1	14
4. Tallow acids, still distillate	3	2.9	25
5. Tallow acids, still residue	1	4.5	2000
6. Tallow	2	5.1	30
7. Fat from broiler feed	4	2.5	8000

and 3, respectively. Presence of chick edema disease was determined by bioassay (2) using a special basal ration. Organic halogen in the unsaponifiable matter (assumed to be chlorine) was determined by microcoulometric gas chromatographic analysis of 50 mg portions of unsaponifiable matter without prior fractionation by alumina chromatography. Both toxic and nontoxic fats contained widely variable amounts of unsaponifiable matter and organic chlorine. Sources refer to individual manufacturers. Gas chromatograms of unsaponifiable matter from the

[3] Supplied by Dr. N. R. Artman, Procter and Gamble Co., Cincinnati, Ohio.

Table 3. Data on nontoxic fats

Component	Manufacturer	% Unsaponifiable Matter	Organic Cl in Unsaponifiable Matter, ppm
1. Cottonseed oil (CSO)	8	0.6	80
2. Cottonseed oil	8	0.5	13
3. CSO foots, still residue	6	12.1	5
4. CSO fatty acids	2	0.4	31
5. Vegetable oil foots	5	2.2	28
6. Tallow fatty acids, still residue	0	2.2	7
7. Corn oil	7	0.6	150

other toxic fats and from nontoxic fats obtained without prior fractionation by alumina chromatography were similar; most of the organic halogen eluted in 2–4 minutes, and there were no peaks with R_A values greater than 3. Since components with R_A values of 5 or more are usually present in the unsaponifiable matter of toxic fats at very low levels, a concentration step by alumina chromatography is necessary prior to microcoulometric analysis.

Fractionation of Unsaponifiable Matter on Alumina Prior to Microcoulometric Analysis.—The 7 toxic and 7 nontoxic fats were then analyzed by procedures essentially as described above. At first, 6-foot, and later, 3-foot chromatographic columns were used in the microcoulometric gas chromatograph. With shorter columns, faster elution permitted analysis of 12 samples each working day, and results were comparable to those obtained with the conventional 6-foot columns. Because 10–100 mg portions of sample were repeatedly injected, all components of the microcoulometric gas chromatograph were cleaned every 2–4 weeks as required. Gas chromatographic columns were replaced each 1–2 months. In cases where samples contained large amounts of unsaponifiable matter, larger alumina columns were used for the column chromatography so that the ratio of alumina to unsaponifiable matter was at least 20 to 1. In these cases appropriately larger amounts of eluting solvents were also used.

Fractions obtained by adsorption chromatography on alumina of unsaponifiable matter were analyzed by microcoulometric gas chromatography. Gas chromatograms of polar fractions from toxic fats (eluted with 5% and 25% ethyl ether in petroleum ether) all showed peaks with R_A values greater than 5. No peaks with R_A values greater than 5 were found in gas chromatograms of these fractions from the nontoxic fats.

Portions of alumina fractions 2 and 3 equivalent to only about 50 g of the nontoxic still residues (Table 3, samples 3 and 6) were chromatographed in the Dohrmann instrument because of the presence of a large amount of crystalline material in these fractions. The infrared spectrum of this material (isolated by recrystallizing from petroleum ether) resembled that of dipalmitone. An additional cleanup procedure must be developed for routine analysis of 100 g samples of such still residues. Additional examination of unsaponifiables from 2000 g portions of three nontoxic vegetable oils (Table 3, samples 1, 2, and 7) failed to reveal chromatographic peaks with R_A values greater than 3.

Table 4. Slow-eluting peaks in microcoulometric gas chromatograms from analysis of toxic fats

No.	Toxic Fat	Manufacturer	$R_A \geq 5$
1	Tallow acids, still distillate	1	6, 9, 12, 21
2	Tallow acids, still residue	1	6, 9, 10, 14
3	Tallow acids	1	6, 9, 10, 18
4	Tallow	2	6, 9, 12
5	Tallow acids, still distillate	2	6, 9, 10, 18
6	Tallow acids	3	6, 10

Table 4 lists the slow-eluting peaks found in gas chromatograms from 6 of the 7 toxic fats. Figure 1 shows chromatograms of alumina fraction 3 from three still distillates, each of which was obtained from a different manufacturer of commercial fatty acids. A similar pattern of slow-eluting peaks sug-

gests that a common complex contaminant may be responsible for the presence of chick edema factor in fats. Each peak probably represents a complex mixture of closely related compounds. In fact, when polar alumina fractions from several toxic fats were further fractionated by additional column chromatography on alumina, such purification often resulted in partial resolution of the corresponding peaks into at least 2 components.

The fat from a chick edema-producing sample of broiler feed (toxic sample 7) contained over 400 ppm chlordane, and this sample required special treatment because large quantities of chlordane eluted in alumina fractions 2 and 3. Although the R_A of chlordane = 1, the large amounts present produced overloaded chromatograms which interfered with gas chromatographic detection of other components. A portion of combined fractions 2 and 3 was molecularly distilled in a "cold finger" pot still for 2 hours at 85°C and 50 μ pressure. The chlordane was volatile under these conditions and was eliminated from the residue which was analyzed in the Dohrmann instrument. Peaks with R_A values greater than 5 were then found in the chromatograms.

These results indicated that alumina chromatography of unsaponifiable matter followed by microcoulometric gas chromatography of appropriate fractions might be used as a screening procedure to detect chick edema factor in fats and fatty acids. Additional work on cleanup procedures is required before this technique can be applied routinely to examination of 100 g samples of low-grade fats, such as still residues containing large amounts of material that elute in alumina fractions 2 and 3.

Effect of Alumina Activity and Column Dimensions on Adsorption Chromatography.—Alumina activity was found to affect the rate of elution of substances from toxic fats which are responsible for the gas chromatographic peaks of $R_A = 5$ and greater which are characteristics of toxic samples. Various batches of Merck alumina used for this work were found to vary in activity from

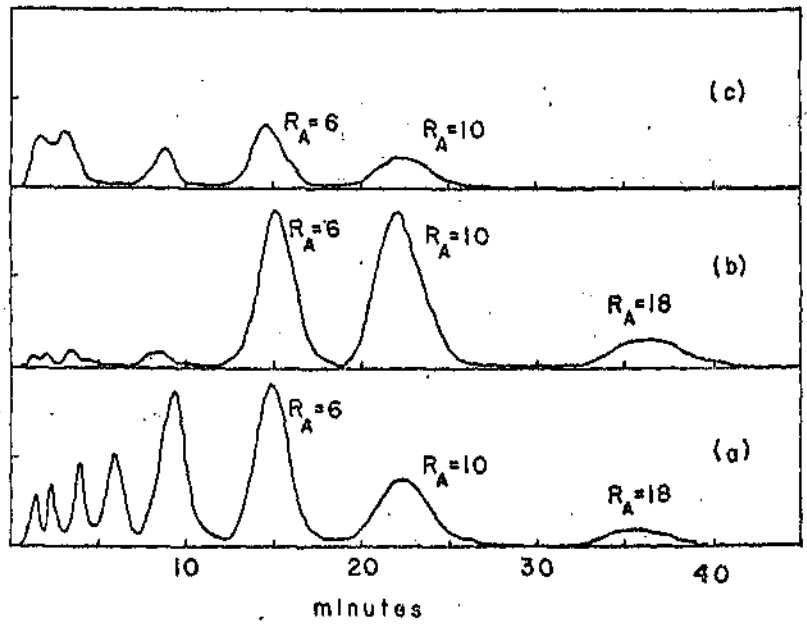

Fig. 1—Microcoulometric gas chromatograms of alumina fraction 3 isolated from toxic batch still distillates obtained from 3 manufacturers of commercial fatty acids. The fractions were isolated from (a) 5 g, (b) 5 g, and (c) 20 g of fat.

Brockmann activity I to activity II. Activities were determined by observing the rate of travel of solutions of specific pairs of azo dyes (13, 14). Using activity I alumina, unsaponifiable matter from toxic fat or from toxic fat added to USP cottonseed oil was eluted from the columns so that the characteristic slow-eluting peaks were found in gas chromatograms of alumina fraction 3. With activity II alumina, these peaks were found in chromatograms of alumina fraction 2.

Generally, the alumina used in this work was not standardized; it required gas chromatographic analysis of alumina fractions 2 and 3. Standardization of the alumina should permit elution of the slow-eluting compounds in one fraction, reducing the number of samples required for gas chromatography. For example, Merck alumina heated 48 hours at 200°C had a Brockmann activity I, and all the slow-eluting peaks from several toxic fats examined were found in chromatograms of fraction 3. Work is continuing on a procedure for standardizing alumina in a simple and reproducible manner.

Column dimensions also were found to affect the elution of characteristic substances from toxic fats. When activity I alumina was used, these substances were eluted in fraction 3 from a 25 × 300 mm column, whereas they eluted in fraction 2 from a 30 × 300 mm column.

Effect of Column Temperature and Flow Rate on Gas Chromatography of Slow-eluting Components of Toxic Fats.—Studying the gas chromatographic behavior of chlorinated pesticides, Burke and Johnson (15) found that varying the column temperature and/or the carrier gas flow rate resulted in variations of relative retention times of the pesticides. Similar variations in relative retention times were observed with the slow-eluting components of toxic fats. R_A values, however, were affected more by variations in temperature than in flow rate. A toxic substance isolated from triolein (6) had the following R_A values at 246°, 250°, and 252°C: 5.6, 4.9, and 4.7. An inactive analogue isolated from the sample had the following R_A values at these temperatures: 9.7, 8.0, and 7.8.

Because of the design of the microcoulometric gas chromatograph used for this work, whereby oven temperatures are controlled only by a variable transformer, line voltage fluctuations result in continuous variation of column temperature. A variation of ± 1°C within a 1-2 hour period is the best stability to be expected. Even with these variations, however, the instrument is suitable for detection of slow-eluting materials in toxic fats because of the large difference in retention times between these slow-eluting materials and the chlorinated pesticides and other fast-eluting chlorinated materials found in all fats examined.

Analysis of Bioassay Collaborative Samples.—A recent collaborative study of the AOAC bioassay method for detection of chick edema disease (12) indicated that the lower limit of sensitivity was obtained with a test sample containing 1.56% of a toxic fat in USP cottonseed oil. One hundred g portions of this sample, the toxic fat, and the original cottonseed oil were analyzed. Extracted unsaponifiables were chromatographed on 50 g alumina as described above, and the 5% and 25% ethyl ether eluates (fractions 2 and 3) were gas chromatographed. Chromatograms of fraction 2 from the cottonseed oil without and with added toxic fat are shown in Figs. 2 and 3, respectively. Peaks with R_A values greater than 1.5, including the $R_A = 6$ and $R_A = 10$ peaks, are due to the toxic fat. A chromatogram of alumina fraction 2 from 100 g of test sample containing 0.78% toxic fat in

Table 5. Analysis of USP cottonseed oil containing various levels of added toxic fat; microcoulometric gas chromatography of alumina fraction 2

Toxic Fat Added, %	Disc Integrator Response (No. of Pen Strokes)	
	$R_A = 6$	$R_A = 10$
0.00	0	0
0.78	6	2
1.56	14	6
3.12	32	13
4.68	32	12
6.24	47	28

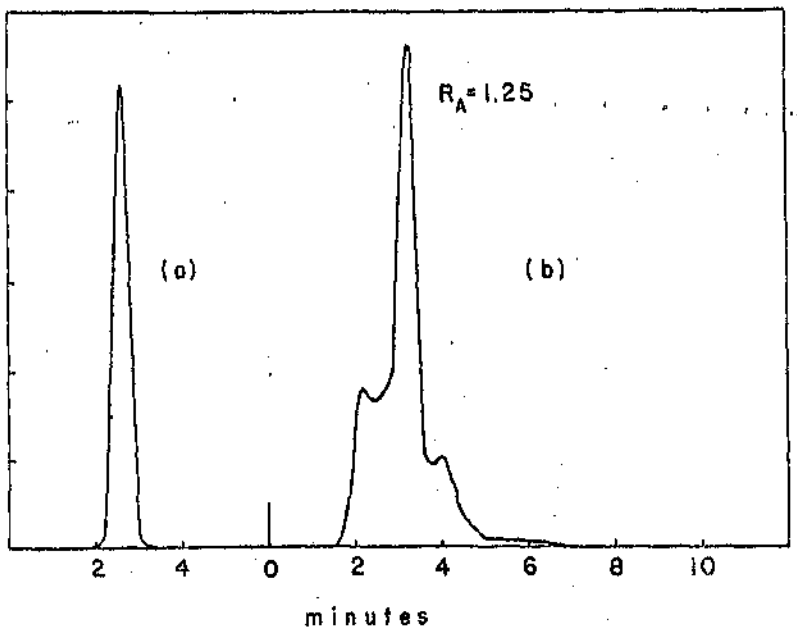

Fig. 2—Microcoulometric gas chromatograms of (a) aldrin standard (1 μg), and (b) alumina fraction 2 from USP cottonseed oil without added toxic fat.

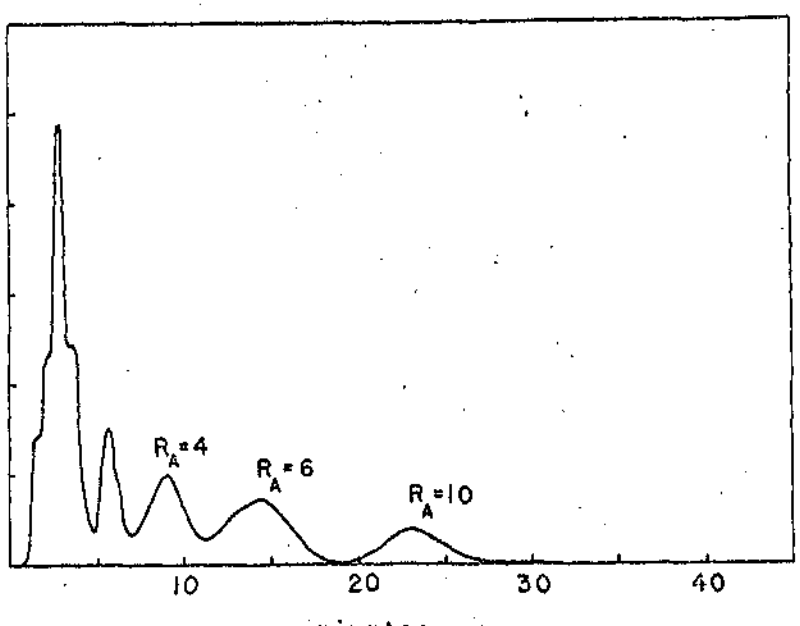

Fig. 3—Microcoulometric gas chromatogram of alumina fraction 2 from USP cottonseed oil containing 1.56% toxic fat.

the USP cottonseed oil is shown in Fig. 4. The $R_A = 6$ and $R_A = 10$ peaks can still be definitely detected at this level of toxic fat, which was half of that found to be at the lower limit of detection of the AOAC bioassay.

Samples of USP cottonseed oil containing various levels of added toxic fat up to 6.24% were analyzed. The disc integrator response of the $R_A = 6$ and $R_A = 10$ peaks are shown in Table 5. The integrator response (number of pen strokes) is approximately proportional to the level of added toxic fat in the cottonseed oil. Each stroke is equivalent to about 0.05 μg of organic halogen.

Analysis of Commercial Oleic and Stearic Acids and Derivatives.—Twelve food grade oleic acids were examined by the chromatographic method. The examination of 9 of these samples for chick edema toxicity was reported earlier (9). Gas chromatograms of alumina fractions from a nontoxic and a toxic acid are shown in Figs. 5 and 6, respectively. Chromatographic peaks of $R_A = 6$ and greater from the toxic sample are shown in Fig. 6. These peaks were present in alumina fractions 2 and 3. The "peak" with $R_A = 6$ in the chromatogram from alumina fraction 1 is believed to be an artifact due to overloading of the coulometer.

Results of analysis of the 12 oleic acids, compared with the chick bioassay using a special basal ration (2), are shown in Table 6. Hydropericardium activity, the primary index of the presence of chick edema factor, was estimated as described by Firestone and co-workers (9). Six samples were positive by both the bioassay and chromatographic methods. One sample (No. 9) was negative by the regular bioassay when fed at the usual level of 16% in the diet. However, a positive response was obtained when *extracted unsaponifiable matter* was fed at a level equivalent to 6 times that present in the normal test diet.

Sample 10 was negative by the chick bioassay, but gave a weak positive response by the chromatographic method. A small chromatographic peak with $R_A = 10$ was obtained from alumina fraction 2. In comparing bioassay activity and peak areas of slow-eluting components of the other toxic oleic acids, it would be expected that the level of chick edema factor in sample 10

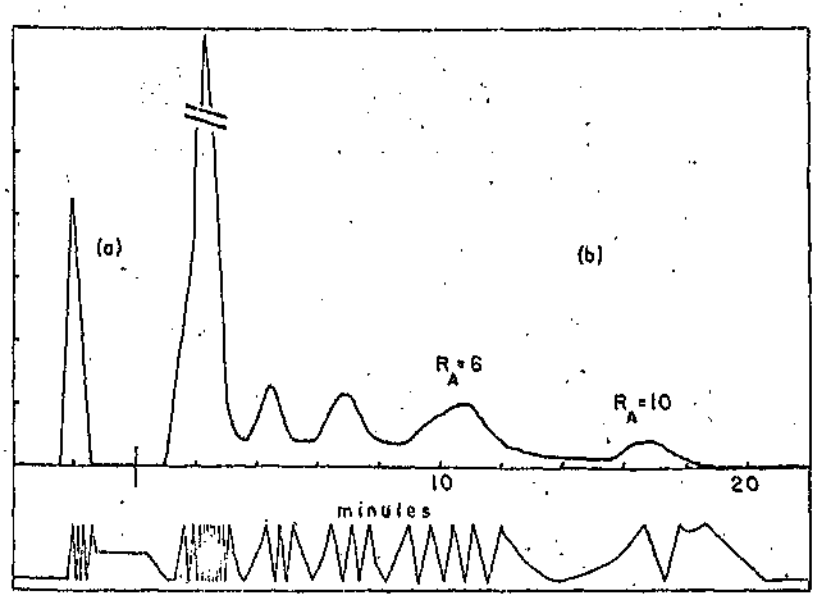

Fig. 4—Microcoulometric gas chromatograms of (a) aldrin standard (1 μg), and (b) USP cottonseed oil containing 0.78% toxic fat. Also shown are disc integrator pen strokes.

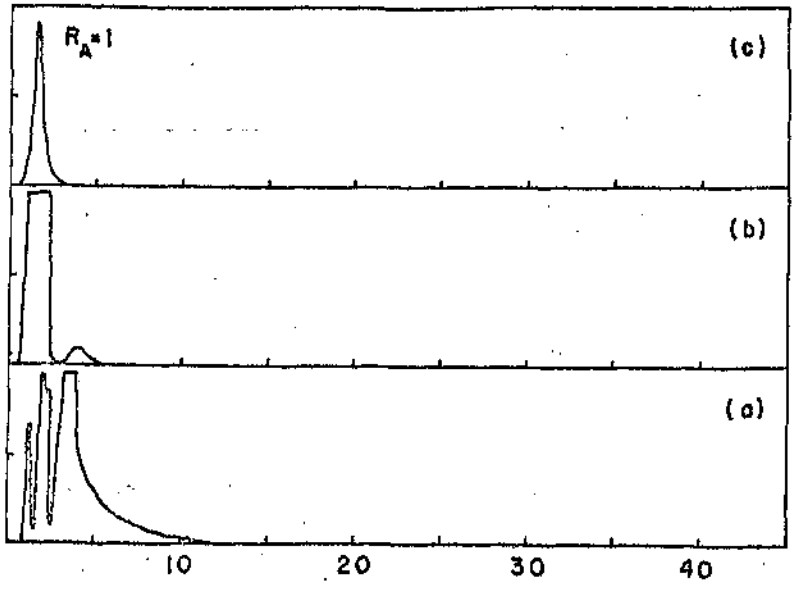

Fig. 5—Microcoulometric gas chromatograms of (a) alumina fraction 1, (b) alumina fraction 2, and (c) alumina fraction 3 from a non-toxic commercial oleic acid.

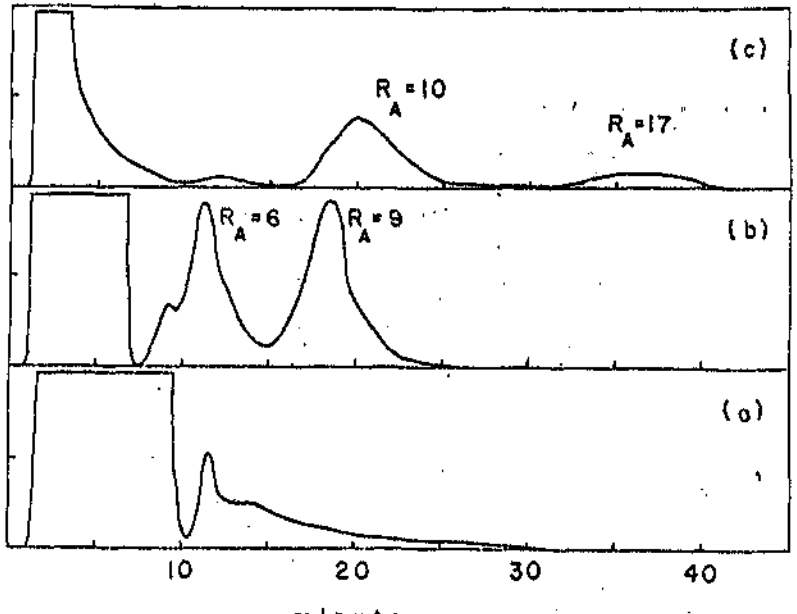

Fig. 6—Microcoulometric gas chromatograms of (a) alumina fraction 1, (b) alumina fraction 2, and (c) alumina fraction 3 from a toxic commercial oleic acid.

Table 6. Analysis of commercial oleic acids

Sample No.	Bioassay: Hydropericardium Activity (0)	Chromatographic Analysis, Relative Peak Area (Integrator Strokes)		
		R_A = 5–6	R_A = 9–10	R_A = 15–18
1	+0.2	74	150	9
2	+3	340	90	8
3	+0.3	98	196	104
4	+0.1	118	156	9
5	+0.2	46	88	14
6	—			
7	—			
8	—			
9	+0.1[b]	106	105	25
10	—		0.6	
11	—			
12	—			

[a] Each stroke is equivalent to about 0.05 μg organic halogen.
[b] Unsaponifiable matter fed at a level equivalent to 6 times that present in the normal test diet.

might be below that detectable by the bioassay.

In comparing hydropericardium activity and gas chromatographic response (relative peak area), no parallel relationship was found between the bioassay response of the toxic oleic acids and the chromatographic response. It should be emphasized, however, that the slow-eluting compounds in toxic samples represent both toxic and relatively nontoxic materials, and most of the gas chromatographic response is probably due to relatively nontoxic substances. Nevertheless, microcoulometric gas chromatographic detection of slow-eluting compounds appears to be an effective screening tool for segregating questionable samples and diverting them to nonedible uses or for further testing by bioassay.

The gas chromatographic response of the $R_A = 9$ peak present at widely different levels in 2 oleic acids was checked by analysis of individual samples on different days, using different gas chromatographic columns for each run. Results are shown in Table 7. Sample 1 contained a low level of material responsible for the $R_A = 9$ peak. The differences in integrator response are due both to variation in coulometer response and to inaccuracies of disc integrator response at low halogen levels. The larger amounts of material in sample 2 responsible for the $R_A = 9$ peak were distributed in alumina fractions 2 and 3 to varying extents depending upon the alumina activity. The total integrator response from both alumina fractions was fairly constant, however, varying from 136 to 169 integrator pen strokes.

In addition to the oleic acids, a number of derivatives of oleic acid (triolein, glycerol monooleate, etc.) were analyzed. Nine of ten samples were negative by both the bioassay and chromatographic analysis. One toxic sample, a triolein, gave gas chromatograms with peaks of $R_A = 6$, 10, and 19.

Ten stearic acids and derivatives examined were negative by the bioassay and chromatographic method.

Analysis of Animal and Vegetable Fats and Commercial Vegetable Oil Fatty Acids.—Fifteen animal and vegetable fats were examined. Three toxic animal tallows were positive by the chromatographic method.

Table 7. Response[a] of $R_A = 9$ peak from 2 oleic acids

Run No.	Sample 1		Sample 2		
	Fraction 2	Fraction 3	Fraction 2	Fraction 3	Total
1	0.2	0	20	116	136
2	0.8	0	96	48	144
3	0.6	0	168	0.5	169
4	5.0	0	100	50	150

[a] Disc integrator response in number of disc integrator pen strokes.

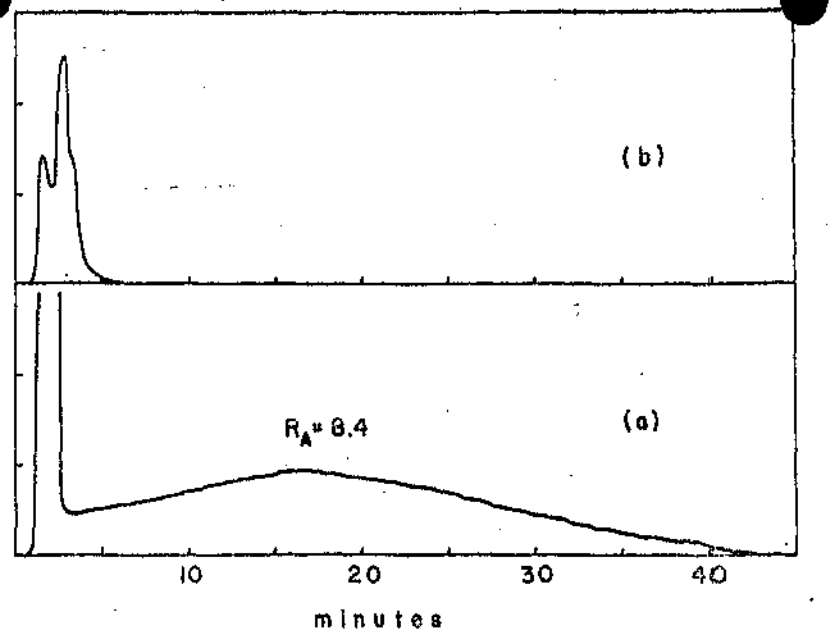

Fig. 7—Microcoulometric gas chromatograms of (a) alumina fraction 2, and (b) alumina fraction 3 from a growth-depressing cottonseed oil which did not produce chick edema disease.

Ten vegetable fats (including cottonseed oil, corn oil, peanut oil, safflower oil, and soybean oil) were examined, and were negative by the bioassay and chromatographic method. Chromatograms from several of the vegetable oils showed broad bands with no definite peaks. Chromatograms of alumina fractions 2 and 3 from one of these samples, a USP cottonseed oil, are shown in Fig. 7. The broad band in chromatogram (a) is typical of that found in the other oils. This sample depressed the growth of young chickens, but did not produce symptoms of chick edema disease. The broad band in chromatogram (a) with a maximum at 8.4 is not characteristic of a toxic fat. No additional work has been done to identify the substances causing these broad bands.

Twelve commercial vegetable oil fatty acids, nontoxic by the chick edema bioassay, were examined. These samples included fatty acids from coconut, cottonseed, corn, palm, soybean, and tall oils. Eleven of the samples were negative by the chromatographic method, but one of the samples (a tall oil fatty acid) gave a small chromatographic peak with $R_A = 5$, indicative of a toxic sample.

Acknowledgments

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The Injection of Chemicals into the Yolk Sac of Fertile Eggs prior to Incubation as a Toxicity Test

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The increasingly large number of food additive chemicals introduced into the market each year has necessitated the development of rapid and reliable methods for the evaluation of their toxicity. Toxicologic studies of all these chemicals by the usual methods using animals are very difficult, and such studies sometimes give inconclusive results.

The toxicity of some chemicals, and especially of food additives, may be determined by injection of the chemical into the yolk sac of fertile eggs prior to incubation and subsequent observation of the effects of the chemical on the embryonic development of the chick. This appears to be a promising method in that it may be carried out much more economically in terms of money and space than would be possible with larger animals. Hundreds of chicken embryos may be observed in a minimum of space, and over a comparatively short period of time. The feasibility of using such large numbers is valuable also in the statistical evaluation of toxicity data.

A review of the literature shows how little work has been done in this field except in a fragmentary way on isolated cases. Most of the reports refer to injections of chemicals made after the fourth or eighth day of incubation and examination of the embryos killed before they hatch.

The earliest work that we have found in the literature was by Féré (1893). During ten years after this date he published about sixty-seven papers; a review article (Féré, 1899) contains a summary of many of his studies. His work consisted mainly of injection before incubation, but the eggs were usually opened on the third day of incubation. His interest was mainly in the teratogenic effect of chemicals.

Since 1900 other articles have appeared in the literature, but most of them concern the effects of one or two chemicals on a small number of embryos. One of the most informative papers is that of Ridgway and Karnofsky (1952) in which they report experiments on compounds containing fifty-five of the elements, mainly the metallic ones, generally injected at either the fourth or eighth day of embryonic development to study toxicity and teratogenic effects. Hamburger and Hamilton (1951) have described an elegant method for determining the stages of development of the chick embryo. More recent investigations employing the chick embryo to study the toxicity of various chemicals have been reported by Kemper (1962), Platt *et al.* (1962), and Goerttler (1962). Finally, the classic books of Romanoff and Romanoff (1949) and Romanoff (1960) contain a wealth of information on the avian embryo.

Preliminary reports of this investigation have been presented by Marliac (1962) and McLaughlin and Mutchler (1962).

EXPERIMENTAL

The fertility and hatchability of eggs and the livability of chicks are dependent on a complex interrelationship of ecological factors, among which are the genetic background and the age of the mated birds, the nutritional status and general management of the flock, and seasonal variations. In view of this the initial phase of our work, which started in 1959, was devoted to a study of the hatchability of our supply of White Leghorn eggs[1] under conditions existing in our laboratories. The data accumulated during two years for control eggs showed that the hatchability of these eggs was, in fact, consistent, reproducible, and very high. The possibility of a seasonal variation occurring in responses to compounds introduced into the eggs was also examined by repeated testing of several chemicals at all seasons of the year. No important variation was detected.

Selection of eggs. Eggs to be injected are first candled in order to discard those that are defective and to outline with a pencil the exact location of the air cell. In our laboratory 9% of 5000 eggs had to be discarded: 2% cracked, 4% with improperly calcified shells, 1.5% with a tremulous air cell, 1% with the air cell in the wrong place, and 0.5% with blood clots. After the elimination of such defective eggs, the hatch of control eggs averages 95%. A further restriction is based on the weight of the eggs: all those weighing less than 52 g or more than 63 g are rejected. After

[1] Truslow Eggs, Chestertown, Maryland.

handling, the eggs are randomized in order to avoid series of infertile eggs in any one experiment.

The initial experiment with a given chemical is for range-finding and is performed at two or more concentrations of the chemical with 10 eggs per level. On the basis of this information, 20 or more eggs are injected with the appropriate amount of the chemical.

If the chemical proves to be nontoxic, the experiment is repeated with the minimum number of eggs that will give a reliable and reproducible value for the hatchability. In the case of a toxic chemical, additional eggs are injected to determine the specific effects of the chemical. The total number of eggs used for a chemical depends upon the data obtained initially and upon the kind of information desired. Hence, data for some chemicals are based on less than one hundred eggs, whereas data for others are based on several hundred eggs.

Technique of injection. The injections of pure chemicals, chemical solutions, or suspensions are made at volumes up to 0.10 ml. When necessary, dilutions are made with solvents such as water, propylene glycol, corn oil, peanut oil, or other nontoxic solvents.

In order to avoid contamination, the injections are carried out in an Isolator Box[2] with a sterile atmosphere created by using formaldehyde vapors (produced by mixing 2 g of potassium permanganate and 50 ml of 37% formalin). During the period of a year, more than fifteen hundred noninjected eggs were exposed to formaldehyde vapors; no toxic effect was noticed. After exposure to these vapors for 30 minutes, the eggs are ready for injection.

The large end of the egg is wiped with a sterile gauze pad moistened with a 70% alcohol solution, and a hole is drilled in the shell in the center of the surface over the air cell (Fig. 1). Care must be taken not to damage the shell membrane with the point of the drill[3]; this is to avoid, if possible, contact of the air with the egg membrane. Fine particles of shell are removed with an aspirator to prevent the needle from carrying them into the yolk.

Immediately before the injection, each egg is shaken with a quick twist of the wrist. Since the germinal disc occasionally sticks to the air cell and it is possible to damage it with the needle, this movement will allow the disc to float free in the egg.

[2] Kewaunee Scientific Company, Adrian, Michigan.
[3] Burgess Vibrocrafters Inc., Grayslake, Illinois.

The needle (hypodermic, 1 inch long, either no. 22 or no. 27, depending on the viscosity of the liquid to be injected) is inserted horizontally through the air cell into the yolk (Fig. 1). Care must be taken in withdrawing the needle to avoid damaging the vitelline membrane since such damage could cause the yolk to spread out in the albumen. If the end of the needle has some yolk on it, the injection is not satisfactory. The needle should be wiped with a sterile gauze pad between each injection. As soon as the egg has been injected, the hole in the shell is covered with a small piece of Scotch tape, care being taken not to cover the entire air cell.

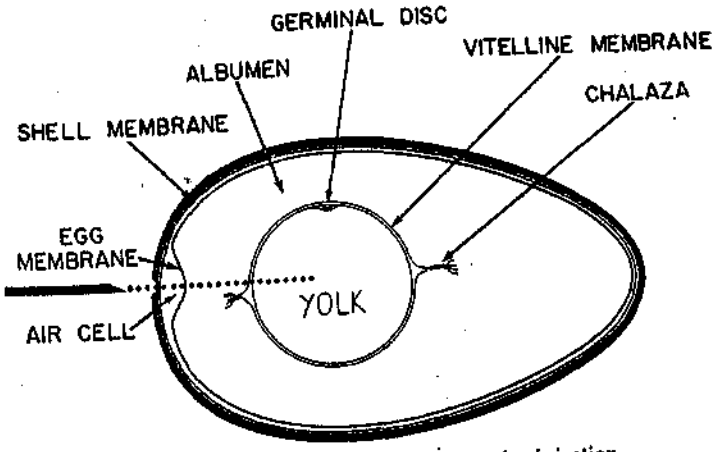

FIG. 1. Diagram of egg and position for injection.

Incubation and hatching. The injected eggs are put into the incubator trays with the large end up; the trays are placed in the incubator,[4] which automatically rotates hourly and is maintained at an optimum temperature of 38°C and a relative humidity of 60%. The eggs are candled on the fifth day of incubation and every day thereafter. Clear eggs and dead embryos are removed for examination. On the seventeenth day of incubation the fertile eggs are transferred to the hatcher[5] and kept at a temperature of 37°C until they hatch.

EVALUATION OF DATA

The injection of the chemical into the egg may produce one of four possible results: (1) the chemical is highly toxic at the level injected, and

[4] Humidaire Incubator Co., New Madison, Ohio; model no. 50, capacity 450 eggs.
[5] Brower Manufacturing Co., Quincy, Illinois.

all embryos are killed during the first 20 hours of incubation (before the two-somite stage); (2) the chemical is toxic but allows a number of embryos to develop only up to a certain point, and some possibly even to hatch; (3) the chemical has little effect on the hatch; and (4) the chemical has no effect on the hatch or on the posthatch development of the chick ("no effect" level).

If the chemical appears to be highly toxic and all the embryos are killed, the experiment is repeated with smaller doses of the chemical until some hatch is obtained. If the chemical is toxic, but allows the embryos to develop for a longer period of time, dead embryos are examined pathologically and the chicks that do hatch are examined for eye damage, color of the feathers, weight, length of the legs, form of the beak and of the rump, hematologic changes, and condition of the internal organs (liver, kidneys, heart, gall bladder, and spleen).

It is advisable in all cases to observe the chicks for a period of a few weeks in order to detect any delayed effects. Since as much as 30% of the yolk remains at the time of hatching and is absorbed during the first 7 days thereafter, effects of a toxic chemical may first be observed at this time. There may be weight retardation, death during the first week, or the appearance of nerve damage occurring as late as 2–6 weeks after hatching.

The toxicity of a chemical is evaluated mainly from the percentage of hatch at varying dosages of the chemical as compared to noninjected (control) eggs, from a study of the embryonic development of the eggs that fail to hatch, and from a study of the appearance and development of the chicks that do hatch. However, several other factors must also be considered in this evaluation: these are specific gravity, solubility, coagulating effect, pH, and the ionic concentration of the chemical tested.

If the chemical has a high specific gravity, there is the possibility of its settling out in the bottom of the egg and thereby giving a value of apparently low toxicity.

The solubility is quite important since the availability of the chemical for utilization in the chick embryo is partially dependent on its solubility in the egg. However, since egg yolk is an emulsion, solubility problems are somewhat minimized. In the case of insoluble chemicals that are injected as suspensions, it is also necessary to consider particle size in the evaluation of toxicity data.

In order to have a toxic effect, the chemical must come in contact with the embryo either directly or indirectly through the bloodstream. Chemicals such as the lower aliphatic alcohols have a coagulating effect on the protein, and this coagulation may decrease the availability of the chemical as well as some yolk nutrients, and thereby alter the response of the embryo.

If the pH is highly acidic or basic, a pH effect differing from the true toxic effect of the chemical may be obtained due to interference with the normal acid-base equilibrium in the egg.

The ionic concentration is also important for a similar reason. The observation of increasing toxicity with increasing concentration of a chemical should be interpreted cautiously, since highly concentrated solutions may upset the physical equilibrium of the yolk by causing osmotic effects.

Finally, the introduction of a chemical into the yolk may cause a special type of toxicity because it destroys, alters, or combines with essential nutrients such as vitamins and minerals.

EXPERIMENTAL DATA

Twenty-five thousand eggs have been used in our laboratory during the past three years to test more than 100 chemicals with the following results: (1) nontoxic chemicals injected at an appropriate level allowed the embryo to develop and to hatch as did the controls; (2) toxic chemicals produced effects at dose levels which may be compared to those producing effects in feeding experiments using animals; and (3) this technique often provided toxicologic information which had not been shown by conventional methods.

TABLE 1
NONTOXIC CHEMICALS

Chemical	Solution injected: Concentration	Solution injected: Quantity (ml)	Per cent hatch
Water (boiled)	—	0.05	95
Propylene glycol	Undiluted	0.05	95
Corn oil	Undiluted	0.05	90
Peanut oil	Undiluted	0.05	90
Sodium chloride	0.9% in water	0.05	90
	5.0% in water	0.05	70
	5.0% in water	0.10	30
	10.0% in water	0.05	20
Dextrose	5.0% in water	0.05	90

Table 1 lists the results obtained with some chemicals in common use in food and shows the dosages used and the percentages of hatched chicks.

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TABLE 2
CHEMICALS WITH A HIGH ORDER OF TOXICITY

Chemical	Solution injected		Per cent hatch	Remarks
	Concentration	Quantity (ml)		
Lead acetate	10% in water	0.05	0	—
	2% in water	0.05	0	Hydrocephalus in embryos that failed to hatch
	0.5% in water	0.05	20	—
Mercuric chloride	1% in water	0.05	0	All embryos died at the beginning of incubation
Thiourea	10% in water	0.05	0	Some embryos lived up to 34 days
	5% in water	0.05	20	Hatched on the 28th day of incubation; growth retardation
Triorthocresyl phosphate	Undiluted	0.02	0	Most of the developing embryos died on the 15th day of incubation
	Undiluted	0.01	40	Hatched chicks showed growth retardation and developed paralysis 2–6 weeks after hatching
p,p'-Diaminodiphenylmethane	10% in ethanol	0.05	30	Leg damage; beak deformity (short mandible)
Sodium selenite	0.2% in water	0.05	0	—
	0.1% in water	0.05	20	—
	0.1% in water	0.02	80	—
Dibutyl-tin-dilaurate	Undiluted	0.01	0	Beak deformity
Aroclor 1242 (chlorinated polyphenyls)	Undiluted	0.025	0	—
	Undiluted	0.01	5	Beak deformity (short upper beak); + edema; growth retardation

These data, supplemented by an examination of the nonviable eggs and an autopsy of the chicks that hatched, have not indicated any hazard from their use in food. However, it must be pointed out that any chemical, added at a sufficiently high concentration, may have some toxic effect. Since our data and those reported in the literature indicated safety, this phase of the study was not carried beyond a preliminary examination to ascertain that nontoxicity also was shown for these chemicals by the chick embryo technique. This is of theoretical as well as practical importance for the evaluation of any new technique to be used in toxicologic studies.

Table 2 lists our results with several chemicals which have been shown to be highly toxic to animals. In each case there is not only the low percentage of hatch at a low level of the chemical tested, but there are also congenital abnormalities and other responses that raised extremely serious questions as to the safety to the consumers of any food contaminated with these chemicals.

Lead acetate resulted in no hatch at a level of 1 mg per egg. Autopsy of the dead embryos showed extensive brain damage, as has been reported by de Franciscis and Bocalatte (1962) and by Karnofsky and Ridgway (1952).

Mercuric chloride showed no hatch even at a level of 0.5 mg per egg.

Thiourea is a known carcinogen with a basic effect on the thyroid gland. The hatch time was delayed with increasing amounts of this chemical. At a level of 5 mg per egg no chicks hatched and the embryo required 35 days to develop to the stage normally attained at 20 days. At 2.5 mg per egg some chicks hatched, but most of them had to be helped out of the shell. This effect has been reported also by Yushok (1950).

Cavanagh (1954) reported that triorthocresyl phosphate (TOCP), a well-known plasticizer for nonfood use, causes paralysis when fed to adult chickens. We observed this paralysis in some chicks which hatched from eggs injected with 10 mg of the undiluted chemical.

The compound *p,p'*-diaminodiphenylmethane has been reported by Zylberszac (1951) to cause cirrhosis of the liver in rats. We found this chemical to be extremely teratogenic at a level of 5 mg per egg; more than 90% of the chicks had a short mandible and leg damage consisting of a severe bending of the tibia and a general shortening of the bones of the leg.

Sodium selenite proved to be highly toxic. At a level of 0.1 mg per egg, no embryos developed to more than the 5-day stage.

Dibutyl-tin-dilaurate showed no hatch at a level of 10 mg per egg. The majority of the embryos, which did not live more than 15 days at this

TABLE 3
CHEMICALS WITH AN INTERMEDIATE ORDER OF TOXICITY

Chemical	Solution injected: Concentration	Solution injected: Quantity (ml)	Per cent hatch	Remarks
Acetone	Undiluted	0.05	70	
n-Butanol	Undiluted	0.01	95	* At these levels: corneal opacity, cataract, cleft palate, nerve and kidney damage
		0.02*	70	
		0.03*	20	
		0.04	0	
Carbon tetrachloride	Undiluted	0.05	40	
		0.10	0	
Diethylene glycol	Undiluted	0.05	80	
		0.10	55	
Ethyl acetate	Undiluted	0.05	35	
		0.10	15	
Ethyl alcohol	Undiluted	0.05	95	
		0.10	60	
		0.30	0	
Ethylene glycol	Undiluted	0.05	80	
		0.10	65	
Di-2-ethylhexyl phthalate	Undiluted	0.05	95	
Heptachlor	1% in propylene glycol	0.05	75	
		0.10	65	
Hydrochloric acid	4% in water	0.05	80	
Isopropanol	Undiluted	0.05	35	
		0.10	15	
Malathion	Undiluted	0.05	60	Short legs; bleaching effect on chick down
		0.10	0	
Methanol	Undiluted	0.05	90	
		0.10	65	
Styrene	5% in 80% ethanol	0.05	85	

level, had a short and/or flexed mandible; in addition, some embryos showed subcutaneous edema.

Aroclor 1242 gave no hatch at a level of 25 mg per egg. At a level of 10 mg per egg, one chick hatched out of 20 injected eggs, but died 2 days later. Some embryos, which were examined after they died, showed beak deformities (often a short upper beak), edema, and growth retardation.

Table 3 lists preliminary data on the toxicity of some chemicals which have been shown to be toxic in some degree to animals, and which may be found in some processed foods. Included in this group are various solvents, plasticizers, and insecticides. Some of the chemicals on this list require further study, including observation of the chicks until they reach maturity, before they may be classified as to low or high toxicity.

DISCUSSION

The injection of chemicals into the yolk of fertile eggs prior to incubation is a method which can be advantageously used as an element in the evaluation of the safety of food additive chemicals and drugs, and which could be used to screen new products and eventually to correlate their toxicity with that of similar products already tested.

If one considers that a chemical injected into the yolk may be compared to a substance which has the power to cross the placental barrier, this technique, in addition to being an embryonic feeding study, assumes further importance in that it is also a reproduction study. The unfortunate experiences recently suffered with chemicals that have teratogenic effects in humans, and the failure of conventional testing methods to produce this effect in animals, emphasize the urgent necessity for new methods of analysis. Preliminary work that we have done in this area has given satisfactory results (Verrett and McLaughlin, 1963).

Since this represents a system in which the chemical is in direct contact with the embryo throughout development, it is more than likely that any toxic or teratogenic effects would be readily observed. However, there is always the possibility that the chicken will not be a species susceptible to a particular compound, just as it has been shown that the other commonly used species of animals do not respond to all chemicals in a similar manner. It is also possible for the chicken to be more sensitive to a chemical than other species.

Finally, this technique may be applied also to the study of the synergistic effects of chemicals. Results of experiments in our laboratory on

the potentiation of a few pesticides have been very encouraging (Marliac and Mutchler, 1963).

SUMMARY

An evaluation of toxicity by injection of the chemical into the yolk sac of fertile eggs prior to incubation gave the following results:

Water, propylene glycol, corn oil, peanut oil, isotonic saline solution, and isotonic glucose solution showed no toxicity or a very low order of toxicity.

Mercuric chloride, lead acetate, selenium, triorthocresyl phosphate, *p,p'*-diaminodiphenylmethane, thiourea, Aroclor 1242, and dibutyl-tin-dilaurate showed a high order of toxicity and/or teratogenic effects at certain levels.

Acetone, methanol, ethanol, *n*-butanol, diethylene glycol, ethylene glycol, isopropanol, di-2-ethylhexyl phthalate, hydrochloric acid, carbon tetrachloride, ethyl acetate, malathion, heptachlor, and styrene showed an intermediate order of toxicity.

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Use of the Chicken Embryo in the Assay of Aflatoxin Toxicity

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The possibility of using the chicken embryo as a test organism for the assay of aflatoxin toxicity has been investigated and found to be feasible. The injections of test solutions were made before incubation, in fertile White Leghorn eggs, by either of two routes: yolk or air cell. The development of the embryos was observed for the full 21 day incubation period. The vehicle for all injections was propylene glycol.

The injection of solutions of pure aflatoxins B_1 and G_1, and of extracts of aflatoxin-producing mold cultures indicated that the chicken embryo was sensitive to these compounds. A dose-response was exhibited in that the toxicity of the samples was related to the mortality at the time of hatching.

Extracts of aflatoxin-free peanut products were found to be nontoxic to the chicken embryo. The addition of aflatoxin B_1 to such uncontaminated extracts produced the expected toxicity in the embryos. The injection of extracts from contaminated peanut products resulted in a toxic response that correlated well with that obtained by injection of pure aflatoxin B_1 solutions at the same dose levels, and in most instances the chemical analysis was confirmed. The presence of aflatoxins G_1, B_2, and G_2 had no apparent effect on the toxicity due to aflatoxin B_1, at the levels at which they occurred in the particular samples tested.

The separation of aflatoxin B_1 from contaminated extracts by thin-layer chromatography, and its subsequent elution from the plates and injection into the eggs, confirmed that the toxicity of these extracts was due primarily to their aflatoxin B_1 content.

The sensitivity of the chicken embryo to aflatoxins was reported in 1962 by Platt, *et al.* (1) who observed that preparations of "groundnut toxin" injected into the yolk of 5-day old chicken embryos caused deaths, and that the quantities required were about 1/200th of those required for a positive result in the day-old duckling.

An investigation of the feasibility of using the chicken embryo for a bioassay of aflatoxin toxicity is currently underway in our laboratories. The preliminary results reported here consider only the general systemic toxicity of the aflatoxins to the chicken embryo. At the present time, the studies are not sufficiently complete to verify whether the aflatoxins produce any specific pathological lesion in the embryos.

Experimental

The sensitivity of the chicken embryo to aflatoxins was studied by injecting the following: (*1*) solutions of pure aflatoxin B_1 and pure aflatoxin G_1; (*2*) extracts of aflatoxin-producing mold cultures; (*3*) extracts of raw peanuts, roasted peanuts, peanut meal, and peanut butter; and finally (*4*) aflatoxin B_1 obtained from crude extracts by elution from thin-layer chromatographic plates.

The solutions were injected into fertile White Leghorn eggs before incubation. Groups of at least 20 eggs were used at each dose level of a sample, and each sample was injected at two or more levels when there was sufficient material available. More than 8,000 eggs have been used in these studies to date.

The injections into the eggs were made by one of two routes: into the yolk, or into the air cell. The technique for injection into the yolk has been described previously (2). The volume injected into the yolk was 0.05 ml or less in all cases. For injections into the air cell, a hole of about 5 mm diameter was drilled in the shell over the air cell. The solution was then deposited on the egg membrane by a syringe, and the hole was sealed with adhesive cellophane tape. The eggs were allowed to remain undisturbed in a vertical position (air cell up) for about an hour to let the material disperse. The volume injected into the air cell was restricted to 0.04 ml or less.

The solvent used for all injections was propylene glycol, which was known, from previous investigations (3), to be nontoxic in the eggs at the levels used. However, eggs were periodically injected with this solvent in appropriate amounts and incubated with the aflatoxin-injected eggs. Noninjected controls and drilled-only controls were also included in the experiments to provide the necessary data on the background mortality.

The eggs were incubated (2) and candled daily from the fourth incubation day on, at which times all nonviable embryos were removed and examined grossly.

Results and Discussion

Purified Aflatoxin Solutions and Mold Culture Extracts.—The toxicity of solutions of crystalline aflatoxin B_1 and aflatoxin G_1 to the chicken embryo was first determined.

The toxicity of aflatoxin B_1 to the chicken embryo was greater when injected via the air cell route than when injected into the yolk. Figure 1 contains plots of the mortality at 21 days due to the injection of several dose levels of aflatoxin B_1 by both the yolk and the air cell routes. The LD_{588} obtained were 0.048 and 0.025 μg for the yolk and air cell routes, respectively.

Aflatoxin G_1, which was injected into the yolk only, showed a lower toxicity to the chicken embryo than that obtained with

aflatoxin B_1. The injection of 1.0 μg of aflatoxin G_1 produced a mortality of 60% (at 21 days), while 2.0 μg was required to produce a mortality of 90%.

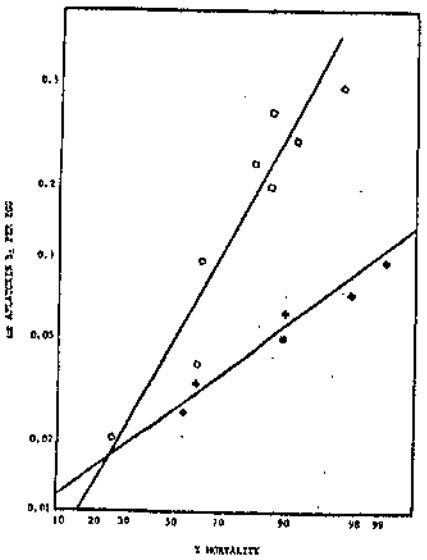

Fig. 1—Toxicity of aflatoxin B_1 in the chicken embryo: *mortality at 21 days.* LD_{50}: *yolk, 0.048 μg; air cell, 0.025 μg.* Open squares: yolk injection. Closed squares: air cell injection.

Examination of nonsurviving embryos from eggs injected with aflatoxin B_1 by either route revealed a severe growth retardation in most cases. In addition, edema, hemorrhage, underdevelopment of the mesencephalon (in embryos that died before the seventh day), mottled and granular liver surface, short legs, and slight clubbing of the down were also observed in many of these embryos.

Extracts of several cultures of aflatoxin-producing molds were used to determine the sensitivity of the chicken embryo to combinations of aflatoxins B_1, G_1, B_2, and G_2. The concentrations of these four constituents in the extracts were known from prior chemical analysis. The extracts were injected into eggs by both routes, in amounts designed to contain specific levels of aflatoxin B_1, to compare their toxicities to those of solutions of the pure aflatoxin B_1 at the same dose levels.

The toxicities of these extracts to the embryos showed a good correlation with the standard solution of aflatoxin B_1. There was no apparent alteration in the toxicity of aflatoxin B_1 due to the presence of aflatoxins B_2, G_1, and G_2 at the levels at which they occurred in these extracts.

Aflatoxin-free Extracts of Peanut Products.—The usefulness of the chicken embryo in a bioassay of aflatoxins in peanut products depends on whether the constituents of aflatoxin-free peanut product extracts are inherently toxic to the embryos.

To determine the toxicity of these materials, extracts of raw peanuts, roasted peanuts, peanut meal, and peanut butter, which were found to be free of aflatoxins by chemical analysis, were injected into eggs by both the yolk and air cell routes. In most of these experiments the equivalent of original peanut product injected ranged from 1 to 3 g per egg. The toxicity was low for all of these extracts; in general, it was equal to or only slightly higher than that of the background, which ranged from 0 to 20% mortality.

One experiment was carried out with an extract of aflatoxin-free raw peanuts, injected by both the yolk and air cell routes, in quantities corresponding to a raw peanut equivalent of 1, 2, 4, and 8 g per egg. The toxicity observed for the 8 g level was not significantly higher than that observed for the 1 g level or that of the background.

In the same experiment, known quantities of the pure aflatoxin B_1 were added to this raw peanut extract, and injected at the same levels and by both routes. This was done to determine whether the aflatoxin B_1 toxicity could be masked or enhanced by the presence of increasing amounts of peanut material. Separate groups of eggs were injected with corresponding amounts of the standard aflatoxin B_1 solution for comparison. The results of this experiment indicated that as little as 25 ppb of aflatoxin B_1 in the original raw peanuts could be easily detected by administration of the extract by either injection route, and that the toxicity was not significantly different from that obtained with the solution of pure aflatoxin B_1 of the same concentration.

This paper was presented at the Seventy-eighth Annual Meeting of the Association of Official Agricultural Chemists, Oct. 19-22, 1964, at Washington, D.C.

Aflatoxin-contaminated Peanut Products.—Extracts from raw or roasted peanuts, peanut meal, and peanut butter, which were shown to contain aflatoxins by chemical analysis, were injected into eggs by both routes. The amount of extract to be injected in the egg was calculated on the basis of the aflatoxin B_1 content determined chemically, irrespective of the amounts of aflatoxins B_2, G_1, and G_2 present. In most instances the results corroborated the chemical analysis, since the toxicity was comparable to that obtained with the injection of equivalent amounts of the pure aflatoxin B_1 solution.

Aflatoxin B_1 Obtained by Thin-Layer Chromatography (TLC).—In order to confirm that the toxicity of the contaminated extracts was primarily due to their aflatoxin B_1 content, separate portions of some extracts were subjected to TLC and the resulting aflatoxin B_1 spots were removed from the plates, eluted, dissolved in propylene glycol, and injected into eggs.

Separate experiments were performed to confirm that no toxic materials were derived from the TLC process itself. TLC "blanks" injected into eggs in a similar manner had a very low toxicity and were comparable to background.

The toxicities of these eluted aflatoxin B_1 spots from more than 20 extracts of a variety of peanut products correlated very well with that of standard aflatoxin B_1 solution injected at the same dose level, and verified that the toxicity of these extracts was, in fact, primarily due to aflatoxin B_1.

Embryo Age and Aflatoxin Toxicity.—The toxicity of aflatoxin B_1 to the chicken embryo at various stages of incubation was also investigated. Single injections into the yolk were made up to the fourth incubation day, and air cell injections were made in embryos from 1 to 18 days old.

These experiments revealed that, with both injection routes, the embryonic sensitivity to the aflatoxin decreased rapidly as the embryo age increased, and the maximum toxic effect was obtained with pre-incubation injections.

Evaluation of Sample Toxicity.—In the course of these studies it was also observed that the toxicity of aflatoxin B_1 injected at the higher dose levels was apparent early in the incubation period, since most of the embryos did not survive beyond the eighth to tenth day. With lower dose levels, it is necessary to continue observations for the duration of the 21 day period, since many embryos survive longer than the tenth day but fail to hatch. In these instances an evaluation of the toxicity of a sample on the basis of survivors at 8 or 10 days would be premature, and the true toxicity of a sample might be underestimated.

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The Role of "Toxic Fat" in the Production of Hydropericardium and Ascites in Chickens

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SUMMARY

When "toxic fat" was added to the diet of experimental birds at concentrations from 0.25 to 5.0% for 35 to 150 days, edema of the myocardium, skeletal musculature, and lungs; hydropericardium; ascites; and foci of lymphoytes in the myocardium and epicardium were observed. Appreciable changes were not observed in the total serum protein levels, albumin: globulin ratio, electrolyte balance, or in the nonprotein nitrogen levels of the blood. There was dilation, edema, and lymphocytic infiltration of the heart. The myocardial mitochondria were vacuolated and shrunken. An increase in venous pressure was also noticed. The fluid imbalance observed in birds that consumed toxic fat did not result from a decrease in total blood proteins or from an alteration in the albumin: globulin ratio, but was associated with cardiac decompensation and increased capillary permeability.

Schmittle *et al.*[19] were the first to incriminate some fats as responsible for the production of hydropericardium and ascites in young chickens. Subsequent studies[9,18] have demonstrated a reduction in growth rate, retarded sexual development, and increased mortality in pullets that have consumed toxic fat. A marked reduction was observed in the hatchability of eggs from hens fed toxic fat.[24] Turkeys and ducks appeared to be less susceptible than chickens to the detrimental effects of this fat in the diet.[4] Fat-soluble tissue extracts from chickens fed toxic fat were capable of producing hydropericardium and ascites when added to the diet of other birds.[6] Associated with the transudate, Sanger *et al.*[16] observed necrosis of the liver, subepicardial hemorrhage, and lymphocytic infiltration of muscle fibers in chickens consuming toxic fat. In addition to these changes, Simpson *et al.*[20] also noticed bile duct hyperplasia and proliferation of the endothelium in the parenchymal tissues. The unsaponifiable portion of some batches of fat was found responsible for the toxicity.[2,10,17,22] Repeated passages of this fraction through alumina and silica gel columns led to the isolation of a crystalline factor of unknown structure which was able to produce anasarca.[8]

The primary aim of this study was to investigate the mechanism by which toxic fat produces anasarca. Preliminary studies suggested that a vascular or cardiac injury may be responsible for the transudation of fluid into tissues. In the hope that some clarification of the mechanism might be obtained, arterial perfusions, recordings of hemodynamic changes, and ultrastructural myocardial studies were undertaken.

MATERIALS AND METHODS

In experiment 1 (acute), 100 White Leghorn cockerels, 1 day old, were separated into 5 groups of 20, placed in heated batteries, and fed rations containing 0, 0.5, 1.0, 3.0, and 5.0% toxic fat* for 35 days. Because the exact chemical nature of toxic fat is not known, the amount of the toxic material undoubtedly varies in fats from different sources. The fat used in these experiments was from the same source, identical shipments, and had the same LD_{50} when fed to day-old chickens.

In experiment 2 (chronic), forty-eight 4-week-old cockerels of comparable weight were given, for 150 days, a ration** containing 0, 0.25, and 1.0% toxic fat. The birds were fed and watered daily. Throughout the trial period the general appearance, food consumptions, and mortality were spleen, pancreas, kidneys, adrenal glands, skeletal muscle, brain, and bone marrow were fixed in 10.0% neutral formalin, embedded in paraffin, sectioned at 7 μ, and stained with hematoxylin and eosin. Frozen sections were prepared from the livers and kidneys and stained with Sudan IV to demonstrate neutral fats. At the termination of the trials, blood was obtained from the cephalic vein, complete

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** McMillen Feed Mills, Fort Wayne, Ind.

blood counts were conducted on a portion of the sample, and the remainder was allowed to clot. After centrifugation the serum was saved for total protein,[9] electrolyte concentration,[7] and nonprotein nitrogen determinations[5] and electrophoretic studies.[21]

In experiment 1, surviving birds were used for vascular perfusion studies. Immediately after they were killed, the thoracic aorta was perfused with 5.0% dextrose to remove blood, and 1.0% silver nitrate was injected to outline the "cement substance" between the endothelial cells of the mesenteric capillaries. Slides were prepared of the mesenteric vessels and examined for alteration of interendothelial silver precipitate in birds consuming toxic fat.

Five control and 5 test birds given the ration containing 1.0% toxic fat were selected for hemodynamic studies after 150 days on trial. Venous pressures were determined by making an incision through the skin over the jugular vein. The vein was dissected away from the surrounding tissue, and 2 ligatures were placed around the vessel. The superior legature was made secure, and a small incision was made into the vessel. A No. 5 cardiac catheter was placed in the vein and made secure by the inferior ligature. The birds were placed under a fluoroscope and the exact position of the catheter determined. The catheter was attached to a 3-mm. water manometer filled with physiologic saline solution, thus enabling pressures to be determined in the venae cavae and right ventricles.

At the termination of experiment 2, tissues were obtained for electron microscopy by severing the cervical spinal cord and exposing the heart by removal of the sternum. Sections were taken from the left ventricle and cut into blocks of approximately 1 mm. with a razor blade. The tissues were immediately fixed in 1.0% osmium tetroxide, dehydrated, and embedded as outlined by Palade.[15] Thin sections were cut on a cicrotome.[1] Tissues were examined with an electron microscope[2] operating at 50 kv.

RESULTS

In experiment 1, birds given rations containing 0 to 1.0% toxic fat survived, whereas those consuming rations with 3.0 and 5.0% toxic fat had a 25.0 to 55.0% mortality, respectively (Table 1). As the level of toxic fat in the diet

TABLE I.—EFFECTS OF TOXIC FAT CONSUMPTION ON CHICKENS

No. birds	Toxic fat in diet (percent)	Days on trial	No. died	Ascites	Hydropericardium
20	0	35	0		
20	0.5	35	0		
20	1.0	35	0		
20	3.0	35	2	20	20
20	5.0	35	11	20	20
12	0	150	0	0	0
12	0.25	150	1	1	4
12	0.5	150	4	0	8
12	1.0	150	6	2	11

was increased, there was a corresponding decrease in growth rate. The control birds averaged 348 Gm., whereas those given rations with toxic fat averaged 176 Gm., at the end of 35 days. Hydropericardium and ascites were not observed in the chickens fed rations containing 0 and 0.5% toxic fat. Pericardial fluid volumes ranged from 0.5 to 5.0 ml. in the birds given rations with 1.0 to 0.5% fat. Ascites was also observed in these groups, but the volume was not determined due to the partial congulation of the transudate.

In experiment 2, 6 birds fed 1.0%, 4 fed 0.5%, and 1 fed 0.25% toxic fat in the ration died. The mean survival time for the groups was 80 ± 20, 100 ± 14, and 135 days, respectively. The control group gained an average of 16.5 Gm., whereas the survivors of the group fed the ration containing 1.0% toxic fat gained 14.9 Gm. per day. The weight gain of the surviving birds of the

[1] Porter-Blum microtome, Ivan Sorvall, Inc., Norwalk, Conn.
[2] RCA, EMU-3G, Radio Corporation of America, Camden, N.J.

groups fed rations containing 0.5 and 0.25% toxic fat was almost comparable to that of the controls. Hydropericardium was observed in 11 and ascites in 7 birds fed rations containing 1.0% toxic fat. Eight of 12 birds on the 0.5% toxic fat ration had hydropericardium but were free of ascites. The average volume of pericardial transudate in the test birds was 13.5 ml. No appreciable difference was noticed in the organ weights of the test and control birds, with the exception of the testes and hearts of the birds in experiment 2. The average weight per testis of birds in the control group was 14.9 Gm., whereas that of the 0.25% group was 6.6 Gm., the 0.5% group was 4.1 Gm., and the 1.0% group was 3.9 Gm. The hearts of birds of the control group averaged 15.0 Gm., and those of birds in the test groups averaged 23.0 Gm. and were markedly dilated in most cases (Fig. 8).

Tissue sections of the various organs were examined microscopically. The birds with hydropericardium had fibrinous deposits and foci of lymphocytes on the visseral pericardium. The muscle fibers were separated by edematous fluid. A number of the small myocardial artieries were surrounded by lymphocytes. There were also foci of lymphocytes between the myocardial fibers (Fig. 12). The lungs were congested and had a moderate amount of peribronchial lymphoid hyperplasia. Pulmonary edema was found in those birds which died during the experiments. Livers from birds with marked ascites frequently had thickened capsules. In many cases there was coagulation of the transudate on the convex surface of the liver which formed a false capsule (Fig. 3). There was moderate fatty infiltration of the hepatic cells in birds given toxic fat in experiments 1 and 2 (Fig. 4). Extensive lymphoid hyperplasia around the periportal areas was found consistently (Fig. 5). Five of the test birds in experiment 2 also had myeloid hyperplasia scattered throughout the parenchymal tissue of the liver.

Microscopic examination of the testes of the birds given toxic fat in experiment 2 revealed a reduction in size of the seminiferous tubules. The Sertoli cells and spermatogonia appeared normal, but there was a reduction of the primary and secondary spermatocytes. These maturing cells were reduced to the point where no spermatids and spermatozoa were observed. Cells in the testes of the test birds appeared normal. The major difference between testes

TABLE 2.—SERUM PROTEIN STUDIES ON CHICKENS FED TOXIC FAT

Toxic fat in diet (percent)	Serum protein (Gm./100 cc.)	Albumin (Gm./100 cc.)	Globulin (Gm./100 cc.)	A/G	Nonprotein nitrogen (mg./100 cc.)	Sodium (mEq./liter)	Potassium (mEq./liter)
0	3.36	1.06	2.30	46	21.7	143	5.5
0.5	3.24						
1.0	3.14						
3.0	3.08						
5.0	3.06	0.94	2.12	44	20.7	146	5.3

of birds in test and control groups was a lack of spermatogenesis and a marked reduction in tubular size (Fig. 6,7) in testes of test birds.

Total blood proteins were determined by the micro-Kjeldahl method. The control group of experiment 1 had an average value of 3.36 Gm./100 cc. The 0.5, 1.0, 3.0 and 5.0% groups had values of 3.24, 3.14, 3.08, and 3.06 Gm./100 cc. of protein, respectively (Table 2). In experiment 2, it was found that the groups given rations containing 1.0% toxic fat had an average of 4.1 Gm./100 cc., the 0.25% group averaged 4.5 Gm./100 cc., and the controls had an average value of 4.8 Gm./100 cc. of serum protein. The protein level of the ascitic fluid of the experimental birds was 1.7 Gm./100 cc., with no appreciable difference in the levels of the various groups. The albumin: globulin ratio was determined by paper electrophoresis, and little difference was found in the protein ratio of the various groups.

Sodium, potassium, and nonprotein nitrogen levels were determined on serum samples from 6 birds given rations containing 5.0% toxic fat and 6 control birds. No appreciable difference was found in the nonprotein nitrogen levels of the 2 groups, with the controls averaging 21.5 mg./100 cc. and the test birds 20.7 mg./100 cc. The sodium values were 146 mEq./liter in the experimental

and 143 mEq./liter in the control birds. The potassium levels were 5.3 mEq./liter in the test birds and 5.5 mEq./liter in the control birds.

When 1.0% silver nitrate solution was perfused in the thoracic aorta and the mesenteric capillaries examined microscopically, a difference was found in the cement substance located between the endothelial cells of the test and control birds in experiment 1. The control group had uniform diamond-shaped bands of silver precipitate between the capillary endothelial cells, whereas the bands in the test birds were irregular and indistinct (Fig. 9,10).

Catheterization of the heart revealed the mean right ventricular pressure of the control birds to have an average value of 15.3 Cm. of water, whereas the test birds had an average of 21.3 Cm. of water. The pressures in the inferior vena cava averaged 5.5 cm. of water in the control birds and 7.1 cm. of water in the test birds (Table 3).

TABLE 3.—HEMODYNAMIC ALTERATIONS DUE TO TOXIC FAT CONSUMPTION IN CHICKENS

Toxic fat in diet (percent)	Mean right ventricular pressure (cm. H_2O)	Mean superior vena cava pressure (cm. H_2O)	Mean inferior vena cava pressure (cm. H_2O)
0	15.4	5.6	5.2
0	15.3	5.4	5.3
0	15.2	5.2	5.6
0	15.0	5.8	5.8
0	15.0	5.4	5.7
5.0	22.2	7.4	7.0
5.0	20.0	7.0	7.1
5.0	21.0	7.2	6.9
5.0	25.5	6.5	7.2
5.0	20.4	6.8	7.4

Preliminary data obtained from electron micographs of heart muscle from 10 test and 10 control birds of experiment 2 indicted that the major changes occurred in the mitochondria of the cardiac muscle cells. In some cases the mitochondria were markedly shrunken and vacuolated with indistinct cristae. In the birds with more advanced cases, many of the mitochondria had disappeared, leaving large areas devoid of any organelle.

Discussion

There was normal development of all organs with the exception of the testes of birds in experiment 2, which were markedly reduced in size. Kumaran and Turner[10] outlined the tubular and spermatogenic development in chickens at various states of maturity. It would appear that the consumption of toxic fat in these experiments retarded testicular development by approximately 2 months in the 6-month-old roosters. Despite the reduction in testicular size, secondary sex characteristics such as comb size and body conformation were unaffected.

In earlier studies,[1] young birds were killed every other day for 3 weeks to determine when hydropericardium or ascites developed. Hydropericardium was usually noticed about the 16th day after feeding toxic fat in the ration was initiated, without ascites or pulmonary edema. Fluid accumulation in the lungs and peritoneal cavity invariably developed later. When the concentration of toxic fat in experiment 2 was reduced to a low level, the birds failed to develop ascites, but hydropericardium was a consistent observation.

The most prominent lesions at necropsy were hydropericardium, ascites, and pulmonary edema. A number of procedures were conducted to resolve what caused the anasarca. Birds with ascites and hydropericardium in many cases had higher serum protein levels than those in the control group. The albumin: globulin ratios of experimental and control birds were approximately comparable. The nonprotein nitrogen and electrolyte levels of the blood were within normal range in the test groups. There was a reduction in the cement substance in the experimental birds with hydropericardium and ascites. Since a large quantity of fluid escapes between the endothelial cells rather than through them[23] any alteration in the cementum would affect the permeability of the vessels.[16] Whether this alteration in the cement substance of the capil-

laries was associated with hypoxia of the endothelial cells resulting from cardiac decompensation or from a direct effect of toxic fat on the capillary membrane remains to be clarified.

Hemodynamic studies revealed an increase in right ventricular and vena cava pressures in the experimental birds. Associated with these pressure changes were dilatation and hypertrophy of the right side of the heart of birds given toxic fat. Pulmonary edema, cardiac dilatation, and increased venous pressure are indicative of cardiac decompensation.

What do these data reveal in regard to the causes for the excessive extravascular fluids? The lack of any appreciable change in the total blood protein and albumin: globulin ratio would indicate that the liver was producing adequate amounts of albumin. There were no marked alterations in kidney function, because the serum albumin and nonprotein nitrogen levels were comparable in the 2 groups, which suggests that the kidney tubules and adrenal cortex were not affected by toxic fat ingestion. The microscopic observations fortify the biochemical data, because the alterations in the liver, kidneys, and adrenal glands of test birds did not appear of sufficient magnitude to account for the anasarca.

Since myocardial fibers are very active, there is a constant demand on the respiratory enzymes located in the numerous mitochondria between the myofibrils. A rather high metabolic demand is implied, because the ratio of mitochondria in cardiac muscle to skeletal muscle is approximately 500:1.[11] Any significant alteration in the mitochondria would affect the respiratory enzymes and sooner or later lead to cardiac failure. Certainly this would appear to be a logical explanation for what occurs in birds consuming toxic fat. As the heart becomes less efficient, there will be an increase in hydrostatic pressure in the veins and capillaries, with a predisposition for the extravasation of fluid into the tissues and body cavities.

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Summario in Interlingua

Le Rolo de "Grassia Toxic" in le Production de Hydropericardio e Ascites in Gallinas

Quandro "grassia toxic" esseva addite al dieta de aves experimental a concentrationes de 0,25 a 6,0 pro cento durante 35 a 150 dies, le sequente alterationes esseva observate: (1) Edema del myocardio, del musculatura skeletic, e del pulmones; (2) hydropericardio; (3) ascites; e (4) focos de lymphocytos in le myocardio e le epicardio. Appreciablle alterationes non esseva observate in le nivellos de proteina total in le sero, in le proportion albumina a globulina, in le balancia electrolytic, e in le nivellos de nitrogeno non ligate a proteina in le sanguine. Esseva notate dilatation, edema, e infiltration lymphocytic del corde. Le mitochondrios myocardial esseva vacuolate e contrahite. Un augmento del tension venose esseva etiam notate. Le imbalancia de liquido observate in aves que consumeva grassia toxic non resultava ab un declino in total proteinas de sanguine o ab un alteration in le proportion albumina a globuina sed esseva associate con discompensation cardiac e un augmento del permeabilitate capillar.

Industrially Acquired Porphyria

Twenty-nine patients working in a chemical factory engaged in the manufacture of 2,4-dichlorophenol (2,4-D) and 2,4,5-trichlorophenol (2,4,5-T) exhibiting features of chloracne were studied for the presence of porphyria cutanea tarda. In 11 cases urinary uroporphyrins were elevated.

Two of these patients who showed evidence of acquired porphyria with chloracne were hospitalized. The features of chloracne as well as the clinical and laboratory features of acquired porphyria have been discussed. There appeared to be an etiologic but not quantitative relationship between the chloracne in workers engaged in the manufacture of 2,4-D and 2,4,5-T and porphyria cutanea tarda of the acquired type. It is our feeling that either the finished chemicals or some intermediate are responsible for both diseases.

Since Waldenstrom first implied that porphyria cutanea tarda might be acquired, a growing number of chemials have been implicated in the pathogenesis of this disease. These chemicals have included alcohol, sedatives, fungicides, etc.[1-10] While treating a severe outbreak of chloracne in a factory which manufactures 2,4-D and 2,4,5-T, a number of workers were noted to have hyperpigmentation, hirsutism, fragility of the skin and vesiculobullous eruptions on exposed areas of skin, together with cutaneous findings of chloracne. Investigation revealed evidence of porphyria cutanea tarda of varying degrees of severity in 11 out of 20 workers investigated. Porphyria cutanea tarda has never before been described as related to chloracne, nor has it been ascribed to

industrial exposure in the United States. This outbreak is therefore of interest in adding more evidence to the growing concept that porphyria cutanea tarda may be an acquired disease occurring after various insults to the liver. Three cases were studied in detail.

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REPORT OF CASES

CASE 1.—A 48-year-old white male who was employed at the factory for three years as a chemical operator. His work brought him into intimate contact with the suspected chemicals. His past history included two attacks of biliary colic prior to 1953. He was never a heavy user of alcohol. A diagnosis of cholecystitis had been made and a cholecystectomy was performed early in 1953. After this he came to work at the factory in question. In 1956 he began to notice some darkening of his skin and suffered right upper quadrant pain. A diagnosis of common duct obstruction was made, and this patient was operated on again in January of 1956. An unsuccessful attempt was made to probe the common duct, and no further operative procedure was done. During the postoperative course, this man received 2 gm of barbiturates. The patient stated that his urine had turned "the color of Coca-Cola" at least one year prior to the second operation. That spring, an eruption of bullae appeared on the face, ears, and hands. These lesions could be produced either by exposure to the sun or by pressure. In addition to the vesicular eruption, the patient noted progressive darkening of his skin and marked hirsutism, especially over the temples. Inspection of the urine revealed a Coca-Cola coloration and, under the Wood's Light, a brilliant red fluorescence. The exact laboratory data on this patient are no longer available except for the presence of quantitatively markedly increased excretion of urinary porphyrins including uroporphyrins, coproporphyrins, and porphobilinogen.

This man is now alive and well and apparently is suffering minimal if any symptoms of porphyrim cutanea tarda. His present job does not entail the use of any chemicals. He has failed to present himself for further testing.

CASE 2.—This is a 60-year-old white male who has been employed in the factory for seven years as a welder. In the course of his work, which consisted of welding tanks and pipes, he was brought into frequent and prolonged contact with chemicals. He was admitted to the Newark Beth Israel Hospital for investigation. He stated that three months prior to admission, he had noted an increased darkening of the skin, thickening of the eyebrows, and a darkening and reddening of his urine. His family history and his past medical history were unrevealing, except for moderately heavy alcohol intake for some years.

Physical examination revealed numerous comedones and small epidermoid cysts and furuncles the face, chest, and shoulders. There was intense grayish-brown hyperpigmentation with a purplish tint on the exposed surfaces of the face, neck, chest, and hands and moderate hypertrichosis of the temples. The scalp hair showed a lusterless, dull silver color change. The liver edge was palpable about 3 cm below the right costal margin and was smooth and nontender. The remainder of the physical examination was within normal limits. A casual urine specimen revealed a strong tea color with a deep fluorescence, reddish, under the Wood's light.

Laboratory studies revealed increased urinary uroporphyin, coproporphyrin, and urobilinogen excretion. There was no demonstrable porphobilinogen. The feces showed increased uroporphyrins and coproporphyrins. All porphyrin determinations were qualitative and done by the Watson-Schwartz method. Other significant findings included an elevated serum glutamic oxaloacetic transaminase ranging between 41 and 51 units on five different days. Serum glutamic pyruvic transaminase on corresponding days ranged between 53 and 64 units. The sulfobromophthalein retention was 6% in 30 minutes. The erythrocyte sedimentation rate (Westergren) was 94 mm in the first hour. All other studies, which included complete blood count, bleeding and clotting time, urinalysis, glucose tolerance test, serum bilirubin, blood urea nitrogen, total

proteins, albumin-globulin ratio, serum cholestrol, alkaline phosphatase, phthalein flocculation, and thymol turbidity, serum electrolytes, and CO, combining power, as well as serological tests for syphilis were all within normal limits. Electrocardiograms and chest x-rays were normal. A liver biopsy was performed and the specimen immersed in isotonic saline. It fluoresced intensely under the Wood's light. The red pigment diffused out into the saline, so that the entire tube fluoresced. The microscopic examination of the liver specimen revealed parenchymal cell regeneration and hemofuscin deposition. A skin biopsy from clinically hyperpigmented postauricular skin showed a normal epidermis except for the dermoepidermal border, where there was a striking deposition of brown granular pigment. In addition, there was mild infiltrate of small round cells in the dermis. No sebaceous glands were visible in the sections. Shortly after discharge from the hospital in June, 1963, the patient was treated for a chromic trichophytosis of the feet with griseofulvin 0.5 gm twice daily. About four days after the onset of this treatment, a severe vesiculobullous eruption on the dorsal surface of the hands appeared. The griseofulvin was stopped, but the eruption progressed for another two weeks. Healing time was very prolonged, and at present, residual atrophic scarring is visible on both hands (Fig 1). In the scars occasional milia are seen.

SUMMARY OF DATA FOR 26 WORKERS WHOSE URINE WAS TESTED FOR PORPHYRINS

Patient	Chloracne[1]	Hyperpigmentation	Hirsutism	Urine Uroporphyrins	Chemical[2] Contact	Skin Fragility
1	Severe	Mild	Moderate	Pos.	Moderate	Pos.
2	Mild	None	None	None	Severe	Neg.
3	do	Mild	do	do	do	Do.
4[3]	Severe	do	do	do	do	Pos.
5	Mild	None	Mild	do	do	Do.
6	do	do	None	Pos.	Moderate	Neg.
7	do	Mild	do	None	Moderate	Do.
8	Severe	Moderate	Severe	do	do	Do.
9	Mild	Mild	Mild	do	do	Do.
10	Moderate	Moderate	Moderate	do	Severe	Do.
11	Severe	do	do	do	Moderate	Do.
12	None	None	None	do	Severe	Do.
13	do	do	do	do	do	Do.
14	do	do	do	Pos.	Moderate	Pos.
15	Moderate	Mild	Moderate	None	do	Do.
16	do	Moderate	Marked	do	Severe	Neg.
17	Mild	Mild	None	do	Mild	Do.
18	Moderate	Moderate	Marked	do	do	Do.
19	Severe	Marked	None	Pos.	do	Do.
20	Mild	Mild	Moderate	Pos.	do	Do.
21	None	None	None	None	do	Do.
22	do	do	do	do	do	Do.
23	Mild	do	do	do	do	Do.
24	None	do	do	do	do	Do.
25	do	do	do	Pos.	do	Do.
26	do	do	do	do	do	Do.

[1] Severity of chloracne is judged on the presence of comedones, epidermoid cysts, and furuncles and pustules.
[2] The extent of exposure is difficult to truly judge because of such variables as personal hygiene and work habits.
[3] Brief period of employment.

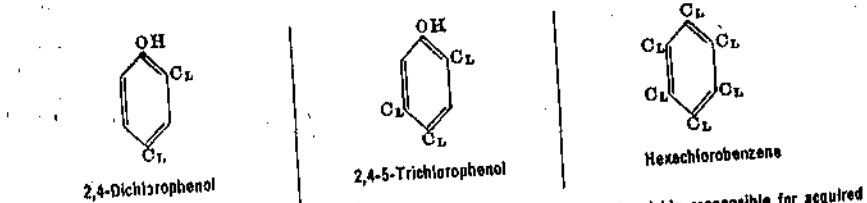

Figure 3.—Comparison of structural formulae of weed killer manufactured and fungicide responsible for acquired prophyria cutanea tarda. Note the similarities

CASE 3.—This is a 48-year-old white male employed at the factory for eight years mixing batches of chemicals. During the past two years, he had developed hyperpigmentation of the exposed skin of the face and hands. There was

ked hirsutism which involved the temples. The dull silvery tint of the hair was visible. He stated that in the past he had had episodes of blistering of the exposed skin. He also had noticed that his urine was dark on voiding. His family history was noncontributory. The physical examination revealed an intense hyperpigmentation of the face, neck, and hands. There was severe hirsutism involving the eyelids, eyebrows, and lateral aspects of the forehead (Fig 2). Comdones and small epidermoid cysts were very prominent, and there were numerous furuncles scattered over the entire body. The remainder of the physical examination was within normal limits except for prolapsed hemorrhoids. The following laboratory studies were within normal limits: complete blood cell count, urinalysis, bleeding and clotting time, prothrombin time, blood glucose tolerance test, urea nitrogen, cholestrol, bilirubin, alkaline phosphatase, total protein and albumin-globulin ratio, cephalin flocculation, thymol turbidity, serum electrolytes including sodium potassium, chlorides, CO_2 combining power, calcium, and phosphorus. The serum glutamic oxaloacetic transaminase on five successive days ranged between 39 and 56 units while the serum glutamic pyruvic transaminase on corresponding days ranged between 47 and 72 units. The sulfobromophthalein retention was 8% in 30 minutes. The electrocardiogram was normal. The chest x-ray revealed a diffuse nodular infiltration of both lungs due to pneumoconiosis. This was consistent with the patient's history of having worked a number of years as a coal miner. The plain film of the abdomen was negative. The urine revealed a negative Watson-Schwartz test. The urine failed to fluoresce under the Wood's light. The erythrocyte sedimentation rate (Westergren) was 24 mm in the first hour.

A liver biopsy was performed and the specimen immersed in saline. Under the Wood's light the specimen and saline in which it was immersed fluoresced faintly. On microscopic examination, the liver biopsy showed evidence of liver cell regeneration and hemofuscin deposition. A skin biopsy showed brown granular pigmentation as the basal margin of the epidermis. There was a mild chronic inflammatory infiltrate scattered through the dermis. No sebaceous glands were visible in the sections.

Since the man's chloracne has been so severe, he had been removed from contact with chemicals two years prior to his admission to the hospital. This probably was responsible for the failure to prove qualitative chemical evidence of porphyrins in the urine. It also may indicate that acquired porphyria cutanea tarda is reversible.

SCREENING TESTS

Twenty-six additional men working at this chemical factory were studied on an ambulatory basis. In addition to routine urinalysis, each urine specimen was tested for uroporphyrin by the Watson-Schwartz method. Eight out of the 26 manifested significantly increased excretion of urinary uroporphyrins by the Watson-Schwartz method. If the three cases described in the case reports above are added, this is a total of 11 cases of porphyria cutanea tarda of varying degrees of severity out of 29 patients tested, or 37+% (Table).

COMMENT

Hyperpigmentation in these workers was limited to the sun-exposed areas of the head, neck, and hands. It was more frequently observed in the Negro patients involved. The degree of hyperpigmentation was roughly proportional to the severity of the chloracne. The hyperpigmentation varies from mild redness in extremely fair individuals to dark gray intense dusky bronzing of the skin. The degree of hirsutism was also proportional to the severity of the chloracne. This too was quite variable in degree but always involved the temples between the lateral half of the eyebrow and the temporal hair of the scalp. The hirsutism in a few cases, notably case 3, extended beyond this and involved both the upper and lower eyelids. The hair was of approximately the same texture and density as that of the eyebrows.

The occupational environment of these men consists of a group of basic chemicals including acetic acid, phenol, monochloracetic acid and sodium hydroxide, plus the finished products 2,4-D and 2,4,5-T as well as many unknown intermediary products. It is known that one of the intermediaries is a highly volatile chlorinated phenolic ether which contains six chlorine atoms. This particular compound, because of its volatility, is strongly suspected of being a possible causal agent. Porphyria has been described in many cases as a

result of ingestion of hexachlorbenzene,[2,3] chemically a closely related compound (Fig 3). This would lend support to the concept that porphyria cutanea tarda is not necessarily genetically produced, unless the genetic defect is an extremely common one.

An analysis of the table of the 26 surveyed workers (Table 4) reveals: the severity of chloracne does not usually correspond to the degree of exposure to chemicals (patients 1, 8, 11, 19 or patients 2, 3, 5, 10, 12, 13, 16). The severity of prophyria does not usually correspond to the degree of chemical exposure (patients 7, 20, 25, 26 or patients 2, 3, 4, 10, 12, 13, 16). The severity of chloracne does not usually correspond to the presence of porphyria (patients 1, 4, 8, 11, or patients 5, 20, 25, 26). Therefore it would appear that there is some individual susceptibility to these disease. It has been observed in general that: (1) Patients with adolescent acne tend to get worse chloracne: (2) Possibly previous liver damage (alcoholism, etc) predisposes to porphyria. Also there must be in these cases some etiologic relationship between chloracne and porphyria since a relatively large number of both diseases began to appear and have persisted at the same time.

On the basis of the elevated transaminase levels and the histological signs of liver cell regeneration in the liver biopsies, it may be assumed that the basis of the distrubed porphyrin metabolism is a hepatotoxic effect of one or more of the chemicals in this factory environment. The synergistic roles of other known liver toxins such as alcohol and barbiturates, or griscofulvin (1 case), cannot be overlooked.

We would like to express appreciation for the help offered by Dr. Donald J. Birmingham of the division of Industrial Dermatology of the U.S. Public Health Service, to Dr. Marcus Key, and to Dr. Norman Olivier.

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Electron Microscopic Alterations in the Liver of Chickens Fed Toxic Fat*

Toxic fat is the name applied to certain fats that produce hydropericardium, hydrothorax, and ascites when added to the diet of chickens.[8, 12, 20] It has been demonstrated that the toxic fraction is associated with the unsaponifiable portion of the fat.[10, 12] The toxicity can be increased by repeated passages through silica and alumina gel columns.[6, 24] Crystalline preparations of the toxic fraction have been prepared, but the chemical nature of the compound remains to be identified.[12]

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The following alterations that resulted from the consumption of toxic fat have been reported. Sanger, Scott, Hamdy, Gale, and Pounden[10] observed focal necrosis of the liver, epicardial hemorrhage, and lymphocytic infiltration of the cardiac muscle. Simpson, Pritchard, and Harms[21] reported bile duct hyperplasia and proliferation of the endothelial lining of the smaller blood vessels. Allen and Lalich [4] observed testicular hypoplasia. Marked dilation and edema of the myocardium and altered right ventricular and vena cava pressures have been demonstrated.[3]

The present experiment was initiated to determine the morphologic effect of toxic fat upon the liver of chickens and to correlate these findings with the development of anasarca.

EXPERIMENTAL PROCEDURE

One hundred sixty-eight 1-day-old White Leghorn-New Hampshire chickens were divided into groups of 48 and 120 chickens. One-half of the chickens from each group were given a commercial diet containing 3.0 per cent toxic fat (Emery Industries, Inc., Cincinnati, Ohio), while the remaining chickens received a comparable diet which contained 3.0 per cent corn oil. In the group of 48 chickens, three control and three experimental chickens were killed every other day for 16 days and sections of the liver were obtained for electron microscopic evaluation.

The group of 120 chickens remained on the toxic fat diet until the LD_{50} was established. From the survivors, blood was obtained for hematocrit,[16] hemoglobin,[8] total serum protein,[13] serum electrophoretic pattern,[20] and electrolyte studies.[11] The ascitic fluid was also collected for total protein and electrophoretic pattern determinations. All chickens that died during the course of the experiment as well as those sacrificed at its completion were necropsied. Portions of the liver were fixed in 10 per cent buffered formalin for 24 hours, dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Other portions of the liver were sectioned on a cryostat and stained with Sudan IV for neutral fats. Additional paraffin-embedded tissues were stained with aniline blue and phosphotungstic acid-hematoxylin.[5]

Small sections of liver from the 48 chickens of the first group plus 20 control and 20 experimental chickens of the second group were obtained at the time of death, cut in small cubes, and fixed in Caulfield's[7] and Millonig's fixatives.[10] These tissues were dehydrated through a graded series of ethanol and embedded in an Araldite-Epon mixture.[17] Thin sections of approximately 0.5 μ were cut on an ultramicrotome for light microscopy and stained by the toluidine blue method.[20] Ultrathin sections were placed directly on 400-mesh, uncoated copper grids, stained with uranyl acetate, and examined with an RCA EMU-3G electron microscope.

TABLE 1.—ALTERATIONS IN THE PERIPHERAL BLOOD OF CHICKENS CONSUMING TOXIC FAT

No. of chicks	Toxic fat in diet	Hematocrit	Hemoglobin	Na	K	Serum protein	Serum albumin
	Percent	*Percent*	*gm. per 100 ml.*	*mEq./liter*	*mEq./liter*	*gm. per 100 ml.*	*Percent*
30	0.0	31.0	10.0	160	5.4	3.4	64
30	3.0	18.0	6.0	162	5.9	2.3	36

RESULTS

When 3.0 per cent toxic fat was added to the diet of young chickens 50 per cent died within 15 days. Approximately 24 hours prior to death, the chickens became listless and moved only when agitated. Because of the marked abdominal distention the chickens assumed a ducklike gait when forced to walk. Moist rales were present in the lungs and considerable amounts of clear to blood-tinged fluid were observed in the oral cavity of the experimental chickens at the time of death.

On the 15th day, blood studies were made on the surviving chickens. Those fed toxic fat showed a reduction in several of the blood components (Table 1). Hemoglobin levels decreased from 10.0 gm. per 100 ml. to 6.0 gm. per 100 ml., and hematocrits were reduced from 31.0 per cent to 18.0 per cent. There was a decrease in total serum protein from 3.4 gm. per 100 ml. to 2.3 gm. per 100 ml.

and a shift in the albumin-to-globulin ratio of the treated chickens. Similar electrophoretic patterns were obtained on the ascitic and pericardial fluids, although the total protein of these fluids was only 1.3 gm. per 100 ml. Blood electrolytes were not appreciably altered.

GROSS AND MICROSCOPIC OBSERVATIONS

A large quantity of colorless, semiclotted fluid accumulated in the subcutaneous tissue and the pectoral, thigh, and lower leg muscles were pale and edematous. The abdominal cavity of each experimental chicken contained approximately 40 ml. of ascitic fluid. The liver was somewhat mottled with rounded margins and a thick gelatinous material resembling coagulated plasma was firmly attached to the capsule in 25 per cent of the chickens that had received toxic fat. The kidneys were pale and swollen. All of the organs within the abdominal cavity appeared edematous. There was marked distention of the pericardial sac with a clear, slightly yellow fluid. Five to 10 ml. of pericardial fluid were obtained from each chicken. There was a noticeable dilation and hypertrophy of the right side of the heart and the myocardium was pale, with no gross lesions evident. The lungs were extremely edematous and the tracheobronchial tree was filled with blood-tinged, foamy fluid. The brain was edematous but free of other gross lesions.

The major microscopic alterations were observed in the liver. Its capsular surface was covered by a thick layer of homogeneous, eosinophilic material containing numerous fibrin strands. Although the general architecture of the liver parenchyma was maintained, numerous foci of necrosis were observed (Fig. 1). These lesions varied in size, from only two or three cells to a major portion of the lobule. There was a fairly sharp line of demarcation between the viable and dead cells. Immediately adjacent to these necrotic foci, the cytoplasm of the parenchymal cells was quite vacuolated, but otherwise not remarkable.

THIN SECTIONS FOR LIGHT MICROSCOPY

Better visualization of the histologic alterations was obtained from tissues embedded in the Araldite-Epon mixture than was possible using the conventional paraffin embedded preparations. The parenchymal liver cells of the control chickens stained uniformly with toluidine blue. Their nuclei were dark blue and the cytoplasm was much lighter and granular. The degenerating parenchymal liver cells of the toxic fat chickens were of two types on the basis of their affinity for toluidine blue. One cell type had a decided affinity for the dye so that both the cytoplasm and nucleus were very dark (Fig. 2). Many of these cells were shrunken, distorted and contained large morphologic features of the organelles were fairly well maintained in the cells obtained during the first 5 days of the experiment. However, the organelles were in very close apposition as a result of what appeared to be shrinkage of the cells and loss of the cytoplasmic matrix. Although the external mitochondrial membranes were quite irregular, the shape and distribution of the cristae remained unaltered. The membranes of the endoplasmic reticulum were in close apposition and had abundant ribosomes along the outer surface. Free ribosomes were dispersed throughout the cytoplasm. In the dark cells, the Golgi complex, lysosomes, and microbodies were usually sparse and only vaguely discernible. Only isolated areas within the cytoplasm contained glycogen granules. Numerous myelin-like structures appeared to arise from the cytoplasmic membranous systems of these cells. The nuclei of these dark cells had approximately the same electron density as the cytoplasm. The paired nuclear membranes appeared as one wide, irregular dense band containing few discernible pores. Except for the extreme density of the nucleoplasm, the chromatin material and nucleoli resembled those in the control cells.

From the 7th through the 15th day of the experiment, the dark parenchymal liver cells became increasingly electron-dense, the intercellular spaces wider and myelin-like bodies more abundant along the plasmalemmae. The cytoplasmic organelles became more difficult to visualize. Numerous oval to oblong clear vacuoles of variable size developed in the cytoplasm. Myelin-like bodies, identical to those along the plasmalemma, were present throughout the cytoplasm. At this time it was difficult to visualize the existence of a distinct nuclear and cytoplasmic separation because of the electron density and the

absence of any distinct nuclear membrane. Eventually these cells developed into dark, shrunken, fairly homogeneous masses which exhibited little morphologic resemblance to liver parenchymal cells (Fig. 6).

The second type of parenchymal cell in the degenerative areas was large and very electron-lucent. An isolated cell of this variety was seen most frequently within a group of the previously described dark cells. The plasmalemmae of these cells were in close apposition to the adjacent dark cells. The microvilli projecting into the space of Disse were shorter and less abundant than those of the dark cells (Fig. 7). Many of the organelles were markedly distorted. Small segments of the endoplasmic reticulum were scattered throughout the cytoplasm. The majority of these membranes contained ribosomes along their outer borders. Numerous free ribosomes were also scattered throughout the cytoplasm. The mitochondria were large and disorted. Their external membranes were quite irregular and formed many bulblike projections from the surface of the mitochondria. Small vesicles were apparent between the external and internal mitochondrial membranes. The enlarged cristae practically filled the interior of the mitochondria (Fig. 6). Large, clear vacuoles and cytoplasmic degenerative bodies were abundant throughout the cytoplasm while glycogen granules were very sparse in the light cells. The Golgi complex consisted of collapsed vesicular sacs dispersed in small groups throughout the cytoplasm. The mitochondria were large *and distorted*. Their external chromatin material quite evenly dispersed throughout the nucleoplasm.

During the terminal portion of the experiment, the large electron-lucent cells developed openings in their plasmalemmae. Their cytoplasmic organelles were observed in the sinusoids, space of Disse, and in the intercellular spaces.

The endothelial cells which line the sinusoids showed similar changes to those previously described in the dark parenchymal cells. The endothelial changes were most frequently observed adjacent to a large focus of dark cells. The long, slender cytoplasmic processes of the endothelial cells became extremely dark with the cytoplasmic and nuclear structures *becoming indistinguishable*. Large fragments of these endothelial cells were frequently seen in the sinusoidal spaces. Similar changes were observed in the bile duct epithelium. It was not uncommon to see two or three extremely electron-dense cells which had lost all their internal structure adjacent to a number of seemingly normal epithelial cells (Fig. 8).

DISCUSSION

The gross lesions produced in chickens fed toxic fat have been well documented, yet the mechanism by which this fat produces ascites, hydropericardium, hydrothorax, and edema has not been clarified. Vascular lesions and cardiac decompensation have been mentioned as possible causes of the massive accumulation of extravascular fluid in chickens that have eaten toxic fat.[1] In this experiment the liver was investigated to determine its association with the accumulation of extravascular fluid in chickens whose diets contained toxic fat.

There was a decided reduction in the total blood protein of the toxic fat chickens. The electrophoretic patterns of their sera showed the drop in protein resulted from a decrease in the serum albumin. When the liver tissues were examined microscopically the explanation for the decrease in serum albumin became apparent. There were numerous areas of focal necrosis throughout the liver of the experimental chickens.

There is no clear explanation of why some parenchymal liver cells shrink and become extremely electron-dense, while others appear to swell and become very electron-lucent. Undoubtedly each of these changes is a degenerative process which eventually terminates in the death of the cell. One could postulate that these two cell types perform different functions and because of this functional difference, the reactions of these cells to this toxic material were manifested differently. Such changes in size and lucency, however, are not unique for toxic fat but resemble those observed in hepatic lesions produced by monocrotaline and x-irradiation.[2] Further studies will be necessary to clarify the differing responses of various parenchymal cells to this toxic substance.

The accumulation of large quantities of extravascular fluid in the tissues and body cavities of chickens that have consumed toxic fat can be attributed at least in part to an alteration in the permeability of the vascular bed. The high level of protein in the ascitic and pericardial fluid and the electrophoretic

pattern which resembled that of blood serum would be substantiating data for the above statement. Extensive necrosis of the endothelial cells of the liver would also be related to altered capillary permeability. These observations would confirm a previous report[3] regarding the toxic effect of this fat upon the vasculature of chickens.

SUMMARY

Three per cent toxic fat was added to the diet of 1-day-old chickens. Fifty per cent of the chickens died within 15 days. There was a reduction in the hemoglobin, hematocrit, serum protein, and a shift in the albumin-to-globulin ratio of these chickens. The gross lesions in these chickens included hydropericardium, dilation of the heart, pulmonary edema, ascites, and subcutaneous edema. When the tissues were examined microscopically the most distinct lesions were necrosis of the parenchymal cells, bile duct epithelium and endothelial cells of the liver. The liver lesions were evaluated electron microscopically and the morphologic changes reported.

The reduction in total serum protein and shift in the albumin-to-globulin ratio of the chickens that consumed toxic fat were attributed to liver necrosis. The decrease in serum albumin may have predisposed to a portion of the extravasated fluid. However, altered permeability of the vascular bed following toxic fat consumption appeared to be the major predisposing factor for the accumulation of large quantities of extravascular fluid.

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Chick Edema Factor: Some Tissue Distribution Data and Toxicologic Effects in the Rat and Chick.* (31029)

The chick edema factor (CEF), responsible for a large number of deaths in the broiler industry in the fall of 1957, was traced to the unsaponifiable matter (unsap) of the fat used in the broiler rations. It since has been crystallized (1,2,3) and its structure proposed as that of a hexachlorohexahydrophenathrene (2). It is known that in the toxic fat, a mixture of related compounds can be found, some toxic and some relatively nontoxic.

To lay the groundwork for a study of the specific physiological effects of pure CEF compounds, a few short studies have been completed to learn the distribution of the toxic material in the body and which organs were primarily affected.

Experimental. Adult rats and day-old White-Rock chicks were used. Because the pure material was not available in sufficient quantity, we used, from the toxic fat, the unsap which represented 38% of the original toxic fat and was estimated to contain at least 10 ppm CEF. The unsap was force fed because the animals' food intake was drastically curtailed when it was mixed in the diet. All animals were offered water and commercial feed *ad libitum*.

Table I shows the experimental plans and dosage levels employed for all 3 Trials. Feed consumption and fecal and urinary excretion were measured for the rats. Body weights were recorded in all experiments. All animals, upon sacrifice, were examined grossly for pathology and selected organs were weighed and frozen. Hydropericardial fluid (HPF) volume was measured in the chicks.

In addition to the examination for gross effects, the presence of CEF material in various tissue was determined. Adrenals, kidneys, and livers were assayed in the rats; livers only have been analyzed in the chicks. To obtain a picture of the amount of material being absorbed and perhaps excreted, analyses of the feces and urine of rats and of the combined chick excreta were made.

TABLE I.—EXPERIMENTAL PLAN OF TRIALS

Trial	Species	Number of animals	Group	Dosage (per kg) Unsap (ml/day)	Dosage (per kg) CEF (estimated (μg/day)
I. 14 days	Rat	2	High	2.0	18
		2	Low	1.0	9
II. 6 days	do	4	High	2.0	18
		4	Low	1.0	9
		4	Control [1]	0	0
III. 6 days	Chick	6	High	5.4	43
		6	Low	1.1	18
		6	Control [1]	0	0

[1] Control animals were not intubated.

The assay method for CEF is being reported in detail elsewhere.‡ In short, the sample is homogenized with water and saponified with alcoholic KOH. The unsaponifiable portion is extracted with petroleum ether and chromatographed first on an alumina column. The eluate obtained with 25% ethyl ether in petroleum ether, following prior elution with petroleum ether and 5% ethyl ether in petroleum ether, is concentrated and chromatographed on 500 μ thin layer silica gel plates with 3% ethyl ether in petroleum ether. The silica gel in the area of R_f 0.80–1.00 is removed and eluted with ethyl ether, the solvent removed, and the residue is redissolved in isooctane for gas chromatography. We used an F & M Model 400 gas chromatograph with an electron capture detector and a U-tube column, 3 ft × 6 mm (o.d.) × 4 mm (i.d.), packed with

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‡ T. C. Campbell and L. Friedman in press, J. AOAC, "Chemical Assay and Isolation of Chick Edema Factor."

3% SE-30 on ANAKROM ABS (Analabs). The operating conditions were: temperatures, column oven 180°C, EC detector 200°C, flash heater 240°C; gas flows, helium carrier gas, 60 cc/min; argon, 10% methane purge gas 180 cc/min. The limit of detection by this method is approximately 0.2 ppb, assuming a sample size of 5 g and a sensitivity of the EC detector of 5×10^{-11} g.

The so-called CEF components can be seen as a gas chromatographic pattern of peaks shown in Fig. 1. This pattern is that of a highly concentrated material isolated from the toxic unsap. It is similar to a material isolated by Yartiff *et al* and kindly supplied to us by Firestone (3). We have numbered the peaks 1 through 8 as shown. Peak 4a was not seen in the Firestone preparation.§

Results and discussion. Table II shows the effects upon body weights, feed conversion, and feed consumption for the rats. The results for the vital organ weights and HPF volume are shown in Table III. There was no apparent gross pathology in either species with the exception of HPF and some ascites and subcutaneous edema in the chicks.

While only the chicks develop hydropericardium, apparently both are equally sensitive to liver weight increase, as shown in Table III. In the case where each species was maintained on CEF for 6 days, (Trials II and III), the rats

TABLE II.—GROSS EFFECTS IN RATS

Trial	Group	Percent body wt. change	Feed intake depression [1] (percent)	Dry matter dig. coeff. (percent)
I. 14 days	High	−15	—	76
	Low	−8.5	—	78
II. 6 days	High	−6.6	−38	72
	Low	−3.7	−29	75
	Control	−1.0	—	77

[1] Depression below control animals.

TABLE III.—ORGAN WEIGHTS EXPRESSED AS PERCENT OF BODY WEIGHTS

Trial	Group	Heart	Liver	Spleen	Kidney	Adrenals
II. Rats	High	.34	[2] 4.08	.20	.90	[3] .21
	Low	.32	[2] 4.08	.20	.82	.18
	Control	.31	3.36	.19	.76	.14
					HPF (ml)[1]	
III. Chicks	High	.86	[2] 3.85	.61	[3] .65	
	Low	.70	3.15	.54	[3] .12	
	Control	.78	2.73	.65	.04	

[1] HPF—hydropericardial fluid (ml).
[2] Significant statistically at 1 percent probability level.
[3] At 5 percent probability level (Hogben L test)(5).

(0 μg/kg/day) showed an increase over controls of 21% while the chicks (10 μg/kg/day) showed an increase of 15%. (The increase in liver weight in the chicks was not due to moisture or fat.)

Also, in Table III, neither heart nor spleen weights in either species are significantly affected. In rats, there appears to be a slight increase in kidney weights, though not statistically significant, and a highly significant increase of 50% in adrenal weights for animals on the high level. Whether this adrenal weight increase is simply a non-specific stress effect from intubation is not known, although it would seem that this cannot be entirely responsible, since the high level group showed an increase of nearly twice that of the low level group.

Some other observations which have been made on previously studied birds in this laboratory are of interest. Hematocrit values are depressed. Of a total of 48 birds, we have observed that, with an average of 0.08 ml HPF in control

§ The chromatogram for the Firestone preparation is presented elsewhere.

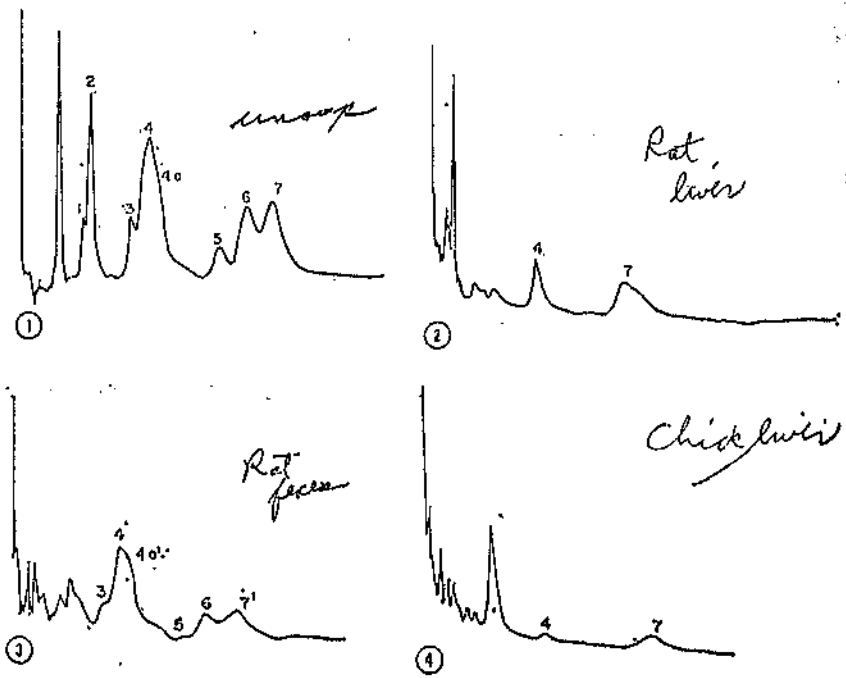

FIGURE 1.—Toxic CEF components found in unsap used in this study.
FIGURE 2.—Rat liver extract showing 2 CEF peaks.
FIGURE 3.—Rat feces extract showing CEF peaks, with altered Nos. 4a and 7.
FIGURE 4.—Chick liver extract showing 2 CEF peaks. (Large peak just before No. 4 found to be contaminant leached from liner of sample vial.)

birds (considered normal), there was a packed cell volume of 33.0%, while for diseased birds having 0.78 ml HPF, the packed cells volume was 27.9%.

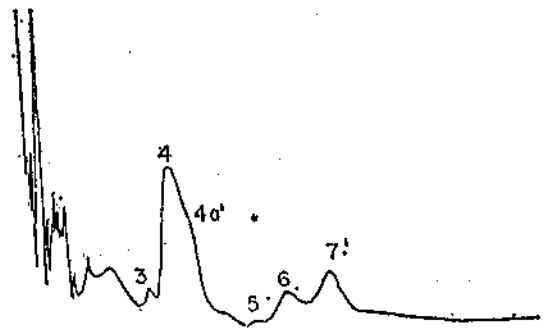

FIGURE 5—Chick feces extract showing CEF peaks, with altered Nos. 4a and 7.

Also, we observed that whereas control birds will show disappearance of a given amount of an I.V. injected dose of T-1824 dye (Evans Blue) from their vascular system of approximately 1%/min, poisoned birds will show a disappearance of approximately 2%/min, supporting the observation of Allen (4), that the permeability of the vascular wall appears to be increased.

Distribution of the CEF in animal body.—Of the rat tissues and samples examined, CEF was detected only in the liver and feces. Fig. 2 through 5 show typical chromatograms of purified extracts of feces and liver of both species.

Control animals not receiving CEF did not show these peaks. These chromatograms show that only peaks 4 (or 4a?) and 7 were present in the liver. In the fecal extracts all peaks were found, with the exception that instead of peaks 4a and 7 showing their original retention times, these were slightly increased in each case and have been designated 4a′ and 7′.

These retention times increases in the fecal components were measured by noting their retention times in relation to their neighbors, as shown in Table IV. Whether the 2 components of the liver are the products of components 4 and 7 or the original unaltered substances cannot be accurately determined from these chromatograms, since the rest of the CEF chromatographic pattern is missing. It may be concluded, however, that there is a selective absorption of peaks 4 (or 4a) and 7, with metabolism by the liver and excretion into the intestine.

It appears, on the basis of the tissues analyzed, that the liver is the target organ. Furthermore, the pattern of CEF chromatographic peaks presented here, Nos. 4 and 7 are the key components. We have succeeded in partially separating the CEF components such that 4a and 7 crystallized together, indicating a certain chemical as well as physiological similarity.‡ There is no way of differentiating 4 from 4a with regard to its absorption and metabolism on the basis of the available evidence.

Summary. Unsaponifiable matter isolated from a toxic fat and containing an estimated 10 ppm chick edema factor (CEF) was force-fed daily to adult rats at levels of 2.0 cc and 1.0 cc/kg body weight/day in 2 studies of 14 and 6 days, respectively. Feed consumption, body weight, and digestibility were depressed. Heart and spleen weights were unaffected, kidney weights seemed to be slightly increased, and adrenal and liver weights were significantly increased. In the chick, typical hydropericardium, ascites, and subcutaneous edema were observed. There were no significant changes in heart or spleen weights. Liver weights were significantly increased. The rat was as sensitive as the chick to CEF according to increase in liver weight. Of the 8 or 9 CEF components shown to be in this unsap, 2 (Nos. 4 and 7) were found to be absorbed and located in the liver, while the other components were not

TABLE IV.—CHANGES IN CHROMATOGRAM RETENTION TIMES OF CEF PEAK NOS. 4a AND 7

Material analyzed	No. of runs	Relative retention times $R_{7:4}$	$R_{4a:4}$
Firestone Standard	31	[a] 1.10±.01	4a not found
Unsap [1]	2	1.11±.01	1.22±.01
Feces [2]	13	1.14±.03	1.27±.04

[1] Unsaponifiables administered to animals in this study.
[2] Includes feces of 11 individual rats and the composite feces of 2 groups of 6 chicks each.
[3] The deviation includes the total range of values.

detected. In place of Nos. 4 and 7, there were 2 new peaks in the feces with slightly increased retention times. This suggests that the 2 active CEF components are metabolized in the liver and excreted into the intestine *via* the bile, both in the chick and the rat. No CEF-like material was found in kidneys, adrenals, or urine.

STUDIES ON THE METABOLISM OF CHICK EDEMA FACTOR: DISTRIBUTION IN CHICK TISSUES

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The distribution of chick edema factor in chick tissues following consumption of rations containing toxic fat has been of considerable interest. Chick

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[6] Received January 13, 1966. P.S.E.B.M., 1966, v121.

edema factor was detected in chicken tissues by electron capture gas chromatography, and an estimation of the concentration in various tissues was made by evaluation of gas chromatographic response.

Thirty samples consisting of homogenized tissues and parts from three groups of chickens, submitted by the Division of Nutrition, were examined for chick edema factor by a recently developed method.[1] One group had been fed a ration containing 3% of a reference toxic fat. Another group had been fed a ration containing unsaponifiables equivalent to 3% of the toxic reference fat. The last group received a ration free of toxic fat. The weights of tissue samples ranged from 0.4 to 98.4 grams. The samples were received in glass stoppered Erlenmeyer flasks, in ethanol.

DETERMINATION

(1) Extraction of unsaponifiable matter from animal tissue (Modification of AOAC Official Method 26.071).

Quantitatively transfer an alcoholic solution of the homogenized tissues to a 24/40 round bottom flask, add ethyl alcohol to give a final volume of 4 ml/gram tissue, but not less than 50 ml. Add 2 ml KOH solution (3 + 2) per gram of tissue.

Saponify by boiling with occasional swirling on a steam bath for one hour under reflux air condenser. Transfer alcohol soap solution while still warm to separator using water (equivalent to twice the volume of ethyl alcohol). Rinse saponification flask with the same volume of ethyl ether and transfer to separator. Shake vigorously, let layers separate and clarify, breaking any emulsion by adding up to 1/20 volumes of alcohol and swirling gently. Drain lower layer and pour ether layer through top into a second separator containing water (2 ml/g tissue), but not less than 20 ml. Make two more extractions of soap solution with ethyl ether (8 ml/g tissue). Rinse pouring edge with ethyl ether and add rinsings to second separator.

Rotate combined ether extracts gently with the H_2O (violent shaking at this stage may cause troublesome emulsions). Let layers separate and drain aqueous layer. Wash with two additional portions of H_2O (2 ml/g tissue), shaking vigorously. Then wash ether solution two times with alternate portions (2 ml/g tissue) of K_2CO_3 and H_2O. If emulsion forms during washing, drain as much of aqueous layer as possible, leaving emulsion in separator with ether layer and proceed with next washing. Wash final solution with H_2O until washings are neutral to phenothalein.

Transfer ether solution to erlenmeyer, rinsing separator and its pouring edge with ether, adding rinsings to main solution. Dry ether solution by adding anhydrous Na_2SO_4 (1 g/g tissue) and swirling vigorously ca 1 min. Let solution stand 10 min. Decant ether solution through glass funnel containing pledget of pre-rinsed cotton in neck and holding 25 gr anhydrous Na_2SO_4 into another erlenmeyer containing boiling chips. Wash first erlenmeyer with ether and transfer to second erlenmeyer.

Evaporate most of solvent on steam bath and transfer to 100 ml (preweighed) extraction flask. Evaporate residual solvent on steam bath under N. Dry flask to constant weight and obtain weight of unsaponifiable matter.

Proceed with fractionation of unsaponifiable matter on alumina and cleanup of alumina fraction 3 as directed in method (1), analyzing the residue by electron capture gas chromatography as directed.

RESULTS AND DISCUSSION

All residues except for liver samples were initially taken up in 100 ul of iso-octane and 5 ul injected. The liver samples were taken up in 250 ul of iso-octane and 1 ul injected. Examination of the chromatograms indicated the presence of a small contaminant (Ra 11.1) in the reagents used in the cleanup of the negative control samples.

Six out of 10 samples were "toxic" from each group of chicks fed toxic fatty material. The liver sample in each group was the most "toxic", containing roughly 83% of the total "toxicity" as indicated in Table I. The terms "toxic" or "toxicity" as used here to describe results of gas chromatographic analyses refers to the amount of material in an individual tissue which produces characteristic peaks at Ra 12 and 22 resulting from feeding the toxic fat. Of the pooled adrenals, bone, brain, heart, intestine, kidney, liver, skeletal muscle, skin, and testes that were examined, only the bone, heart, intestine, kidney, liver, and skin showed peaks characteristic of the presence of chick edema factor. No characteristic peaks were found in the adrenals, brain, skeletal muscle, and testes; perhaps the concentration was too low to be detected in these tissues.

The toxic fat as well as the toxic unsaponifiables used for this study exhibited 4 characteristic GLC peaks of Ra = 10.6, 12.4, 18.6, and 21.6 (See Table 2). The intestine chromatograms exhibited greatly diminished 10.6, 18.6 and 21.6 peaks, and a peak appeared at 12.0 in place of the 12.4 peak of the toxic fat and toxic unsaponifiables. The 10.6 and 18.6 peaks were not evident in chromatograms from the other tissues exhibiting characteristic peaks, and the 12.0 peak was the major characteristic peak. In addition, the bone, heart, kidney and skin extracts from the chicks fed toxic fat (Group A) exhibited a 12.4 peak which occurred as a shoulder on the major 12.0 peak.

These results suggest a selective absorption of the chlorinated components of the toxic fat; this is most clearly indicated by the diminished 10.6 peak in the intestine extract which is completely absent in the other tissue extracts. The appearance of peaks at 12.0 or 12.0 and 12.4 in place of the 12.4 peaks of the toxic fat can also be explained by selective absorption and deposition of individual components if we recognize that the GLC peaks observed each represent more than a single component. Campbell and Friedman (2) have also observed selective absorption of chick edema factor components in rats as well as in chicks. However, these authors only detected chick edema factor in the liver and feces.

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TABLE I.—RELATIVE % OF CHICK EDEMA FACTOR IN POSITIVE TISSUES[1]

Tissue	Group A (3 percent toxic fat)	Group B (unsap.≅ 3 percent toxic fat)
Bone	7.1	4.8
Heart	0.6	1.2
Intestine	2.4	2.4
Kidney	2.4	1.8
Liver	82.8	83.6
Skin	4.7	7.2

[1] The relative percent of CEF in each positive tissue was estimated by adjusting the volume of each sample so that a 5 ul injection of each would yield a chromatogram exhibiting the major peak (Ra 12) with a height of ca 6 cm. The following formula was used to calculate percentages:

$$\% \text{ CEF} = \left(\frac{\text{volume of sample}}{\text{Total volume of all samples in group}}\right) \times 100$$

TABLE II.—RETENTION TIMES (RA)[1] OF CHARACTERISTIC OF CHICK EDEMA FACTOR COMPONENTS IN TOXIC FAT ADDED TO DIET AND ISOLATED TISSUES

[Barber Colman Model 5360 gas chromatography; 7 foot 1/4 id glass column packed with 2½% SE52 silicone gum rubber on 60–80 mesh Gas Chrom Q; 3 x 10-8 amperes full scale; detector voltage, 30; injector temp., 240° C; column temp. 200° C; detector temp., 210° C]

Sample	Group A (Fed 3% toxic fat)	Group B (fed unsap. ≅ (3% toxic fat)
Toxic fat	10.6, 12.4, 18.6, 21.6	
Unsaponifiables (from toxic fat)		10.6, 12.4, 18.6, 21.6
Adrenals	None	None.
Bone	12.0, 12.4, 21.6	12.0, 21.6
Brain	None	None
Heart	12.0, 12.4, 21.6	12.0
Intestine	10.6, 12.0, 18.6, 21.6	10.6, 12.0, 21.6
Kidney	12.0, 12.4, 21.6	12.0, 21.6
Liver	12.0, 21.6	12.0, 21.6'
Skeletal Muscle	None	None
Skin	12.0, 12.4, 21.6	12.0, 21.6
Testes	None	None.

Ra =
[1] Retention time relative to aldrin.

Light and Electron Microscopic Observations in *Macaca mulatta* Monkeys Fed Toxic Fat

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SUMMARY

Thirty-six *Macaca mulatta* monkeys were given a diet that contained 0.125 to 10.0% of a fat capable of producing hydropericardium, ascites, and death in chickens. There was an inverse relationship between the concentration of toxic fat in the diet and the survival time of the monkeys. The monkeys given the greatest level of toxic fat had the mean survival time of 91 days, and the monkeys given the lowest level and the mean survival time of 445 days. During the last 30 days of life, the monkeys developed generalized subcutaneous edema, ascites, hydrothorax, and hydropericardium. There were decreases in erythrocytes, leukocytes, total serum protein values, and altered albumin:globulin ratios. There was also cardiac dilatation and myocardial hypertrophy and edema. Experimental monkeys had reduced hematopoiesis and spermatogenesis, degeneration of the blood vessels, focal necrosis of the liver, and gastric ulcers. It was proposed that toxic fat exerted its injurious effects upon the parenchymal cells of the liver, endothelium, and myocardium with subsequent development of generalized anasarca.

Fats from plant and animal sources have been used to increase the caloric level of diets for animals. As a result of increased demands by feed manufacturers for low-cost fats, almost every available source of these products has been utilized. Certain fats were found to be extremely toxic to poultry, and hundreds of thousands of chickens died or were killed after they were fed diets containing these fats. Results of experiments indicated that young chickens developed hydrothorax, pulmonary edema, ascites, and subcutaneous edema in 1 or 2 weeks when there was toxic fat in their diet.[1,2,16,17] The accumulation of large quantities of extravascular fluid in chickens seemed to result from altered permeability of the vascular bed, cardiac decompensation, and liver necrosis.[1,2]

Chemical studies on toxic fat indicated that the toxic fraction was located in the unsaponifiable portion.[6,20] By repeated passages through alumina and silica gel columns, crystalline preparations of the toxic fraction were prepared[6]; however, the chemical composition of the compound was not determined.

Before the cause of this intoxication of poultry was established, many chickens that had been fed toxic fat were processed for human consumption. Since that time, the clinical, histologic, and electron microscopic changes that occurred in the intoxicated chickens have been enumerated.[1,2,16,17] Data are not available, however, concerning the effects of toxic fat on primates. Since the chemical composition of this fat is unknown, the possibility exists that it may once again adulterate various edible fats. This is a report on experiments undertaken to determine the effect of toxic fat on lower primates. The results of the experiments may be helpful in postulating the effects that toxic fat might have in man.

MATERIALS AND METHODS

Since the chemical nature of toxic fat was unknown and chemical procedures were not available to determine the toxicity, a biological assay was performed on the fat used in the experiments. When the diet of 1-day-old chickens contained 3.0% toxic fat, 50.0% died within 15 days.

In the initial experiment, 16 *Macaca mulatta* monkeys (av. weight, 4.2 kg.) were allotted to 4 groups and fed diets containing 0 (control), 1.0, 5.0, and 10.0%, respectively, of toxic fat. In the 2nd experiment, 20 *M. mulatta* monkeys (av. weight, 6.0 kg.) were allotted to 4 groups and fed diets containing 0 (control), 0.125, 0.25, and 0.5%, respectively, of toxic fat. The toxic fat was combined with corn oil to obtain similar fat intake levels in all groups of monkeys. The following data were obtained at 1-month intervals: total serum protein value,[7] serum electrophoretic pattern,[18] complete blood count,[19] prothrombin time,[13] serum bilirubin value,[10] cholesterol level,[4] serum electrolytes,[3] blood urea nitrogen value,[14] and body weight. Observations were made each day on the general appearance and amount eaten. Needle biopsies of the liver were performed each month throughout the experiment to study sequential changes that occurred in hepatic tissues.

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All monkeys were necropsied immediately after death. When possible, the monkeys were killed immediately prior to their anticipated death to ensure procurement of fresh tissue for light and electron microscopy. Tissue sections from heart, lung, liver, spleen, mesenteric lymph node, sternal bone marrow, skeletal muscle, testis, gastrointestinal tract (3 zones of stomach and every 10 cm. of intestinal tract), skin, adrenal gland, pancreas, kidney, cerebrum, cerebellum, pituitary gland, thyroid gland, parathyroid gland, and urinary bladder were fixed in 10.0% neutral formalin, dehydrated, embedded in paraffin, sectioned at 6 μ, and stained with hematoxylin and eosin stain. Frozen sections from liver and heart were stained with Sudan IV for neutral fats.

Sections of liver and heart were taken from each monkey, cut into small cubes, and fixed in Millonig's[11] and Caulfield's[5] fixatives. These tissues were subsequently dehydrated through a graded series of ethanol and embedded in a plastic resin mixture.[12] Sections of the tissues were cut on an ultramicrotome, placed on 400-mesh uncoated copper grids stained with uranyl acetate, and examined with an electron microscope.*

TABLE 1.—TERMINAL HEMATOLOGIC CHANGES IN MONKEYS FED DIETS CONTAINING TOXIC FAT

Group	No. of monkeys	Total serum protein (Gm./100 ml.)	Serum albumin (%)	Packed cell volume (%)	White blood count x10³/cmm.	Red blood count x10⁶/cmm.
Controls	9	7.5	61	41	6.8	6.5
Fed toxic fat	27	5.4	35	16	3.0	2.5

RESULTS

In the initial experiment, monkeys in the groups fed diets containing 5.0 and 10.0% of toxic fat had the mean survival time of 91 days. The monkeys in the group fed the diet with 1.0% toxic fat had the mean survival time of 169 days.

In the 2nd experiment, the monkeys survived for a much longer period. The monkeys in the groups fed 0.5, 0.25, and 0.125% toxic fat had mean survival times of 202, 274, and 445 days, respectively.

There were considerable differences in survival times of monkeys in the various groups; however, the major clinical and pathologic changes were similar and occurred during the terminal 30 days regardless of whether the monkey survived for less than 4 months or longer than 1 year. Therefore, the data from the experimental monkeys will be presented collectively.

HEMATOLOGIC EVALUATIONS

Total serum protein values of the monkeys were reduced approximately 2.0 Gm./100 ml. during the experiment (Table 1). The mean percentage of serum albumin in the monkeys fed toxic fat on the last determination prior to death was 35%, whereas that of the control monkeys averaged 61%. There was a gradual decrease in the cellular elements of the blood during the experiments. Packed cell volumes were reduced from 32% to 16% during the last 30 days of life. These findings were substantiated by the total red blood cell counts which averaged 2.5 million/cmm. of blood immediately before the monkey died. The hemoglobin values followed a similar course, with the mean value of 6.0 Gm./100 ml. of blood being obtained in the last 30 days of life. Comparable observations were recorded for white blood cell counts; these averaged 3,000 white cells/cmm. of blood on the last hematologic evaluation of the experimen-

* RCA EMU-3G Electron Microscope, Radio Corporation of America, Camden, N.J.

al monkeys. Prothrombin times, serum bilirubin values, serum electrol[illegible] blood urea nitrogen values, and cholesterol levels of serum were not changed appreciably during the experiment.

CLINICAL OBSERVATIONS

The major changes in the monkeys were the development of generalized alopecia and subcutaneous edema 1 to 2 months before death. Edema, first noticed around the lips and eyelids, progressed to the remainder of the face and eventually involved the subcutaneous tissue of the trunk and extremities. Especially obvious was the marked edema of the scrotum and sheath which developed during the last few weeks of life and, in some monkeys, partially obstructed the flow of urine. During the last month of life there was decreased food consumption and the subsequent loss of body weight was frequently as much as 1 kg. Diarrhea developed in 75% of the experimental monkeys during the last few days of life. Results of bacteriologic cultural examinations of feces were negative for pathogenic enteric organisms.

GROSS AND MICROSCOPIC FINDINGS

The findings at necropsy substantiated the presence of extensive subcutaneous edema in over 75% of the monkeys fed toxic fat.

Heart.—Dilatation of the heart was especially obvious on the right side. This was further clarified when the circumference of the valves was determined. The mean tricuspid and mitral valve circumference of the experimental monkey hearts was 55 and 45 mm., respectively; in contrast, the tricuspid and mitral valves of the control monkey hearts averaged 40 to 36 mm., respectively. Hypertrophy of the cardiac muscle was also apparent in the experimental monkeys. The hearts of the experimental monkeys were 0.55% of the body weight, whereas hearts of the control monkeys were 0.30%. Microscopically, the muscle fibers were distinctly separated by fluid. Individual muscle cells were hypertrophic, and their nuclei were enlarged, distorted, and hyperchromic (Fig. 1). There were no distinct valvular lesions in hearts of the experimental monkeys.

Lungs.—Lungs of experimental monkeys were not altered appreciably. Isolated areas of atelectasis, congestion, edema, and fibrosis were observed. The proliferation of fibrous connective tissue was associated with the presence of lung mites *(Pneumonyssis simicoli)*.

Liver.—Livers of experimental monkeys were small, firm, and moderately yellow. On microscopic examination, moderate distortion of the architecture was found. Many parenchymal cells were enlarged, multinucleated, and had only moderate affinity for stain, whereas other cells were small and markedly hyperchromic. There was also focal necrosis of the parenchymal cells in the centrilobular zone (Fig. 2). Many parenchymal cells contained vacuoles in their cytoplasm which stained positively for neutral fat when frozen sections were prepared. Small areas of fibrous connective tissue occurred in the periportal area; however, they did not alter the architecture appreciably.

Spleen.—Spleens of experimental monkeys averaged only 0.074% of the body weight, and those of the control monkeys were 0.13%. Microscopically, the germinal centers were surrounded by only a narrow zone of lymphocytes, the blood sinuses were practically devoid of cells, and the trabeculae were especially prominent.

Mesenteric Lymph Nodes.—Lymph nodes were light tan and edematous. Microscopically, the germinal centers were surrounded by a narrow band of lymphocytes. The medullary cords were indistinct, and the sinuses were filled with proteinaceous fluid.

Sternal Bone Marrow.—Grossly, the bone marrow resembled coagulated plasma. Microscopically, only a small number of hematopoietic cells were seen in the marrow, and those were approximately equally divided between the myeloid and erythroid series (Fig. 3). Most of the bone marrow was composed of fatty tissue and proteinaceous fluid. The blood vessels and sinuses contained only a limited number of cells.

Skeletal Musculature.—The skeletal muscle was pale and edematous. Microscopically, the muscle bundles and fibers were widely separated by fluid, but otherwise seemed normal.

Testes.—Grossly, the testis seemed normal; however, when examined microscopically, active spermatogenesis was not found. The seminiferous tubules had abundant spermatogonia and Sertoli cells, but only a limited number of primary spermatocytes. There were no spermatids or mature spermatozoa. Interstitial tissue was moderately edematous, but the Leydig cells did not seem affected.

Gastrointestinal Tract.—In 18 of the 27 experimental monkeys, marked hypertrophy of the gastric mucosa occurred in the fundic and pyloric regions. In the same areas, small gastric ulcers penetrated the mucosal layer. (Fig. 4). In 6 monkeys, inflammatory changes in the intestinal tract were seen. Results of bacteriologic evaluations of these lesions indicated no pathogenic organism that could have been associated with the gastrointestinal disturbance. Microscopically, the hyperplastic gastric mucosa was seen to form many large, interdigitating folds. Adjacent to these proliferative areas, the mucosal lining was eroded, and the underlying tissue was necrotic. Large numbers of polymorphonuclear leukocytes were in the necrotic tissue and underlying musculature. Blood vessels of the edematous submucosal and muscular layers of the stomach adjacent to the ulcerated areas were free of obstruction.

Enteritis in 6 monkeys had caused moderate denudation of the intestinal mucosa and considerable hemorrhage into the lumen. The mucosal lining near the base of the crypts was intact, and the underlying musculature was normal.

Skin.—There was marked edema of the dermal layer of the skin, causing disarray of the collagen fibers. The epidermal layer was comparable in the control and experimental monkeys. There was an absence of any detectable change in the hair follicles of the monkeys given toxic fat.

The adrenal gland, pancreas, kidneys, cerebrum, cerebellum, pituitary gland, thyroid gland, and urinary bladder of the experimental and control monkeys were comparable grossly and microscopically.

ELECTRON MICROSCOPIC CHANGES

The extent of electron microscopic change in the liver correlated well with the level of toxic fat in the diet and the duration of toxic fat consumption. An early change in the parenchymal cells was the disruption of the orderly arrangement of the granular endoplasmic reticulum. The cisternal spaces were dilated, and the loss of ribosomes from the outer surfaces of the cisternae resulted in an apparent increase in the smooth endoplasmic reticulum. As a result of mitochondrial swelling, the cristae seemed shorter and less abundant than those of the control monkey hepatic cells (Fig. 5 and 6). Cytosomes of variable content and size were moderately prevalent in the cytoplasm. Small vesicles and flattened lamellae comprised the relatively small Golgi complex. Fat vacuoles were abundant throughout the cytoplasm (Fig. 6). The nuclei contained distinct nucleoli and abundant chromatin dispersed throughout the nucleoplasm.

Results of hepatic biopsies made a few weeks before death, and experimental tissues obtained at the time of death, indicated distinct alterations. Many parenchymal cells were shrunken and electron dense (Fig. 7). These dark cells were seen in various stages of degeneration. In some cells, the cytoplasmic organelles were still visible despite the extremely dense matrix. Vacuoles were dispersed between the cellular organelles. Myelin bodies were abundant in the cytoplasm, along the plasmalemma, and in the intercellular spaces. The nuclei were also electron dense, and the nuclear envelope was only vaguely discernible. Other dark cells had lost all resemblance to normal parenchymal cells,

being devoid of discernible cytoplasmic organelles and nuclei. Microvilli were extremely prominent and abundant along the plasmalemma adjacent to these space.

The lighter cells (Fig. 8) seemed to follow an entirely different course in their degenerative process. The cytoplasmic organelles were quite distinct in the abundant matrix. There was marked disruption of the endoplasmic reticulum, with only short fragments being scattered throughout the cytoplasm. Abundant free ribosomes were quite evenly dispersed between the organelles. The external contours of the mitochondria were frequently irregular, and the matrix was moderately electron dense. Occasionally, bulblike projections were formed by the external mitochondrial membrane. The plasmalemmae were irregular, and the microvilli were short and sparse. Occasionally, there were myelin bodies along the plasmalemmal surface. In some instances, the light cell plasmalemmae had ruptured, and organelles were dispersed throughout the extracellular space.

Bile duct epithelium was affected markedly in monkeys fed diets containing toxic fat. Many of the epithelial cells were so electron dense that the cytoplasmic organelles were difficult to visualize, and their nuclear membranes were irregular and extremely dense. The interlocking plicae of adjacent cells were widely separated. As a result of the shrunken condition of the epithelial cells, microvilli on the luminal surface of the plasmalemmae seemed thin and elongated.

Endothelial cells in some areas of the liver had a distinct resemblance to the dark parenchymal and bile duct epithelial cells. The dark endothelial cells were shrunken, and their internal structures were distorted. There were widened fenestrations between the endothelial cells. Changes in the cytoplasmic organelles were comparable with those observed in the parenchymal cells. Occasionally, large cytoplasmic sequestra from the endothelial cells were observed in the lumen of the vessels.

Heart.—The main differences between hearts of control and experimental monkeys were the dilatation of the intercellular spaces and the wide dispersal of the myofibrils in the latter. Between the widely separated groups of myofibrils (Fig. 9), there were mitochondria, occasionally a segment of sarcoplasmic reticulum, and abundant matrix. Usually, only the Z lines could be readily visualized. Cytosomes were much more abundant in the muscle cells of experimental monkeys and were usually found near the nucleus (Fig. 10). In more than 75% of the hearts of the experimental monkeys there was a distinct swelling of the mitochondria. The cristae were widely separated, and the mitochondrial matrix was abundant (Fig. 11). Large myelin figures were observed within and surrounding many mitochondria. Myocardial nuclei, components of the transverse tubular system, as well as elements of the sarcoplasmic reticulum, seemed unaffected. There was a noticeable separation of intercalated disks in many of the experimental monkeys (Fig. 12). The various bands formed by the myofibrils were often masked due to the abundance of edematous fluid in the tissue.

Many of the endothelial cells appeared electron dense and shrunken, with distortion of the nuclei and cytoplasmic organelles (Fig. 13). In most instances, the organelles were in close apposition as the result of the cell shrinkage. There were cytoplasmic myelin bodies in many of the cells. Occasionally, intercellular stromal cells had changes similar to those in the endothelial cells.

DISCUSSION

Toxic fat consumption had a decided effect upon hematopoiesis and spermatogenesis. Myeloid, erythroid, and lymphoid elements of the peripheral blood were markedly reduced. Germinal centers in the lymph nodes and spleen and the islands of hematopoietic cells in the marrow were extremely sparse. Inhibited spermatogenesis was also observed in the monkeys fed toxic fat. Similar

observations have been reported in chickens fed toxic fat.[3] The exact mechanism by which toxic fat exerts its inhibitory effects upon hematopoiesis and spermatogenesis has not been determined, and additional research will be required to elucidate these points.

The relationship of toxic fat consumption by monkeys and the development of moderate to extensive alopecia has not been established. Periodically, under normal conditions, there is a loss of hair by most monkeys. However, only in isolated instances has alopecia become as extensive as that of the monkeys given toxic fat.

An interesting and not well-understood lesion was the development of gastric ulcers in more than 66% of the monkeys fed toxic fat. The microscopic appearance of the ulcers was similar to that observed in man and lower animals, and determination of the etiologic factors was equally evasive. The direct effect of toxic fat upon the gastric mucosa and underlying vasculature must be considered; however, vascular lesions associated with the ulcers were not observed in the monkeys examined.

The most noticeable lesion produced by toxic fat consumption was the accumulation of large quantities of fluid in the tissue spaces and body cavities. When the blood protein values were tested, a decrease in total serum protein and a reversal in the albumin:globulin ratio were found (Table 1). The decrease in serum protein values, particularly albumin, could not be attributed to altered renal function because there was no increase in blood urea nitrogen or albumin in the urine. The most logical explanation for the decrease in serum protein would be related to the changes observed in the liver. Focal areas of necrosis and degeneration were observed in the livers of all experimental monkeys. Examination of electron micrographs of these degenerative areas substantiated the light microscopic observations. As a result of the altered functional status of the hepatic parenchymal cells, there was a decrease in albumin production. This would, in turn, alter the osmotic pressure of the blood sufficiently to produce stasis of fluid in the extravascular spaces.

Another important aspect of the problem was the effect of toxic fat upon the vasculature. Many of the hepatic and myocardial vessels were dark, shrunken, and seemed to have undergone degenerative changes. Since these vascular changes were not detected when the tissues were examined with the light microscope, it is likely that other vessels throughout the body had undergone similar changes. These observations would correlate well with the vascular changes recorded in chickens that have consumed toxic fat.[4] As a result of cellular shrinkage, the intercellular spaces were widened and there was a subsequent increase in the porosity of the vessel. This porosity would eventually lead to an increase in vessel permeability and predispose to the accumulation of extravascular fluid. There was cardiac dilatation and hypertrophy in all of the monkeys that consumed toxic fat. When the myocardium was examined microscopically, there was hypertrophy of the individual muscle cells and marked interstitial edema. Both of the latter have been commonly reported in patients with cardiac insufficiency. As the heart became less efficient, there was likely a gradual increase in hydrostatic pressure on the venous side which eventually produced stasis of fluid in the tissue spaces. An increase in right side of the heart and vena cava pressure similar to that postulated for the monkeys has been reported in chickens fed toxic fat.[1]

Reduced osmotic pressure of the blood, increased capillary permeability, and cardiac insufficiency have been incriminated as the causes for development of anasarca in monkeys fed toxic fat. It is difficult to determine at this time which of these entities was responsible. The most likely explanation is that all 3 play a major role, with each becoming the paramount entity at a particular stage of the disease.

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CALCULATED DIETARY INTAKES OF CHICK EDEMA FACTOR (CEF) FROM DATA PUBLISHED BY ALLEN AND CARATONS (AM. J. VET. RES, 28, 1513-26 (1967))

Dietary level of toxic fat (Percent)	Mean survival time (days)	CEF intake per animal per day (ug.)	Total CEF intake (ug.)
MONKEYS (MACACA MULATTA)[1]			
0.125	445	0.225	100
0.25	274	0.45	123
0.50	202	0.90	182
1.0	169	1.80	304
5.0	90	8.0	720
10.0			
CHICKS (DAY-OLD)[2]			
3.0	15	0.36	5.4

[1] Calculations based on average daily food consumption of 225 gm. for a 5.0-kg. monkey (F. Sperling, personal, communication, June 12, 1969).
[2] Calculations based on average daily food consumption of day-old chicks (personal studies, see Flick, et al., Poultry Science, 45, 630-36 (1966)).

Note on an Improved Cleanup Method for the Detection of Chick Edema Factor in Fats and Fatty Acids by Electron Capture Gas Chromatography

By PAUL NEAL (Division of Food Chemistry, Food and Drug Administration, Washington, D.C. 20201)

The electron capture GLC screening test for chick edema factor (1, 2) has been modified by replacing the saponification step with a sulfuric acid cleanup (3, 4) which permits a 50% reduction in sample cleanup time.

The modified procedure involves treatment of 2.5 g sample with sulfuric acid, fractionation of the petroleum ether extract from the sulfuric acid treatment on an alumina column, and sulfuric acid cleanup of the third alumina fraction, followed by electron capture GLC. Gas chromatographic peaks with retention time versus aldrin of 10-25 are indicative of the presence of chick edema factor.

Table 1. Comparison of sulfuric acid cleanup with saponification for detection of chick edema factor by electron capture gas chromatography (ECGLC)

Sample[a]	ECGLC Analysis[b]	
	Saponification	H_2SO_4 Cleanup
Low positive reference fat (1.5% toxic fat in USP Cottonseed Oil)	10.1 (45), 11.8 (48), 17.6 (65), 20.4 (60)	10.1 (18), 11.8 (14), 17.6 (40), 20.4 (34)
Toxic animal tallow	10.2 (74), 11.9 (82), 18.8 (94), 20.6 (98)	10.2 (56), 11.9 (42), 18.8 (85), 20.6 (90)
Toxic oleic acid	10.1 (147), 11.8 (23), 17.8 (>500), 20.4 (200)	10.1 (98), 11.8 (16), 17.8 (>500), 20.4 (154)
Toxic glyceryl monooleate	10.1 (50), 11.8 (23), 17.8 (200), 20.4 (82)	10.1 (46), 11.8 (14), 17.8 (258), 20.4 (98)
Vegetable oil soapstock (nontoxic)	10.4 (trace), 13.5 (trace)	10.4 (trace), 13.5 (trace)
Oleic acid (nontoxic)	10.4 (trace), 13.5 (trace)	10.4 (trace), 13.5 (trace)
Cottonseed oil (nontoxic)	10.4 (23), 13.5 (22)	10.4 (trace)
Blank	10.4 (trace), 13.5 (trace)	10.4 (trace), 13.5 (trace)

[a] Toxic and nontoxic refer to results of AOAC chick bioassay (AOAC *Official Methods of Analysis*, 10th Ed., 1965, 26.087–26.091).

[b] The first values (without parentheses) refer to retention time of peaks at 200°C vs. aldrin; the values in parentheses refer to peak area which is equal to retention time (cm) × peak height (cm).

Method

Reagents and Apparatus

Rinse all glassware with appropriate solvent before use. Do *not* use polyethylene containers to store solvents (5).

(a) *Petroleum ether.*—Reagent grade; redistill in glass between 30° and 60°C (available from Burdick and Jackson Laboratories, Muskegon, Mich.).

(b) *Carbon tetrachloride.*—Distilled-in-glass.

(c) *Celite.*—Johns-Manville #545, acid-washed. Wash well with petroleum ether and dry.

(d) *Filter paper.*—#540 S&S Blue Ribbon, or equivalent.

Determination

Sulfuric acid cleanup.—Dissolve 2.5 g fat in 10 ml CCl_4 in 400 ml beaker (heat, if necessary). Add 10 ml concentrated H_2SO_4 and then 20 g Celite; mix with heavy glass stirring rod during additions and stir until homogeneous mixture is obtained. Add 125 ml petroleum ether, mix well, let solids settle, and filter the supernatant liquid through filter paper in 90 mm conical funnel. Repeat with additional 125 ml portion of petroleum ether. Evaporate combined petroleum ether filtrate to 5 ml for alumina column fractionation.

Complete determination as outlined in the method of Higginbotham *et al.* (1).

Results and Discussion

GLC retention times and peak areas for negative, positive, and blank samples were compared for both the sulfuric acid and the saponification methods; see Table 1. Results are comparable as indicators of toxic material. Gas chromatographic peak heights were lower in some cases with the sulfuric acid cleanup; however, the presence of toxic factor was clearly indicated in the low positive reference material. The nontoxic cottonseed oil samples would have been judged toxic by the saponification method because of the relatively large GLC peak of R_a 13.5, a peak detected at low levels in gas chromatograms from blanks and other nontoxic samples; see Table 1. Small peaks of R_a 10–25 were observed in both procedures in the blank and nontoxic samples. However, they did not interfere with identification of the toxic fats and differentiation of toxic from nontoxic samples. Gas chromatograms of blank and low positive reference samples after saponification and preliminary sulfuric acid treatment are shown in Figs. 1 and 2.

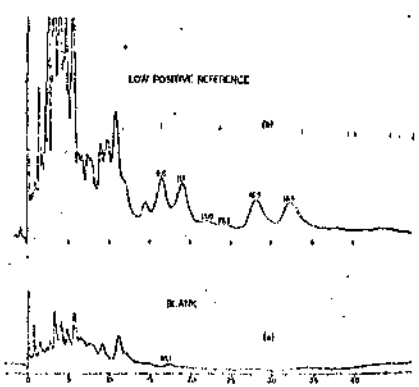

Fig. 1—Gas chromatograms of (a) blank and (b) low positive reference fat after cleanup with saponification.

GLC conditions: 7′ × 4 mm i.d. glass column packed with 2.5% SE–52 on 60–80 mesh Gas Chrom Q at 205°C. Amount injected: 1/50th of alumina column fraction 3.

Acknowledgments

The author expresses sincere thanks to David Firestone for guidance and encouragement throughout the development of this project and to Richard Staaf who performed many of the analyses.

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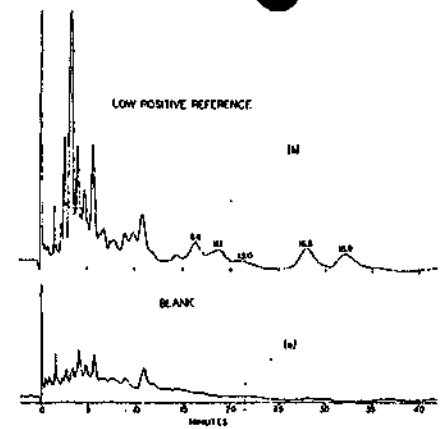

Fig. 2—Gas chromatograms of (a) blank and (b) low positive reference fat after cleanup with sulfuric acid treatment. See Fig. 1 for GLC conditions.

(3) Davidow, B., *ibid.* 33, 130–132 (1950).

(4) Dingle, J. H. P., *Analyst* 90, 638 (1965).

(5) Burke, J., and Giuffrida, Laura, *This Journal* 47, 326–342 (1964).

Reprinted from the *Journal of the Association of Official Analytical Chemists*, Vol. 50, December 1967.

(1) The official, first action GLC-microcoulometric method for chick edema factor, 26.092–26.096, was changed by adding the following to 26.092:

(g) *Ethyl ether for alumina chromatography.*—Ether (not >2% alcohol) or absolute ether (not >0.01% alcohol) (available from Burdick and Jackson Laboratories).

(2) The official, first action electron capture method for detection of chick edema factor, *This Journal* 50, 216–218(1967) was changed by addition of the following to (b) in the *Determination* section:

After ". . . 26.094" in line 6 add "(using ether specified in 26.092(g)" (item (1) above).

(3) The following rapid screening method for detection of chick edema factor was adopted as official, first action:

PRINCIPLE

Samples are subjected to preliminary H_2SO_4 cleanup and extd with petr. ether. Ext. is purified on Al_2O_3 column and examined by electron capture GLC, after addnl H_2SO_4 cleanup. Gas chromatographic peaks with retention time relative to aldrin of 10–25 are indicative of chick edema factor.

REAGENTS AND APPARATUS

(a) *Petroleum ether.*—Redistd in glass, b.p. 30–60° (available from Burdick and Jackson Laboratories, 1953 S. Harvey St., Muskegon, Mich. 49442).

(b) *Ethyl ether for alumina chromatography.*—Ether (not >2% alcohol) or absolute ether (not >0.01% alcohol) (available from Burdick and Jackson Laboratories).

(c) *Carbon tetrachloride.*—Redistd in glass (available from Burdick and Jackson Laboratories).

(d) *Celite.*—No. 545, acid-washed. Wash well with petr. ether and dry at room temp.

(e) *Aldrin standard soln.*—0.1 μg/ml. See *Reagents and Apparatus*, section (a), JAOAC 50, 216(1967).

(f) *Chick edema factor low positive reference sample.*—1.5% reference toxic fat in USP cottonseed oil. (Available from Division of Food Standards and Additives, Food and Drug Administration, Washington, D.C. 20204).

(g) *Activated alumina.*—See *Reagents and Apparatus*, revised 26.092(b), JAOAC 50, 216(1967).

(h) *Alumina chromatographic column.*—To *dry* chromatographic tube, 17 mm o.d. (14.5 mm i.d.) × 250 mm long, fitted at bottom with coarse porosity fritted glass disk and Teflon stopcock (tube without fritted disk but holding glass wool plug in bottom may be used), add redisd petr. ether, *dried* before use with anhyd. Na_2SO_4, until column is ⅔ full. Weigh 15 g Al_2O_3 and transfer to column in small portions, tapping tube as Al_2O_3 settles. When last portion of Al_2O_3 settles and air bubbles stop rising to surface, add 5 g anhyd. Na_2SO_4. Drain excess petr. ether so that it is just above upper surface of Na_2SO_4.

(i) *Gas chromatographic column.*—Glass, 5–7′ × ¼″ i.d., packed with 2.5% SE-52 silicone gum rubber on 60–80 mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa. 16801). Coat support with substrate as follows: Weigh 2.5 g silicone gum rubber stationary phase and dissolve in 300 ml CH_2Cl_2-toluene (1 + 1), heating to dissolve. Add 97.5 g Gas Chrom Q and let stand 10 mm with occasional gentle stirring. Dry in rotary evaporator held in 50° bath. Pack coated material into chromatographic column by adding small amts while vibrating column at packing level with Vibro-graver tool (Fisher Scientific Co., Pittsburgh, Pa. 15219). Fill to within 1″ on exit side and 3″ on entrance side, and fill remaining space with silanized glass wool. Condition column at operating pressure 2–5 days at 250°.

(j) *Gas chromatograph with electron capture detector.*—See *Reagents and Apparatus*, (e), JAOAC 50, 216(1967).

DETERMINATION

(a) *Preliminary sulfuric acid cleanup.*—Dissolve 2.5 g fat in 10 ml CCl_4 in 400 ml beaker; mix with heavy glass stirring rod while adding 10 ml H_2SO_4. Add 5 g anhyd. Na_2SO_4 and stir well while adding 20 g Celite, until homogeneous mixt. is obtained. Add 125 ml petr. ether, mix well, let solids settle, and filter supernatant thru paper in 90 mm conical funnel. Repeat with addnl 125 ml petr. ether. Evap. combined petr. ether filtrate to 5 ml for Al_2O_3 column fractionation.

(b) *Fractionation of petroleum ether filtrate by alumina chromatography.*—Dry solvents prior to use by shaking with anhyd. Na_2SO_4. Transfer petr. ether filtrate from (a) to Al_2O_3 chromatographic column, using total of 15 ml petr. ether. Let liquid level fall to just above top of Na_2SO_4. Elute sample with 100 ml petr. ether (fraction 1), 50 ml 5% ether in petr. ether (fraction 2), and 100 ml 25% ether in petr. ether (fraction 3). Relatively fast flow rates of ca 8–9 ml/min give satisfactory results. Keep liquid level above top of Na_2SO_4 at all times. Discard fractions 1 and 2, and collect fraction 3 in 125 ml erlenmeyer. Add several boiling chips and evap. to dryness on steam bath. Transfer residue with petr. ether to 10 ml g-s. graduate and evap. petr. ether soln to 3 ml.

(c) *Sulfuric acid cleanup of alumina fraction 3.*—See *Determination*, section (c), JAOAC 50, 217(1967).

(d) *Electron capture gas chromatography of petroleum ether extract.*—See *Determination*, section (d), JAOAC 50, 217(1967).

(4) The official, first action method for methyl esters of fatty acids, 26.055–26.059, with changes in *This Journal* 49, 231–232 (1966), was revised as follows:

(A) 26.058(c), revised third paragraph, *This Journal* 49, 232(1966), after ". . . to obtain calibration factor." insert "Reference mixts simulating most fats and oils may be obtained from Applied Science Laboratories, Box 440, State College, Pa. 16801; Supelco, Box 581, Bellefonte, Pa. 16823; and Lipids Preparation Laboratory, Hormel Institute, Austin, Minn. 55912)."

(B) 26.059, change to read as follows: "Two single detns of major components (>5%) performed in 1 laboratory shall not differ by >1.0 percentage unit. Two single detns performed in different laboratories shall not differ by >3.0 percentage units."

(5) The official, final action lead-salt ether method for determination of saturated and unsaturated fatty acids, 26.040, was changed as follows:

(A) Change first paragraph, first sentence to read: "Accurately weigh 10 (for plant fats used in common household cooking oils) or 20 g sample into 200 . . ."

(B) Add the sentence "Reserve ether filtrate (contains ether-sol. Pb soaps)." to the end of the second paragraph.

(C) Fifth paragraph, line 8, after ". . . HCl-free", insert "(no ppt with $AgNO_3$)."

(D) Revise the sixth sentence of the fifth paragraph to read: "Distill ether, avoiding any loss of fatty acids, and heat over steam bath to constant wt under controlled flow of N to prevent oxidation of fatty acids. Cover steam bath with towel to prevent splashing H_2O into erlenmeyer."

(E) Paragraph 6, line 1, insert "reserved" after "Transfer". Line 6, add "Repeat HCl hydrolysis until no more $PbCl_2$ is pptd." after ". . . into beaker."

(F) Change paragraph 7, lines 2 and 3 to read, ". . . until HCl is removed (no ppt in wash H_2O with $AgNO_3$). Dehydrate ether with ca 2 g anhyd. Na_2SO_4 and transfer ether soln . . . ."

(G) Change paragraph 8 to read: "Det. in duplicate 1 numbers of 0.2–0.3 g oil from unsatd fatty acid fraction, and from entire satd fatty acid fraction. (1 number of satd acid fraction is due to presence of some unsatd acid.)"

(6) The following gas-liquid chromatographic method for butylated hydroxyanisole (BHA) (121006) and butylated hydroxytoluene (BHT) (128370) in corn and rice breakfast cereals was adopted as official, first action.

APPARATUS

(a) *Gas chromatograph.*—Barber-Colman Model 5000, or equiv., with H flame ionization detector and strip chart recorder. Establish following operating conditions: temps—column 160°, detector 210°, flash heater 200°; N flow rate, sufficient to elute BHT in 3–4 min from QF-1 column and elute BHA in 3–4 min from Apiezon column; H flow rate, ca 40 ml/min for Apiezon L and ca 25 ml/min for QF-1; air flow rate, ca 340 ml/min; electrometer sensitivity; 500 × (5×10^{-10}) amp full scale deflection) with 5 mv recorder. Adjust H and air flow rates, if necessary.

Adjust electrometer sensitivity so 0.1 μg BHA gives ca 50% deflection. Repeat injections until constant peak heights are obtained on successive injections of identical vol. of std mixt.

Order of appearance on Apiezon column (4′): BHA, BHT, di-BHA. Order of appearance on QF-1 column (6′): BHT, BHA, di-BHA.

(Reprinted from Nature, Vol. 220, No. 5168, pp. 702–703, November 16, 1968)

Chemical and Toxicological Evaluations of Isolated and Synthetic Chloro Derivatives of Dibenzo-*p*-dioxin

ONE of the toxic compounds known as hydropericardium factor (chick oedema factor) is 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin[1]. Because it is highly improbable that these very toxic compounds are naturally occurring components of the fats from which they have been isolated, the question of their origin has aroused speculation.

A clue to the possible origin of hydropericardium factor is found in a report by Tomita *et al.*[2] of the synthesis of polyhalodibenzo-*p*-dioxins: chlorophenols and their salts, when heated, undergo condensation reactions and form chlorinated derivatives of dibenzo-*p*-dioxin. Because of this observation, we studied the pyrolysis of a number of commercially available chlorophenols which are widely used in agriculture and industry, and present results of some preliminary experiments which suggest that hydropericardium factor could arise from certain chlorophenols.

The commercial chlorophenols used in this work were pyrolysed in accordance with the general procedures described[2]. Benzene extracts of the reaction mixtures were fractionated by passing them through an alumina column. Residues of the benzene effluents were extracted with petroleum ether at room temperature, and the petroleum ether was then removed. The resulting products were sufficiently pure in most cases for examination by electron capture gas-liquid chromatography (GLC) and biological testing by the chicken embryo assay[3]. Table 1 shows retention times of the GLC peaks on a 7 foot, 2·5 per cent '*SE-52*' column at 200° C and the results of the chicken embryo assay. Individually isolated hydropericardium factors of known chemical structures were not available for use as standards; consequently, a concentrate from the unsaponifiable fraction of a certain commercial toxic fatty acid material was used as a GLC reference. The unsaponifiable fraction of the fatty acid material was known to contain trace amounts of the hydropericardium factor. The toxic fatty acid material, a by-product obtained from the manufacture of oleic and stearic acids, has been used as a reference in all our previous chemical and biological work[4].

Table 1. EXAMINATION OF CHLOROPHENOL PYROLYSATES

Sample pyrolysed*	R_e at 200° C of components† in pyrolysate	Chicken embryo bioassay: µg/egg‡	Chicken embryo bioassay: Per cent mortality§
2,4-Dichlorophenol (R)	1·0	500·0	70
2,4,5-Trichlorophenol (T)	3·5, 6·5	0·25	100
2,4,6-Trichlorophenol (R)	2·6	5·0	60
2,3,4,6-Tetrachlorophenol (T)	8·9, 10·0, 11·2, 17·4, 20·0, 35·0	1·0	100
Pentachlorophenol (R)	35·0	5·0	27
Chlorinated dibenzo-*p*-dioxin	1·0, 1·8, 3·5, 6·6	0·05	100
Reference toxic fat components	1·0, 1·8, 3·6, 6·6, 8·0, 10·0, 11·2, 17·4, 20·0, 36·0	5·0	100

* (R) = reagent grade; (T) = technical grade.
† R_e = retention times of peaks relative to the retention time of aldrin.
‡ 1 µg = 20 p.p.b. in the egg. Sample injected into the air cell of fresh fertile eggs before incubation. Solvents used: ethanol, acetone and chloroform.
§ Embryonic mortality at 21 days. The mortality of non-injected and solvent-injected controls was 10–15 per cent.

The chromatogram of components from the toxic fatty acid material contained a number of peaks of widely varying retention times; four of these peaks had R_e values greater than 10. According to a current electron capture GLC test[4], the presence of one or more GLC peaks with R_e values of 10 or more indicates that hydropericardium factor is present in a fat. The pyrolysate from technical grade 2,3,4,6-tetrachlorophenol was the only product that showed a peak pattern indicating the presence of hydropericardium factor; the peak pattern resembled that displayed by the long retention time components of the reference toxic fat. When tested by the official chick bioassay[5] for hydropericardium factor, the mixture produced the disease at a dietary level of 0·1 p.p.m.

In addition, dibenzo-*p*-dioxin was chlorinated directly in order to prepare 2,3,7,8-tetrachlorodibenzo-*p*-dioxin[1]. This material proved to be extremely toxic in the chicken embryo assay (Table 1), and provided a source for isolation of a trichloro as well as the tetrachlorodibenzo-*p*-dioxin.

The technical grade 2,3,4,6-tetrachlorophenol pyrolysate, chlorinated dibenzo-*p*-dioxin, and components from the reference toxic fat were each fractionated by preparative GLC (thermal conductivity detection), using a 12 foot × 4 mm column at 250° C packed with 10 per cent '*DC-200*' on 60/80 mesh 'Gas Chrom Q'. Isolated components from the 2,3,4,6-tetrachlorophenol pyrolysate and from the chlorinated dibenzo-*p*-dioxin were examined by electron capture GLC and infrared spectroscopy, and were also analysed by mass spectrometry to determine molecular weight and number of chlorine atoms per molecule. Results are recorded in Table 2. The components isolated from the reference toxic fat have not yet been examined spectroscopically.

Table 2. COMPONENTS ISOLATED FROM CHLORINATED DIBENZO-*p*-DIOXIN AND 2,3,4,6-TETRACHLOROPHENOL PYROLYSATE

Mixture	GLC peak No.	R_e at 200° C	Molecular weight	No. of Cl atoms
Chlorinated dibenzo-*p*-dioxin	1	1·8	286	3
	2	3·5	320	4
2,3,4,6-Tetrachlorophenol pyrolysate	1	8·0	388	6
	2	10·0	388	6
	3	11·2	388	6
	4	11·6	388	6
	5	17·4	422	7
	6	20·0	422	7

Infrared spectra of individual components exhibited characteristic absorption bands in the region of 1,330–1,280 cm⁻¹ attributed to asymmetric stretching vibration of C–O–C in the dibenzo-*p*-dioxin ring. The two principal compounds isolated from the chlorinated dibenzo-*p*-dioxin were 2,3,7-trichlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The infrared spectrum of the trichloro compound has bands at 870 cm^{-1} and 805 cm^{-1} (1,2,4-substituted benzene), and 860 cm^{-1} (1,2,4,5-substituted benzene). The infrared spectrum of the tetrachloro compound has a strong band at 868 cm^{-1} (1,2,4,5-substituted benzene). The infrared spectrum of the tetrachloro compound matched the spectrum of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin published by Tomita *et al.*[2]. Chicken embryo assay showed that the tetrachloro compound was more toxic than the trichloro compound.

The first four peaks in the chromatogram from the tetrachlorophenol pyrolysate were caused by positional isomers, each containing six chlorine atoms; the fifth and sixth peaks were caused by positional isomers, each containing seven chlorine atoms. A seventh peak in the chromatogram, observed when the sample injection was overloaded, had a retention time identical to that of a sample of octachlorodibenzo-*p*-dioxin prepared by pyrolysis of pentachlorophenol. The presence of pentachlorophenol in technical grade 2,3,4,6-tetrachlorophenol accounts for the formation of the two heptachloro isomers and the octachlorodibenzo-*p*-dioxin.

A fourth hexachloro isomer, in addition to the three expected from pure 2,3,4,6-tetrachlorophenol, is probably caused by the presence of a tetrachlorophenol other than the 2,3,4,6-isomer in the starting material. Infrared spectra of peaks 3 and 4 from the 2,3,4,6-tetrachlorophenol pyrolysate were very similar to published spectra of two hydropericardium factors isolated from a contaminated fat[6].

The components isolated by preparative GLC from the reference toxic fat, the chlorinated dibenzo-*p*-dioxin, and the 2,3,4,6-tetrachlorophenol pyrolysate were analysed by electron capture GLC at 200° C and by the chicken embryo assay. These results are shown in Table 3. The quantity of material in each fraction from the reference toxic fat was estimated on the basis of the weight of sample injected into the preparative gas chromatograph and the number of fractions collected. The amounts of material isolated from the chlorinated dibenzo-*p*-dioxin and 2,3,4,6-tetrachlorophenol pyrolysate were rough estimates based on comparison of GLC peak areas of isolated components and the starting mixtures. Amounts of individual components in the mixtures were estimated from normalized peak area calculations. The data suggest that one hexa and one hepta-isomer are less toxic than the other isomers. Work is now under way to prepare individual compounds of high purity in sufficient quantities for additional chemical and biological testing.

Table 3. GLC ANALYSIS AND EGG EMBRYO ASSAY OF ISOLATED COMPONENTS

Reference toxic fat components: R_e at 200° C	Reference toxic fat components: Chicken embryo assay* per cent mortality	Components from chlorinated dibenzo-*p*-dioxin and 2,3,4,6-tetrachlorophenol pyrolysate: R_e at 200° C	Chicken embryo assay†: µg/egg	Chicken embryo assay†: Per cent mortality
1·0	20			
1·8	20	1·8‡	0·5	15
3·2	100	3·5‡	0·2	100
5·1	10			
6·5	100			
8·9	60	8·0	2·0	30
10·0	60	10·0	1·0	100
		11·2	1·0	100
11·6	100	11·6	0·5	100
17·1	60	17·4	2·5	15
20·0	100	20·0	1·25	100
35·0	40			

* It was estimated that about 20 µg of material was injected into each egg.
† See footnotes 3 and 4, Table 1.
‡ Components from chlorinated dibenzo-*p*-dioxin.

Reference toxic fat components with low R_e values (less than R_e 8·0) could be due to chloro-organic pesticides and/or lower chlorodibenzo-*p*-dioxins. Additional work is required to identify the compounds responsible for the peaks with low R_e values. The peaks with high R_e values (those equal to or greater than 8·0) correspond to those of the hexachloro and heptachloro positional isomers isolated from the pyrolysate of 2,3,4,6-tetrachlorophenol. The overall results of this work suggest that chlorophenols could be precursors of hydropericardium factor. Commercial chlorophenols are widely used for such diverse applications as contact herbicides, defoliants and termite control agents, as well as for control of microbial attack in the manufacture of a number of products. There are many opportunities for fats and fatty acids, the only products in which hydropericardium factor has been found, to become contaminated. When crude fats and tallows are subjected to heating operations (hydrolysis, distillation) in the production of commercial fatty acids, residues of commercial chlorophenols present in the fat might be converted in part to chick oedema factors. Additional work is required to determine whether hydropericardium factors are formed from residual chlorinated phenols during production of commercial fatty acids. This laboratory is developing methods for detecting chlorophenols in fats and fatty acids.

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Internal Preliminary Report Analysis of Commercial Chlorophenols for Trace Amounts of Their Condensation and Plymerization Products

By G. R. Higginbotham and John Ress

Laclair (1) has reported an ultraviolet absorption study of the components of technical pentachlorophenol, separated by vacuum sublimation, and disclosed only pentachlorophenol (PCP), 2.3.4.6-tetrachlorophenol, and an unidentified, dark brown, high melting, "chlorophenol" containing 58.3% chlorine. The unidentified material was presumed to be a polymerization product produced durlug process of manufacture. Tomita *et al.* (2) have demonstrated that chlorphenols and their salts, when heated, undergo condensation reactions and form chlorinated derivatives of dibenzo-p-dioxin (chick edema factors, CEF).

Recently, we examined the unsaponifiable fraction of a number of commercial chlorophenols and obtained results which suggest that the impurities of chlorophenols consists of chloro derivatives of dibenzo-p-dioxin in addition to other components which have not been identified.

Thirteen chlorophenol samples were analyzed for chick edema factor. The samples were carried thru the 2.5 gram saponification procedure and examined by electron capture GLC after Al_2O_3 column chromatography and H_2SO_4 extraction [JAOAC, *50*, 884 (1967)]. GLC peaks indicative of chick edema factor were observed in most of the chromatograms from the thirteen samples.

Six of the thirteen samples were selected for further study. The unsaponifiables from each sample were extracted and chromatographed on an Al_2O_3 column, according to A.O.A.C. Method 26.092–26.094. The unsaponifiable fractions were submitted to the egg embryo biassay. Two components (Ra 6.3 and Ra 11.6) were isolated by preparative GLC from the unsaponifiable fraction of Eastman's technical grade 2,3,4,6-tetrachlorophenol. The components were examined by mass spectrometry and submitted to the egg embryo bioassay. Results are tabulated below.

Table I shows the amount of unsaponifiables isolated from 100 g samples of six representative commercial chlorophenols. In every case the amount of CEF in the total sample is estimated to be less than 0.3%. If a lipid sample is contaminated with pre-formed chick edema factor,* high levels of poly chlorophenols would also be expected to be present in the sample.

Results from the chick embryo assay are recorded in Table II. Table III summarizes the results obtained on two components isolated from 2,3,4,6-tetrachlorophenol. The molecular weight and the number of chlorines for each component are not consistent with a chloro derivative of dibenzo-p-dioxin.

The Ra values of these components are not consistent with known chick edema factors. The compounds may be photodecomposition products of technical grade 2,3,4,6-tetrachlorophenol.

Further work will be required to characterize the unsaponifiables from commercial chlorophenols. The overall results of this preliminary study emphasize the need for a rapid method for polychlorophenols in fats and fatty acids. The unsaponifiable and CEF (hexa-, hepta- and octachlorodibenzo-p-dioxin) content of a number of reagent and technical grade commercial chlorophenols are shown in Table 4. CEF content was determined by GLC using a synthetic CEF mixture as a reference material. CEF was found in all the chlorophenols examined, varying from a trace (ca 0.001 μg/g) to 205 μg/g.

* Pre-formed chick edema factor is defined here as a group of chloro derivatives of dibenzo-p-dioxin initially present in a sample before it is subject to any type of heat treatment.

References

[1] Laclair, J. B., Anal. Chem. *23*, 1760 (1951).
[2] Chem. Abstr. *53*, 1315d (1959).

TABLE 1.—EXAMINATION OF CHLOROPHENOLS FOR PRE-FORMED CHICK EDEMA FACTORS

Sample (100 g.)	Wt. Unsap., mg.	Wt. Al_2O_3 Fr. 3	Percent CEF[1]
2, 3, 4, 6-Tetrachlorophenol (Eastman)	[3] 126. 5	109. 2	0. 11
2, 4, 5-Trichlorophenol (Pract.)	223. 7	11. 9	0. 012
Pentachlorophenol (tech.)	323. 8	228. 5	0. 23
2, 4, 6-Trichlorophenol (Reagent)	2. 0	0. 9	0. 001
2, 3, 4, 6-Tetrachlorophenol (Baker)	[2] 135. 8	120. 2	0. 12
2, 4-Dichlorophenol (Baker)	12. 2	0. 3	

[1] Estimate based on weight of Al_2O_3 Fr. 3 and assuming that the fraction consists only of CEF.
[2] A second extraction of the unsaponifiables (after acidification) of the soaps afforded 5.0 mg of material.
[3] A second extraction of unsaponifiables gave 9.8 mg of material.

TABLE 2.—CHICK EMBRYO ASSAY

Sample (unsaponifiables)	μg/egg	Percent mortality
2,4,5-Trichlorophenol (T)	10.0	100
	0.55	30
2,3,4,6-Tetrachlorophenol (Baker)	20.0	100
	0.60	15
Pentachlorophenol (T)	10.0	100
	0.6	55
2,3,4,6-Tetrachlorophenol (Eastman)	4.8	70
2,4,6-Trichlorophenol (R)	50.0	40
2,4-Dichlorophenol (T)	15.0	13

TABLE 3.—EXAMINATION OF TWO COMPONENTS FROM THE UNSAPONIFIABLE FRACTION OF TECHNICAL 2,3,4,6-TETRACHLOROPHENOL

R_a of GLC component	Mol. wt.	No. of Cl atoms	Chicken embryo assay	
			μg/egg	Percent mortality
6.23	408	7	2.0	0
11.8	442	8	1.0	0

TABLE 4.—EXAMINATION OF COMMERCIAL CHLOROPHENOLS FOR CHICK EDEMA FACTORS (HEXA-, HEPTA- AND OCTACHLORODIBENZO-P-DIOXINS)

No.	Compound[a]	mg. Unsap.	μg/g. of CEF[b]
1	2,4-Dichlorophenol (R)	2.0	0.018
2	2,4-Dichlorophenol (T)	2.4	0.219
3	2,5-Dichlorophenol (R)	0.8	0.008
4	2,4,5-Trichlorophenol (T)	32.5	trace
5	2,4,6-Trichlorophenol (T)	12.3	trace
6	2,4,6-Trichorophenol (R)	1.9	0.013
7	2,3,4,6-Tetrachlorophenol (T)	22.5	205
8	2,4,5-Trichlorophenol (R)	3.0	0.021
9	2,3,4,6-Tetrachlorophenol (T)	35.0	96.5
10	Pentachlorophenol (T)	67.1	121
11	Pentachlorophenol (R)	1.4	0.167
12	Dowcide B (sodium 2,4,5-trichlorophenate (T)	1.8	trace
13	Dowcide G (sodium pentachlorophenate (T)	25.6	47.0
14	Reference toxic fat	223	3.0

[a] R=reagent grade. T=technical grade.
[b] Hexa-, hepta- and octachlorodibenzo-p-dioxins.

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The Identification and Crystal Structure of a Hydropericardium-Producing Factor: 1,2,3,7,8,9-Hexachlorodibenzo-*p*-dioxin

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A crystalline material, isolated from a contaminated animal feed fat, and capable of producing hydropericardium in chicks, was shown by solution of its crystal structure to be 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin ($C_{12}O_2H_2Cl_6$). The triclinic unit cell has the dimensions $a=7{\cdot}952\pm0{\cdot}005$, $b=9{\cdot}379\pm0{\cdot}01$, $c=9{\cdot}433\pm0{\cdot}01$ Å, $\alpha=92{\cdot}35°\pm0{\cdot}20°$, $\beta=92{\cdot}39°\pm0{\cdot}20°$, $\gamma=109{\cdot}92°\pm0{\cdot}30°$. The calculated density is 1·958 g.cm^{-3} for $Z=2$, compared with 2·01 g.cm^{-3} measured for the bulk material. A statistical treatment of the 1158 measured reflections indicated a center of symmetry; the space group was therefore assumed to be $P\bar{1}$. The structure was solved by the symbolic addition method of Karle & Karle. The nearly planar molecules are almost parallel to the (0$\bar{1}$1) crystallographic planes. No unusual bond lengths or angles were found. The structure was refined to $R=10{\cdot}5\%$.

Introduction

The isolation, chemical analyses, and spectroscopic data on the hydropericardium toxic factor (HPTF) material have been described by Wootton, Artman & Alexander (1962), and by Wootton & Courchene (1964). One of the active fractions of material isolated was that called α-3·17, where this nomenclature refers to the vapor phase chromatographic behavior as described by Wootton *et al.* (1962). Wootton and his colleagues proposed that HPTF was a chlorinated hexahydrophenanthrene with the empirical formula $C_{14}H_{10}Cl_6$. Following the molecular identification herein reported, Wootton (1966) showed that a synthetic hexachlorinated dibenzo-*p*-dioxin, whose physical properties are remarkably similar to the isolated α-3·17 material, does indeed produce the hydropericardium condition in chickens.*

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Experimental

Two types of crystals were isolated from a warm benzene–hexane solution of the α-3·17 material. The bulk of the crystalline material appeared to differ in phase from the material used for this study. No crystals of the bulk phase were found to be satisfactory for single-crystal studies, and only two crystals of the studied phase were isolated. Measured *d*-spacings of X-ray powder patterns taken of the bulk phase material did not match *d*-spacings calculated from the unit cell of 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin. However, when the bulk phase was heated to just below the melting point (230°C) a phase change occurred. Measured *d*-spacings from X-ray powder patterns of the transformed bulk phase match the calculated *d*-spacings of 1,2,3,7,8,9-hexachlorodibenzo-*p*-dioxin reasonably well. Therefore, it was assumed that the material used for this crystal structure determination was a high temperature phase of the bulk crystalline material known as α-3·17 HPTF.

The single crystals used were diamond shaped and had the approximate dimensions $0{\cdot}18\times0{\cdot}10\times0{\cdot}08$ mm ($a\times b\times c$).

The unit-cell parameters were determined from single-crystal data using a General Electric single-crystal orienter and Ni-filtered Cu $K\alpha$ radiation ($\lambda=1{\cdot}5418$ Å). The parameters of the triclinic cell chosen according to Dirichlet (Bulashov & Ursell, 1957) are as follows:

$a=7{\cdot}952\pm0{\cdot}005$ Å $\quad\alpha=92{\cdot}35\pm0{\cdot}20°$ $\quad\varrho_c=1{\cdot}958$ g.cm^{-3}

$b=9{\cdot}379\pm0{\cdot}01$ $\quad\beta=92{\cdot}39\pm0{\cdot}20$ $\quad\varrho_o=2{\cdot}01$ g.cm^{-3}

$c=9{\cdot}433\pm0{\cdot}01$ $\quad\gamma=109{\cdot}92\pm0{\cdot}30$ $\quad Z=2$

$V=662{\cdot}8$ Å^3

where ϱ_o was measured for the bulk phase by flotation.

Two-dimensionally integrated equi-inclination Weissenberg data were collected for the *a*-axis zones, 0*kl*–4*kl*, and for the *b*-axis zones *h*0*l*–*h*5*l* using the multiple-film technique (one pack each of four films, Eastman Kodak No-Screen). Intensity data were recorded for both crystals, reduced separately, then compared, edited, and averaged. Absorption corrections were made separately for each crystal using Busing & Levy's general absorption correction program as modified by Jeffrey (1964).

Owing to the very tiny crystals, and in part to the integration, very long exposures of approximately 150 hours were required to obtain satisfactory multiple-film data. The entire Weissenberg camera was placed inside a plastic bag and a helium atmosphere was provided to reduce background due to air scattering. Of the 3030 possible reflections, 1158 (38%) were recorded; 397 of these reflections had intensities less than a minimum threshold value and were classified as 'less-thans'. The intensities of most of the reflections were measured by a Joyce Loebl microdensitometer scanning at right angles to the longer integration direction. The weakest reflections were estimated visually. A standard intensity strip was prepared and used for the visually estimated intensities. To ensure that both types of intensity data were on the same scale, a sufficient number of medium intensities were measured both visually and by the densitometer. Radiation damage effects were found to be negligible by retaking data for earlier crystal settings.

Statistical treatment of the intensity data by Ramachandran & Srinivasan's (1959) modification of the method of Howells, Phillip & Rogers (1950) indicated a center of symmetry. The space group was assumed, therefore, to be $P\bar{1}$ (C_i^1) and this assumption was confirmed during the direct method calculations.

Solution and refinement of the structure

Initially we knew the weight of the molecule and the number of chlorine atoms per molecule, and we knew that the molecule possessed some aromatic character. Attempts to solve the structure from the three-dimensional Patterson map were not successful. The symbolic addition method of Karle & Karle (1963, 1966) was then employed.

The phases were determined for the 251 most intense reflections in terms of four algebraic quantities, a, b, c, g. A summary of the calculation of the unitary structure factors or *E*-values used for this determination is compared with theoretical values and is as follows:

Quantity	Non-centrosymmetric	Centrosymmetric	$C_{12}H_2O_2Cl_6$	Karle *et al.* (1964) 3-Tadolyl-acetic acid
$\langle\|E\|\rangle$	0·886	0·798	0·772	0·769
$\langle\|E^2-1\|\rangle$	0·736	0·968	0·970	0·934
$\langle\|E\|^2\rangle$	1·000	1·000	1·000	1·033
$\|E\|>3{\cdot}0$		0·3%	0·4%	0·2%
$\|E\|>2{\cdot}0$		5·0%	4·5%	3·3%
$\|E\|>1{\cdot}0$		32·0%	30·8%	36·1%
			1·158 reflections	1·289 reflections
			761 non-zero	865 non-zero
			397 unobserved ('less-than')	424 zero

* The composition for the structure reported here, namely $C_{12}O_2H_2Cl_6$, agrees well with unpublished microchemical analyses performed by Professor Wolfgang J. Kirsten, University of Uppsala, Uppsala, Sweden, at a very early stage of this structure work.

The overall temperature factor was 2·24.

A summary of the sixteen cases that resulted from the sign permutation of the four algebraic assignments is as follows:

	Case	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	g	−	−	+	+	+	+	−	+	−	+	−	+	+	−	−	+
	f	−	+	−	+	−	−	+	+	−	+	−	+	−	−	+	+
	h	+	+	+	−	−	−	−	+	+	+	+	−	−	−	−	+
	n	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
No. refl. generated	+	119	183	114	153	142	116	118	251	121	121	115	125	121	122	128	122
	−	132	68	137	98	109	135	133	0	130	130	136	126	130	129	123	129
No. incorrect signs		117	132	125	132	133	129	130	129	121	128	104	130	35	61	120	117
% wrong sign		46·6	52·6	49·8	52·6	53·0	51·4	51·7	51·4	48·3	51·0	41·5	51·7	13·9	24·3	47·8	46·6
Objections to choice		✓	✓	✓	✓	✓	✓	✓†						*			

✓ Electron density peaks were found at centers of symmetry when the special sections $x=0$ and $x=\frac{1}{2}$ were computed.
† It is very unlikely that all 251 most intense reflections would be positive.
* This correct case was selected after examining three-dimensional electron density maps for cases 9 to 16.

Case 13 was chosen over the other seven acceptable choices because of the appearance of a chemically reasonable structure in the electron density plot in an (0$\bar{1}$4) plane. It had already been determined that the molecule had to be oriented approximately in alternate (0$\bar{4}$4) planes, based on (*a*) the early analysis of the Patterson map, (*b*) the very high intensity of 022, (*c*) electron densities calculated from models based on the Patterson vectors, and (*d*) packing considerations. A nearly planar hexachlorinated phenanthrene structure was initially fitted to the *E*-map peaks located near an (0$\bar{1}$4) plane. An *R* value of 50% was obtained for the initial trial coordinates and a three-dimensional electron density map suggested that the phenanthrene skeleton should be changed to an anthracene one with some changes made in carbon and chlorine assignments resulting in, ideally, a planar *1,2,3,7,8,9-hexa*-chloroanthracene with *mm* symmetry. This change in the structure dropped the *R* value to 35%. The plane of the molecule was originally in alternate (0$\bar{4}$4) planes but required some tilting and when these changes were made the *R* value dropped to 24%. One cycle of least squares reduced the residual to 19%. At this point the two bridging atoms were recognized as oxygens, since their relative electron densities were 30% higher than those for the carbon atoms and the individual temperature factors for these atoms had gone negative. Substituting oxygen atoms for these carbon atoms in the proposed model resulted in an *R* value of 16%. There was a careful editing of the data, especially of those reflections classified as 'less-thans', at the $R=16\%$ stage of refinement. This editing consisted of deleting a few doubtful reflections recorded near the edge of a film, correcting transcription errors, and reclassifying some of the 'less-thans' to observed reflections on the basis of visual estimations of the weakest reflections.

Table 1. *Final parameters and their standard deviations*

The fractional coordinates have been multiplied by 10^4 and the temperature factors by 10^2; the standard deviations are in parentheses. The anisotropic temperature factors of the chlorine atoms are in the form

$$\exp[-\frac{1}{4}(B_{11}h^2a^{*2}+2B_{12}ha^*kb^*+B_{22}k^2b^{*2}+\ldots)]$$

	X	*Y*	*Z*	*B* or B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
Cl(1)	−0099 (6)	3158 (6)	5185 (6)	287 (20)	376 (29)	487 (26)	149 (21)	−035 (17)	−068 (22)
Cl(2)	−0145 (6)	0579 (6)	2934 (5)	362 (23)	378 (30)	417 (27)	025 (22)	−162 (19)	−043 (22)
Cl(3)	3314 (7)	−0257 (6)	2621 (5)	487 (26)	411 (30)	376 (26)	163 (24)	−056 (21)	−084 (24)
Cl(4)	9493 (6)	7375 (6)	10547 (5)	372 (23)	457 (30)	347 (24)	171 (23)	−018 (18)	−105 (22)
Cl(5)	6188 (6)	8434 (6)	10752 (5)	395 (24)	375 (29)	309 (23)	076 (22)	034 (18)	−024 (21)
Cl(6)	2919 (6)	6936 (6)	8629 (5)	282 (21)	421 (29)	473 (27)	148 (21)	028 (18)	−102 (23)
C(1)	1762 (24)	2601 (22)	4879 (20)	354 (41)					
C(2)	1718 (21)	1502 (20)	3979 (18)	262 (35)					
C(3)	3286 (25)	1118 (22)	3853 (20)	386 (43)					
C(4)	4753 (24)	1920 (23)	4673 (21)	370 (42)					
C(6)	7712 (23)	5516 (21)	8394 (19)	321 (39)					
C(7)	7706 (20)	6572 (19)	9388 (16)	219 (34)					
C(8)	6219 (27)	7051 (24)	9503 (22)	427 (45)					
C(9)	4754 (21)	6238 (19)	8539 (17)	249 (35)					
C(11)	3292 (23)	3471 (21)	5778 (19)	325 (39)					
C(12)	4776 (24)	2934 (22)	5629 (19)	369 (42)					
C(13)	6226 (21)	4811 (19)	7461 (18)	240 (34)					
C(14)	4752 (20)	5271 (19)	7591 (17)	213 (32)					
O(5)	6312 (15)	3742 (14)	6496 (13)	355 (27)					
O(10)	3266 (15)	4591 (14)	6713 (13)	334 (27)					

The *R* value dropped to 13% during the next least-squares refinement. Fixing the chlorine atoms and refining only the carbon skeleton resulted in an *R* value of 11·8% at which time the anisotropic temperature refinement on the chlorine atoms was undertaken and gave the final *R* of 10·5%. Of the 397 'less-than' reflections only 42 calculated were larger than their threshold values and none by more than 36%. A weighting function similar to that given by Hughes (1941) was chosen so as to have little dependence on F_o; it was taken as $\sqrt{w}=K/(F_o+53{\cdot}4)$ for $F_o>F_m$ and $\sqrt{w}=K/(F_m+53{\cdot}4)$ for $F_o\leq F_m$, where $F_m=21{\cdot}4$, and the quantity minimized was $\Sigma w(F_o-F_c)^2$. 'Less-than' reflections having $F_c>F_o$ (threshold) contributed like an ordinary reflection, but those having $F_c<F_o$ did not influence the refinement at all. A full-matrix least-squares procedure was used throughout the refinement, and in the last cycle all parameter shifts were less than $\frac{1}{4}\sigma$. In a difference map calculated at the $R=11{\cdot}8\%$ stage, the residual electron density ranged from −0·8 to +0·6 e.Å^{-3}, and the maxima and minima did not show any chemically or structurally significant features.

The final position and thermal parameters are given in Table 1. The observed and calculated structure factors are listed in Table 2. In this work, the scattering factors are taken from *International Tables for X-ray Crystallography* (1962); those for carbon are by Freeman (1959); those for oxygen by Berghuis, Haanappel, Potters, Loopstra, MacGillavry & Veenendaal (1955); and those for chlorine by Dawson (1960).

Table 3. *Distances of atoms from least-squares planes*

Distances for atoms not defining the plane are marked with an asterisk; s.d. is the standard deviation of the atoms defining the plane. Under the (0$\bar{1}$4) heading are listed the deviations from the (0$\bar{1}$4) plane containing the molecule. The planes are defined in direct space by equations $Px+Qy+Rz=S$.

	All	C+O	(0$\bar{1}$4)
Cl(1)	−0·10	−0·10*	0·33*
Cl(2)	−0·11	−0·12*	0·10*
Cl(3)	0·00	−0·03*	−0·25*
Cl(4)	−0·16	−0·18*	−0·45*
Cl(5)	−0·05	−0·06*	0·12*
Cl(6)	0·13	0·13*	0·54*
C(1)	−0·02	−0·03	0·15*
C(2)	−0·06	−0·08	0·02*
C(3)	−0·02	−0·05	−0·16*
C(4)	0·06	0·03	−0·17*
C(6)	0·00	−0·03	−0·25*
C(7)	−0·05	−0·07	−0·21*
C(8)	−0·01	−0·03	0·03*
C(9)	−0·01	−0·02	0·13*
C(11)	0·06	0·05	0·13*
C(12)	0·05	0·03	−0·09*
C(13)	0·05	0·02	−0·10*
C(14)	0·06	0·05	0·12*
O(5)	0·08	0·05	−0·17*
O(10)	0·09	0·08	0·25*
Origin	1·57*	1·55*	1·67*
s.d.	0·07	0·05	0·00
P	−1·1589	−1·1086	0·0000
Q	−5·9655	−6·0031	−6·6618
R	6·8500	6·8404	6·6618
S	1·5675	1·5497	1·6655

Discussion

The molecule is nearly planar and the molecular plane is tilted 8° to the (0$\bar{1}$1) plane. Deviations of atoms from the (0$\bar{4}$4) plane essentially containing the molecule and

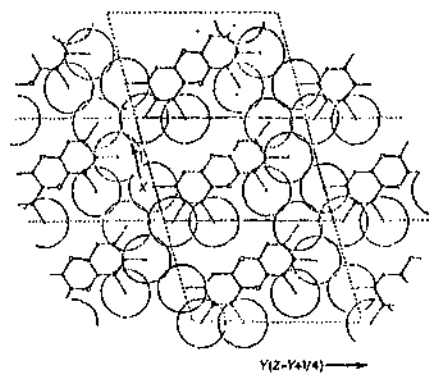


Fig. 1. Molecular packing in the (0$\bar{3}$4) plane containing the molecule. Large shaded circles are Cl, solid circles are C, and open circles are O.

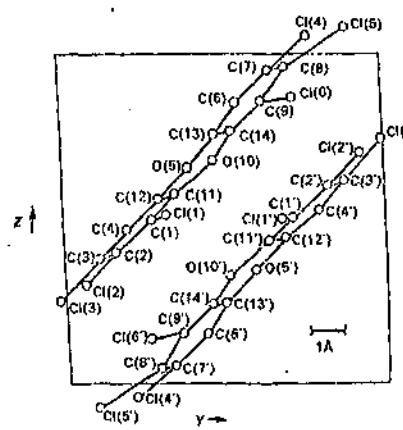
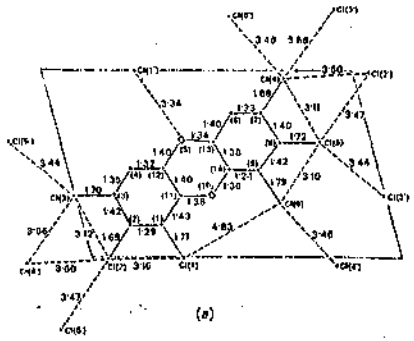


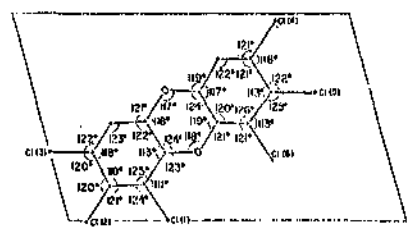
Fig. 2. Projection onto *YZ* plane.

from the least-squares planes of the entire molecule and of the carbon-oxygen skeleton are given in Table 3. The molecule appears to be slightly bowed in the middle and slightly twisted about a line from Cl(3) to Cl(5). The packing arrangement of chlorines 4, 5, and 6 appears to be more crowded than that for chlorines 1, 2, and 3. This packing difference could account for the slight twist of the molecule.

Fig. 1 pictures the molecular packing in the (0$\bar{3}$4) plane containing the molecule, and Fig. 2 gives a projected view of two adjacent molecules related by the center at $(\frac{1}{2},\frac{1}{2},\frac{1}{2})$. Intermolecular distances in this (0$\bar{3}$4) plane of less than 4·0 Å are shown in Fig. 3. Between centrosymmetrically related molecules there are a number of Cl(*n*)–Cl(*n'*) and equivalent Cl(*m*)–Cl(*n'*) distances of 4·0 Å or less. From the parent molecule to the one related by the center at $(\frac{1}{2},\frac{1}{2},\frac{1}{2})$ the distances are Cl(2)–Cl(4′) = 3·85 Å, Cl(3)–Cl(5′) = 3·66 Å, and Cl(3)–Cl(6′) = 3·83 Å; by the center at $(0,0,\frac{1}{2})$–Cl(1)–Cl(3′) = 3·84 Å; by the center at $(0,\frac{1}{2},\frac{1}{2})$–Cl(1)–Cl(1′) = 3·39 Å and Cl(2)–Cl(6′) = 3·98 Å; and by the center at $(\frac{1}{2},\frac{1}{2},1)$–Cl(4)–Cl(6′) = 4·00 Å. The least-squares planes of the two adjacent molecules related by the center at $(\frac{1}{2},\frac{1}{2},\frac{1}{2})$ are 3·13 Å apart; between these two molecules the shortest interatomic distance is 3·30 Å from a C(12) to an O(10).



(*a*)



(*b*)

Fig. 3. (*a*) Interatomic distances. Primed atoms are on neighboring molecules in the same plane. $\sigma_{C-C} = 0{\cdot}025$ Å; $\sigma_{C-Cl} = 0{\cdot}019$ Å; $\sigma_{C-O} = 0{\cdot}022$ Å. View corresponds to Fig. 1. (*b*) Bond angles. $\sigma = 2{\cdot}0°$.

Fig. 3 indicates the bond distances and angles. The mean standard deviations are as follows: $\sigma_{C-C} = 0{\cdot}025$ Å, $\sigma_{C-Cl} = 0{\cdot}019$ Å, $\sigma_{C-O} = 0{\cdot}022$ Å; for angles $\sigma = 2{\cdot}0°$. The bond distances are not significantly different from those found by Davydova & Struchkov (1962) and Gafner & Herbstein (1962) for 1,4,5,8-tetrachloronaphthalene where molecular over-crowding results from the presence of many chlorine atoms substituted on adjacent aromatic positions. This compound belongs in group (I) according to the classification due to Harnik, Herbstein, Schmidt & Hirshfeld (1954) for compounds that are affected by molecular over-crowding.

An electron density map plotted in the (0$\bar{3}$4) plane containing the molecule is shown in Fig. 4.

The authors wish to acknowledge their appreciation to Dr J. M. Stewart of the University of Maryland, who furnished the X-ray 63 computer program and provided much information on its use. In addition, we wish to thank Dr Lyle Jensen for a number of helpful discussions on the use of the X-ray 63 computing system and on approaches to the solution of the structure in general.

We wish to express our appreciation to Drs Jerome and Isabella Karle who provided assistance in applying the direct method for determining the phases of a number of the most intense reflections.

Thanks are due to Mr Robert Gloss who obtained part of the data and provided the computer programs used in generating the relations between reflections necessary for applying the direct method.

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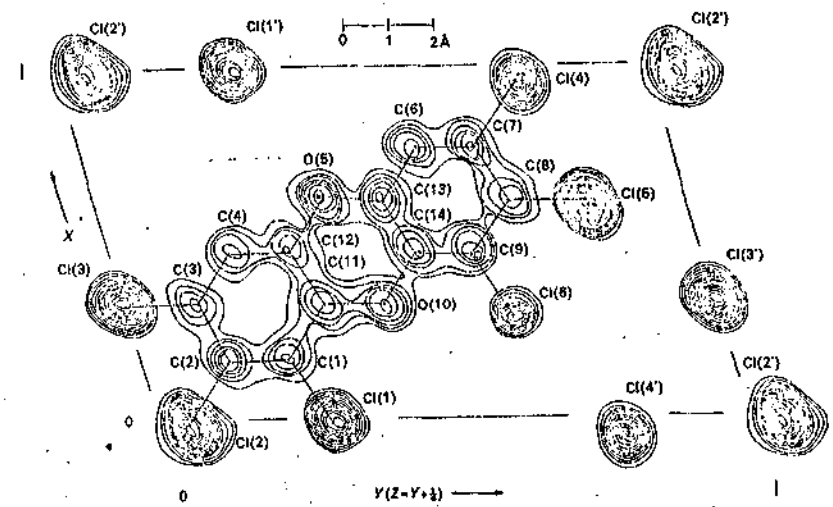

Fig. 4. Electron density in the (0$\bar{3}$4) plane containing the molecule. Contours are at 1 e.Å^{-3} starting at 2 e.Å^{-3}. The × marks are projections onto (0$\bar{3}$4) from the electron density maxima, which in most cases are a short distance from (0$\bar{3}$4).

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Clinical Picture and Etiology of Chloracne

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Chloracne is the name for forms of occupational acne which develop as a result of intoxication with certain chlorinated aromatic compounds. The name dates back to Herxheimer who described the first case in 1899 and still assumed that in analogy to bromine and iodine, the acneiform eruptions are the result of free chlorine as the etiological toxin. This view proved to be incorrect. The proposed designation "perna disease" of Wauer, Teleky and others based on the finding that this clinical picture occurred more frequently under the influence of *per*chlorinated naphthalenes also does not go to the heart of the matter, since other chlorinated aromatics in addition to chlorinated naphthalenes are also etiologically important.

The *clinical picture of symptoms* primarily affects the skin. Beyond this, internal organs may be affected and nervous system and emotional disorders may appear.

The skin symptoms are in the regions of the follicles. Comedones, resulting from a follicular hyperkeratosis, predominate and frequently are so numerous that hardly a single follicle remains untouched and the affected region of the skin obtains a dirty-gray appearance. In addition, at the peak of the disease, fairly large sebaceous cysts, inflammatory nodules, pustules and furuncles appear and in some of the patients, large spots or patches of pigmentation appear in regions exposed to light. Preferential sites are the face as well as the exposed areas of the throat and neck. Frequently, the external ear, especially, the ear lobes, are involved where small cysts can be easily palpated. In more pronounced forms, changes can also be found on the back, chest and extremities and in males, on the genitalia. Hands and feet usually are not involved. It is not rare that the symptoms of acne are preceded by a dermatitis with erythema and edema. In this phase of the condition, photosensitivity frequently exists which evidently contributes to the development of dermatitis and the mentioned pigmentations (S. Braun, Grimmer).

Generally, a *differential diagnosis* is not particularly difficult. The primary problem is to define the condition compared to other forms of occupational acne and acne vulgaris, which is generally possible with consideration of the clinical aspect, localization and especially the patient's history. Acneiform dermatoses caused by tars, pitch and mineral oils are found primarily on the extremities and trunk, while the face is more rarely involved. The predominance of inflammatory changes, such as folliculitis and furuncles in oil and tar acne and of comedones in chloracne are other characteristic features. Drug-caused acneiform exanthemas due to iodine, bromine or cortisone also have a picture differing from chloracne.

The *course* is eminently chronic. In spite of intensive local and general therapy, recidivism may occur even years after the elimination of the causal toxins. Healing frequently takes place with extensive pitted, permanently disfiguring cicatrization (Schmidt and Boslet).

The skin is not the only indicative region of intoxication with chloracne-causing substances. Damage of internal organs is not rare, with the *liver* being in the foreground. Several authors have reported on grave damage of hepatic parenchyma accompanied by icterus and functional disorders, including a number of fatal cases of acute atrophy of the liver (see reviews of W. Braun and A. Risse-Sundermann). The pronounced liver-toxocity of chloracne-causing substances was also confirmed in animal experiments (Bennett, Drinker and Warren; Hofmann, Oettel; Schulz).

In addition to liver damage, changes in the kidneys, pancreas, gastrointestinal tract and myocardium can also be observed, although much more rarely.

Nervous system and psychological disorders were found primarily among workers occupied in the production and processing of chlorinated phenols (Trubant et al.). General fatigue, weakness of the legs, headache, attacks of vertigo, paresthesias, muscle pain, tendency to orthostatic collapse, local paresis and disturbed sensibility, anomalies in reflexes as well as an autonomic syndrome with lowered drive, depression, reduced power of recall and concentration, disturbed sleep, irritability, loss of appetite, reduced libido and impotence have been reported as the most frequent neurological and psychopathological symptoms of intoxication which become manifest often only several months after it occurs (Bauer, Schulz and Spiegelberg). With regard to the question of distinguishing the latter from psychoneurotic obsessions (wish for compensation), reference is made to the discussions of Spiegelberg.

Etiology.—When we review the literature, we find that periods of greater incidence of chloracne have existed in the last 60 years, which can be correlated with industrial development. W. Braun has described these relationships in his monograph.

The first cases were observed near the turn of the century when chlorine and hydrochloric acid began to be produced by the electrolytic route. At that time, the condition occurs primarily among workers having the assignment to clean the so-called hydrochloric acid towers, but only when tar was used as the protective coating of the walls. Although the causal toxin could not be determined at the time, it can be assumed on the basis of our present knowledge that the reaction products of chlorine and aromatic components of the tar must be considered as etiological factors of this condition.

The next period of increased chloracne frquey coincides with the introduction of so-called halogenated waxes. These are mixtures of highly chlorinated naphthalenes and diphenyls with a waxy consistency and a number of valuable properties. They are water-repellent, nonflammable, resistant to acids, are a good dielectric and are not pest-promoting. The halogenated waxes developed during the first world war at that time were used primarily for the manufacture of gas masks. Numerous cases of chloracne occurred in the manufacturing plants.

In the middle twenties, these halogenated waxes were used in the mining industry as a water-repellent and nonflammable insulation for detonators. The high incidence of diseases observed in detonator manufacturing plants has been described by Teleky.

The next massive occurrence is related with the rise of the electrical and radio industry. Chlorinated naphthalenes and diphenyls were in more widespread use for the insulation of wires and condensors at the start of the thirties. Several hundred cases including one fatality with liver atrophy became known especially in the United States.

With the entry of the United States in the second world war, the field of application of these materials expanded also into ship building; this had the following reason: It was found that the halogenated waxes were in the position to insulate ships from the dangerous weapon of German magnetic mines. Consequently, large quantities of these materials were used in American shipyards for the impregnation of ship hulls. Mass incidences of chloracne with several fatalities were the results.

In spite of all negative experiences, the use of chlorinated naphthalene waxes did not stop after the last war. Chloracne cases of greater or lesser frequency were repeatedly observed in the electrotechnical and cable industry (Braun, Grimmer, Risse-Sundermann). From the pathogenic aspect, it is of interest to note an observation of Herzberg of 7 patients who developed intestinal symptoms and acneiform dermatoses following the use of industrial chlorinated greases for frying.

The question of the relationships between chemical structure and acne-producing effects of chlorinated naphthalenes is the subject of several experimental studies. Teleky as well as Drinker and Warren arrived at the conclusion about thirty years ago that the toxicity of the molecules increases with an increasing number of chlorine atoms on the ring. Later experiments conducted by Sebley and Kligman with human subjects and with the use of several chlorinated naphthalenes showed that penta- and hexachloronaphthalenes produced the strongest effects; compounds with 1 to 3 as well as 7 and 8 chlorine atoms were far less toxic of inactive. We confirmed this finding in animal experiments using rabbit ears (Schulz 1965).

In the last 10–15 years, halogenated waxes have become less important as etiological factors of chloracne. Evidently, this is related with the *fact* that they are no longer as important industrially and have been replaced by synthetics of the most diverse nature in most fields of application.

In the fifties, the incidence of chloracne was observed in entirely different sectors of industry i.e. in the *production and processing of chlorinated phenols.* Reports of group involvements have been published from at least three

industrial plants in Western Germany. Baader and Bauer as well as Brinkman described 17 workers of a plant in Nordrhein-Westfalen who developed the typical skin symptoms as well as damage of the internal organs and central nervous system disorders in the production of pentachlorophenol.

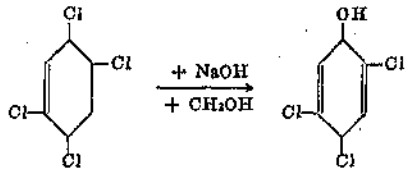
A larger number (about 60 cases) of similar disorders were recorded several years later in the region of southwest Germany among workers occupied in the production of of trichlorophenol (Hergt, Oettel, Hofmann). Approximately at the same time, 31 workers in a Hamburg plant became ill after working with industrial 2,4,5-trichlorophenol, an intermediate of the synthesis of trichlorophenoxyacetic acid, a weed killer.

Siliar high incidences of the disease occurred a few years ago in chemical plants of the Netherlands and the U.S. during analogous production processes.

The high frequency of cases in Hamburg led to studies of the etiology. They were conducted by us together with Dr. Sorge, the former manager of the chlorophenol plant.

Rabbit ears were used as the biological substrate on which symptoms corresponding to human chloracne can be produced by local painting as demonstrated by Hofmann and Neumann with chloronaphthalenes. The results, which have been reported earlier (Schulz 1957; Kimmig and Schulz 1957), can be briefly summarized as follows:

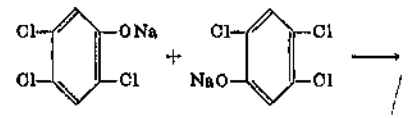
First, it was found that it was not possible to produce changes in the rabbit ear in the form of chloracne with the use of the chemically pure compound in contrast to the technical grade of 2,4,5-trichlorophenol used in the plant. Pure 1,2,4,5-tetrachlorobenzene also was inactive. The toxic factor therefore must have formed as a byproduct during the alkaline hydrolysis of tetrachlorobenzene into trichlorophenol.



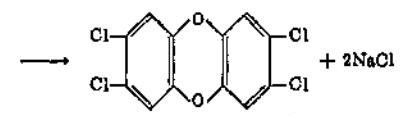
1,2,4-tetrachlorobenzene 2,4.5-trichlorophenol

Alkaline hydrolysis of 1,2,4,5-tetrachlorobenzene into 2,4,5-trichlorophenol.

Since the isolation of well-defined compounds from the distillation residue of trichlorophenol was unsuccessful, a number of especially synthesized substances were investigated which might have formed as a byproduct of the cited saponification process on the basis of theoretical considerations. The majority of investigated compounds proved to be inactive. Only dibenzofurans with 3 and 4 chlorine atoms (diphenylene oxides) and 2,3,6,7-tetrachlorodibenzodioxine (tetrachlorodiphenylene dioxide) led to the characteristic changes on the rabbit ear already in low concentrations. Moreover, it was demonstrated that 2,3,6,7-tetrachlorodibenzodioxine had formed by the following reaction route in the industrial process of alkaline hydrolysis of 1,2,4,5-tetrachlorodibenzene.



Sodium salt of 2,4,5-trichlorophenol



2,3,6,7-tetrachlorodibenzodioxine
(2,3,6,7-tetrachlorodiphenylene dioxide)

Under the conditions of a salt fusion in a solvent-free state, 2 molecules of sodium trichlorophenolate form 1 molecule 2,3,6,7-tetrachlorodibenzodioxine with the elimination of 2 molecules of NaCl. Dr. Sorge synthesized the compound and in addition, isolated it from the distillation residue of industrial trichlorophenol.

Animal experiments conducted with tetrachlorodibenzofuran and 2,3,6,7-tetrachlorodibenzodioxine showed an extremely high toxicity of these compounds. Even concentrations of 0.001–0.005% of tetrachlorodibenzodioxine led to severe reactions on the rabbit ear after local application. On human skin in a self-experiment, two applications of 10 γ of the substance produced the symptoms characteristic of chloracne. On the rabbit ear, tetrachlorodibenzofuran showed an activity which was about 10 to 20 times less pronounced. Moreover, the unexpectedly high hepatotoxic action is worthy of note, particularly after tetrachlorodibenzodioxine. Single oral doses of 20–50 γ/kg body weight regularly produced lethal liver necrosis, while doses of 10 γ/kg were lethal for about 50% of the rabbits.

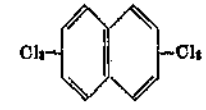
On the basis of these chemical and toxicological findings, it is justified to conclude that 2,3,6,7-tetrachlorodibenzodioxine played an important role in the etiology of the cases of chloracne which occurred during the industrial production of trichlorophenol. It cannot be ruled out, however, that other, as yet unknown chlorinated aromatics of highly toxic properties may form during the industrial process under certain conditions. The results of the study are an example that materials which form only in small amounts as byproducts of large-scale syntheses can be of importance in occupational medicine. If such toxic byproducts can be uncovered and their mechanism of formation can be elucidated, this will create an important prerequisite for successful prophylaxis. In our special case, the plant succeeded in avoiding the formation of highly toxic byproducts by modifying the production process.

Our animal experiments were extended to other chlorinated aromatics to which other authors ascribed a chloracne-causing action on the basis of clinical observations. (Reviews of these compounds in the monograph of W. Braun.) Neither benzenes and phenols with 1 to 6 chlorine atoms nor chlorinated diphenylethers produced an effect in animal experiments. It seems indicated to assume, therefore, that neglected toxic byproducts were of decisive etiological importance in these cases rather than the main products.

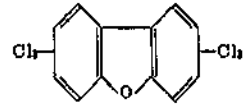
In connection with the acne produced by chloronaphthalenes, the question arose whether toxic byproducts rather than the chloronaphthalenes themselves might not be considered as the true toxins (Oettel). In the production of industrial naphthalene by fractional distillation of tar, the presence of other aromatic compounds deriving from the tar apparently cannot be ruled out. In the following chlorination process, such substances might then also undergo CL-substitution. These question prompted us to carry out animal experiments on rabbit ears using chemically pure chloronaphthalenes of different degrees of chlorination specially synthesized for this purpose.*

In agreement with the findings of Shelley and Kligman, we found that naphthalenes containing 5-6 chlorine atoms have a chloracne-producing effect. The necessary concentrations, however, were about 100 times higher than those of tetrachlorodibenzofuran (diphenylene oxide) about about 1000 times higher than for 2,3,6,7-tetrachlorodibenzodioxine (tetrachlorodiphenylene dioxide).

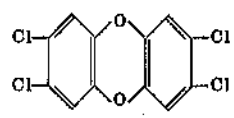
On the basis of the present state of the art, therefore, the chloracne-producing activity of the following compounds appears to be sufficiently demonstrated or at least highly probable:



Naphthalenes containing 5–6 chlorine atoms:



Dibenzofurans (diphenylene oxides) with higher degrees of chlorination:



2,3,6,7,-tetrachlorodibenzodioxine:

In conclusion, it should be noted that the toxicodermatosis represented by chloracne results from intoxication with certain chlorinated aromatics. The causally responsible compounds partly involve highly toxic substances which can cause damage in various internal organs, especially the liver and nervous system, in addition to the skin. Since the skin does not always represent the only manifestation site, it is recommended that thorough internal and neurological as well as psychiatric examinations be made in cases of suspected chloracne.

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Report on Methodology for Chlorinated Aromatics in Fats, Oils, and Fatty Acids

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ABSTRACT

The official, first action electron capture GLC (EC-GLC) method for chick edema factor (polychlorodibenzo-p-dioxins) have been reviewed. This general procedure, which underwent collaborative study in 1967, has undergone several minor modifications which result in better recoveries of polychlorodibenzo-p-dioxins and increased specificity in interpretation of the gas chromatographic results. The EC-GLC method can be used as a screening test, or where a typical pattern of GLC peaks is obtained as a preliminary test, but confirmatory tests are needed to demonstrate structure and toxicity of polychlorodibenzo-p-dioxins. Preliminary work with combined GLC-Mass Spectrometry indicated that this technique might provide a suitable test, if adequate sample cleanup can be accomplished. A chicken embryo assay has been developed to the point where toxicity can be observed in three to five days after injection of eggs.

A preliminary procedure has been developed for isolation and gas chromatography of chlorophenols in fats and fatty acids. Polychlorophenols have been found to be precursors of chlorodibenzo-p-dioxins. The use of a non-specific microbiological test for chlorophenols employing the *Bacillus megaterium* was evaluated. Chlorophenols were found to produce uniformly graded growth inhibition of the test organism in the range 1–100 μg.

The widespread use of toxic organochlorine compounds in agriculture and industry requires development of sensitive methods for their detection in a wide variety of commodities. In addition, it is equally important that methods be developed to detect toxic breakdown or conversion products of organochlorine compounds. One of the most urgent needs in the fat and oil industry is for a rapid and specific method for polychlorodibenzo-p-dioxins (chick edema factors) in fats, oils, and fatty acids. The official, first action, microcoulometric and electron capture methods for chick edema factors (CEF) are essentially screening procedures (1,2,3). Both methods, at present, require a rather time consuming three-week chick bioassay (4) for confirmation.

The purpose of this report is to review the current status of chemical and biological methods for chlorophenols and chlorinated dibenzo-p-dioxins in fats, oils, and fatty acids.

CEF consists of a mixture of chlorinated dibenzo-p-dioxins which occur occasionally as a trace contaminant in fats. Recently, a communication (5) from this laboratory reported the results of a preliminary study which demonstrated the possibility that CEF could arise from residues of pentachlorophenol and 2,3,4,6-tetrachlorophenol in fats and fatty acids. Chlorophenols and their salts, when heated, undergo condensation reactions and form chlorinated derivatives of dibenzo-p-dioxin. The following equation illustrates this condensation reaction.

Technical grades of pentachlorophenol contain ca 10% of 2,3,4,6-tetrachlorophenol which also undergoes thermal condensation reactions and forms hexachloro derivatives of dibenzo-p-dioxins. The condensation of 2,3,4,6-tetrachlorophenol with pentachlorophenol forms two heptachloro derivatives of dibenzo-p-dioxin.

An electron capture GLC method has been developed for pentachlorophenol and 2,3,4,6-tetrachlorophenol in fats, oils, and fatty acids (6). However, recov-

eries of the two volatile polychlorophenols were low and varied over a wide range; nevertheless, the method appears to be satisfactory for qualitative measurements at the 0.5 ppm level. Several samples of oleic acid known to contain CEF were analyzed and were found to be contaminated with residues of pentachlorophenol. The method requires further study.

The official electron capture and microcoulometric methods for CEF were developed before their chemical structures were known. The methods are screening procedures and are based on the observation that toxic fats contain a number of chlorinated components (now known to be polychlorodibenzo-p-dioxins) which have greater retention times than chlorinated pesticides. The electron capture method has received wide acceptance. It is approximately 2000 times as sensitive and requires less sample than the microcoulometric method. In addition, electron capture gas chromatographic equipment is simpler and is in general use in many laboratories.

Recently, it has come to our attention that a number of laboratories that routinely use the electron capture method for control work are not aware of improvements (3) that have been made in the original procedure (2). Recent changes which have not been published include a minor modification of the H_2SO_4 cleanup step and a slight modification of the procedure for packing the GLC column. The modified method, which includes these changes, replaces all existing GLC methods for the chemical assay of CEF.

METHOD

Reagents and Apparatus

Rinse all glassware with appropriate solvents before use. Do not use polythylene containers to store solvents.

(1) Concentrated H_2SO_4.—Reagent grade.

(2) Petroleum Ether.—Reagent grade, redistilled in glass between 30 and 60°C (Available from Burdick and Jackson Laboratories, 1953 S. Harvey St., Muskegon, Mich. 49442).

(3) CCl_4.—Distilled in glass. (Available from Burdick and Jackson Laboratories, Muskegon, Mich.)

(4) Anhydrous Na_2SO_4.—Analytical reagent grade.

(5) Ethyl ether.—Analytical reagent grade (not >2% alcohol) or absolute ether (not >0.01% alcohol).

(6) Iso-octane.—Distilled in glass (Available from Burdick and Jackson Laboratories, Muskegon, Mich.)

(7) Standard aldrin solution.—Dissolve aldrin in iso-octane to make 0.05 μg/ml solution.

(8) Chick edema factor low positive reference sample.—1.5% reference toxic fat in USP cottonseen oil or other suitable vegetable oil. (Prepare from reference toxic fat available from the Division of Pesticides, Bureau of Science, Food and Drug Administration, Washington, D.C. 20204).

(9) Activated Al_2O_3 (Fisher No. A-540, do not substitute).—Activate 100 g portions by heating 4 hours at 260°C. Transfer without cooling to dry container and close tightly. Check activity of Al_2O_3 by analysis of the low positive reference sample, examining Al_2O_3 fractions 2 and 3. With sufficiently activated Al_2O_3, chick edema factor elutes predominantly or entirely in Al_2O_3 fraction 3 as indicated by the gas chromatograms. (Chromatogram should show a series of GLC peaks with Ra values between ca 8 and 45).

(10) Al_2O_3 chromatographic column.—To a *dry* chromatographic column, 17 mm o.d. x 250 mm long, fitted at bottom with coarse porosity fritted glass disk and Teflon stopcock (a column without the fritted disk but holding a glass wool plug in the bottom may be used), add redistilled petroleum ether, *dried* prior to use with anhydrous Na_2SO_4, until column is ⅔ full. Weigh 15 g Al_2O_3 and transfer to column in small portions, tapping the column as Al_2O_3 settles. When last portion of Al_2O_3 settles and air bubbles stop rising to surface of

solvent, add 5 g anhydrous Na_2SO_4. Drain excess petroleum ether so that it is just above upper surface of Na_2SO_4.

(11) Gas chromatographic column.—Glass, 6–7′ long x ¼″ i.d., packed with 2½% SE 52 silicone gum rubber on 60/80 mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa. 16801). Coat the support with substrate as follows: Weigh 2.5 g of the silicone gum rubber stationary phase and dissolve in 300 ml of 1:1 methylene chloride-toluene, heating to dissolve. Add 97.5 g of support material to liquid mixture and let stand 10 minutes with occasional gentle stirring. Dry in rotary evaporator. Apply vacuum to the chromatography column, and pack the coated material into the column by adding small amounts while tapping the column at the packing level after each addition. Fill to within 1″ on the exit side and 3″ on the entrance side, and fill the remaining space with silanized glass wool. Condition the column at operating pressure at 250°C for 2–5 days.

(12) Gas chromatograph with electron capture detector.—A tritium source concentric type detector is recommended. Operate instrument in accordance with instructions of manufacturer, and obtain a stable baseline before carrying out analyses. Choose an operating voltage (ca 50–80 volts) that will cause between 0.6 and full scale deflection for 0.1 mg of aldrin (2 μl of standard aldrin solution) at a sensitivity setting of 1 x 10^{-9} AFS. Keep the column temperature at 200° ±1°C, and adjust nitrogen flow rate so that aldrin elutes in 1–1.5 min. (8–4 min. per in. chart speed). Inject 2 μl of the standard aldrin solution before injection of each reference or test sample.

DETERMINATION

(*a*) Analysis of 1.5% reference toxic fat in USP cottonseed oil.—Dissolve 2.5 g of the 1.5% reference toxic fat in 10 ml of CCl_4 in a 500 ml glass stoppered Erlenmeyer flask. Proceed with determination as described below in sections (b), (c) and (d). Take up residue from (d) in 250 μl iso-octane and inject 5 microliters of reference solution (equivalent to 50 mg of the original sample) into calibrated gas chromatograph. The resulting gas chromatogram should exhibit a series of GLC peaks with Ra ca 8–45, depending on operating conditions. Peaks at Ra 8–13 are due to hexachlorodibenzo-p-dioxin isomers, 2 peaks at Ra 17–22 are due to the 2 heptachlorodibenzo-p-dioxin isomers, and a peak at Ra 35–45 is due to octachlorodibenzo-p-dioxin.

(*b*) Preliminary sulfuric acid cleanup.—Dissolve 2.5 gm of fat in 10 ml CCl_4 in 500 ml glass stoppered Erlenmeyer. Add 10 ml conc. H_2SO_4, stopper, and shake for 30 sec. Add 125 ml of petroleum ether, stopper and shake vigorously for ca one minute. Allow layers to separate and decant supernatant liquid into a 500 ml erlenmeyer, avoiding transfer of lower layer. Repeat extraction with additional 125 ml portion of petroleum ether. Evaporate the combined petroleum ether extracts to 5 ml for Al_2O_3 column fractionation.

(*c*) *Fractionation of Petroleum ether filtrate by alumina chromatography.*—Solvents must be dried prior to use by shaking with anhydrous Na_2SO_4. Transfer petroleum ether filtrate from (b) to Al_2O_3 chromatographic column using total of 15 ml petroleum ether. Let liquid level fall to just above top of Na_2SO_4. Keeping liquid level above top of Na_2SO_4 at all times, elute sample with 100 ml of petroleum ether (fraction 1), 50 ml of 5% ethyl ether in petroleum ether (fraction 2), and 100 ml of 25% ethyl ether in petroleum ether (fraction 3). Relatively fast flow rates of ca 8–9 ml/min give satisfactory results. Ground glass stoppered separatory funnels (250 ml) are satisfactory reserves for eluting solvents. Discard fractions 1 and 2, and collect fraction 3 eluate in 125 ml Erlenmeyer. Add several boiling chips and evaporate solvent to dryness on steam bath. Transfer residue with petroleum ether to 10 ml graduate cylinder equipped with a ground glass stopper, and evaporate petroleum ether solution to 3 ml.

(*d*) *Sulfuric acid cleanup of alumina fraction 3.*—Add 2 ml of concentrated H_2SO_4 to graduated cylinder containing 3 ml petroleum ether solution from (c), stopper and shake vigorously for 30 sec. Allow layers to separate and decant petroleum ether layer into 10 ml beaker avoiding transfer of H_2SO_4 layer. Add 2 ml petroleum ether to cylinder, swirl vigorously, allow layers to separate, and decant petroleum ether layer into beaker. Add ca 0.5 g of solid $NaHCO_3$ to beaker and stir ca ½ min. Let stand five minutes and decant petroleum ether layer into clean 2 or 4 dram vial. Wash $NaHCO_3$ with 2 ml petroleum ether and decant washing into vial. Evaporate solvent under N.

(*e*) *Electron capture gas chromatography of petroleum ether extract.*—Take up residue in 250 μl iso-octane (redistilled in glass), stopper vial tightly and rotate so that solvent wets sides of vial. Inject 1 microliter of sample solution (equivalent to 10 mg fat) into calibrated gas chromatograph. Gas chromatographic peaks with Ra 8–45 are indicative of the presence of chick edema factor. Compare Ra values of sample peaks with Ra values of peaks from reference toxic fat. See (a) for identification of peaks. If peaks indicative of chick edema factor are not observed, inject 5 μl of sample solution (equivalent to 50 mg fat) into gas chromatograph. Check reagents for possible interferences by running a blank with each set of samples. The chromatogram from the blank should show a smooth low baseline from Ra 8—Ra 45. (Types of samples found by experience to be generally free of components characteristic of toxic fats may be examined by initial injection of 5 μl of solution.)

DISCUSSION

Analysis of the low positive reference sample serves as an overall check on instrument performance and sample cleanup. The chromatogram from the low positive reference fat should show a distinct peak pattern as illustrated in Figure 1. The lower chromatogram (B) represents an injection equivalent to 50 mg of the original low positive reference fat. Chromatogram (A) represents a mixture of synthetic polychlorodibenzo-p-dioxins prepared by pyrolysis of 2,3,4,6-tetrachlorophenol and pentachlorophenol. As stated previously, peaks 1 through 4 are due to four positional isomers of hexachlorodibenzo-p-dioxins. The isomer associated with the small shoulder (peak 3) is probably caused by the presence of a tetrachlorophenol other than the 2,3,4,6-isomer in the starting material. Peaks 5 and 6 are due to two positional isomers of heptachlorodibenzo-p-dioxin. Peak 7 is due to octachlorodibenzo-p-dioxin.

Aldrin is used to calibrate the instrument sensitivity for chick edema factor analyses. The similarity of detector response vs. applied voltage for aldrin and an extract from the 1.5% reference toxic fat (low positive reference sample) is illustrated in Figure 2.

CHEMICAL AND BIOLOGICAL CONFIRMATORY METHODS

The need for rapid chemical and biological confirmatory tests has led to investigation of mass spectrometry as well as two biological toxicity assays. Preliminary work has suggested that rapid EC-GLC screening for chick edema factors can be carried out initially; if the presence of chick edema factors is indicated, then larger portions of sample would be fractionated and cleaned up for chemical and biological confirmation.

MASS SPECTROMETRY

Results of a preliminary investigation of combined GLC-mass spectrometry (GLC-MS) indicated that the use of this technique might be suitable if adequate sample cleanup can be accomplished. A 7-foot coiled glass column packed with 2.5% SE-52 on 60–80 mesh Gas Chrom Q was used with an Atlas CH-4 mass spectrometer and single-stage Llewellyn (silicone membrane) separator to

examine standards and an extract from a 2.5 g sample (positive for chick edema factor by EC-GLC). The GLC oven temperature was 220°C with a helium flow at 75 ml/minute. Injection temperature was 235°C and silicone membrane temperature was about 150°C. MS sensitivity setting was 32 x at 40 PA.

GLC retention times as well as molecular weight and number of chlorine atoms in the molecule were determined for a standard mixture and an extract from the test sample. Two μl of 10μl solution from the test sample was injected; it was estimated that 2 μl test sample contained 0.4 μg of hexa-, hepta- and octachlorodibenzo-p-dioxins in addition to other unidentified constituents. A summary of results are in Table 1. Comparison of fragmentation pattern and relative abundance of the ions from standards and sample might afford additional specificity; impurities in the test sample prevented such evaluation at this time.

CHICKEN EMBRYO ASSAY

Extracts from a reference toxic fat and several test samples were subjected to the chicken embryo assay (7); 111 g samples of fat were fractionated according to AOAC (1965) 26.093–26.094, and alumina fraction 3, and cleaned up with sulfuric acid (JAOAC (Changes in Methods) *50*, page 217, section (c) (1967)). Small portions of each extract was retained for EC-GLC analysis and the remainder, in chloroform solution, was subjected to the chicken embryo assay (10–15 eggs per sample were tested by injection of portions of the sample extract in the air cell) at three levels equivalent to ca 40, 30 and 10 g starting sample. Assay results are shown in Table 2. These results indicate that the chicken embryo test can provide a sensitive indication of toxicity as well as a measure of specificity due to observations of localized and genralized edema. In many instances evidence of toxicity can be observed (by periodic candling) in 3–5 days.

B. megaterium TOXICITY TEST

The use of a non-specific biological test employing the *Bacillus megaterium* was evaluated (8). This test involves observation of inhibition of a seeded petri dish holding a *B. megaterium* spore suspension in agar medium. Filter paper discs of sample extracts are placed on the surface of the agar plates and inhibition zones are observed after 18 hour incubation at 37°C.

Five samples (111 g each) were fractionated according to the general procedure of AOAC (1965) 26.093–26.094. These test samples consisted of two highly toxic fats, one low toxic reference material (1.5% TEF in USP cottonseed oil), one nontoxic oil, and a reagent blank. In addition to the three alumina fractions obtained with petroleum ether, 5% ethyl ether in petroleum ether and 25% ethyl ether in petroleum ether, a fourth fraction eluted with 400 ml of 100% ethyl ether was obtained. The four alumina fractions were cleaned up twice with sulfuric acid according to JAOAC (Changes in Methods) *50*, 0.217 Sect. C (1967). The residues of fractions 2,3, and 4 were spotted on filter discs (7.5 mm diameter) at two levels equivalent to 2.5 and 54 g starting sample. EC-GLC analysis of alumina extracts indicated that chick edema factors (polychlorodibenzo-p-dioxins are predominantly concentrated in alumina fraction 3. In addition, solvent blanks, a synthetic reference standard consisting of hexa-, hepta- and octachlorodibenzo-p-dioxin (CDPD), and a sample of technical grade 2,3,4,6-tetrachlorophenol (2,3,4,6-TCP) were spotted at the following concentrations: 0.01 μg, 0.1 μg, 0.5 μg, 1.0 μg, 10.0 μg, and 100 μg.

After incubation, inhibition was observed in six cases as shown in Table 3. The sensitivity of the *B. megaterium* test for CEF appears to be limited to 100 ppm and even then only a very small zone of inhibition was noticed. It appears, however, that a rapid confirmation test for chlorophenols can be developed at levels of ca 2–3 ppm or less. These are the low levels at which aflatoxin B_1 shows inhibition of *B. megaterium*.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Mr. Joseph Barandy, Drew Chemical Company, Boonton, N.J. and Dr. E. N. Gerhardt, Emery Industries, Inc., Cincinnati, Ohio for suggesting modifications of the H_2SO_4 cleanup procedure which have resulted in improved recoveries of chick edema factor.

The contribution of the following members of the Food and Drug Administration are gratefully acknowledged: to Dr. M. J. Verrett for the chicken embryo assays; to Joseph N. Damico and Robert P. Barron for the mass spectrometric analyses; to Robert M. Eppley for the *B. megaterium* tests; and Thomas J. Dols for his helpful discussions concerning GLC operating parameters.

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TABLE 1.—MASS SPECTROMETRIC ANALYSIS OF ISOLATED COMPONENTS

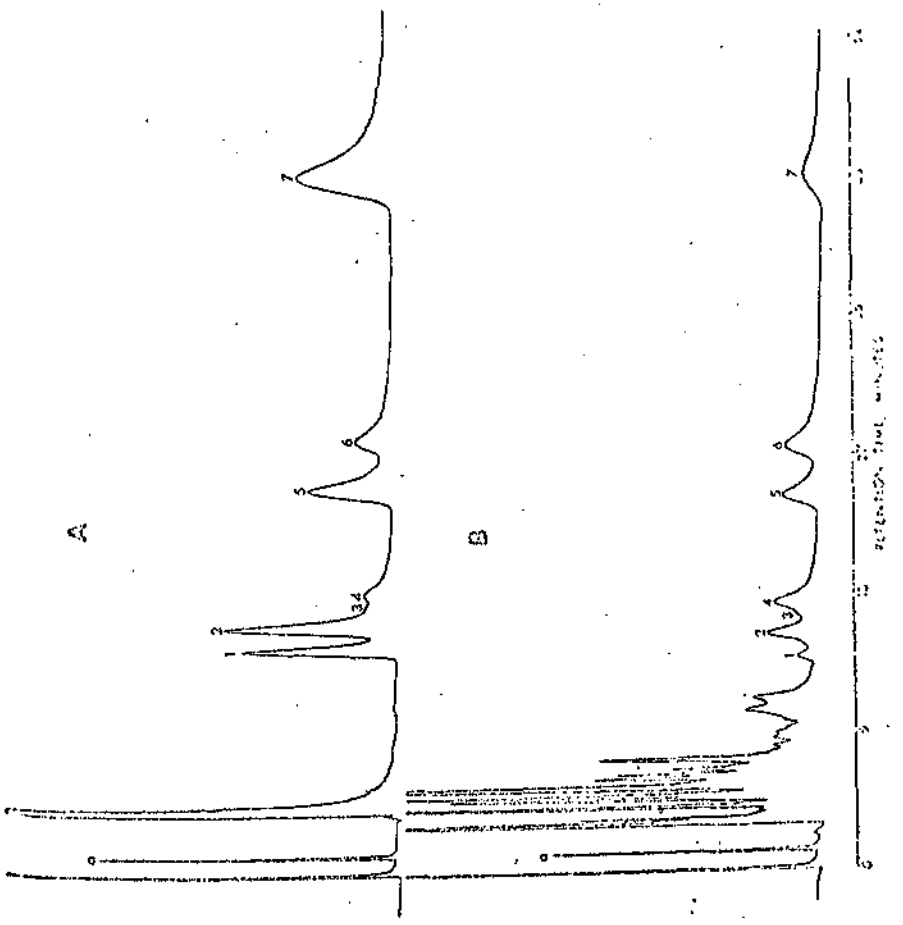
	GLC peak no.	Identity	Molecular weight found	No. of Chlorine atoms indicated
Standard mixture	1	Hexachlorodibenzo-p-dioxin	388	6
	2	do	388	6
	3	Heptachlorodibenzo-p-dioxin	422	7
Test sample	1	Hexachlorodibenzo-p-dioxin	388	6
	3	Heptachlorodibenzo-p-dioxin	422	7

TABLE 2.—CHICKEN EMBRYO ASSAY OF EXTRACTS

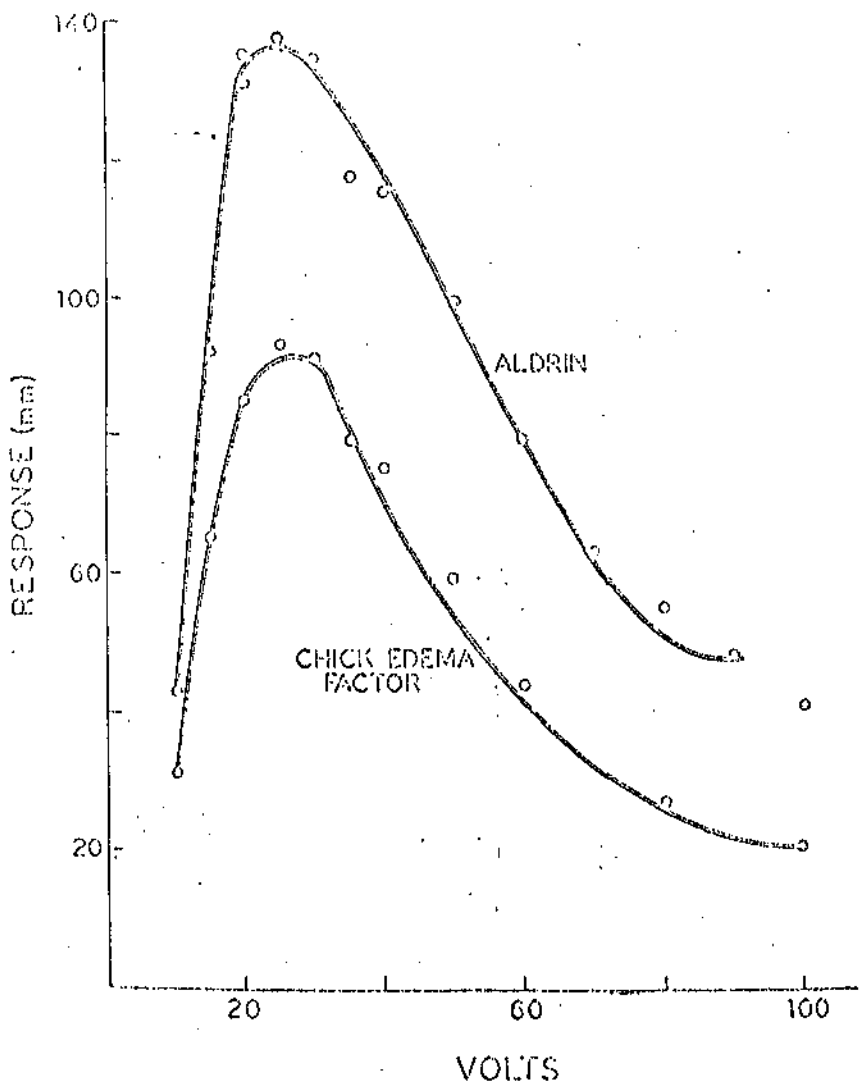
Sample	EC-GLC analysis for chick edema factor	Estimated level of hexa-, hepta- and octachloro-dibenzo-p-dioxins in fat, in ppm	Percent mortality chicken embryo assay	Assay observations
Reference toxic fat	positive	2.4	100	edema observed.
Test fat No. 1	do	0.1	73	Do.
Test fat No. 2	do	0.7	100	Do.
Test fat No. 3	do	0.6	93	Do.
Reagent blank	negative		30	
Chloroform solvent	do		20	
Control eggs	do		7	

TABLE 3.—RESULTS FROM B. MEGATERIUM TEST

Sample	Observation
Nontoxic USP cottonseed oil Fr. 4 (2.5 g extract)	Barley visible around disc.
TEF-F797,- Fr. 2, (2.5 g extract)	Do.
100 μg synthetic (CDPD) reference standard	Do.
1 μg 2,3,4,6-TCP	Do.
10 μg 2,3,4,6-TCP	16 mm inhibition zone.
100 μg 2,3,4,6-TCP	36 mm inhibition zone.



45-382 O - 70 - 23



SECTION 121.1070 FATTY ACIDS

The food additive fatty acids may be safely used in food and in the manufacture of food components in accordance with the following prescribed conditions:

(a) The food additive consists of one or any mixture of the following straight-chain monobasic carboxylic acids and their associated fatty acids manufactured from fats and oils derived from edible sources: Capric acid, caprylic acid, lauric acid, myristic acid, oleic acid, palmitic acid, and stearic acid.

(b) The food additive meets the following specifications:

(1) Unsaponifiable matter does not exceed 2 percent.

(2) It is free of chick-edema factor or other factors toxic to chicks, as evidenced during the bioassay method for determining the chick-edema factor as prescribed in paragraph (c) (2) of this section.

(c) For the purposes of this section:

(1) Unsaponifiable matter shall be determined by the method described in section 26.049 of the Official Methods of Analysis of the Association of Official Agricultural Chemists, Ninth Edition (1960).

(2) Chick-edema factor shall be determined by the bioassay method described in the Journal of the Association of Official Agricultural Chemists, Volume 44, page 146 (1961). The presence of chick-edema factor shall be determined by a comparison between the mean log of the pericardial fluid volumes of a test group and of a concurrent negative control group. The significance of the difference in pericardial fluid volumes between the test group and the negative control group is determined by calculating a t value according to the formula: The test sample is judged to contain chick-edema factor if the calculated t exceeds 1.3 and the mean log of the pericardial fluid volume obtained from the negative control group multiplied by 100 is less than 1.1461.

$$t=\frac{\bar{x}_t-\bar{x}_c}{\sqrt{\frac{s_t^2}{n_t}+\frac{s_c^2}{n_c}}}$$

where:

$\bar{x}_t$ and $\bar{x}_c$ are the means of the log of the pericardial fluid volumes of the test and control groups, respectively;

n_t and n_c are the number of chicks in the respective groups;

s_t^2 and s_c^2 are the variances of the test and control groups, respectively.

The variances are calculated as follows:

$$s^2=\frac{n\Sigma x^2-(\Sigma x)^2}{n(n-1)}$$

where:

Σx is the sum of the logs of the pericardial fluid volumes;

Σx^2 is the sum of the squares of the log of the pericardial fluid volumes for either the test t or control c group date.

(3) "Other factors toxic to chicks" referred to in paragraph (b) (2) of this section shall be determined during the course of the bioassay test described in subparagraph (2) of this paragraph on the basis of chick deaths or other abnormalities not attributable to chick-edema factor or to the experimental conditions of the test.

(d) It is used or intended for use as follows:

(1) In foods as a lubricant binder and as a defoaming agent in accordance with good manufacturing practice.

(2) As a component in the manufacture of other food-grade additives.

(e) To assure safe use of the additive the label and labeling of the additive and any premix thereof shall bear in addition to the other information required by the act the following:

(1) The common or usual name of the acid or acids contained therein.

(2) The words "food grade," in juxtaposition with and equally as prominent as the name of the acid.

SECTION 121.1071 SALTS OF FATTY ACIDS

The food additive salts of fatty acids may be safely used in food and in the manufacture of food components in accordance with the following prescribed conditions:

(a) The additive consists of one or any mixture of two or more of the aluminum, calcium, magnesium, potassium, and sodium salts of the fatty acids conforming to § 121.1070.

(b) The food additive is used or intended for use as a binder, emulsifier, and anticaking agent in food in accordance with good manufacturing practice.

(c) To assure safe use of the additive, the label and labeling of the additive and any premix thereof shall bear in addition to the other information required by the act the following:

(1) The common or usual name of the fatty acid salt or salts contained therein.

(2) The words "food grade," in juxtaposition with and equally as prominent as the name of the salt.

Title 21—FOOD AND DRUGS

Chapter 1—Food and Drug Administration, Department of Health, Education, and Welfare

SUBCHAPTER B—FOOD AND FOOD PRODUCTS

PART 121—FOOD ADDITIVES

Subpart D—Food Additives Permitted in Food for Human Consumption

FATTY ACIDS

The Commissioner of Food and Drugs has received a petition (FAP 6A2003) from Fatty Acid Producers' Council, Division of the Soap and Detergent Association, 295 Madison Avenue, New York, N.Y. 10017, proposing that § 121.1070, the food additive regulation providing for safe use of fatty acids in food and in the manufacture of food components, be amended:

A. To provide for the use of a screening method for determining the presence of chick-edema factor in the fatty acids that, within certain conditions, may be used in lieu of the bioassay method prescribed by paragraph (c) (2), and

B. To delete references to "other factors toxic to chicks" from the section.

From the available information it can be concluded that the anomalies presently identified as due to other toxic factors, which may be evidenced during the bioassay method for determining chick-edema factor, are directly associated with the same conditions or substances producing chick-edema factor, and the proposed physicochemical method is adequate as a screening test for detecting the chick-edema factor complex of toxicants.

Based on the information sbumitted in the petition, and other relevant material, the Commissioner has concluded that the regulation should be amended as petitioned. In addition, the references identifying the chick-edema bioassay procedure was updated to refer to the Official Methods of Analysis of the Association of Official Agricultural Chemists.

Therefore, pursuant to the provisions of the Federal Food, Drug, and Cosmetic Act (sec. 409(c) (1), 72 Stat. 1786; 21 U.S.C. 348(c) (1)), and under the authority delegated to the Commissioner by the Secretary of Health, Education, and Welfare (21 CFR 2.120; 31 F.R. 3008), § 121.1070 (b) (2) and (c) (2) and (3) are amended to read as follows:

SECTION 121.1070 FATTY ACIDS

* * * * *

(b) * * *

(2) It is free of chick-edema factor:

(i) As evidenced during the bioassay method for determining the chick-edema factor as prescribed in paragraph (c) (2) of this section; or

(ii) As evidenced by the absence of chromatographic peaks with a retention time relative to aldrin (R_A) of five or more using the gas chromatographic-microcoulometric method prescribed in paragraph (c) (3) of this section. If chromatographic peaks are found with R_A values of five or more, it shall meet the requirements of the bioassay method prescribed in paragraph (c) (2) of this section for determining chick-edema factor.

(c) * * *

(2) Chick-edema factor shall be determined by the bioassay method described in Official Methods of Analysis of the Association of Official Agricultural Chemists, 10th Edition (1965), sections 26.087 through 26.091.

(3) The gas chromatographic-microcoulometric method for testing fatty acids for chick-edema shall be the method described in Official Methods of Analysis of the Association of Official Agricultural Chemists, 10th Edition (1965), sections 26.092 through 26.096, except that the following procedure is substituted for that described in section 26.092(b):

Activated alumina.—(Fisher No. A540 or equivalent.) Activate 250-gram portions by heating 4 hours at 260°C. Transfer without cooling to dry container and close tightly. Use within 1 week after preparation. Check activated Al_2O_3 by analysis of a reference standard by examining fractions 2 and 3. Chick-edema factor should elute in Al_2O_3 fraction 3 as indicated by the gas chromatogram. (A sample of the reference standard may be obtained on request from the Bureau of Science, Food and Drug Administration, Washington, D.C. 20204.)

* * * * *

Any person who will be adversely affected by the foregoing order may at any time within 30 days from the date of its publication in the FEDERAL REGISTER file with the Hearing Clerk, Department of Health, Education, and Welfare, Room 5440, 330 Independence Avenue SW., Washington, D.C. 20201, written objections thereto, preferably in quintuplicate. Objections shall show wherein the person filing will be adversely affected by the order and specify with particularity the provisions of the order deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought. Objections may be accompanied by a memorandum or brief in support thereof.

Effective date. This order shall become effective on the date of its publication in the FEDERAL REGISTER.

(Sec. 409(c) (1), 72 Stat. 1786; 21 U.S.C. 348(c) (1))

Dated: August 18, 1966.

J. K. KIRK,
Acting Commissioner of Food and Drugs.

[F.R. Doc. 66-9263; Filed, Aug. 24, 1966; 8:47 a.m.]

Table 12 Embryotoxicity of Chlorophenols, Dibenzo-p-dioxins (Chick edema)

Estimated Dose Levels to Produce Indicated Mortality (Percent)

↓Compound	Mortality ≈	Phenols (Dose in mg) 100	50	0	Pyrolysis* Products (Dose in mg) 100	50	0	Unsaponifiable* Fraction (Dose in mg) 100	50	0
2,4-Dichlorophenol		>2	1.5	0.4	>0.5	0.25	0.01	-	-	1.5×10^{-2}
2,4,5-Trichlorophenol		-	0.05	0.01	2.5×10^{-5}	5×10^{-6}	2.5×10^{-6}	$\sim 5\times10^{-3}$	2×10^{-3}	5×10^{-4}
2,4,6-Trichlorophenol		-	1.0	0.2	0.05	0.005	2.5×10^{-3}	-	.05	-
2,3,4,6-Tetrachlorophenol		2.5	1.0	0.2	1×10^{-3}	2×10^{-4}	1.0×10^{-4}	6×10^{-3}	1.2×10^{-3}	6×10^{-4}
Pentachlorophenol		1.5	0.7	0.4	-	2.5×10^{-2}	-	$>1\times10^{-2}$	6×10^{-3}	1×10^{-3}
2,4-Dichlorophenoxyacetic acid			1.0	<0.1						
2,4,5-Trichlorophenoxy-acetic acid										
Bionetics		1.0	0.25	< .05						
Dow #120449			1.0	~0.5						

Polychlorodibenzo-p-Dioxins *

Dibenzo-p-dioxin		0.5	
Chlorinated dibenzo-p-dioxin	2.5×10^{-5}	1×10^{-5}	2.5×10^{-6}
Trichloro dibenzo-p-dioxin	2×10^{-3}	2×10^{-4}	5×10^{-5}
Tetrachloro dibenzo-p-dioxin	2×10^{-4}	5×10^{-5}	-
Hexachloro isomers (4)			
a. Most toxic	5×10^{-4}	1.25×10^{-4}	-
b. Least toxic	-	2×10^{-3}	1×10^{-3}
Heptachloro isomers(2)			
a. Most toxic	1.25×10^{-3}	2.5×10^{-4}	-
b. Least toxic	-	-	1.25×10^{-3}

Unpublished data of J. Verrett
* Many of these are mixtures
Code: 1 mg = 20 ppm
1 µg = 20 ppb

Air Cell Injections
Pre-incubation

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PRELIMINARY REPORT ON TERATOLOGY STUDIES WITH DIOXIN USING GOLDEN HAMSTERS

Compound	Amount Intubated Days 6 - 10	Fetl Litter	Total # of Fetl	# Born Alive	Terata	# Dead	Avg. Wt. of Fetus
Control	0	8.6	43	41	None	2 - 1 ED, 1 LD	1.55
Chlorodibenzo-p-dioxin (21% trichloro, 53% tetrachloro, synthesized in FDA)	9.1 µg/kg/day	10.3	62	11	eye anomalies	51 - 4 ED, 47 LD	1.38
	2.0 µg/kg/day	10.8	43	41	gastro-intestinal hemorrhage	2 - 2 ED	1.47
	0.5 µg/kg/day	10.5	63	62	gastro-intestinal hemorrhage	1 - 1 ED	1.66
	0.13 µg/kg/day	11.6	58	56	None	2 - 2 ED	1.58

ED - Early Dead, LD - Late Dead

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FROM: Dr. T.F.X. Collins, Mr. W.H. Hansen, Dr. C.H. Williams
Residue Toxicology Branch
Division of Pesticide Chemistry & Toxicology
Office of Pesticides & Product Safety

PRELIMINARY REPORT ON TERATOLOGY STUDIES WITH 2,4,5-T SAMPLES USING GOLDEN HAMSTERS

Compound	Amount Intubated Days 6 - 10	Foti Litter	Total # of Foti	# Born Alive	Terata	# Dead	Avg. Wt. of Fetus
Control	(16)	12.3	211	198	None	13 - 12 ED, 1 LD	1.79
2,4,5-T (Pure) (Recrystallized)	100 mg/kg/day (9)	12.4	87	38	3[a]	49 - 49 ED	1.46
	80 mg/kg/day (5)	13.2	66	50	gastro-intestinal hemorrhage	16 - 6 LD, 10 ED	1.52
	40 mg/kg/day (6)	14.0	84	79	gastro-intestinal hemorrhage	5 - 1 LD, 4 ED	1.51
2,4,5-T (Pure) (Recrystallized and Extracted)	100 mg/kg/day (4)	13.3	53	16	None	37 - 36 ED, 1 LD	1.66
	150 mg/kg/day (4)	10.3	41	10	None	31 - 29 ED, 2 LD	1.66
2,4,5-T Dow) (0.5 ppm dioxin)	100 mg/kg/day (5)	10.8	54	11[b]	gastro-intestinal hemorrhage	43 - 43 ED	1.64

[a] One had right hind limb deformity, in 2 the fusion in the skull was not complete.
ED - Early Dead, LD - Late Dead.

[b] All alive were from one litter.

FROM: Dr. T.F.X. Collins, Mr. W.H. Hansen, Dr. C.H.Will
Residue Toxicology Branch
Division of Pesticide Chemistry & Toxicology
Office of Pesticides & Product Safety

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE,
Research Triangle Park, N.C., December 4, 1969.

DR. J. MCLAUGHLIN,
Food and Drug Administration,
Washington, D.C.

DEAR DR. MCLAUGHLIN: I am sending you under separate cover a sample of 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) from the supply that was used at Biometics Research Labs, Inc. for the Teratogen Screening Study for NCI.

Sincerely,

K. DIANE COURTNEY, Ph.D,
Pharmacology and Toxicology Branch,

THE DOW CHEMICAL COMPANY,
Midland, Mich., February 9, 1970.

M. JACQUELINE VERRETT, *Bureau of Science, Division of Toxicology, Department of Health, Education, and Welfare, Food and Drug Administration, Washington, D.C.*

DEAR DR. VERRETT: The complete assay on the sample of 2,4,5-trichlorophenoxyacetic acid (Dow production batch 120449) which we recently sent to you is as follows:

	PERCENT
2,3,7,8-tetrachlorodibenzo-p-dioxin = (0.5 ppm)	
2,6-dichlorophenoxyacetic acid = <0.02	
2,5-dichlorophenoxyacetic acid = 0.42	
2,4-dichlorophenoxyacetic acid = 0.05	
2,3,6-trichlorophenoxyacetic acid = 0.55	
2,4,6-trichlorophenoxyacetic acid = <0.1	
Bis-(2,4,5-trichlorophenoxy)acetic acid = 0.4	
3 isomers of dichloromethoxy phenoxyacetic acid = 2.9	
2,4,5-trichlorophenol = 0.23 (Max.)	
sodium chloride = 0.035	
2,4,5-trichlorophenoxyacetic acid = Balance	
Freezing Point = 152.9°C	
Total assay by acid titration = 100%	

Sincerely,

GEORGE E. LYNN
Government Regulatory Relations,
Dow Life Sciences.

MARCH 2, 1970

CHLORINATED DIBENZO-P-DIOXIN STANDARD (F768)

1. We have supplied Dr. C. Rilliams with a mixture of tri- and titrachlorodibenzo-p-dioxins (F768) prepared by direct chlorination of dibenzo-p-dioxin at room temperature for two and one-half hours (see memo of Ress to Campbell 1/30/70 and memo of Firestone to Campbell 2/5/70).

2. This mixture, previously analyzed by EC-GLC and found to contain about 50% tetrachlorodibenzo-p-dioxin (GLC peak area), was reexamined by GLC with flame ionization detection (6 foot glass column; 200°C; 3% OV-101; Packard GC model 871), which gives a more accurate determination of composition. By this latter analysis, it was determined that the chlorination mixture F768 consists of two components as follows:

(*a*) 38% 2,3,7-trichlorodibenzo-p-dioxin
(*b*) 62% 2,3,7,8-tetrachlorodibenzo-p-dioxin

MARCH 26, 1970.

SAMPLES FOR CHICKEN EMBRYO TESTING

1. The following samples were delivered to Dr. Verrett on 3/17/70 for chicken embryo testing:

Our. No.	Identification	Remarks
F871	2,3,7,8-tetrachlorodibenzo-p-dioxin, Dow, pure (ca. 95% by GLC)	0.0878 mg in 10 ml of acetone.
F877	2,7-dichlorodibenzo-p-dioxin, Dow, pure (ca. 99% by GLC)	0.3806 mg in 10 ml of acetone.
F883	2,3,7,8-tetrachlorodibenzo-p-dioxin, FDA, prepared by Dr. Pohland (99.5% pure by GLC).	0.1735 mg in 10 ml of acetone.
F881-A-1	2,4,5-trichlorophenoxyacetic acid; Dr. Williams' purified sample; further extracted 3 x with petr. ether and 4 x with 1+1 petr. ether—diethyl ether.	1.712 grams.
F881-B	Sylvex; Dr. Williams' purified sample; further extracted 3x with petr. ether and 4x with 1+1 petr. ether—diethyl ether.	2.519 grams.

D. FIRESTONE, *Head,*
Fats and Oils Section.

MARCH 26, 1970.

CHLOROPHENOL SAMPLES

1. About 3 grams each of the following chlorophenols were delivered on 3/23/70 to Dr. Verrett (at her request) for chicken embryo testing.

Our No.	Identification	EC-GLC anal PPB *CEF
F888-sub A	Pentachlorophenol (purified) Aldrich Chemical Co	167.
F888-sub B	2,3,4,6-Tetrachlorophenol (technical) Eastman Chemicals	96,500.
F888-sub C	2,4-Dichlorochlorophenol (purified) Eastman Chemicals	16.
F888-sub D	Ortho-chlorophenol (purified) Fisher Scientific	No analysis.
F888-sub E	2,4,5-Trichlorophenol (technical) Eastman Chemicals	Trace.
F888-sub F	2,4,5-Trichlorophenol (reagent) Dow Chemical Co	Trace.

*Hexa-, hepta-, and octachlorodibenzo-p-dioxins.

J. RESS,
Fats and Oils Section.

I. Cleft Palate. 21-day Embryo. 0.02 micrograms 2,3,6,7-Tetrachloro-dibenzo-p-dioxin.

II. Abnormal (incomplete) Development of Eyelid. 21-day Embryo. 0.0025 micrograms 2,3,6,7-Tetrachloro-dibenzo-p-dioxin.

III. Edematous Cyst Covering Rump. 21-day Embryo. 0.02 μg (micrograms), 2,3,6,7-Tetrachloro-dibenzo-p-dioxin.

Senator HART. The hour being 12:25, I suggest we recess until 2:15.

(Whereupon, at 12:25 p.m., the hearing was recessed, to reconvene at 2:15 p.m. this same day.)

AFTERNOON SESSION

Senator HART. We resume this afternoon to hear first from Dr. Julius Johnson of the Dow Chemical Co. If a corporate entity can have a spirit attached to it, the comparing is a distinguished corporate constituent of mine.

STATEMENT OF DR. JULIUS E. JOHNSON, VICE PRESIDENT AND DIRECTOR OF RESEARCH, THE DOW CHEMICAL CO.; ACCOMPANIED BY ETCYL BLAIR, DIRECTOR OF DOW AGRICULTURAL CHEMICAL RESEARCH; V. K. ROWE, DIRECTOR OF THE DOW TOXICOLOGICAL LABORATORY; AND GEORGE LYNN, DIRECTOR OF GOVERNMENT REGULATORY RELATIONS OF THE DOW CHEMICAL CO.

Dr. JOHNSON. Thank you, Senator Hart.

I have with me Dr. Blair, director of Dow Agricultural Chemical Research, Mr. Rowe, director of our Toxicological Laboratory, and Mr. George Lynn, director of our Government Regulatory Relations.

Senator HART. Thank you. You are all welcome.

Doctor, you have given us a statement. We will order it printed in full in the record and as you go along, if there is any extension or summation you care to make, the record will contain the full statement in any event.

Dr. JOHNSON. Senator Hart, the policy decision has already been made and announced this morning. I will, however, with your permission, read my testimony, all except the last part which deals with some historical matters that already appears in the record.

Then, if you would permit, I would like to make some additional comments which may be appropriate to the process of shortening the time interval between the discovery of a suspected toxic phenomena and taking appropriate action.

I would also like to refer back to some earlier work done under Senator Ribicoff's guidance in his committee and quote at least one passage from that work published in 1966, which I think is appropriate to the issue, if I may do so.

Senator HART. By all means.

Dr. JOHNSON. Thank you.

Mr. Chairman, I am Julius E. Johnson, vice president and director of Research and Development of the Dow Chemical Co., Midland, Mich. I also served as a member of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health, May 8, 1969, to November 7, 1969, chairman, Emil M. Mrak.

I have with me George Lynn, director of Government Regulatory Relations of the Dow Chemical Co. V. K. Rowe, director of the Dow Toxicological Laboratory and Etcyl Blair, director of Dow Agricultural Chemical Research, are also present to assist if necessary.

This statement is concerned with the herbicide 2,4,5-trichlorophenoxyacetic acid, which has often been referred to as 2,4,5-T and

the chemical intermediate 2,4,5-trichlorophenol used in the manufacture of 2,4,5-T.

An announcement was issued October 29, 1969, by Dr. Lee Dubridge of the Office of Science and Technology which referred to birth defects observed in tests by the Bionetics Laboratories using 2,4,5-T in various dosage ranges in mice and rats.

This announcement preceded the final report of the Panel on Teratology of the Mrak Commission appointed by Secretary Finch which, since May 8, 1969, had been reviewing the effects of pesticides upon health and the quality of environment. At the time, October 29, 1969, members of the Mrak Commission had not seen the Bionetics report on teratology.

Following the announcement by the Office of Science and Technology, I became particularly concerned because Dow is a manufacturer of this herbicide. Consequently, I made a diligent effort to trace the source of samples used and learned that the 2,4,5-T sample came from the Diamond Alkali Co. (which no longer makes 2,4,5-T).

Moreover it was learned that 2,4,5-trichlorophenol also tested by the Bionetics Laboratory came from Coleman-Mathison-Bell who had obtained the sample from McKesson-Robbins who in turn had procured it from the Dow Chemical Co.

2,4,5-trichlorophenol is used as an intermediate in the manufacture of 2,4,5-T. Hence, the quality of 2,4,5-T is related to the quality of its intermediate 2,4,5-trichlorophenol. The chemical process used by Dow for manufacture is as follows:

1,2,4,5-tetrachlorobenzene is hydrolyzed in a solution of methanol and sodium hydroxide in water to form sodium 2,4,5-trichlorophenate. This is in turn reacted with sodium monochloroacetate to form sodium 2,4,5-trichlorophenoxyacetate. The solution is acidified to precipitate and recover the 2,4,5-trichlorophenoxyacetic acid.

Since 1950 we have been keenly aware of the possibility of a highly toxic impurity being formed in 2,4,5-trichlorophenol as a side reaction under conditions of elevated processing temperatures. The most sensitive toxic reaction observed in humans to this impurity was manifested by a condition known as chloracne, a skin disorder mostly prevalent on the face, neck, and back.

It is similar in appearance to severe acne often suffered by teenagers. We also knew that if the impurity was present in the 2,4,5-trichlorophenol it could be carried forward to the end product, 2,4,5-T. It is not formed during the manufacture of Dow 2,4,5-T from the 2,4,5-trichlorophenol, nor does it form on storage even at high temperatures. To avoid the impurity in 2,4,5-T it is necessary to keep it out of the 2,4,5-trichlorophenol.

Our early control test was a bioassay. This consists of applying a solution of the material to the inner surface of a rabbit's ear and observing for the typical skin response described in a paper published in 1941 by Dow scientists. I wish to insert in the record at this point the paper entitled "The Response of Rabbit Skin to Compounds Reported to have cause Acneform Dermatitis," by E. M. Adams, D. D. Irish, H. C. Spencer, and V. K. Rowe, published in Industrial Medicine, January 1941.

Senator HART. It will be printed.

(The information follows:)

The Response of Rabbit Skin to Compounds Reported to Have Caused Acneform Dermatitis

E. M. Adams, D. D. Irish, H. C. Spencer,
and V. K. Rowe,
Biochemical Research Laboratory,
The Dow Chemical Company
Midland, Michigan

THOSE of us acquainted with the industrial field have recognized the need of an experimental method for studying skin irritation. We would profit greatly by knowing the potential skin hazards of a substance before it is put into use; we would be able to take proper precautions in the cheapest and most satisfactory manner and many undesirable incidences could be avoided.

In the literature there are many instances of irritation tests upon the skin of animals, but apparently there has not been a comprehensive study. In an attempt to develop an experimental method, we began about six years ago to study the responses of rabbits' skin to various types of substances. We considered the possibility that if enough were known of these responses to different types of compounds, particularly to those with which there has been considerable human experience, then these responses could be organized to form the basis of an experimental method.

Acneform dermatitis, characterized by such lesions as folliculitis, comedones, nodules, papules, pustules, and inflammatory changes, has been reported arising from exposure to quite varied substances including petroleum oils and greases, shale oil, paraffin, zinc oxide, chlorine, tars, pitches, chlorinated diphenyls, chlorinated naphthalenes, and crude chlorinated phenols.[1, 2, 4, 5, 6, 7, 8, 9, 10, 13, 15, 14, 16, 18, 17, 18, 19, 20, 24, 25, 26, 27, 28, 29, 30, 32, 33, 34, 35] The recent occurrence in this country of such an acneform eruption, [4, 9, 12, 16, 28] sometimes called "chloracne," has attracted particular interest, and we included in our animal studies five types of substances known to cause the reaction. Today we wish to describe the unusual response of the rabbits' skin to these materials and to consider its possible significance.

Experimental Part

IN OUR experiments, materials have been applied to the inner surface of the ear of albino rabbits and to the shaven belly. The undiluted materials have been used as well as solutions of various concentrations in olive oil, paraffin oil U. S. P., propylene glycol, ethanol, and water. Liberal applications were made on the ear without any covering. The applications on the abdomen were made in a small cotton pad which was covered by a large bandage of filter cloth held in place by adhesive tape. Applications were made once a day, five days a week, for four weeks or until a marked reaction resulted.

The responses obtained following the application of some hundreds of test substances are easily arranged according to type.

Certain of the strongest irritants produce a rapid destruction of the tissue (necrosis), without the skin having an opportunity to show an active response. Irritants with milder and slower actions than this have some effect upon the tissues, as a result of which we see certain responses on the part of the tissue. Most irritants have resulted in responses in the rabbits' skin which tend to develop rapidly and to subside in a short time. This relatively rapid response, which we have termed a simple irritation or reaction, may include, depending upon the severity, any of the following: hyperemia, congestion, inflammation, exfoliation, edema, blistering, sloughing, exudation, crustation, necrosis, induration, hair loss. Microscopically one may see hyperemia, congestion, hemorrhage, edema, blistering, leucocytic infiltration, sloughing, and various degenerative changes.

One type of response has been observed, however, which requires a somewhat longer interval in which to become apparent, and which has a much more prolonged course. This latent reaction is a proliferative response which may possibly occur in any of the structures of the skin, but that about which we are particularly concerned now is epithelial hyperplasia, with its resultant thickening of the skin, follicle enlargement and sequellae.

Naturally responses vary to some extent, and we have observed various combinations of these reactions, depending upon the substances applied to the skin and the intensity of action.

For purposes of classification we have arbitarily divided the proliferative response into the following five groups according to intensity:

1. Least detectable.
2. Very slight.
3. Slight.
4. Moderate.
5. Severe.

While there are naturally no sharp breaks between these, and some overlapping occurs, division was rather easy and has been very useful.

Least detectable epithelial hyperplasia: This degree of response is manifest as an increased prominence of the hair follicles on the inside of the ear. The little dots that one sees on the inside of the ear simply become slightly larger. After exposures are ended this enlargement regresses in a short time, leaving the skin apparently normal.

This degree of response is commonly seen as part of a mild simple irritation which is maintained by repeated exposures. Thus far we have been unable to attach a particular significance to this intensity of reaction.

Very slight epithelial hyperplasia: This reaction appears on the ear as a slight enlargement of the hair follicles, which protrude and become hard, causing the ear to feel rough. The thickness of the ear may be increased. A very slight scaly exfoliation may accompany this degree of response, but seldom is there any detectable hyperemia or hair loss. On the abdomen one seldom sees any gross evidence of hyperplasia.

Slight epithelial hyperplasia: In this reaction the ear increases in thickness to about twice normal and feels slightly stiffened and "leather-like." There is some hyperemia, scaly exfoliation, and hair loss. The hair follicles become slightly enlarged, raised and hard. On the abdomen there may be a slight thickening of the skin and an exfoliation, but enlargment of the follicles is not apparent.

Moderate epithelial hyperplasia: This reaction consists of a thickening of the ear to 3 to 4 times normal as a result of which it is quite stiff and leathery. The follicles on the ear become moderately enlarged, raised and hard, causing the surface of the ear to feel like the coarsest of sand paper. After a time the protruding hard masses can be easily expressed by the finger-nail or by bending the ear. At times the enlarged follicles are not apparent until after considerable exfoliation has occurred. A moderate hyperplasia is usually accompanied by a slight to moderate hyperemia. Exfoliation of a granular or scaly type is of moderate intensity and hair loss is nearly complete. After a number of weeks the ear is completely denuded of hair, slightly pitted, with a slight or moderate hyperemia and possibly some exfoliation. The abdominal skin may show a greater simple irritation than does the ear; hyperemia, edema, and even sloughing and exudation have occurred. Hyperemia is usually maintained during the course of thickening. The abdominal skin finally becomes hard and stiff, followed by a marked scaly and granular exfoliation, which persists for weeks.

Severe hyperplasia: This reaction is usually preceded by a marked simple irritation, including even necrosis; however, there may be only hyperemia and edema. As a severe hyperplasia progresses, a marked hyperemia is evident until obscured by the thickened epithelium. The thickness of the ear is increased to many times normal, ears at least 1 cm. thick having been formed. As a result they become very stiff, hard, and heavy. Exfoliation at first has a granular consistency, later flaky, and persists for months. The enlarged hair follicles are buried under the thickened epithelium and become apparent only after considerable exfoliation has occurred. From them large masses of keratin may be expressed leaving pits that may reach 2 to 3mm. in width.

On the abdomen the hardened mass of epithelium cracks and lifts off in large pieces like portions of a cast. Often beneath these is a soft, cheesy, foul-smelling material, which soon dries and comes off revealing a markedly exfoliating skin beneath.

The exfoliation often has a granular consistency at first, which later becomes flaky. There is a complete hair loss.

This proliferation of the epithelium seems to progress only to a certain extent, even with repeated applications of the provoking agent. The slowness and persistence of this latent reaction is to be emphasized. The maximum of a severe hyperplasia usually has occurred in the neighborhood of two weeks, the largest amount of exfoliation around four weeks, and a scaly exfoliation and hyperemia have persisted for months.

Although we make exposures upon both ear and belly, the skin of the ear appears to respond in the most satisfactory manner. There the mildest reactions are more apparent and the enlarged follicles are more easily seen. As a rule the abdominal skin shows a more marked simple irritation.

Histology

MICROSCOPIC examinations were made using 10% formalin as fixative, paraffin for imbedding, and hematoxylin-eosin as stain.

The slightest hyperplastic response is shown by a very slight increase in thickness of the epithelium and the development of small projections (like papillae) of but a few cells in size. The early stages of more severe responses show increasing degrees of thickening of the surface and follicular epithelium. Numerous projections reach downward from the surface epithelium, nearly to the cartilage of the ear. The follicular epithelium spreads outward and downward, often completely engulfing hair follicle and sebaceous glands. Apparently there is also a hyperplasia in the corium. Accompanying this hyperplasia, one may see congestion, even occasional hemorrhages, edema, and leucocytic infiltration.

Later the rate of proliferation apparently lessens and those changes resulting in keratinization become more evident. As those changes leading to keratinization progress from the lowermost layer of the epithelium, which constitutes a basal layer markedly displaced from the original, large masses of material are thrown off. Thus in one section of abdominal skin we see a thick layer of partly keratinized and degenerate tissue being thrown off above a flat, normal-appearing stratum corneum. At the hair follicles most of the tissue undergoes complete keratinization, forming the hard plugs that may be expressed. Completely engulfed follicles and glands are destroyed as the hyperplastic epithelium is keratinized and thrown off.

The sebaceous glands have seemed to be inactive. One sees them, apparently normal, being engulfed by proliferating epithelium. Some glands, of normal size and appearance, are seen opening into the pits or cysts; others are seen with their

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ducts extending through large masses of keratinized epithelium.

Sections taken at a late stage show an atrophic or very slightly thickened surface epithelium and numerous large pits surrounded by slightly hyperplastic epithelium. The corium may still be thicker than normal.

Ultimately there is a tendency for the pits to broaden out and become shallower, and one sees a very irregular atrophic epithelium.

Discussion

THERE are certain points which indicate a relationship between this reaction observed in the rabbit and the acneform dermatitis of man. First, the reaction in the rabbit was produced by 5 types of substances known to cause an acneform dermatitis in man. They were chlorinated diphenyls, chlorinated naphthalenes, chlorinated diphenyloxides, crude chlorinated phenols, and petroleum oils. A few other types of substances have produced the epithelial hyperplasia, but there has been no exposure of these on man. Wacker and Schmincke[21] reported the experimental production of epithelial hyperplasia with various oils, fats, and paraffin. Sachs,[25] and others, apparently, have produced the identical epithelial hyperplasia in rabbits with a number of dyes. In his review of the pertinent literature, Sachs states that the most common dermatosis arising from exposure to aniline and coal tar dyes is eczema; however, warty growths and acneform dermatitis have also occurred. Thus it appears probable that the development of an outstanding hyperplastic response of the rabbits' skin is specific for those substances capable of causing an acneform dermatitis in man, and possibly, the related papular and warty eruptions.

Secondly, by gross and microscopic examination, the enlarged follicles produced in the rabbit resemble the comedones, nodules, and cysts of the dermatitis in man. In both cases there is a relatively large pit or cyst whose walls are composed of epithelium and which contains varying amounts of keratinized epithelium, and at times hair, hair follicles, and debris.

In both the rabbit and in man there is hyperplasia of the epithelium. Proliferative changes have not been stressed in descriptions of the human reaction and probably have not been seen to a greater extent because tissues were taken at relatively late stages of the reaction. There are reports of increased numbers of mitoses and of thickening of the rete Malpighii.[3, 24, 30, 34, 35] Prosser White[34] describes acanthosis in the "primary papule" and considers one important factor in the production of oil folliculitis to be the chemical irritant causing auxetic cell growth. The ability of tar to cause active mitosis is well known.[11] Bornemann's[3] first case, examined at a late stage, showed more mitoses than normal and slight thickening; but in his second case, examined at an earlier stage, the thickening of the epithelium was much more marked.

Although mention is often made of sebaceous cysts in descriptions of the acneform dermatitis only two instances were found of the specific mention of sebaceous glands in descriptions of the microscopic picture. Jones and Alden[12] reported slight edematous changes in a few glands they saw; Curgil and Acton[6] said that the sebaceous glands were unaffected. Bornemann[3] felt that the cysts were of sebaceous origin but admitted the difficulty of proof, and his description shows them to be essentially epithelial structures.

These facts, together with our experimental results, indicate that the so-called "sebaceous cysts" of the acneform dermatitis in man are directly the result of an epithelial hyperplasia. Their content of sebaceous-like material is probably due to the occurrence of inflammatory and degenerative changes in the mass of epithelial tissue. Of course, the retention of sebum may also occur, and influence the picture to some extent, but this appears to be a secondary reaction. Bacterial infection may be a factor influencing the nature of the reaction.

Conclusions

THERE have been a number of hypotheses concerning the formation of this acneform eruption in man.[34, 35] We feel that evidence shows this acneform dermatitis to be the visible response of the skin to an irritant acting upon it from the exterior, and that this response takes the form of first epithelial hyperplasia, second inflammatory and degenerative changes, and finally regenerative processes.

And in conclusion, it is possible that this apparently unusual response of the rabbits' skin offers us an experimental method which will indicate the ability of substances to produce an acneform dermatitis in man.

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34. WHITE, R. PROSSER: The Dermatergoses, H. K. Lewis and Co., Ltd., London, 1934, pp. 195-241.
35. Occupation and Health, International Labour Office, Geneva, 1930. See Chlorine, Coal Tars, Paraffin, Petroleum Oils, Pitch, Shale Oil Industry.

Dr. JOHNSON. Thank you.

As early as 1944 we were monitoring the oils removed as impurities from the 2,4,5-trichlorophenol process by the rabbit ear test. It is in these waste oils that the impurities are concentrated.

In late 1964 some workmen developed chloracne and our bioassay program showed that the chloracne potential of the waste oil from 2,4,5-trichlorophenol process was building up to a danger point.

This came about from operating changes made to improve production capacity. Exposure to this waste oil was the cause of the acne in the workmen. (This waste is routinely destroyed by incineration at high temperature.)

The plant was summarily shut down. Bioassays of Dow 2,4,5-trichlorophenol and 2,4,5-T being produced at this time were negative. We confirmed that the principal offending impurity was 2,3,7,8-tetrachlorodibenzo-p-dioxin. Technology had advanced by early 1965 to the point where we were able to develop a gas chromatographic method for the tetrachlorodibenzo-p-dioxin with a sensitivity of 1 p.p.m. in 2,4,5-trichlorophenol and 2,4,5-T.

I wish to insert in the record at this point a paper entitled "The Determination of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in 2,4,5-trichlorophenoxyacetic acid by Gas Liquid Chromatography," by the Dow Chemical Co.

Senator HART. It will be printed.

(The information follows:)

DOW THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN 48640

ANALYTICAL METHOD

June 22, 1965 MLW.65.11

THE DETERMINATION OF 2,3,7,8-TETRACHLORODIBENZO-p-DIOXIN IN 2,4,5-TRICHLOROPHENOXYACETIC ACID BY GAS-LIQUID CHROMATOGRAPHY

1. Scope

This method is applicable to the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin in 2,4,5-trichlorophenoxyacetic acid. The dioxin can be detected at the one ppm level with a lower limit of 0.5 ppm possible at optimum operation conditions.

2. Principle

The 2,3,7,8-tetrachlorodibenzo-p-dioxin is separated from the 2,4,5-trichlorophenoxyacetic acid by means of an extraction with chloroform. The chloroform extract is concentrated and then chromatographed. The 2,3,7,8-tetrachlorodibenzo-p-dioxin in the sample is measured and compared to a known standard.

3. Safety Precautions

2,3,7,8-Tetrachlorodibenzo-p-dioxin is capable of causing a severe delayed skin response (chloracne) upon minimal contact. Samples suspected of containing any of this compound should be handled so as to prevent all skin contact and inhalation. Wear impervious gloves (rubber, polyvinyl chloride, etc.) at all times when contact is a possibility. Clean all equipment with acetone followed by a chloroform wash. Dispose in such a manner as to prevent all skin contact, any potentially contaminated equipment or materials which are not readily cleaned with chloroform, i.e., towels, gloves, etc.

4. Apparatus

(a) Gas chromatograph, Aerograph A-600-D with flame ionization detector, Wilkins Instrument and Research, Inc., Walnut Creek, California, or equivalent.

(b) Recorder, -0.05 to +1.05 millivolt, full span, one-second full response time.

(c) Syringe, Hamilton microliter, No. 701N, or equivalent.

(d) Syringe, Multifit 5 cc, Becton, Dickinson and Company, or equivalent.

(e) Syringe, Yale 1/4 cc, Becton, Dickinson and Company, or equivalent.

(f) Centrifuge
(g) Injector insert, Pyrex glass for A-600-D. Available from Wilkins Instrument and Research, Inc., Walnut Creek, California (Note 11a).
(h) Column, 1/8-inch O.D., 0.081-inch I.D., stainless steel tubing, five feet in length packed with reagent 5(c).

5. Reagents

(a) Solid support, Chromosorb W, 60/80 mesh, Johns-Manville.
(b) Partitioning agent, SE-30, Silicone gum rubber-methyl (Note 11b).
(c) Column packing, five percent by weight of SE-30 on 60/80 mesh Chromosorb W. Available from Wilkins Instrument and Research, Inc., Walnut Creek, California.
(d) Carrier gas, nitrogen, commercial grade.
(e) Chloroform, ACS grade.
(f) 2,3,7,8-Tetrachlorodibenzo-p-dioxin, available from The Dow Chemical Company, Midland, Michigan.
(g) Sodium hydroxide, 1 N solution. Dissolve 40 grams of reagent grade sodium hydroxide in one liter of water.

6. Chromatographic Conditions

(a) Oven temperature, 225°C.
(b) Inlet temperature, 260°C.
(c) Carrier gas flow rate, 75 ml. per minute as determined by the moving soap bubble technique.
(d) Attenuation, such that a response of at least 50% of scale is obtained from a 1.0 microliter sample of a standard containing 100 micrograms of 2,3,7,8-tetrachlorodibenzo-p-dioxin in one milliliter of chloroform.

7. Preparation of Standard

(a) Again read Section 3.
(b) Weigh, using a micro-balance, one milligram of 2,3,7,8-tetrachlorodibenzo-p-dioxin into a ten ml. volumetric flask.
(c) Dilute to the mark with chloroform.
(d) Inject a 1.0 microliter sample into the chromatograph. See Figure I for a typical chromatogram.

8. Procedure

(a) Weigh 10.0 grams of the sample into a four-ounce bottle.
(b) Add 20.0 milliliters of chloroform and shake for one hour.
(c) Place the solution in a centrifuge tube and, with proper balancing, centrifuge for five minutes.
(d) Using an eye-dropper, draw off as much of the clear chloroform layer as possible into a two-ounce bottle.

(e) Add 25 ml. of 1 N sodium hydroxide to the chloroform extract and shake for 15 minutes (Note 11c).
(f) Centrifuge for five minutes.
(g) Using a five-milliliter syringe, draw off as much of the bottom chloroform layer as is possible into a small vial. Note this volume.
(h) Evaporate to dryness in a hood.
(i) Take up with chloroform to 5.0% of the volume noted in step (g). This final solution represents ten grams of sample per ml. of chloroform.
(j) Inject 1.0 microliter into the chromatograph and measure the response of the 2,3,7,8-tetrachlorodibenzo-p-dioxin. Figure II shows a representative chromatogram.

9. Calculations

Let:

A = The area of the 2,3,7,8-tetrachlorodibenzo-p-dioxin in the sample.

B = The attenuation of the chromatograph for the sample.

C = The micrograms per milliliter of the 2,3,7,8-tetrachlorodibenzo-p-dioxin in the standard.

D = The area of the response from the 2,3,7,8-tetrachlorodibenzo-p-dioxin in the standard.

E = The attenuation of the chromatograph for the standard.

Then,

$$\text{ppm of 2,3,7,8-tetrachlorodibenzo-p-dioxin} = \frac{A \times B \times C}{D \times E \times 10}$$

10. Accuracy

The accuracy of this method is $\pm$ 5%, or less, relative.

11. Notes

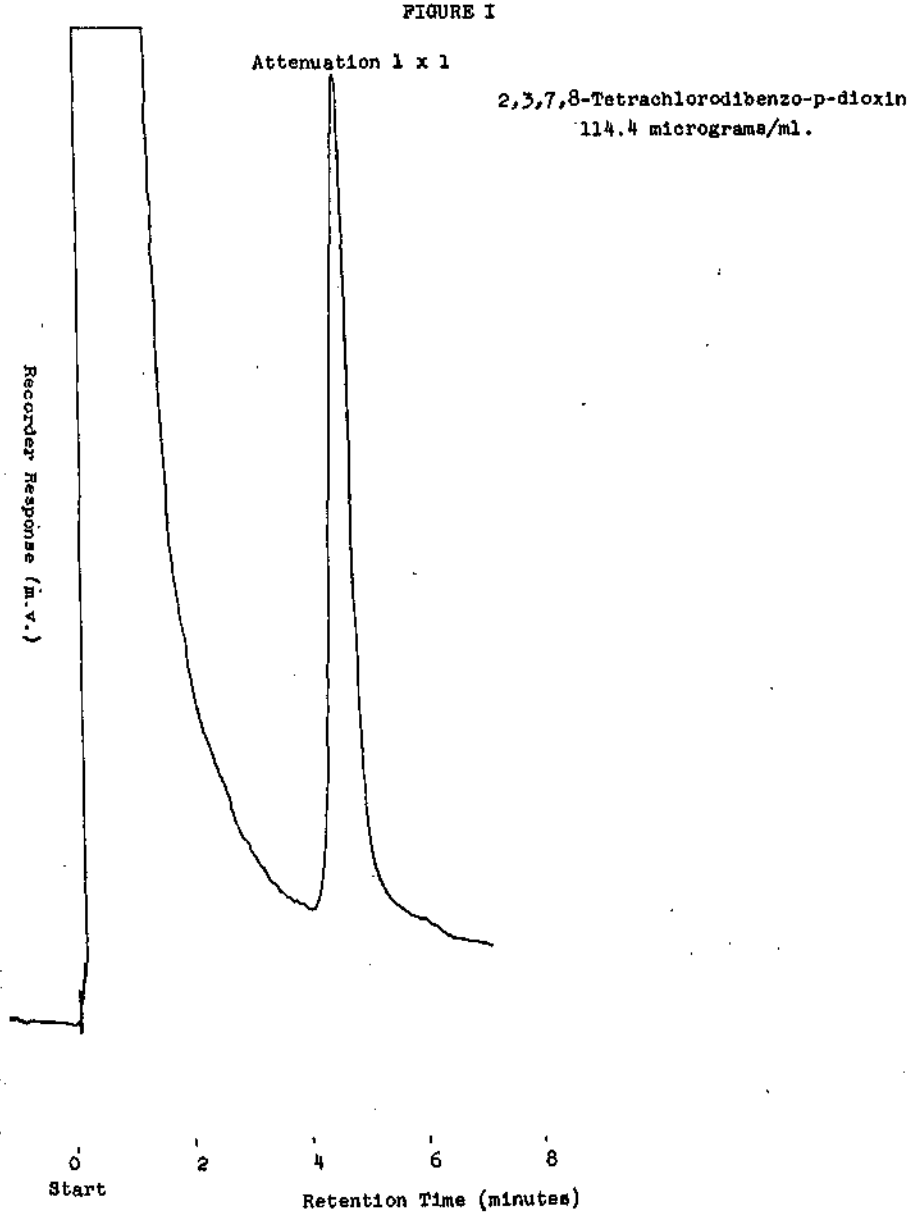
(a) Glass inlet liners have been found to be necessary to provide reproducible results.

(b) Silicone FS-1265 (fluro) has been found to work well as a stationary phase. It also is available from Wilkins Instrument and Research, Inc., Walnut Creek, California.

(c) Any 2,4,5-trichlorophenoxyacetic acid which has dissolved in the chloroform extract must be removed as it will interfere with the chromatographic analysis of the 2,3,7,8-tetrachlorodibenzo-p-dioxin.

* * * * * * * * * * * *

FIGURE I



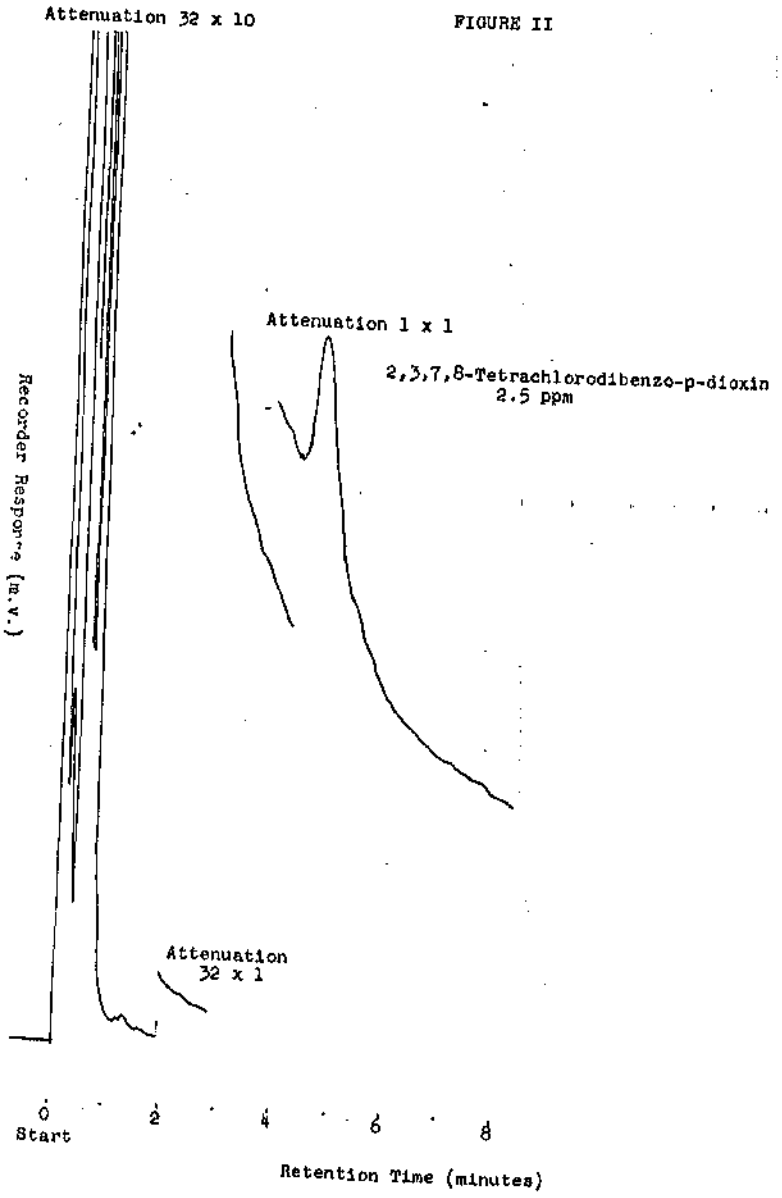


FIGURE II

Dr. JOHNSON. Thank you.

Senator Hart, the 2,4,5-trichlorophenol plant was redesigned to insure, insofar as possible, the production of a product containing a minimum of the tetrachlorodibenzo-p-dioxin. By so doing we were able to control the quality of Dow 2,4,5-T.

By May 1965 we had the technology to establish a manufacturing specification of no detectable 2,3,7,8-tetrachlorodibenzo-p-dioxin in 2,4,5-trichlorophenol and 2,4,5-T, using an analytical method sensitive to 1 p.m.m. While the plant was being rebuilt, we purchased 2,4,5-trichlorophenol and 2,4,5-T on the basis of this specification.

The new plant came on stream in 1966 and since that time Dow 2,4,5-trichlorophenol and 2,4,5-T have met this specification, and most has contained less than 0.5 p.p.m. of the 2,3,7,8-tetrachlorodibenzo-p-dioxin.

I apologize for repeating these long chemical names but the position and number of chlorines is important.

Senator HART. The day will come when I can pronounce them, even if I can't understand them. I can't do either yet.

Dr. JOHNSON. When the difficulty was encountered in 1964 we notified the Michigan Department of Health, the Institute of Industrial Health, University of Michigan, and various other health oriented individuals in private medicine and industry.

In addition we called a meeting which was held in March 1965 to notify other manufacturers of 2,4,5-T of the difficulties encountered. We described to them the nature of the health hazard and shared our test procedures and analytical standards.

With this background—and firsthand experience—it was only natural that my associates and I would inquire about the identity of the sample used for the Bionetics tests. The 2,4,5-trichlorophenol tested was Dow material and the 2,4,5-T was a Diamond Alkali sample.

It is important to emphasize that 2,4,5-trichlorophenol was reported to show no significant increase of anomalies by the Bionetics Laboratory, but the sample of 2,4,5-T did display a significant increase of anomalies.

This prompted examining our past records of tests run in 1964. The records of analytical determinations of different supplies showed that samples of Diamond Alkali 2,4,5-T in fact did contain tetrachlorodibenzo-p-dioxin up to levels of 16 ppm. It should be emphasized at this point that Diamond Alkali has since stopped manufacturing 2,4,5-T.

I presented the essence of the above information to the Mrak Commission November 7, 1969, and showed pictures of the chloracne observed in humans and pictures illustrating the rabbit ear test. Moreover, I stated that the Bionetics test with 2,4,5-T may have been complicated by an impurity in the 2,4,5-T.

I further emphasized the importance of tests using procedures recognized among experts as being valid and meaningful; the importance of representative materials which could be better obtained by consultation with industry; and the importance of knowledge of composition and purity of the materials tested. These points were made in the course of writing the final draft or recommendations of the Mrak Commission.

In view of our knowledge of the low mammalian toxicity of 2,4,5-T and the absence of reports of increased incidence of birth defects in cattle or sheep grazing rangelands sprayed with 2,4,5-T, we found it difficult to believe that any practical hazard existed from the registered uses of 2,4,5-T.

It became important to gain additional evidence as soon as possible as to whether (1) the sample of 2,4,5-T tested by Bionetics was contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin; (2) if so, could the tetrachlorodibenzo-p-dioxin itself be responsible; and (3) would 2,4,5-T of a specification made by Dow cause similar birth abnormalities.

I asked Dr. Dale Lindsay of FDA if a conference could be arranged with appropriate individuals in DHEW to discuss protocols for tests which would be acceptable to their scientists. Dr. Lindsay asked Dr. McLaughlin of FDA to arrange a meeting which was held November 25, 1969. Present at this meeting were:

Dr. I. Mitchell and Dr. R. Bates of the National Cancer Institute; Dr. J. McLaughlin, FDA; Dr. J. E. Johnson; Mr. D. D. McCollister; Dr. V. B. Robinson; and Mr. V. K. Rowe of Dow.

I requested that Dr. Mitchell identify the test procedure by which we could reexamine 2,4,5-T and the suspected contaminant. Dr. Mitchell replied that tests with Sprague-Dawley rats would be the best procedure for reconfirmation and further stated that, for the purpose, it would be superior to a test with mice.

I offered to underwrite the cost of confirmatory experiments in the laboratories of the National Institute of Health, in the laboratories of a third party (independent of Government or Dow) or in the Dow laboratories open to observation at any time by personnel of the Department of HEW.

Dr. Mitchell stated that he would have confidence in the work if it were done in Dow laboratories. We agreed to repeat the Bionetics work with Sprague-Dawley rats using Dow 2,4,5-T of regular production grade. If this study yielded positive results the Bionetics results would be confirmed. If the results were negative it would be necessary to run further tests on graded levels of the contaminant and on refined 2,4,5-T. It was agreed that Dow would provide samples of 2,4,5-T and 2,3,7,8-tetrachlorodibenzo-p-dioxin to the National Institute of Environmental Health Science laboratories at Research Triangle, N.C.

Moreover—Robinson and Rowe of Dow would visit the NIEHS laboratories in order to confer with them concerning the details of the test methods to be used.

On December 1, 1969, I met with Dr. DuBridge and Dr. Buckley of the OST to apprise them of the possibility of a contaminant in the sample tested by Bionetics and also the information known to Dow. At this meeting the same points were discussed as presented to the Mrak Commission. The plan for additional testing as discussed with Drs. McLaughlin, Bates, and Mitchell of DHEW was also presented.

Dr. DuBridge stated that he would be interested in further information as it developed and was willing to consider new evidence when it was available. I promised to report the results of our work.

Information has been supplied primarily through Dr. Burger of the OST.

On December 11, 1969, Dr. V. B. Robinson and V. K. Rowe met with Drs. Falk, Courtney, and Gaylor at the Research Triangle and discussed with them the design of teratological study to be conducted on Dow regular production 2,4,5-T. Agreement on the design of the experiment was easily achieved and was followed in our studies.

At this meeting Dr. Courtney of the NIEHS Laboratory provided a two gram sample of the 2,4,5-T used by the Bionetics Laboratory. This sample was examined at Dow with the following results:

1. Rabbit ear tests showed a positive reaction characteristic of the contaminant.

2. Analysis by gas liquid chromatography indicated the presence of 27 plus or minus 8 p.p.m. of 2,3,7,8-tetrachlorodibenzo-p-dioxin.

In late December Dr. Burger of the OST requested a review of the chemistry of 2,4,5-T production to be presented to Dr. Baldeschweiler, a consultant of the agency. The information for this report was organized by Dr. Blair of Dow and presented at a meeting with the OST on December 29, 1969 in Washington.

Dr. Blair is on my right.

By January 12, 1970, we had made enough progress in the teratological study in rats with Dow production grade 2,4,5-T to make a report to Dr. Egeberg, Assistant Secretary for Health and Scientific Affairs, HEW. Copies were sent to other involved persons in DHEW and USDA. This report showed that the Dow 2,4,5-T of regular production grade did not cause birth defects as determined by gross examination of fetuses. The dosage levels used were selected in consultation with Drs. Falk, Courtney, and Gaylor of NIEHS.

Furthermore, we were able to report to Dr. Egeberg that a pilot study with pregnant rabbits fed the same 2,4,5-T had not caused birth defects. Dr. H. L. Richardson, pathologist, FDA, observed the results of both of these tests.

These preliminary observations were followed by the more time consuming microscopic examinations of the tissues and detailed skeletal examinations. This work confirmed the preliminary findings.

The final report of the study was presented before the Society of Toxicology in Atlanta, Ga., on March 17, 1970. I wish to insert into the record at this point an abstract of this report entitled "Teratogenic Study of 2,4,5-trichlorophenoxyacetic Acid in the Rat" by J. L. Emerson, D. J. Thompson, C. G. Gerbig, and V. B. Robinson, The Dow Chemical Co.

Senator HART. That will be received.

(The information follows.)

A study to determine the embryotoxicity or teratogenicity of 2,4,5-tricholorophenoxyacetic acid containing less than one part per million of 2,3,7,8-tetrachlorodibenzo-p-dioxin has been completed in Sprague-Dawley derived rats. Five treatment groups, each consisting of 25 females were administered 1, 3, 6, 12 or 24 mg/kg/day of the compound via gavage in 0.25% METHOCEL® on days 6 through 15 of gestation. A single group of 50 females received the suspending vehicle and served as controls. The following parameters were examined: clinical observations, maternal body weights (prebreeding and day 20), number and position of fetuses and resorptions, number of corpora lutea, pup weight and sex, gross external examination of pups, and macroscopic examination for intestinal hemorrhage in pups. Two-thirds of each litter were fixed in Bouin's solution and one-third was prepared for alizarin red-S staining

and skeletal examination. Examination of Wilson sections under the dissecting microscope or of alizarin stained skeletons of all fetuses from the 24 mg/kg/day group and an equal number of control fetuses was performed. Representative stained histologic sections through the head, thorax, and abdomen of 10 control and 10 high level fetuses were studied for histopathologic changes.

No clinical or gross pathologic signs of adverse chemical effect were observed in treated dams during the period of treatment or gestation. Similarly, litter size, number of fetal resorptions, birth weights and sex ratio of pups appeared to be unaffected by chemical treatment. Skeletal and visceral examination of high level and control fetuses as well as histopathologic examination of certain fetuses failed to reveal any teratogenic or embryotoxic effects.

The results of this study fail to substantiate the findings reported recently (unpublished data: Bionetics Research Laboratories, Bethesda, Maryland) of serious effects in fetuses obtained from dams given comparable daily doses of 2,4,5-T containing approximately 27 parts per million of the contaminant, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Dr. JOHNSON. Thank you.

In accordance with the plan discussed with the DHEW in December, as soon as the preliminary results of the 2,4,5-T study on rats indicated no fetal anomalies, we proceeded to conduct a teratology study in rats with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Dosages were used which bracketed the levels of the contaminant which were given inadvertently to the rats in the Bionetics study. The results of this experiment indicated that a high of maternal and fetal toxicity was associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Dr. H. L. Richardson of FDA and Dr. C. T. G. King, National Institute of Dental Research, NIH, participated in the observations made on these animals at necropsy at the Dow Laboratories in Midland.

We concluded that the presence of the tetrachlorodibenzo-p-dioxin in the sample tested in the Bionetics Laboratories could have accounted for the observations reported and attributed 2,4,5-T. At this point I wish to insert into the record an abstract of the report as presented to the Society of Toxicology, March 17, 1970, Atlanta, Ga., entitled "Teratogenic Study of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Rat"[1] by G. L. Sparschu, F. L. Dunn, and V. K. Rowe, The Dow Chemical Co.

Senator HART. That will be inserted.

Dr. JOHNSON. Thank you.

The detailed results of these tests were also presented by Dow personnel to the scientists of FDA, NIH, NIEHS, in Washington on February 24, 1970.

In addition to our investigations with laboratory animals, we have also utilized the medical records of Dow employees accumulated throughout their Dow careers.

Our physicians have made an in-depth evaluation of the health of male employees who have been exposed to 2,4,5-T in manufacturing operations for from 6 months to approximately 20 years. From the medical data available, over 50 clinical parameters were selected for statistical evaluation. The control population for this evaluation consisted of 4,600 other individuals for whom similar data were available.

After careful study of this information, it was the conclusion of our medical staff that there was no evidence that exposure to 2,4,5-T had resulted in adverse effects.

[1] See p. 470.

It is our belief that the adverse effects reported by the Bionetics Laboratories were the result of a contaminant—2,3,7,8-tetrachlorodibenzo-p-dioxin—and were not caused by 2,4,5-T.

Moreover it is our belief that 2,4,5,-T produced under specifications requiring less than 1 part per million of 2,3,7,8-tetrachlorodibenzo-p-dioxin present no practical hazard when used in accordance with good agricultural practices.

Now, from here on it is historical.

If I may I would like to add some recent information and this like the information presented this morning, was only obtained this past weekend, as a matter of fact.

The Midland Labs extended the work by Dr. Robinson using the Dow 2,4,5-T on rats. This is the production grade of Dow 2,4,5-T. The dosage of 50 milligrams per kilogram, higher than that recommended in the consultation at NIEHS, was administered. At this dose we found one fetus in 203 viable fetuses with intestinal hemorrhage. At the 100 milligram per kilogram dose level; there was 75 percent mortality of the dams and high fetal mortality.

At this point I should point up that these levels of 2,4,5-T put extreme stress on the physiology of the test animal.

The next experiment involved drinking water supplied to rats. This drinking water was saturated with 2,3,7,8-tetrachlorodibenzo-p-dioxin and in this case no effect was detected in 21 litters of 259 pups.

In a third experiment we studied combinations of pure 2,4,5-T with 2,3,7,8-tetrachlorodibenzo-p-dioxin. In this experiment 2,4,5-T was fed at 50 milligrams per kilogram and maintained at a base level throughout all tests to which graded or incremental dosage of additional 2,3,7,8-tetrachlorodibenzo-p-dioxin were added. Bear in that this base amount of 2,4,5-T was administered orally in a single dose each day.

I will read the list of results. When 50 milligrams per kilogram of pure 2,4,5-T were administered with zero added 2,3,7,8-tetrachlorodibenzo-p-dioxin, no adverse effects were observed in rats.

When one-hundredth of a microgram per kilogram of the tetrachlorodibenzo-p-dioxin was combined with the 50 milligrams of 2,4,5-T, again no effect was observed. When three-hundredths of a microgram per kilogram per day of the tetrachlorodibenzo-p-dioxin was combined, again no effect was observed. When six-hundredths of a microgram per kilogram of the tetrachlorodibenzo-p-dioxin was combined with the 2,4,5-T, we observed 8 percent of gastrointestinal hemorrhages and one cleft palate in 155 fetuses. When 0.125 micrograms of the tetrachlorodibenzo-p-dioxin was combined we had an increase in the resorptions and some gastrointestinal hemorrhage. We also saw subcutaneous edema in three of the 134 fetuses.

When five-tenths and 1.0 micrograms of the tetrachlorodibenzo-p-dioxin were combined with the base level of 2,4,5-T, we observed an increased incidence in the fetal mortality, in resorptions, in gastrointestinal hemorrhages, and in subcutaneous edema, cleft palates were observed in four out of 14 and five out of 15 litters respectively.

Again I should stress the base level of 2,4,5-T was extremely high, approaching the toxic dose; moreover the base level of dioxin was extremely high.

Is there any further comment to be added on that, Mr. Rowe?

Mr. Rowe. I think you covered it very well.

Dr. Johnson. These data were only available yesterday. The microscopic study of soft tissues and skeletal study have not been done. These animals were stressed to the limit of their tolerance of 2,4,5-T and, in addition, to a toxic stress of the tetrachlorodibenzo-p-dioxin.

I personally feel it is important to consider the dosage related to the response with these materials and later I will get into a more definitive discussion of that, if you desire.

Based on the proposed finite tolerance of 2,4,5-T in food but less than the 1 part per million of tetrachlorodibenzo-p-dioxin in the 2,4,5-T the safety factor for humans as derived from animal studies in several thousandfold. This is well in excess of the safety factors judged adequate by toxicologists in some branches of government and many others in the scientific community.

I would like to make a few comments, because the word dioxin has become almost a cause celebre, in order that we get some conception of the amounts involved. If this rather simple illustration is okay, I guess it really isn't very simple but I will try it, if it is all right.

Now, this is assuming what is *not* going to happen. It is assumed that all food ingested by man contained 0.2 part per million of 2,4,5-T. This is not going to happen but I am just making that assumption for purposes of illustration.

The total, 2,4,5-T ingested per day in the food of a person would be three-tenths of a milligram, that is equal to 300 micrograms, a very small amount. If the 2,4,5-T contained 1 part per million of the tetrachlorodibenzo-p-dioxin, the daily food would contain one one-millionth of this 300 micrograms or——

Senator Hart. Doctor, back up.

If the two parts per million——

Dr. Johnson. Two-tenths.

Senator Hart. Had how much dioxin?

Dr. Johnson. One part per million.

May I proceed?

Senator Hart. Yes.

Dr. Johnson. If the two-tenths parts per million of 2,4,5-T in the food of a person contained one part per million of tetrachlorodibenzo-p-dioxin, the food would contain one-millionth of the 300 micrograms or 300 picograms.

A picogram is one-trillionth of a gram and a gram is one-twenty-eighths of an ounce.

If a person ingested this amount of dioxin each day for 100 years, which is an optimistic period, the total amount ingested would be only 11 micrograms. A grain of sugar weighs about 120 micrograms. So this 11 micrograms of tetrachlorodibenzo-p-dioxin would be no more than one-tenth of a granule of sugar and that level affords a 6,000-fold safety factor over the amounts, as we observed, to cause no embryotoxic effects in rats.

I realize that this has nothing but illustrative value, but it is not very much the tetrachlorodibenzo-p-dioxin we are talking about. That is my main point, Senator.

Senator Hart. All right, some who are better equipped than I to handle the technical aspects may pursue it with you. I just confess that I will have to inhale it if not ingest it.

Dr. Johnson. The simplest way to think of it is one-tenth of the weight of a grain of sugar over a lifetime. Senator Hart, I am not sure how you would like to proceed. I have some other remarks to make which get to this question, perhaps in shortening the time interval. Would you prefer for me to answer questions now or at a later time?

Senator Hart. I have a few questions. Suppose we ask a few questions here and if they do not raise items that you intended to discuss, we would welcome the additional reaction.

One very quick one has to do with the test that you report having made on 110 male employees. I am sorry, 130 with no evidence of adverse effect.

Has there been any evaluation made of the effect on female employees or were any exposed?

Dr. Johnson. None were exposed, Mr. Chairman.

Senator Hart. Was that on purpose consciously?

Dr. Johnson. No, the manufacturing plant is not a desirable place for most women to put in their time.

Senator Hart. I am not sure you speak for all women. That is another subject entirely.

Dr. Johnson. This has unraveled far enough.

Senator Hart. I am sorry, I didn't realize there was a vote signal and we must recess to permit me to get to it. So I will be back shortly.

(Recess.)

Senator Hart. Doctor, let us ask these few questions and then if they do not raise all of the items that you would like to make additional comment on, we would welcome your making a comment.

When did you first have reason to believe that a dioxin contaminant in 2,4,5-T could cause chloracne?

Dr. Johnson. Just a moment, sir, and I will get the exact date.

Senator Hart. Yes.

Dr. Johnson. Senator Hart, in regard to the 2,4,5-T, it was 1964 when we first developed an awareness of this possible buildup of the dioxin potential in the product—in October.

Senator Hart. There was no, as far as you were aware, earlier study here or in Europe that identified tetradioxin as causing chloracne?

Dr. Johnson. Not in 2,4,5-T.

Senator Hart. Tetradioxin in some formula had been found to cause chloracne?

Dr. Johnson. Yes. In 1950 the Germans ran into difficulty with chloracne and they isolated the dioxin. This was in the process of manufacture of 2,4,5-trichlorophenol. We were aware of it.

Senator Hart. When did you begin the manufacture of 2,4,5-T?

Dr. Johnson. We began the manufacture of 2,4,5-T in 1948. I should point out in addition, however, that we were monitoring our workers for chloracne since 1941. This was also when the rabbit ear test was developed by Dow.

Senator Hart. If the German study indicated the tetradioxin as a chloracne cause in 1950, would that have suggested to you any test run on the potential presence or dangers of this contaminant in the 2,4,5-T that you were introducing?

Dr. Johnson. Yes, and we monitored products made with 2,4,5-trichlorophenol knowing that the dioxin resided in the intermediate. It did not appear in the 2,4,5-T until 1964.

Senator HART. Did those tests include any tests to determine the carcinogenicity of dioxin in the formula that you were producing?

Dr. JOHNSON. No, sir, they did not.

Senator HART. Then in June of 1964 you were concerned about the chloracne, and in your testimony you say I think that you notified a number of people. You notified the Department of Health of Michigan and the Industrial Health Institute in Ann Arbor and various other health oriented individuals in private medicine and industry, and you had the meeting in March of 1965 notifying other manufacturers. Why not the FDA and the U.S. Department of Agriculture?

Dr. JOHNSON. At that time, Senator Hart, we considered our obligation discharged by removing the dioxin from our product, by notifying health authorities in the State and we thought we had the problem solved.

In retrospect it would have been much preferred had we notified the U.S. Department of Agriculture, the agency that has statutory authority for the registration.

Senator HART. I would agree. It would seem to me, and as I say, it is easier second guessing, that it would have been more appropriate and foremost to notify the agency that registers the product. But what about the 2,4,5-T that you learned in June of 1964 had this contaminant? Is it a practice to make an effort to remove the contaminated product from the shelves? What about the product in the houses and retail channels? What retrieval effort is made? What call back?

Dr. JOHNSON. Senator Hart, according to the procedures we were using at the time we did not produce or sell contaminated 2,4,5-T within the limits of sensitivity we had available for measure.

Senator HART. What was it you were notifying people about in 1964?

Dr. JOHNSON. This was the chloracne problem.

May I add a comment?

Senator HART. Yes.

Dr. JOHNSON. As indicated in the formal testimony, the manufacture of 2,4,5-T, when pushed by temperature or heat, will produce this contaminant; it builds up as a caustic insoluble oil. This problem produced the chloracne which initiated the actions we took.

Senator HART. What percentage of dioxin was in the 2,4,5-T that you produced prior to the correction made at this time?

Dr. JOHNSON. It was—now you are asking about the 2,4,5-T?

Senator HART. I beg your pardon?

Dr. JOHNSON. You are asking about the 2,4,5-T. At the time there was an undetectable amount using the rabbit ear test as an indicator.

It might be important to point out that chromatographic procedures for analysis were developed during the late 1950's and early 1960's and applied with increasing sensitivity. This is a changing background of analytical capability. At the time we were using the rabbit ear test and we did not know that dioxin was present, if any.

Senator HART. As of now, do you believe that the earlier 2,4,5-T that you were producing was safe or unsafe?

Dr. JOHNSON. Safe, because we were monitoring the intermediate 2,4,5-T-chlorophenol and similar products since 1941.

Senator HART. But you really do not know how much dioxin was in it. How can you say that?

Dr. JOHNSON. It was, according to our ability to determine dioxin, at that time, we thought zero.

Senator HART. But you know better now, don't you?

Dr. JOHNSON. We know better now because we have more sensitive methods.

Senator HART. How can you say it was safe earlier when we know now it was not?

Dr. JOHNSON. In our firm, Mr. Chairman, the matter of safety is considered to be related to the dosage. The product as we sold it and monitored it; and, as it was used, according to the label, we are convinced it was safe. There was not sufficient exposure to the tetrachlorodibenzo-p-dioxin, even if it had been there in the amounts known today, one part per million or a half part per million. We are convinced it was safe.

Senator HART. Did you feel any requirement at any time to engage others in making the judgment which you just made about the earlier formula?

Dr. JOHNSON. The answer is no.

Senator HART. Now, do you agree with the position that has been taken, as announced this morning by the three Secretaries?

Dr. JOHNSON. In a matter of practical hazard, an an imminent hazard to health, I do not agree. Under the climate of pressure today, it was a wise decision.

Senator HART. That sounds like you are planning to run for reelection, but you do not want to announce it.

I am reminded that the action taken today was to cancel, not to suspend the nonliquid 2,4,5-T and that means, as I understood the Surgeon General's testimony this morning, the Secretaries do not regard that form of 2,4,5-T as imminently hazardous to health.

Do you want to rephrase your answer so as to respond specifically to the finding on the nonliquid?

Dr. JOHNSON. The nonliquid form I consider to be safe under labeling registrations.

Senator HART. I think the Food and Drug Committee finds it has potential hazard to health and therefore cancels rather than suspends it. Do you agree with that?

Dr. JOHNSON. I do not agree that cancellation is necessary.

Senator HART. Is it your intention to appeal the action?

Dr. JOHNSON. This must be considered. I cannot answer at this time. We have few, if any, products of our own that are nonliquid formulations of 2,4,5-T.

Mr. BICKWIT. Do you have any evidence on the degradeability of 2,4,5-T?

Dr. JOHNSON. Yes. Just one moment, please. The evidence is present in the literature published by the land grant colleges and the U.S. Department of Agriculture, predominantly. A publication by Dr. P. C. Kearney, E. A. Woolson, J. R. Plimmer, and A. R. Isensee, reviews the subject of degradation in a chapter entitled "Decontamination of Pesticides in Soils."

Page 139 indicates the persistence of 2,4,5-T to last 5 months. There are additional references and review articles that, if you like, I could submit for the record. It would take quite a bit of time to read these, but I could do so, Mr. Chairman, if you like.

Senator HART. They will be received.

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Decontamination of pesticides in soils

By

P. C. Kearney,* E. A. Woolson,* J. R. Plimmer,* and A. R. Isensee*

I. Introduction

The soil, as a medium for decontamination, offers a large number of processes by which organic substances can be destroyed. As such, progressive accumulation of organic pesticides would appear to be unlikely. Unfortunately, the chemical and physical properties of certain insecticides and herbicides afford them a degree of stability against the natural destructive processes in soils. The stability of these compounds is best illustrated in a recent summary of persistence data on 12 major classes of pesticides in a number of soil types (Fig. 1) (Kearney *et al.* 1969). Persistence values are expressed in months and each bar represents one or more classes of herbicide or insecticide. Each open space in the bar represents an individual pesticide falling within the larger chemical class of compounds. The length of each bar depicts the time for each class of pesticide to decrease 75 to 100 percent of the amount applied. These values are based on normal rates of application. As anticipated, the organochlorine insecticides are the most persistent pesticides. The organic herbicides persist for a few days or for more than 12 months depending on their respective properties. Only the

* U.S. Department of Agriculture, ARS, CR, Beltsville, Md. Specific mention of trademark instruments does not constitute an endorsement by the U.S. Department of Agriculture over others designed to give similar performances.

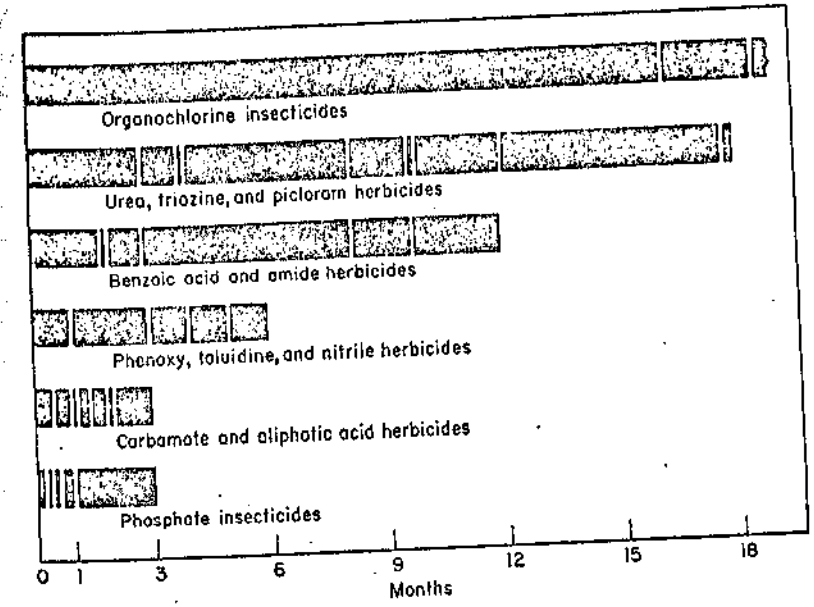

Fig. 1. Persistence of pesticides in soils

major herbicides that persist for a month or longer are shown in Figure 1. The phosphate insecticides do not persist for long periods in most soils. A more detailed picture of organochlorine pesticide persistence is shown in Figure 2. Chlordane and DDT usually persist for several years while heptachlor and aldrin extend their activity through the formation of their respective metabolites, *i.e.*, heptachlor epoxide and dieldrin.

Why are we concerned about pesticide residues in soils? Their effects would appear to be very subtle and not directly related to man or his environment. The need for pure water is obvious, for man directly consumes processed water. Not so with soil, and therefore, why is there concern over soil contamination?

There now exists unequivocal evidence that most plants can absorb and translocate residual pesticides from contaminated soils (Nash 1968). Uptake and translocation have been demonstrated with radio-labeled pesticides incorporated directly into the soil and then seeded with several agronomic crops. Many of the soil variables that influence this uptake process have been studied. For example, increasing the concentration of dieldrin and DDT in soil causes a corresponding increase in the amount of insecticide recovered by the wheat plant. The plant is apparently indiscriminate in its ability to absorb most substances from soils. Therefore a link exists between residual pesticides in soils and man's food chain. In addition, residual pesticides are potential pollutants of water and air.

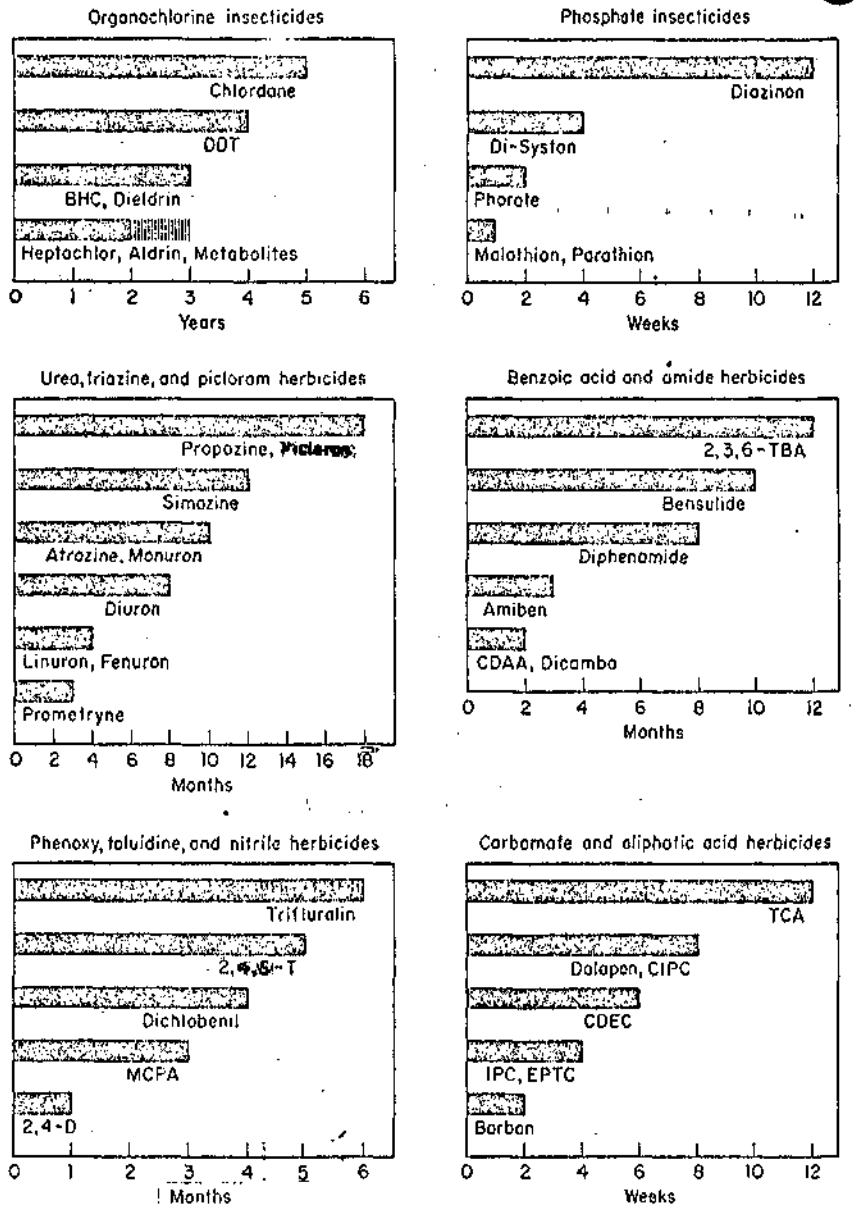

Fig. 2. Persistence of individual pesticides in soils

Now that we have defined the problem and the need for decontamination in soils, what methods are available for removing persistent pesticides? The fate of a pesticide in soils is determined by a number of processes which come under the general heading of physical, biological, and chemical (Fig. 3). Under physical, they include photo-

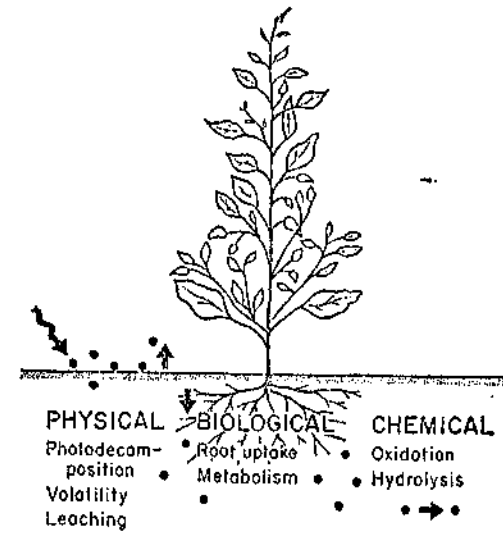

Fig. 3. Processes that determine fate of a pesticide in soil

decomposition, volatility, leaching, and adsorption. Under biological, they include root uptake and microbial metabolism and under chemical, they include oxidation, reduction, and hydrolysis. Several of these are responsible for decomposing pesticides. For example, soil enrichment techniques for the proliferation of specific microorganisms effective in metabolizing foreign substances have been a favorite method for microbiologists. It is conceivable that "catch plants" or plants with a high affinity for certain pesticides could be grown on contaminated soils and then removed after taking up some part of the residual pesticides. It is possible that a combination or several of these methods could be employed to reduce pesticide concentrations in soils.

If each of these processes were active on a pesticide, then soil residues would not exist. If soil microorganisms could be induced to rapidly metabolize DDT to the level of carbon dioxide, then soils would be an ideal medium for decontamination. They don't, and therein lies the problem of reducing soil residues. The work to be reported today deals with two approaches to reducing pesticide residues. The first concerns the use of light to decompose transported pesticides in water and hence its application to irrigation waters and soils. The second concerns the decontamination of field soils containing DDT by flooding and inoculation with microorganisms.

II. Pesticide decontamination in water

A major source of environmental contamination is caused by the movement of materials from their site of application. Pesticides move primarily in the liquid or vapor phase. Pollution of water by organic

compounds is undesirable, contamination by biologically active compounds is potentially dangerous. Two particular situations in which pesticides in water cause concern relate to the waste problem encountered in static or lagoon operations and to irrigation systems. The danger of the latter situation is best illustrated with the water-soluble, mobile herbicide picloram (4-amino-3,5,6-trichloropicolinic acid). Minute amounts of this potent herbicide irrigated on sensitive crops could have disastrous results. Concentrations as little as 10 p.p.b. in soils have a lethal effect on such sensitive crops as soybeans.

What methods are available for removing pesticides in water? The use of energetic radiation (ultraviolet or gamma ray) has been suggested (Mancus *et al.* 1962) for fragmentation or destruction of organics in water. This method should be effective on a large number of pesticides especially in dilute aqueous solutions. Unfortunately, the technology has developed little beyond the experimental stage. Large scale ultraviolet and gamma irradiation techniques are in early technological stages and wider industrial application is the needed stimulus for further development.

We have determined the periods of exposure required to destroy the biological activity of a number of herbicide solutions in a small-scale ultraviolet irradiator. The method may be applicable as a pretreatment for waste waters or as a treatment for contaminated irrigation systems.

The reaction system is a borosilicate glass vessel and holds 250 ml. of the solution to be irradiated (Fig. 4). A quartz, water-cooled, double-walled tube is fitted into this well and is immersed in the solution. A 450 watt Hanovia lamp is suspended in the well. The quartz well transmits a large part of the available energy down to the shortest wavelengths emitted by the lamp.

Solutions of herbicides in water were irradiated (250 ml. at a time) for periods of 5, 10, and 15 minutes. Picloram, 2,4,5-T, bromacil, diphenamid, and 2,3,6-TBA were the herbicides used in the initial experiments. These compounds were chosen because their solubility and persistence are sufficiently high for them to be potential contaminants in irrigation water. Oats were used to bioassay picloram, 2,4,5-T, and diphenamid and cucumber was used for bromacil and 2,3,6-TBA. The treatments consisted of zero, 5, 10, or 15 minute irradiations of the solutions at 1, 5, or 10 p.p.m. concentrations and a control in which no herbicide was added. The time required to destroy the five herbicides is shown in Figure 5 (Plimmer 1968).

A five-minute irradiation greatly reduced the phytotoxicity of picloram and 2,4,5-T at five and 10 p.p.m. and bromacil at one p.p.m. Diphenamid and 2,3,6-TBA required a 10-minute exposure. These initial results indicate that five minutes or less exposure to ultraviolet irradiation of solutions in the range of one p.ppm. would significantly lower their phytotoxicity to plants. More pesticides, under conditions

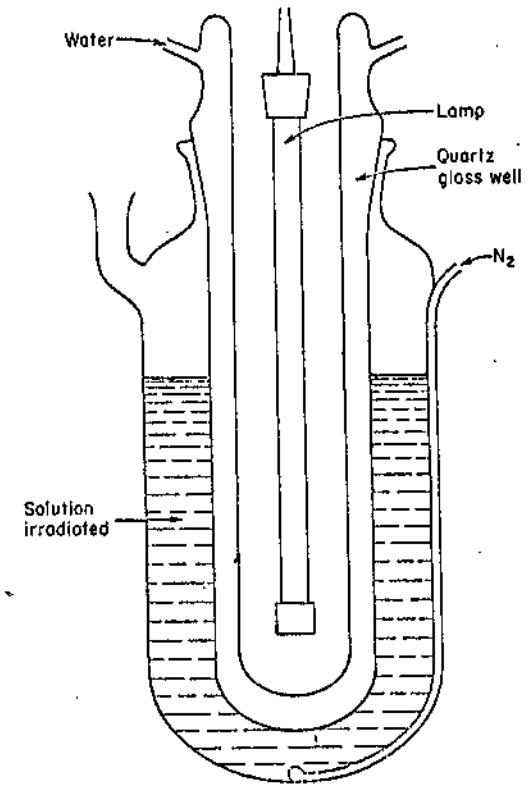

Fig. 4. Photochemical reaction vessel

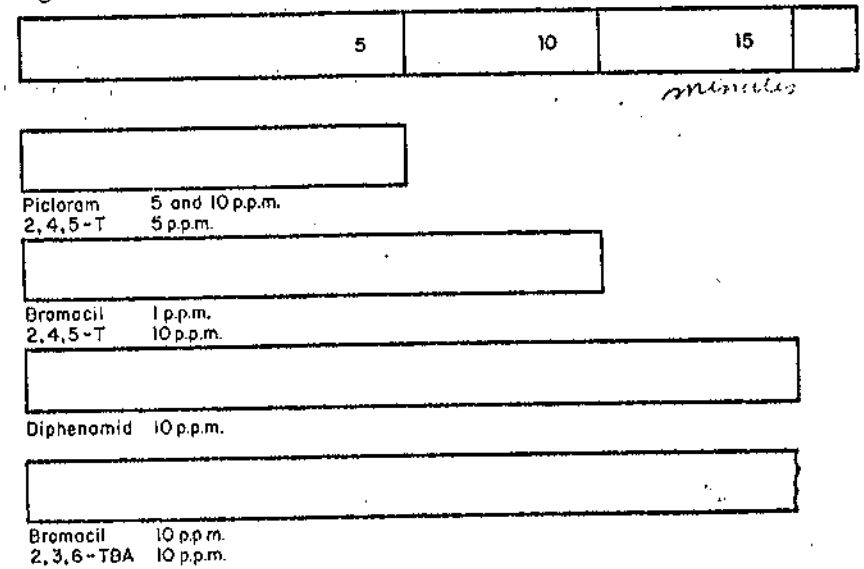

Fig. 5. Times required to destroy herbicidal activity by irradiation

approaching large volumes of water on flow systems need to be investigated before wide application to pesticide decontamination is attempted.

III. DDT decontamination

Turning our attention to field soils, one of the most serious residue problems occurs with organochlorine insecticides. As previously mentioned, DDT persists for several years in most agricultural soils. Complete removal of these residues may be impossible. However, lowering existing residues below some arbitrary threshold level may result in minimal plant residues. Our objective, then, was to find some agent in nature that could attack the DDT molecule. This agent did not necessarily have to cause complete destruction of DDT, but perhaps alter it to a more biodegradable or labile form. Obviously, organisms indigenous to most soils do not possess this agent. Intestinal microorganisms in the rat, however, are able to alter DDT extensively.

Whole cells or cell-free extracts of *Aerobacter aerogenes* catalyze the degradation of DDT *in vitro* to at least seven metabolites (Wedemeyer 1966), previously reported from rats given DDT orally (Peterson and Robison 1964). These reactions proceed by dechlorination, elimination, oxidation, and finally decarboxylation to yield dichlorobenzophenone. Therefore, it occurred to us soils inoculated with *A. aerogenes* may be capable of metabolizing residual DDT.

To test this hypothesis, three soil types were amended with zero, 5, 10, and 20 p.p.m. of DDT. The soils were Lakeland sandy loam, Hagerstown silty clay loam, and Sharkey clay. Four-hundred g. of soil were weighed into pots and DDT was applied in chloroform solution. Since metabolism of DDT by *A. aerogenes* appears to occur most rapidly in still cultures or under partially anaerobic conditions, two-thirds of the soils were flooded to simulate partial anaerobiosis. The water covered the soils to a depth of approximately one inch. One-third of the DDT-treated soils was maintained at field capacity, one-third was flooded, and one-third was flooded and inoculated with *A. aerogenes*.

Cells of *A. aerogenes* from slants obtained from the American Type Culture (ATC 13048) were mass cultured in three percent trypticase soy broth at 36° C. for eight hours. The cells were harvested by high-speed centrifugation, washed, and resuspended in the original volume of fresh broth solution. The cells were incubated for three days in still cultures, harvested again, washed, and concentrated 10-fold in a one percent yeast-extract solution. Aliquots (10 ml.) were added to the flooded soils and mixed into the surface layers. All soils were sampled at weekly intervals. Residual DDT and products were measured by electron-capture gas chromatography. Moist soil samples were extracted with a 3:1 mixture of hexane:isopropanol and injected on to a

column of five percent SE 30 on 100/120 mesh DMCS-treated Chromosorb W. Column temperature was 210° C. with a flow rate of 120 ml./minute. Detector temperature was 215° C.

A total of 18 different parameters could be examined considering there are possible three soils, three concentrations, and two treatments with *A. aerogenes*. Of primary interest is the effect of flooding with and without inoculation. Therefore, let us examine the disappearance of only DDT at the highest rates of application in the three soil types (Fig. 6). Two general trends are apparent. First DDT

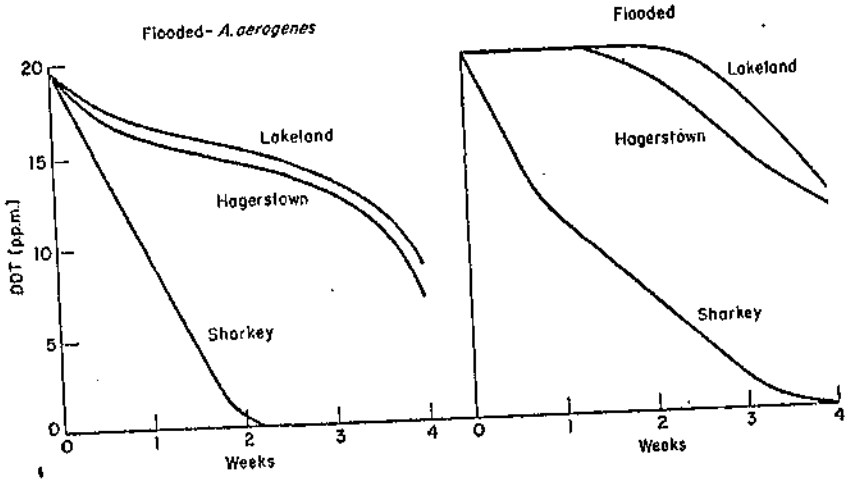

Fig. 6. DDT decomposition in three soils (see text)

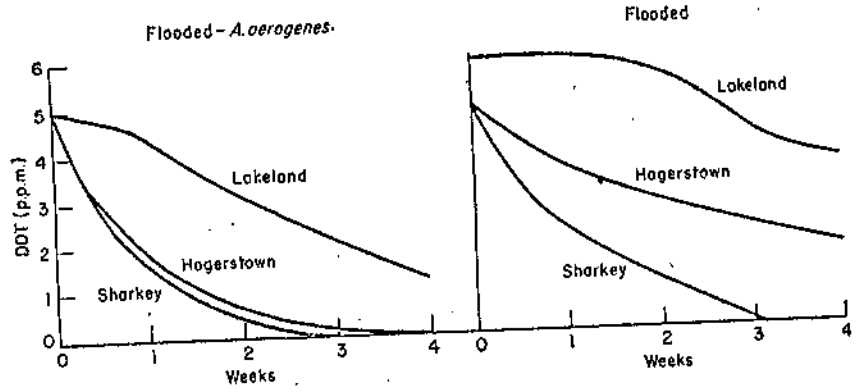

Fig. 7. DDT decomposition in three soils (see text)

disappeared more rapidly in the inoculated soils. Second, complete loss occurred in the Sharkey clay, while lesser amounts were lost from Lakeland and Hagerstown in both flooded series. DDD (TDE) was observed to occur in most soils, but its appearance did not parallel

losses in DDT. In other words, there was a net loss of DDT-DDD in this system and no other products were detected by gas chromatography. Somewhat the same picture is encountered at five p.p.m. of DDT (Fig. 7), with complete loss in the Sharkey clay, more accelerated disappearance in the inoculated soils, and a general trend for total loss of DDT-DDD with time. Recovery values for DDT during the early sampling periods on Lakeland were high and explain the values obtained above five p.p.m. in the flooded soils.

Additional studies were initiated to determine the fate of the DDT in these systems. The experiment using Lakeland soil at five p.p.m. plus DDT-^{14}C was set up in a closed glass system for trapping $^{14}CO_2$. Nitrogen was bubbled through the system and any gaseous carbon dioxide was trapped in base. Samples of the carbon dioxide trapping solution were removed periodically and analyzed for radioactivity. Less than one percent of the total added activity was released in volatile components. This would be in general agreement with results previously reported (Guenzi and Beard 1967).

Therefore, several other alternatives are available to explain the disappearance of the DDT from these soils. A polar metabolite could be present in the aqueous phase and not removed by the hexane:isopropanol extraction, or a metabolite is absorbed on some soil component and not recovered. It is also possible that a volatile metabolite is formed which escapes from the system with time. The presence of a polar metabolite can apparently be ruled out, since only 7 to 28 percent of the added DDT-^{14}C could be detected in the aqueous phase. Subsequent research with ^{14}C-DDT in a similar type of experiment indicated that up to 20 percent of the ^{14}C could not be extracted from flooded soils after a four-week incubation period. The radioactivity is apparently tightly bound to soil particles in some form not recoverable with hot hexane:acetone, ethyl acetate, or ethanol (Kearney and Woolson 1969). Therefore, we must conclude the loss is real and reproducible, although the mechanism is not fully understood.

IV. Conclusions

Radiation as a method for preventing the spread of pesticides in water systems deserves further consideration. Ultraviolet radiation for sterilization processes is in commercial use. High energy radiation has similar applications and its use in food processing has been studied. Ultraviolet radiation has been used for the complete removal of organic materials from sea water samples on a laboratory scale (Armstrong *et al.* 1966). A pebble-bed type of reactor has been described, but not further developed, which appears suitable for continuous ultraviolet irradiation of solutions. A radioactive material is incorporated into impervious ceramic "pebbles" together with a suitable phosphor which emits ultraviolet radiation. A flow system is envisaged

with the pesticide in dilute solution flowing over the ceramic pebbles. In addition to thermal methods of destruction, we suggest that radiation methods be further explored as a simple means of removing pesticides in situations where applicable.

Removal of pesticides from soils is a far more complex process, since the system is static and not conducive to flow-through operations. Several methods have been suggested for reducing residues. The use of calcium polyphosphate on the residual chloro-*s*-triazines has not been successful under field conditions (Harris *et al.* 1968). The use of absorbents (charcoal) for removing toxic materials in replanting certain nursery stock has been successful; extention of this technique to field conditions for atrazine and organochlorine residues has been attempted (Lichtenstein *et al.* 1968). In these situations, however, the cure may be worse than the sickness, since a new and far less understood variable is now being introduced into the soil. Such may be the case with microbial decontamination of DDT by *A. aerogenes*. Many experimental variables would have to be studied before large-scale field studies would be justified.

In the final analysis any decontamination method would have to be economically feasible before it would be acceptable to the farmer. The most promising and yet still unexplored method for reducing soil pesticide residues lies at the molecular level. A thorough understanding of the electronic and steric factors that render a pesticide molecule susceptible to the natural biological degradation pathways is still in early developmental stages. This approach would appear to offer the most challenging chemical method for reducing residues on a continuing basis.

Table I. *Common and chemical names of pesticides mentioned in text*

Bromacil	5-bromo-3-*sec*-butyl-6-methyluracil
DDD	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane
DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
Diphenamid	*N,N*-dimethyl-2,2-diphenylacetamide
Picloram	4-amino-3,5,6-trichloropicolinic acid
2,4,5-T	2,4,5-trichlorophenoxyacetic acid
2,3,6-TBA	2,3,6-trichlorobenzoic acid

Summary

A limited number of methods are available for decontaminating soils and water. Complete destruction of organic herbicides in water may be effected by exposure to intensive high-energy radiation. Measurement of the rates of photodecomposition of picloram, diphenamid, bromacil, 2,3,6-TBA and 2,4,5-T by bioassay techniques is in progress. Experiments with model continuous flow cells indicate rapid destruction of organic dyes. No picloram could be detected in a solution of initial concentration of 1 p.p.m. after 30 minutes or less exposure to high intensity UV irradiation. Where complete removal is not feasible, reduction of existing residues below a level in soils where the

significance of plant uptake becomes minimal may be desirable. Biological alteration of a persistent pesticide to a more degradable form is another method of reducing residues. DDT residues in three soils (Sharkey clay, Hagerstown silty clay loam, and Lakeland sandy loam at rates of 5, 10, and 20 p.p.m.) were reduced in flooded soils and flooded, enriched soils inoculated with *Aerobacter aerogenes*. Losses were most rapid in the inoculated Sharkey and Hagerstown soils receiving the lowest rate of DDT application during the first week. Parallel experiments conducted with ring labeled DDT-^{14}C showed no $^{14}CO_2$ evolved from the inoculated soils. Conventional chromatographic and radiometric techniques indicated a conversion of DDT to DDD and a reduction of the total DDT-DDD residue in soils with time.

Résumé *

Décontamination de pesticides dans les sols

On dispose d'un nombre limité de méthodes décontamination des sols et des eaux. On peut effectuer une destruction des herbicides organiques dans l'eau par exposition à des radiations intenses de haute énergie. La mesure des taux de photodécomposition du piclorame, du diphenamide, du bromacile, du 2,3,6-TBA et du 2,4,5-T par bio-essais est en progrès.

Des expériences à l'aide de cellules à courant continu indiquent une destruction rapide des colorants organiques. Aucune trace de piclorame n'a pu être décelée dans une solution qui en contenait initialement 1 p.p.m., après 30 minutes ou moins d'exposition à une irradiation UV de haute intensité. Dans les cas où une élimination complète n'est pas possible, une réduction des résidus présents en dessous d'une certaine limite peut être souhaitable pour les sols où l'importance de l'absorption par les plantes devient minime. La transformation biologique d'un pesticide persistant en une forme plus aisément décomposable est une autre méthode de réduction des résidus. Des résidus de DDT dans trois sols (argile de Sharkey, limon argileux alluvionnaire de Hagerstown et limon sableux de Lakeland aux concentrations de 5, 10 et 15 p.p.m.) ont été réduits dans des sols inondés et des sols inondés enrichis, inoculés avec *Aerobacter aerogenes*. Les pertes ont été plus rapides dans les sols de Sharkey inoculés et les sols de Hagerstown ayant reçu la plus faible concentration en DDT durant la première semaine. Des expériences parallèles avec du DDT marqué ^{14}C n'ont révélé aucun dégagement de $^{14}CO_2$ des sols inoculés. Les techniques chromatographiques et radiométriques conventionnelles ont indiqué une conversion du DDT en DDD et une réduction des résidus totaux de DDT-DDD dans les sols en fonction du temps.

* Traduit par S. Dormal-van den Bruel.

Zusammenfassung *

Dekontamination von Pestiziden im Boden

Eine begrenzte Zahl an Methoden steht zur Verfügung, um Böden und Wasser zu reinigen. Vollständige Zerstörung von organischen Herbiziden in Wasser kann durch Belichtung mit intensiver Strahlung von hoher Energie bewirkt werden. Messungen der Photoabbauraten von Picloram, Diphenamid, Bromacil, 2,3,6-TBA und 2,4,5-T durch Biotesttechniken sind im Fortschreiten begriffen. Experimente mit Modelldurchflusszellen zeigen schnelle Zerstörung von organischen Farben. In einer Lösung mit einer Anfangskonzentration von ein p.p.m. konnte nach 30 Minuten oder weniger Belichtung mit hoch intensiver ultravioletter Strahlung kein Picloram mehr nachgewiesen werden. Da, wo vollständige Entfernung nicht möglich ist, wird die Reduzierung von vorhandenen Rückständen in Böden unter eine Menge, wo die Bedeutung für die Pflanzenaufnahme minimal wird, wünschenswert. Biologische Veränderung eines persistenten Pestizids zu einer abbaufähigeren Form ist eine andere Methode, um Rückstände zu reduzieren. DDT Rückstände in Böden (Sharkey Ton, Hagerstown sandiger Ton-Lehm und Lakeland sandiger Lehm mit Raten von 5, 10 und 20 p.p.m.) wurden in überfluteten Böden reduziert und weggeschwemmt und angereicherte Böden mit *Aerobacter aerogenes* geimpft. Die Verluste waren am schnellsten in den Sharkey und Hagerstown-Böden, welche während der ersten Woche die niedrigste Rate von DDT Behandlung erhalten hatten. Parallele Untersuchungen, welche mit ^{14}C-ring-markiertem DDT durchgeführt wurden, zeigten keine $^{14}CO_2$ Entwicklung in den beimpften Böden. Konventionelle chromatographische und radiometrische Techniken deuteten die Umwandlung von DDT zu DDD an und eine Reduktion des Gesamt-DDT-DDD-Rückstandes in Böden mit der Zeit.

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* Übersetzt von A. Schumann.

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Mr. BICKWIT. Do you have any evidence on the degradeability of dioxin?

Dr. JOHNSON. In terms of photodegradation, we have some evidence —this is not exhaustive—but nevertheless some evidence that would indicate a half life of—excuse me, I will get that in just a moment. This is the type of experiment run in a laboratory in a solvent. The half life was 2½ hours under a typical type of sun lamp. This is only an indicator type of test. The prognosis is in the presence of light, dioxin will degrade. Degradation in soil we do not have information on. We are diligently, however, preparing carefully labeled—radiochemically labeled—2,3,7,8-tetrachlordibenzo-p-dioxin, and supplying this to the U.S. Department of Agriculture for tests as quickly as possible, because this is the fastest way to get the answer.

Mr. BICKWIT. Do the light conditions you are using exist in nature?

Dr. JOHNSON. Primarily in ranges and pastures, yes. Because 2,4,5-T is intercepted on the upper surfaces of weeds and brush more deposits in exposed than in shaded conditions. The predominance would be exposed to light conditions. Obviously some is going into the shade. Under those conditions of lesser light intensities, I can't reply.

Mr. BICKWIT. Is it ultraviolet light you are using?

Dr. JOHNSON. Yes, with a typical sunlamp.

Mr. BICKWIT. Do I summarize your findings correctly when I say you believe 2,4,5-T is degradeable in a matter of months, and with respect to dioxin there is some evidence it is degradeable, but we do not know whether or not it is?

Dr. JOHNSON. Correct. I should again emphasize if specifications are low, the minute amount presents an extremely small exposure.

Mr. BICKWIT. With regard to the calculations that you offered us earlier, you have assumed that a person who ingests 2,4,5-T is ingesting one part per million dioxin. Isn't it possible——

Dr. JOHNSON. May I clarify the statement for the assumption?

Mr. BICKWIT. Sure.

Dr. JOHNSON. The 0.2 parts per million referred to a hypothetical situation.

Dr. JOHNSON. People do not ingest that much, and that is the total dietary intake, and moreover, 2,4,5-T has not been a residue in food. It is, in effect, zero tolerance.

Mr. BICKWIT. I understood that. What I still understand to be your assumption was that any amount of 2,4,5-T, no matter how small, that was ingested, would have one part per million dioxin in it. That was the basis of your calculation.

Dr. JOHNSON. The specification we have set for our own product is less than one part per million. Actually, it is 0.5. The assumption was to make it easy mathematically, on the high side, and some day I will learn not to draw these mathematical analogies. They never quite make the mark.

Mr. BICKWIT. Well, I hope you learn right now. What I was suggesting as a possibility is, that since 2,4,5-T is degradeable, and since we do not know whether or not dioxin is degradeable, that although your product, when sprayed, has 0.5 parts per million of

dioxin, by the time that is ingested it may have one part per thousand, one part per hundred. It may in fact have more dioxin than 2,4,5-T.

Dr. JOHNSON. I am sorry. I don't understand your logic for this escalation of the dioxin in foods.

Mr. BICKWIT. If we have a compound which contains another compound, and the larger compound breaks down, while the smaller compound does not, the smaller compound becomes a greater and greater percentage of the large compound.

Dr. JOHNSON. Well, Dr. Blair has some information that may be useful in this rather hypothetical situation. May he answer?

Senator HART. Yes.

Dr. BLAIR. Actually, in regard to the question the way you phrased it, if there was a degradation of the 2,4,5-T, there would not be a corresponding increase in that amount of dioxin.

Mr. BICKWIT. Certainly not.

Mr. BLAIR. So your question as phrased has no meaning as I tried to interpret it. Unless you mean that possibly as 2,4,5-T degrades, that it would degrade through dioxin, or into a dioxin product. Then, while the percentage would go up, the exact concentration has not gone up.

Mr. BICKWIT. What I am saying is that your calculations are based on the view that the hypothetical view—that we would allow a 0.5 parts per million tolerance for 2,4,5-T. You then conclude, if 0.2 parts per million of 2,4,5-T were ingested, that an extremely minute amount of dioxin would be ingested.

What I am questioning is whether the conclusion follows from that premise?

Dr. BLAIR. Yes, it would be an extremely small amount, and it would not increase with time.

Mr. BICKWIT. What I am suggesting is that the 2,4,5-T could break down so that it complies with the 0.2 parts per million tolerance. In fact, it might even disappear. Yet, we would be ingesting a good deal of dioxin for which there would be no tolerance.

Dr. BLAIR. It is not possible.

Mr. BICKWIT. I don't understand why not.

Dr. BLAIR. How could you ingest 2,4,5-T that contained a tenth of a part per million——

Mr. BICKWIT. It contained that much when it was sprayed.

Dr. BLAIR. Yes, and with time the concentration of dioxin in that environment has not increased one iota.

Mr. BICKWIT. That's right. The amount has not increased.

Dr. BLAIR. But is not possible to ingest more than what is there. It doesn't make sense.

Dr. JOHNSON. Mr. Chairman, could I make a comment?

Senator HART. I wish I could be helpful. I would like to see if a rephasing of the question might not elicit the point that bothers Mr. Bickwit.

Dr. JOHNSON. Do you want to comment first, or have the question rephrased first?

Senator HART. Make your comment.

Dr. JOHNSON. I think Mr. Bickwit is talking about the possibility of the 2,4,5-T degrading in the food consumed, whereas the degradation question only applies to environment.

Mr. BICKWIT. No, I am not.

Senator HART. Try it again.

Mr. BICKWIT. You are assuming that the maximum amount that we would ingest would be 0.2 parts per million in every amount of food, in every iota of food, that we eat. From that you conclude that the amount of dioxin in that food would be one-millionth of 0.2 parts a million, which I admit is an extremely small amount. I am questioning your assumption of whether the amount of dioxin, compared to the amount of 2,4,5-T that is ingested, would be one-millionth merely because dioxin is one-millionth of the 2,4,5-T amount when it is sprayed?

What I am suggesting is that the dioxin consumed may actually be more than the amount of 2,4,5-T consumed, even if it is only one part per million when sprayed.

Dr. JOHNSON. Is the situation that you have in mind that the 2,4,5-T is sprayed?

Mr. BICKWIT. Yes.

Dr. JOHNSON. The 2,4,5-T is sprayed into the environment. This contains one part per million. Then the 2,4,5-T degrades. The dioxin does not. And over a period of time, is there is a buildup of dioxin?

Is this the problem?

Mr. BICKWIT. I have another way of getting at this, perhaps.

If one gram of 2,4,5-T is sprayed on a blueberry—an unlikely assumption—and the 2,4,5-T degrades so that it complies with the tolerance of 0.2 parts per million, you may still have one microgram of dioxin sitting on that blueberry, without any violation of the tolerance of 2,4,5-T. And that one microgram may well be toxic.

Dr. JOHNSON. I would like to find a degradeable material like that. Theoretically, obviously, if the one gram of degradeable material contained the one microgram or one part per million of nondegradeable material, then the one gram degrades, that one microgram would still be sitting there.

Is that the point you are trying to make?

Mr. BICKWIT. That's right.

Dr. JOHNSON. That's right.

Mr. BICKWIT. Well, how then does a 0.2 parts per million tolerance for 2,4,5-T protect us from dioxin?

Dr. JOHNSON. Because the ratios of those two during the process of growing and supplying the food are going to remain essentially the same.

Mr. BICKWIT. You have no evidence for that statement.

Dr. JOHNSON. I know I don't, but you have no evidence for the hypothetical question, either.

Mr. BICKWIT. I do have some evidence, by your own statement, that dioxin is more likely to be nondegradeable than 2,4,5-T.

Dr. JOHNSON. But these are matters of relative rates. You are suggesting an instantaneous degradation.

Mr. BICKWIT. I am using a hypothetical situation, as you were.

Dr. JOHNSON. My purpose in bringing up what appears to have been a rather foolish example was merely to give a feeling for the magnitude of how much dioxin we are talking about.

Mr. BICKWIT. And my contention is that once the assumption on which the hypothesis was based is removed, then it does not give that feeling.

Dr. JOHNSON. I am sorry. I don't agree.

Senator HART. That is one of the fortunate features of having a reporter here. We can all grab the record in the morning to see if we can count out an understanding and agreement of yesterday.

Mr. BICKWIT. You state in your statement that members of the Mrak Commission had not seen the report prior to Dr. DuBridge's October 29 announcement.

Last week Dr. Kotin of NIEHS told us as work was completed it was promptly passed along to the Commission.

Do you mean to imply that the Bionetics report was not complete until this time?

Dr. JOHNSON. I did not receive a copy of the report of the Panel on Teratology until very late in the deliberations.

Mr. BICKWIT. I think that is unfortunate. I wonder if you have knowledge as to why that was so?

Dr. JOHNSON. No.

Mr. BICKWIT. We have have been told that whenever you burn a polychlorinated phenol, dioxin production is possible, or even likely. Could you enumerate a few of your products that contain such phenols?

Dr. JOHNSON. There's trichlorophenol, tetrachlorophenol and pentachlorophenol, and sodium salts thereof.

Mr. BICKWIT. What is a Dowicide product?

Dr. JOHNSON. The products I just mentioned. Since you related it to the phenols, I assume that is what you are talking about.

Mr. BICKWIT. Yes. I wonder if you could furnish us with a list of all such products——

Dr. JOHNSON. Mr. Rowe clarified a point here that our Dowicide trademark applies not only to chlorophenols, but to other antimicrobials.

Mr. BICKWIT. I wondered if we could have for our files a list of the products which you produce which contain polychlorinated phenols?

Dr. JOHNSON. Yes. I prefer the term chlorophenols. Polychlorinated phenols would indicate a polymer. Chlorophenols.

Senator HART. Now I am getting into something that you don't have to be anything to understand except efficient.

Do you have anything to do with Lake St. Claire?

Dr. JOHNSON. I am not really prepared to discuss that in detail, I don't have any direct responsibility for the Sarnia Plant.

Senator HART. I would be disciplined severely by my outdoor friends if I didn't ask what you are going to do about the mercury that has found its way into Lake St. Claire.

Dr. JOHNSON. I might say this, we intend to exercise responsible action.

Senator HART. That is like the lawyer admitting his client is innocent. But I won't push you if you are not prepared.

Dr. JOHNSON. I am not prepared to say any more.

Senator HART. Thank you very much.

Are there any further items that you have?

Dr. JOHNSON. Yes, if I can take a few more minutes.

Senator HART. Time is not a problem.

Dr. JOHNSON. I would like to make a comment or two, again on dioxin before getting into the last part of my testimony.

There have been questions raised about the decomposition of 2,4,5-T and dioxin. 2,4,5-T is unstable at elevated temperatures. We were able to get degradation when 2,4,5-T or dioxin were burned on paper. We were unable to detect any dioxin residue in the smoke. Now, these are very early bits of information, strictly preliminary but I thought I should mention it at this time.

This morning you asked a question about shortening the time interval between the early indication of some possible difficulties and the learning of sufficient truth about a situation so that we can take appropriate action prior to an imminent hazard to health. Hopefully, if we can improve some of our combined procedures between Government and industry, we can shorten that time.

I have a few remarks to make that apply to the scientific community as well as to this interface with Government. Screening tests, as we have heard, (and I commented on some of my own,) I think are of value. As early indicators. But screening tests alone, without the consideration of quantitative data, can be quite misleading and if necessary——

Senator HART. Can be what?

Dr. JOHNSON. Quite misleading and I think it is necessary to develop understanding in meetings where open reports are published early and where scientific information is submitted to the challenge of other scientists; scientists of the universities; government; industry and professionals so these things can be considered in the caldron of open technical debate.

I think we can operate more quickly to shorten this gap if there is more openness about reports. Moreover, I think another procedure for shortening the time is better to understand test procedures. The screening tests are simple but those tests which are substantive enough to justify intelligent action require, the consideration of scientists outside of the generating laboratory, and the review of appropriate methods.

Test methods for regulatory action are—or for clearance of new products, in particular, require consideration. I don't mean we should lock every clearance procedure into a lock step because the development of techniques is a mutable, changing thing. But the confirmation of tests prior to official acceptance as this relates to public policy is important.

The Association of Official Agricultural Chemists has established a useful procedure. When these gentlemen and this association are considering new tests, the topic is identified, the first scientists to work on the test are identified, collaborators are found to work out objective tests and these tests are run in separate laboratories so that confirming methodology can be established.

As a result the test must be reliable; that is, it must give accurate precise and reproducible results when used by qualified analysts. It must be practical; simple. It must be available to all analysts and it must be substantial; that is, supported by collaborative study.

Now, I will say these types of tests apply primarily to clearance procedures or to regulatory tests. I am not saying extreme tests are not in order. But those which support the authority of regulatory backup need this type of scientific consideration.

The American Standards Society also has procedures for standardizing tests. These are physical tests. They involve one or more laboratories in the preliminary study and interlaboratory studies, maybe as many as 10, in the more refined studies, and this results in a standard test method which can be used.

The Department of Commerce, National Bureau of Standards, does an excellent job in developing standards. Moreover they have an industrial associates program which permits the access of industry into these laboratories, for a period of time to work, so that industry representatives can better understand the problems of standards and go back to their companies better informed.

Protocols for clearance of new and existing products, I think are important for the Food and Drug Administration to consider seriously. Again I don't mean standard tests would remain forever, but protocols. Industry needs to know where it stands, industry also need to participate; appropriately and at arm's length, to be sure, nevertheless; to participate with specific information.

The period of time between 1966 and now could have been shortened by sharing appropriate information, and I admit we could have assisted by volunteering earlier. But there seems to be some reverse of togetherness and there seems to be great concern that communication between governmental agencies and industry is suspect and would be misused.

I would hope that one valuable result to come of this would be a continuation of developing science fortified by interest in this important matter of communication.

On the point of developing protocols (and I frankly think the whole subject of teratology requires the vigor of scientific debate among professional peers involving industry, university and government) I would hope the National Academy of Sciences could provide such a forum, if not the National Academy perhaps the New York Academy of Sciences and I intend to help encourage this because it is important. It is important that these ideas are traded openly and published.

I would mention that the World Health Organization, in a document entitled "Principles for the Testing of Drugs for Teratogenicity," the World Health Organization Technical Report, Serial No. 364 or 1967, has the following to say on test animals for teratology, on the subject of the chick embryo, page 7.

The chick embryo contributes greatly to basic embryological knowledge. However, for the screening of drugs for teratogenicity, its use is not recommended. It is too sensitive to a wide range of agents and affords no parallel with the anatomical and physiological relationship existing between pregnant mammals and her conceptus.

I will support the idea of the use of a chick embryo screening tests, early indications, but from that point on additional protocols and understanding, I think, are necessary.

Again in the line of helping to shorten this interval, I think strict standards are necessary in defining matters of safety and public health. No question about it. I don't object to tought regulations. But industry needs to know where it stands and the dialogue is important. The dialogue between agencies can help to shorten this time and I am glad to see distinct improvement in communications between the Department of Health, Education, and Welfare and the Department of Agriculture.

Again I would like to say we are not talking about a locked-in procedure that could be used as an excuse to avoid further responsibility for progress.

Another point; millions have been spent on cancer research. Yet to this day, adequate experiments have not been devised or supported on an adequate scale by government to establish thresholds. By that I mean levels below which no response occurs. Without that type of information—there will be continuing public fear as analytical methods become more and more sensitive.

Senator HART. May I interrupt you there?

Dr. JOHNSON. May I finish the point and come back?

With teratogens, mutagens, radioactivity and teratology, this all applies. In other words, if the dose is low enough can any of these effects be avoided. This is the big question today. It is the question which coupled with sensitive analytical methods makes the Delaney clause a difficult thing to live with.

I would like to make the comment that vitamin A, an essential nutrient, is a teratogen at high doses. So this matter of developing test procedures and a big enough experiment with carcinogens, mutagens, teratogens or low levels of radioactivity, needs to be done on a national scale and supported so that some of this public fear can be avoided and so we know where we stand.

Scientists should put limitations on their speculations and preconceptions and get the facts but also realize we have a job in serving the public policy that does not exactly follow the scientific method.

I hope we can work out quicker procedures to go from early indications to reliable decisions by involving some of the procedures I have talked about. After responding to your question, I would like to make that final statement.

Senator HART. I am not sure I need to ask the question now. I agree completely on the desirability of doing that which our best minds can suggest to shorten the time.

The question I intended to interrupt you with I think you did answer. I was going to ask to what extent in developing these thresholds and having broader sampling and more reliable data, to what extent is that the responsibility of the Federal Government?

Dr. JOHNSON. On the matters of carcinogenicity, teratology, radiation, and mutagenesis, where such public concern is involved, and since this involves a wide variety of suspected material, it is my opinion that the Federal Government should play a major role in supporting this research, both in its own laboratories and in extra-

ural research done in universities *and* in industry to help get diversity of methods and the best thinking of the nation.

Senator HART. All right, then to the extent that the story that we have heard today and heard last week reflects delay and a failure of communication, I would hope that the suggestions you have made and the discussions that we have had all will insure that there will be no delay, there will be no barrier to free communication, and there will be no suspicion attached as agency and producer try to drive through to the answer.

Now, you wanted to remind us of what the Ribicoff committee said.

Dr. JOHNSON. Yes, if I may.

This is read from Report No. 1379 of the 89th Congress, 2d Session "Interagency Environmental Hazards Coordination, Pesticides and Public Policy", a report of the Committee on Government Operations United States Senate, made by its Subcommittee on Reorganization and International Organizations. I will read from page 50. And this puts the scientists on the spot, including me.

The heading of the paragraph is "The Responsibilities of the Scientists" and we are talking about industry scientists, governmental, and university.

"The committee asked the scientific witness for meaningful advice for the Congress, but much of the testimony was inhibited by defense of past positions, employer loyalties, and lack of authority."

Scientists should do as thorough a job of preparing answers on aspects of research administration and planning as they do on the technical details of the work.

The maker of public policy must have alternatives from which to choose. There are always strong vested interests which resist change. Unless the technological situation (in this case, ecology) can be clearly explained and related to public policy issues, the decisionmaker is hardput to recommend any new course. This understanding must be extended also to the citizen. No great social issues have ever been decided until the needs were clear to the man in the street.

Scientists cannot assure that their knowledge will reach the decisionmaker through the normal channels of publication and review in the scientific community. Without shortcutting the classical methods of assessing the truth, there is still an obligation to interpret what is known and replace emotion, rumor, and misconception with a clear explanation of the facts.

The role of the scientist in relation to the legislator is limited to an area somewhere short of the decisionmaking point. Proper use of scientific advice requires considerable effort on both the part of the scientific community and the body politic.

This is the end of my statement.

Senator HART. I am glad you reminded all of us of it.

Doctor, thank you very much.

Do your associates have anything to add in light of the statement?

All right, thank you very much.

Dr. JOHNSON. Thank you.

Senator HART. Next we have Dr. Samuel Epstein, Children's Cancer Research Foundation.

Doctor, we welcome you.

STATEMENT OF DR. SAMUEL S. EPSTEIN, CHILDREN'S CANCER RESEARCH FOUNDATION, INC., AND HARVARD MEDICAL SCHOOL, BOSTON, MASS.

Dr. EPSTEIN. Mr. Chairman, it is a privilege to testify before you today. The subject of my presentation is teratogenic effects of 2,4,5-T formulations.

My professional background and experience, as stated in the attached appendix 1, broadly relate to the study of hazards due to chemical pollutants in the human environment. Relevant to the present testimony is my recent role as Cochairman of the Advisory Panel on Teratogenicity of Pesticides, and as Chairman of the Advisory Panel on Mutagenicity of Pesticides, of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health, HEW, GPO, December 1969.

The Panel on Teratogenicity was composed of outside consultants and representatives of HEW agencies—CPEHS, FDA, NCI, NIEHS, and NIDR. The HEW Panel report on teratogenicity is included in the appendix (II).

A. Teratogenicity as a public health hazard: Potential hazards posed by environmental pollutants and drugs include toxicity or poisoning, carcinogenicity or induction of cancer, mutagenicity or induction of genetic damage, and teratogenicity or induction of developmental abnormalities in the growing embryo.

Although teratogenic effects of various agents have been recognized for several decades, it was only as a reaction to the thalidomide episode of 1962 that a requirement for teratogenicity data became established.

Teratology is the study of congenital malformations. These are generally defined as structural abnormalities which can be recognized at or shortly after birth and which can cause disability or death. Less restrictedly, teratology, also includes microscopical, biochemical and functional abnormalities of prenatal origin.

Congenital malformations pose incalculable personal, familial and social stresses. The financial cost to society of one severely retarded child, computed on the basis of specialized training and custodial care alone, approximates to $250,000 (Oberle, 1969). This figure excludes further costs to society due to deprivation of earnings.

In the absence of a comprehensive national surveillance system, the precise overall incidence of congenital malformations is unknown. This incidence has been variously estimated as ranging between 3 and 4 percent of total live births.

Three major categories of human teratogens have been identified —viral infections, for example German measles; irradiation, for example X-rays; and chemical agents, for example thalidomide and mercury.

B. Methods for teratogenicity testing: teratogenic effects of chemicals and other agents should of course be identified in experimental animals rather than in human beings following accidental or unrecognized exposure. Test agents should be administered to pregnant animals during active organogenesis of their developing embryos. Shortly before anticipated birth embryos should be harvested by caesarean section and examined.

Parameters to be considered in test and concurrent control animals should include the incidence of abnormal litters, the incidence of abnormal fetuses per litter, the incidence of specific congenital abnormalities, the incidence of fetal mortality, maternal weight gains in pregnancy, and maternal and fetal organ/body weight ratios.

Additionally, some pregnant animals should be allowed to give birth in order to identify abnormalities that may otherwise manifest only in the perinatal period.

Agents and their known metabolites should be administered to two or more mammalian species under various nutritional conditions during active organogenesis and by a variety of routes reflecting possible human exposure.

Of interest in this connection is the lack of data in the available literature on teratogenicity testing by the respiratory route. Respiratory exposure is particularly important for pesticide aerosols and vapors.

Agents should be tested at higher dose levels than might be anticipated in humans following high-level accidental exposure, as well as following extensive low-level exposure. This is essential to attempt to reduce the insensitivity of conventional test systems based on very small numbers of animals compared with the millions of humans at presumptive risk.

To illustrate this further, let us assume that at actual human exposure levels, a pesticide induces teratogenic effects or cancer in as many as 1 out of 10,000 humans, then the chances of detecting this in test groups of less than 50 rats or mice exposed at these actual levels would be very low. Indeed, many more than 10,000 rats or mice, depending on their spontaneous incidence of teratogenic effects or cancer, would be required to demonstrate a statistically significant effect, if we assumed that rats and humans have similar sensitivity to the teratogen or carcinogen being studied.

For some teratogens, humans may be less or may be more sensitive than test animals. Meclizine—a drug used for morning sickness in pregnancy—for example, is teratogenic in the rat, but not apparently in a restricted number of humans studied (King, 1965; Yerushalamy and Milhovich, 1965).

With thalidomide conversely, the lowest effective human teratogenic dose is 0.5 mg. per kg. a day. Corresponding values for the mouse, rat, dog, and hamster are 30, 50, 100, and 350 mg. per kg. a day (Kalter, 1968).

Thus humans are 60 times more sensitive than mice, a hundred times more sensitive than rats, 200 times more sensitive than dogs, 700 times more sensitive than hamsters.

Clearly, attempts to determine a safe level for thalidomide, based on animal teratogenicity data, would clearly expose humans to significant teratogenic hazards. Accordingly, it is routine practice to test for teratogenicity and carcinogenicity at a range of concentrations, including those higher than human exposure levels, and extending to maximally tolerated doses (MTD).

Even at MTD levels administered to mice from day 7 of life until sacrifice at 18 months, less than 10 percent of the 140 pesticides tested in the recent Bionetics study were shown to be carcinogenic.

The report of the advisory panel on teratogenicity states unambiguously * * *

Pesticides should be tested at various concentrations including levels substantially higher than those to which the human population are likely to be exposed.

The report also emphasizes the insensitivity of standard test systems imposed by the relatively insufficient numbers of litters conventionally tested.

The report further states * * *

Thus, compounds showing no increase (in birth defects) cannot be considered nonteratogenic.

Epidemiological surveys of human populations may provide post hoc information on geographical or temporal clusters of unusual types or frequencies of malformations following exposure to undetected or untested teratogens in the environment. However, logistic considerations, quite apart from inadequate current surveillance systems, limit the utility of this approach.

It should be emphasized that no major known human teratogen such as X-rays, German measles, mercury, or thalidomide, has been identified by retrospective epidemiological analyses, even in industrialized countries with highly evolved and sophisticated medical facilities.

Prospective epidemiologic surveys on agents previously shown or suspected to be teratogenic, by experimental studies or by retrospective population surveys, are clearly inappropriate.

C. Bionetics studies on teratogenicity of 2,4,5-T: Bionetics Research Laboratories, Inc., of Litton Industries, under a contract from the National Cancer Institute, tested 48 pesticides, including 2,4,5-T and related compounds, for teratogenic effects during 1965–68.

Although the bionetics studies were originally designed for purposes of large-scale screening 2,4,5-T was tested more extensively than any other pesticide. Thus the data on 2,4,5-T may be regarded as more definitive.

The Bionetics Research Laboratory report is included in the appendix (III). A revised and more detailed statistical analysis of these data is summarized in the report of the Advisory Panel on Teratogenicity of Pesticides (appendix IF).

2,4,5-T was tested on repeated occasions from 1965–68 in three strains of mice and in one strain of rats by subcutaneous and/or oral administration over a dose range from 4.6 to 113 mg. per kg. The total numbers of litters tested at each dose level, by each route in all strains and species, excluding C_3H mice in which only one litter was tested, were as follows, and I list these in a table enclosed.

As can be seen, the bulk of the data was obtained with BL6 mice. Due to control variability, the BL6 data have been considered for three time intervals—prior to September 1966, from September to November 1966, and from November 1966 to August 1968.

Data on AK mice were considered for two time intervals—prior to November 1966, and from November 1966 to August 1968.

Data for BL6 mice, AK mice, and Sprague Dawley rats, as derived from the bionetics report, are as follows:

BL6 mice: 2,4,5-T administered on days 6–14 or days 9–17, and mice sacrified on day 18 of pregnancy.

BL6/AK/mice: 2,4,5-T administered on days 6–14, and mice sacrificed on day 18 of pregnancy.

AK mice: 2,4,5-T administered from days 6–15, and mice sacrificed on day 19 of pregnancy.

Sprague Dawley rats: 2,4,5-T administered from days 10–15 and rats sacrificed on day 20 of pregnancy.

In the 4 tables that follow dealing with a wide dose range, the results are expressed as percentages of abnormal fetuses. I would point out those occasions where statistically significant incidence of results was noted.

Major abnormalitities in mice were cleft palates and cystic kidneys, and in rats, cystic kidneys and gastrointestinal hemorrhages. Increased fetal mortality was generally concomitant with these abnormalities. It is of particular interest that 39 percent abnormal embryos with cystic kidneys were seen in rats even at the lowest dose tested. Thus the no effect level was not reached even at 4.6 mg./kg.

Teratogenicity data on 2,4,5-T, as summarized in the bionetics report (appendix III) are quoted in extenso below. Some critical sentences are italicized:

> This compound was given by the oral route to BL6 mice at dosages of 46.6 and 113 mg/kg and to AKR mice at 113 mg/kg. It was given by subcutaneous injection to BL6 mice at dosages of 21.5 and 113 mg/kg and to AKR mice and B6AK hybrids at 113 mg/kg. It was also given subcutaneously to C$_3$H mice at 215 mg/kg, but there were too few of these to merit inclusion in the discussion which follows.
>
> Administration was for eight days (6th through 14th) in most cases; for nine days (6th through 15th) in some; and for five days (10th through 14th) in one case—the details are indicated in the tabulated results. Subcutaneous administration used DMSO as a vehicle; oral used 50 percent honey.
>
> With the single exception of the lowest dosage used (21.5 mg/kg to BL6 subcutaneously) all dosages, routes and strains resulted in increased incidence of abnormal fetuses. The incidence of cleft palate was high at the 113 mg/kg dosage, but not at lower levels. The incidence of cystic kidney was also high except in the AKR strain and in the BL6 mice which received 46.4 mg/kg orally. Fetal mortality was increased in all groups given 113 mg/kg for eight or nine days, but not in mice (BL6) given this dosage for only five days nor in the two groups of BL6 mice given lesser dosages (46.4 mg/kg orally and 21.5 mg/kg subcutaneously.
>
> Most fetal and maternal measurements showed inconsistent changes from which no conclusions can be drawn. In contrast, there was a highly consistent decrease in maternal weight gain in BL6 mice given 113 mg/kg by either route. Lower dosages and the AKR strain showed either no change or a slight increase. All dosages, strains, and routes showed an increase in the maternal liver weight and this led to a further study discussed separately below.
>
> *These results imply a hazard of teratogencsis in the use of this compound. The problems of extrapolation preclude definition of the hazard on the basis of these studies, but its existence seems clear.*
>
> The observed influence of 2,4,5-T on maternal liver weight as mentioned above raised a question as to its effect on the fetal liver. This was answered by a study carried out in BL6 mice using subcutaneous injections of DMSO solutions at a dosage of 113 mg/kg only. The period of administration was lengthened to cover the period from the 9th through 17th day of gestation. Separate control groups were used concurrently. Except for the inclusion of fetal liver weight, measurements were made as previously described.
>
> The fetal livers of the 2,4,5-T treated mice weighed significantly more than those of controls given DMSO only and the weights of the whole fetuses were significantly less. Correspondingly, there was an increase in the fetal liver weight expressed as percent of body weight.
>
> *Other observations were consistent with those reported above. The incidence of abnormal fetuses was unusually high as were those of cleft palate and cystic kidney.*
>
> Because of the potential importance of the findings in mice, an additional study was carried out in rats of the Sprague-Dawley strain. Using dosages of 21.5 and 46.4 mg/kg suspended in 50 percent honey and given by the oral route on the 6th through 15th days of gestation, we observed excessive fetal mortality (almost 80 percent) and a high incidence of abnormalities in the survivors. When the beginning of administration was delayed until the 10th day, fetal mortality was somewhat less, but still quite high even when dosage was reduced to 4.6 mg/kg.
>
> *The incidence of abnormal fetuses was threefold that in controls even with the smallest dosage and shortest period used.* Fetal and maternal measurements showed only occasional instances of significant differences from controls except in the case of maternal liver weight which was consistently increased in all 2,4,5-T treated animals.
>
> *It seems inescapable that 2,4,5-T is teratogenic in this strain of rats when given orally at the dosage schedules used here. These findings lend emphasis to the hazard implied by the results of studies on mice.*

D. Recent reanalysis of the Bionetic data on teratogenicity of 2,4,5-T. More refined and more appropriate additional statistical analyses of these data were presented and discussed in the report of the Advisory Panel on Teratogenicity of Pesticides (appendix II). These are clearly confirmatory of the original conclusions of the Bionetics report on the teratogenicity of 2,4,5-T. Some relevant portions of the HEW panel report are quoted in extenso below:

> Tested more extensively than other pesticides, 2,4,5-T was clearly teratogenic as evidenced by production of statistically increased proportions of litters affected, and increased proportions of abnormal fetuses within litters in both DMSO and honey for both C57BL/6 and AKR mice. In particular, cleft palate and cystic kidneys were significantly more prevalent. In addition, a hybrid strain resulting from a C57BL/6 female and AKR male showed significant increases in anomalies, in particular cystic kidney, when administered at 113 mg/kg of body weight in DMSA.
>
> Additionally, 2,4,5-T was tested in Sprague-Dawley rats. When given orally at dosages of 4.6, 10.0, and 46.4 mg/kg on days 10 through 15 of gestation, an excessive fetal mortality, up to 60 percent at the highest dose, and high incidence of abnormalities in the survivors was obtained. *The incidence of fetuses with kidney anomalies was threefold that of the controls, even with the smallest dosage tested.*

E. Recent studies on teratogenicity testing of relatively pure 2,4,5-T. In view of the fact that the Bionetics study was conducted with a sample of 2,4,5-T which was subsequently shown to contain a relatively high concentration, 27 ppm, of a tetrachloro dioxin contaminant, testing has been recently repeated with relatively pure samples containing less than 1 ppm of this particular dioxin.

The results of these studies were presented by the FDA and NIEHS at a recent conference of February 24, 1970, at the FDA; the Dow Chemical Co. data were presented at the 9th annual meeting of the Society of Toxicology, Atlanta, March 17, 1970.

As can be seen from the data summarized below, purified 2,4,5-T is teratogenic in three species—rats, mice and hamsters. These data should be regarded as preliminary. Confirmatory data on chick eggs are not presented here.

1. Dow Chemical Co. studies (Emerson *et al.*, 1970). 2,4,5-T with 0.5 ppm dioxins, as a probable contaminant, was tested in pregnant

rats by repeated oral administration at doses of 1, 3, 6, 12, and 24 mg./kg.; the maximal dose tested was 24 mg./kg. No embryo deaths or weight losses were noted within the dose range tested. However, at 24 mg./kg. there was a sevenfold increase in the incidence of fetuses with defective ossification of the fifth sternebra; poor sternebral ossification was noted in four out of 103 control fetuses, and in 20 out of 103 fetuses of 2,4,5-T treated groups.

Defective sternebral ossification has been described in the rat as an expression of the teratogenic effects of drugs such as protamine zinc insulin and tolbutamide (Lichtenstein et al., 1951; Dawson, 1954).

2. NIEHS studies: Using the purest sample of 2,4,5-T, made available by Dow Chemical Co., teratogenic effects were induced in Swiss-Webster mice. Cleft palates were noted at dose levels of 150 mg./kg. and scattered abnormalities at 100 mg./kg.; the cleft palate incidence in control mice was essentially zero.

3. FDA studies: Hamsters were injected with five doses of 100 mg./kg./day of various batches of purified 2,4,5-T between days 6–10 of pregnancy. In one of these studies, there was a 66-percent incidence of mortality in 50 fetuses. Of the surviving fetuses, 17 percent had congenital abnormalities—crooked tail, missing limb, and defect in skull fusion. No data was presented on possible effects induced by doses less than 100 mg./kg.

Of additional interest was a report also presented at the same conference on purified 2,4-D, which produced a 22-percent incidence of congenital abnormalities in hamsters at a dose level of 100 mg./kg./day.

I should like now to address myself to the toxicity of dioxins.

Toxicity of dioxins: Rabbit ear skin is highly sensitive to dioxins, repeated application of which can produce chloracne, as a cumulative manifestation of local toxicity. Approximately 0.3 micrograms of the tetra isomer will produce a positive response; "more than 10 micrograms on a surface wipe sample indicates acute hazard" (to man) (Silverstein, 1970).

The acute oral LD–50 dose of tetra dioxin in male guinea pigs is 0.5–1.0 micrograms/kg., and in male and female rats, 22.5 and 45 microgram/kg., respectively. Feeding chicken edema factor diets, containing dioxins, produced cumulative toxicity in monkeys (Allen and Carstein, 1967). Storage of hexa, hepta and octa isomers, as identified by GLC, has been reported in chickens and rats fed chicken edema factor diets (FDA, unpublished). Chronic administration of 2,4,5-T or 2,4-D to dogs produces cumulative toxicity with gastrointestinal haemorrhage, suggestive of cumulative dioxin effects (Drill and Hiratzka, 1953).

TERATOGENICITY OF DIOXINS

1. FDA studies (FDA Conference, Feb. 24, 1970): A mixture of dioxins, 21 percent trichloro and 53 percent tetrachloro isomers, were injected in hamsters between days 6–10 of pregnancy over a dose range from 0.5 to 9.1 microgram/kg. per day. At the highest dose, the incidence of fetal mortality was 82 percent and the incidence of congenital abnormalities, 82 percent. At the 0.5 microgram/kg. dose, there was a 5 percent incidence of abnormalities. The no-effect level was thus not reached at 0.5 micrograms per kg.

2. Dow studies (Sparschu et al., 1970): The tetra dioxin isomer was fed to Sprague Dawley rats between days 6–15 of pregnancy, over a dose range from 0.03 to 8.0 micrograms/kg. per day. There was a marked increase in resorption sites at the 2 microgram level. Gastro-intestinal hemorrhages occurred over a range from 0.125 to 8 micrograms, dose-dependently. Additionally, at the 0.125 microgram/kg. level there was a decrease in male fetal weights.

It should be emphasized that cystic kidneys were not seen at the 0.125 microgram/kg. dose of the tetra isomer or even higher levels. In the Bionetics study, 2,4,5-T at 4.6 mg./kg., containing 25 ppm of the tetra dioxin isomer equivalent to 0.124 microgram/kg., produced a 39 percent incidence of congenital abnormalities with cystic kidneys.

There is thus a clear discrepancy between the teratogenic effects of 2,4,5-T containing 25 ppm of dioxin, and the effects of the equivalent concentration of the same dioxin. It is, however, conceivable that this discrepancy may reflect synergistic interactions between dioxin and 2,4,5-T.

SOME UNRESOLVED PROBLEMS RELATING TO 2,4,5-T AND DIOXINS

1. Chemical composition of 2,4,5-T formulations: Currently used 2,4,5-T formulations contain about 5 percent of known impurities, largely polychlorophenols. Analytic data on a sample of 2,4,5-T (Dow data, on production batch 120449) in the following table substantiates the approximate 5 percent of polychlorophenol impurities in 2,4,5-T formulations as currently used.

There are no available data on the presence and concentration of the more than 60 positional isomers of dioxin, other than the 2,3,7,8-tetrachloro dioxin isomer, in this batch of 2,4,5-T, or in other batches produced for food crop or other purposes in the United States and abroad.

In view of the relatively high concentration of polychlorophenol impurities in 2,4,5-T, it is likely that a wide range of dioxins are also present. *2,4-D and other phenoxy herbicides are similarly chemically uncharacterized.*

The higher positional dioxin isomers, hexa, hepta, and octa, have been identified in 2,4-dichlorophenol, a precursor of 2,4-D. Apart from the presence of dioxins in polychlorophenols, heating of polychlorophenols will produce additional and very high yields of dioxin.

Illustratively, heating 5 g. of pentachlorophenol at 300° C. for 12 hours yielded 1.5 g. of the octa-dioxin isomer (Cowan, 1970). There are no available data on the possible production of dioxins from combustion of 2,4,5-T or 2,4-D. While improved production techniques may well reduce the levels of polychlorophenols and the levels of the 2,3,7,8-dioxin isomer, apart from other isomers, in 2,4,5-T and other phenoxy herbicides, the degree to which this is practical does not yet appear to have been clearly defined.

2. Stability and persistence of dioxins: The extent of usage of 2,4,5-T and other phenoxy herbicides on food crops and for other purposes in the United States and abroad dictates the scale of resulting environmental contamination with 2,3,7,8-dioxin and other

45-362 O—70——27

isomers. The following data are illustrative and (Agricultural Economic Report No. 131, USDA, 1968).

These data reflect deliberate applications of phenoxy herbicides on crops, and do not reflect unintentional crop contamination following the more extensive application of herbicides for brush control or other purposes. There are no available data on the extent of such unintentional contamination. It is, however, well known that phenoxy herbicide dusts may drift for miles, even on nonwindy days, following routine application (Federal Register, 1969). The concentration of phenoxy herbicides in the air in Washington in 1964 reached a maximum of 3.4 microgram/m.^{3}, with an average of 0.045 microgram/m^3 (Bamesberger and Adams, 1966).

These figures probably underestimate the proportional concentration of atmospheric dioxins, in view of their high stability relative to phenoxy herbicides.

I now present a table on calculated dioxin contamination of an acre of soil, following 2,4,5-T application.

CALCULATED DIOXIN CONTAMINATION PER ACRE FOLLOWING 2,4,5-T APPLICATION

Application 2,4,5-T (lbs/acre)	Contamination with 2,3,7,8-Dioxin (mg/acre)	
	Based on 0.5 ppm Dioxin	Based on 25 ppm Dioxin
2.5 (domestic use)	0.57	28
25.0 (export use)	5.70	284

Now, these calculations are based on the 2,3,7,8-dioxin isomer alone, and ignore additional contamination due to other dioxin isomers. The figures for export use should be adjusted to reflect varying concentrations of 2,4,5-T in different formulations.

The high concentration of polychlorophenolic impurities in 2,4,5-T, approximately 5 percent, apart from other sources of polychlorophenols, may result in extremely high yields of dioxins. As mentioned previously, heating of 5 grams of pentachlorophenol at 300° C. for 12 hours results in a yield of 1.5 grams of the octadioxin isomer. Combustion of shrub, brush, timber, or other materials exposed to phenoxy herbicides or other polychlorophenols, may thus liberate high concentrations of dioxins in the atmosphere.

It is thus of interest to examine the data on stability and persistence of dioxins in the environment. The 2,3,7,8-tetra isomer is known to be heat stable up to 800° C. There are, however, no available data on the heat stability of other dioxin isomers. There are also no available data on the stability and persistence of the 2,3,7,8- and other dioxin isomers in soil, water, crops, milk, and animal or human tissues.

Most importantly, there are no available data on the possible accumulation and transmission of 2,3,7,8- and other dioxins in the food chain—air, soil, and water—to plants, brush and crops—to fish, birds and cattle—to man, with attendant accumulation in man.

The heat stability of the tetra isomer, the general lipid solubility of the dioxins, and their cumulative toxicity in experimental animals, all serve to enhance the possibility of food chain transmission of the various dioxin isomers.

3. Teratogenicity of relatively pure 2,4,5-T.

The relatively contaminated 2,4,5-T used in the Bionetic study, containing about 27 p.p.m. of the tetra-dioxin contaminant, induced congenital abnormalities in mice and rats, particularly cystic kidneys, that were not produced by the pure tetra dioxin. Illustratively, the contaminated 2,4,5-T at 4.6 mg./kg./day oral dose level in rats produced a 39 percent incidence of congenital abnormalities. This dose of 2,4,5-T is equivalent to 0.124 micrograms/kg./day of the tetra-dioxin.

However, as reported in the Dow studies, presented at the Society of Toxicology meeting in Atlanta on March 17, 1970, 0.125 micrograms/kg./day of the tetra dioxin did not produce cystic kidneys in rats. This discrepancy may however conceivably reflect synergistic interactions between 2,4,5-T and dioxins.

Additionally, as indicated above, relatively pure 2,4,5-T, containing one p.p.m. of the tetra-dioxin contaminant, was teratogenic in preliminary studies with three species—mice, rats, and hamsters, over a dose range of 24–150 mg./kg. In some of these studies, for example, in hamsters at 24 mg./kg./day, no effect levels were not reached.

Thus, recent studies on relatively pure 2,4,5-T clearly confirm its teratogenecity and lend further emphasis to the following conclusions of the report of the HEW Teratogenicity Panel: "The use of currently registered pesticides to which humans are exposed and which are found to be teratogenic by suitable test procedures in one or more mammalian species should be immediately restricted to prevent risk of human exposure."

All these considerations confirm the following conclusion stated in the Bionetics reports (App. III, p.19): "These results imply a hazard of teratogenesis in the use of this compound, 2,4,5-T. The problem of extrapolation preclude definition of the hazard—but its existence seems clear."

4. TOXICOLOGY OF DIOXINS

The toxicology of dioxins is of particular interest in view of the previously reviewed data on the very high acute toxicity, embryotoxicity, cumulative chronic toxicity of 2,3,7,8-dioxin and related isomers, and also the stability, widespread environmental distribution, and likelihood of accumulation and transmission of any dioxins in the food chain.

There are no data in the available literature on the carcinogenicity or mutagenicity of positional isomers of dioxins. Recent studies on the teratogenicity of dioxins have been largely restricted to the 2,3,7,8-isomer; there are no available experimental data on behavioral or psychopharmacological effects due to dioxins; this would be of interest in view of the possible psychiatric effects described in humans exposed to dioxins. There are no data in the available literature on any toxicological studies on any dioxin isomer following acute or chronic administration by inhalation.

The extreme inadequacy of toxicological data on dioxins clearly precludes consideration of potential human hazards due to dioxins in air, food or water, and consideration of possible safety margins following exposure to dioxins.

I would like to reemphasize the conclusions of the HEW panel on teratogenicity:

"*The use of currently registered pesticides to which humans are exposed and which are found to be teratogenic by suitable test procedures in one or more mammalian species should be immediately restricted to prevent risk of human exposure. Such pesticides, in current use, include Captan; Carbaryl, the butyl, isopropyl, and isooctyl esters of 2,4,-D Folpet; mercurials; PCNB; and 2,4,5-T. The teratogenicity of 2,4,-D, the other salts and esters of both 2,4,-D and 2,4,5-T, and that of IPC should be investigated further.*"

Finally and critically, available data on the toxicology of the dioxins, and more importantly *on the lack of data* on the toxicology—acute and chronic toxicity, carcinogenesis, mutagenesis, and teratogenesis—of the numerous positional isomers of dioxins, indicate an urgent need for restriction of human exposure to dioxins. Similar restrictions should extend to polychlorophenols, polychlorophenolic containing formulations, and their combustion products.

I thank you, sir.

(A list of the references to the statement follow:)

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Senator HART. Thank you, Doctor.

In your summary you refer to 2,4-D among other things, and earlier in your statement you said that 2,4-D produced birth deformities in the hamster. What is your opinion as to the teratogenicity of 2,4-D in comparison to 2,4,5-T?

Dr. EPSTEIN. The findings of the Bionetics study in relation to 2,4-D, were certainly not as conclusive as for 2,4,5-T. But, on the other hand, in the wide range of tests performed in the Bionetics study there were strong suggestions of teratogenic effects. That was as far as one could go. For these reasons the HEW Panel on Teratogenicity clearly recommended that further work should be done on 2,4-D and that a high degree of suspicion should be attached to it.

Moreover, the recent FDA studies on hamsters would seem to confirm the earlier suspicions of the Bionetics studies in mice and would indicate to say the least the need for a high degree of caution and minimally, indicate the need for restriction of further human exposure to 2,4-D pending clarification of these problems.

I think to await further definitive studies before taking action would not be appropriate in the light of the available data.

Senator HART. That gets us back, and we return here all the time, to this balancing of principle.

On the point that Mr. Bickwit was developing with Dr. Johnson, on the business of the cumulative effect of dioxin I take it you regard them as cumulative; is that correct?

Dr. EPSTEIN. On the basis of the available data in the literature, one could say there is a strong suggestion of cumulative effects. The rabbit ear skin test depends on cumulative toxicity and the chick edema effect is cumulative. One can demonstrate the pickup of dioxins in rat and chicken liver. So available data indicate they are persistent and cumulative, although I would regard these data as far from definitive.

Senator HART. What is your feeling as to the adequacy of the evidence? What is the extent of the evidence on the degradability of the dioxins in ultraviolet light?

Dr. EPSTEIN. I would submit that data on strong sunlight and shortwave UV is probably irrelevant. If you expose dioxins to strong sunlight you are exposing material to light which contains short wave ultraviolet in a manner not closely related to normal conditions.

I would like to see studies on conditions of photo-degradability of dioxins on conditions of shade and normal daylight. This is not available.

Senator HART. Well, we always seem to run into the lack of available data in pursuing answers to these questions. Now let's get to the availability of substitutes. What alternatives exist, or are you in position to know, for 2,4,5-T and 2,4-D?

Dr. EPSTEIN. I am obviously not an agricultural expert and we are now talking about crop use, I presume. Does your question relate to crop use of herbicides or total use?

Senator HART. I guess I should ask it totally and if there are areas where there is an alternative, identify that.

Dr. EPSTEIN. With the qualification that I am no expert in this field and not qualified to comment on the relative merits of various

herbicides, I would merely point out they fall into two categories, the pre-emergence and post. For the pre-emergency group, you have amiben, trifluralin, and atrazine, which all seem to be appropriate for use under a wide range of conditions.

As far as post-emergence herbicides, 2,4-D and 2,4,5-T are herbicides with which we are familiar, additionally there are others e.g., endothal, atrazines and dinoseb. Atrazines are pre-emergence and post-emergence herbicides. There is thus a wide range of herbicides for use on both pre- and post-emergence levels.

Senator HART. We have staff inquiry as to whether you have any knowledge as to the cost differences, if any, as between 2,4-D and 2,4,5-T and some of these other items.

Dr. EPSTEIN. I am not competent to answer that.

Senator HART. What tests have been made on the dioxins for genetic damage or for cancer?

Dr. EPSTEIN. There have been no tests made on any dioxin for genetic damage. There have been no tests made on any dioxin for carcinogenicity, either by oral administration or by injection or by inhalation. I must point out that we are talking about very small levels of highly potent compounds and we must remember certain carcinogens are active at less than a part-per-million level. We have no ball park data on this at all. We just don't know whether we are dealing with significant ranges or nonsignificant ranges.

Senator HART. Is the same true with respect to testing of the exposure by inhalation or respiration of the herbicides or dioxins? Early in your paper you remind us of this.

Dr. EPSTEIN. There are no data in the total literature of chronic carcinogenicity or mutagenicity of any pesticide whatever by inhalation. This is a point brought out quite forcefully in the Mrak Panel report. Inhalation is a very significant route of human exposure and the experimental test systems do not reflect recognition of this exposure.

Senator HART. What possible dangers may result from either 2,4,5-T or 2,4-D in noncrop use, and other herbicides in noncrop use?

Dr. EPSTEIN. Well, a wide range of possibilities exist and it is not possible to define many of these.

First of all, you get drifts of the actual 2,4,5-T and 2,4-D on the crop use and I have given data on the extent of this and the concentration of herbicides in Washington. Over and above that, material which is sprayed like timber, pasture, and shrubs may well be burned.

In fact it is quite common to burn shrubs after you spray and we already know if you heat 5 grams of pentachlorophenol at 300 degrees you get 1.5 grams of the octadioxin isomer. So you are putting into the environment material rich in polyphenols and which are likely to be combusted. We have no idea how long these dioxins will persist and these data are just not available.

But there are very real possibilities of formation of significant quantities of dioxins from phenoxy herbicide use. But I must emphasize that similar considerations also obtain to a rather wide range of polychlorophenols which we have not adequately discussed here.

Senator HART. If you had an opportunity to examine the announcement made and reported to us today, with respect to the suspension or cancellation of 2,4,5-T, given the possible danger of the noncrop use that you discussed, do you concur in the action taken? Do you think it goes too far or not far enough?

Dr. EPSTEIN. Do I have to answer that?

Senator HART. Sir?

Dr. EPSTEIN. Do I have to answer that?

Senator HART. Well, you don't have to, but it would be helpful. I am sure it would be accepted as from a person whose qualifications to make the judgment are excellent.

Dr. EPSTEIN. My view then, based on available data and also lack of data on the phenoxy herbicides, is that their use under conditions where humans are exposed is clearly not warranted.

Senator HART. Does that include the application on grazingland? Would you include that use when you say use to which humans would be exposed?

Dr. EPSTEIN. I would submit that phenoxy herbicides should, under no circumstances, be used on the crops. I would submit there is a strong presumption for suspension of their use under any circumstances in the environment.

Mr. BICKWIT. What possible dangers do you see resulting from use of chlorophenol?

Dr. EPSTEIN. Before responding to that, might I just list the categories of use of polychlorophenols? Again, this is an industrial area in which I am not competent to go into any detail. But as far as I can see polychlorophenols are used for a wide range of purposes.

First of all, for treatment of timber; then as slimicides and fungicides; for paints, varnishes, lacquer; laundry starch; for shampoos; paper and paper coatings, including food wrappings; for curing of hides; rendering of fats; production of feeds for animals and for man; and for manufacture of a wide range of pesticides.

Pentachlorophenol is used as a herbicide quite apart from being used on sugarcane, so there is a wide range of use for this in the environment. If you take 5 grams of pentachlorophenol and heat it at 300 degrees centigrade, you form 1.5 grams of the octadioxin.

We are not now talking about a billionth or trillionth part per gram; we are talking about 1.5 gram of the octadioxin isomer. I cannot tell you the degree of resulting hazard because nobody has tested it for carcinogenicity, mutagenicity, either by injection, skin application, or inhalation.

Therefore, your question is unanswerable except to say what little information we have on the dioxins indicate that these are highly toxic. That is as far as one can go.

Senator HART. Well, the data on the risk side, then, in so many of these areas, are a little skimpy, if not nil. So that when agencies have to make this judgment balancing the benefits which are identifiable against the possible health risk, how can they make that kind of judgment in the absence of adequate risk data?

Dr. EPSTEIN. I agree entirely. The concept of balancing risk against benefit is predicated on two assumptions. We usually get a good idea what the benefits are, and secondly we should know as exactly as possible what the risks are.

In this instance we do not know what the risks are, and, therefore, this equation cannot be evaluated here. We cannot discuss benefit if we have no information whatsoever on carcinogenicity, mutagenicity, and teratogenicity.

Senator HART. Well, without answering the hard question of whether you take a hard line and not permit anything on the market until you get the needed data or whether you use the lack of data as the reason for permitting it to go out until the data are available, the long and short of your testimony, and that of others we have heard today, is that it is extremely important to develop and intensify our efforts to get that data. Is not that the obvious message of today?

Dr. EPSTEIN. This is one of the messages, yes. But we have had information on dioxin for 20 years. We have known of the carcinogenicity of DDT since the late 1940's.

Senator HART. Well, if you are being critical, that is fair enough, but wouldn't you agree that whether we are slow in reacting or not, we had better react now?

Dr. EPSTEIN. Yes.

Senator HART. Doctor, thank you very much for this wonderful statement. I perhaps have indicated my own feelings by my interruption. I have had the same reaction on this subject today as we have on so many subjects. If we could stop the world for 7 days and get one part of one set of problems fixed, then crank the world up before stopping it again to move on the rest, in a lifetime we might get most of the things fixed. But you do have a feeling of wondering whether we will manage to move in time with sufficient intelligence to respond to all the problems that confront us.

(The appendices follow:)

APPENDIX I

SAMUEL S. EPSTEIN

Personal:

Born April 13, 1926, Middlesborough, Yorkshire, England.
Naturalized U. S. citizen - Married - Three children

Private address: 33 Powell Street
Brookline, Massachusetts 02146, U.S.A.

Professional address: Children's Cancer Research Fdn., Inc.
35 Binney Street
Boston, Massachusetts 02115, U.S.A.

Qualifications:

1947	B.Sc. (Physiology) London University, England.
1950	M.B.B.S. (Bachelor of Medicine, Bachelor of Surgery) (Double Honors) London University, England.
1952	D.T.M.H. (Diploma of Tropical Medicine and Hygiene, Bacteriology and Parasitology) London University.
1954	D.Path. (Diploma of Pathology) London University.
1958	M.D. (Doctorate of Medicine, Thesis in Pathology and Bacteriology) London University, England.
1963	Dipolomate, in Public Health and Medical Laboratory Microbiology, of the American Board of Microbiology.

Positions Held:

1950	Demonstrator, Morbid Anatomy, Guy's Hospital, London.
1951	House Physician, St. John's Hospital, London.
1952	Postgraduate Student in Tropical Medicine, Pathology Bacteriology and Parasitology, Royal Army Medical College, London.
1952 - 1955	Specialist in Pathology, Royal Army Medical Corps
1955 - 1958	Lecturer in Pathology and Bacteriology, Institute of Laryngology and Otology, University of London.
1958 - 1960	British Empire Cancer Campaign Research Fellow, in conjunction with the Chester Beatty Cancer Research Institute and Tumor Pathologist at the Hospital for Sick Children, Great Ormond Street, London.
1960	Consultant in Pathology, The Memorial Hospital, Peterborough, England.
1961 to date	Research Associate in Pathology and Microbiology, The Children's Hospital Medical Center and the Children's Cancer Research Foundation, Inc., Boston.
1961 to date	Chief, Laboratories of Carcinogenesis and Toxicology, Applied Microbiology and Histology, the Children's Cancer Research Foundation, Inc., Boston.
1962 to date	Senior Research Associate in Pathology, the Children's Cancer Research Foundation, Inc., Boston, and Research Associate in Pathology, Harvard Medical School, Boston.

Awards:

1. Military Awards in Royal Army Medical Corps, 1953
   (a) Montefiore Gold Medal in Tropical Medicine
   (b) Montefiore Prize in Tropical Hygiene
   (c) Ronald Martin Prize in Military Surgery
2. Society of Toxicology, 1969 Achievement Award

Society Memberships:

British Medical Association
Society of Clinical Pathologists
Society for Pathology and Bacteriology
Society for General Microbiology
Society of Protozoologists
Air Pollution Control Association
American Association of Pathologists and Bacteriologists
American Society for Experimental Pathology
American Association for Cancer Research
American Board of Microbiology
Society of Toxicology
Environmental Mutagenesis Society

Committees:

(a) Member, Committee on the Relation of Protozoology to Public Health. The Society of Protozoologists, 1962--1969.
(b) Chairman, Committee on Biological Effects of Air Pollution. Air Pollution Control Association, 1963--1969.
(c) Member, Technical Council of the Air Pollution Control Association, 1963--1969.
(d) Executive Secretary, Environmental Mutagenesis Society, 1969.
(e) Chairman, Committee on Cyclamates and Caffeine, Environmental Mutagen Society, 1969.
(f) Chairman, Committee on Liaison, Environmental Mutagen Society, 1969.
(g) Chairman, 1969, HEW Panel, Mutagenicity of Pesticides
(h) Chairman, 1969, HEW Panel, Teratogenicity of Pesticides
(i) Member, 1969, HEW Panel, Pesticide Interactions
(j) Member, 1969, HEW Panel, Carcinogenicity of Pesticides
(k) Chairman, NINH Panel on Chronic Non-psychiatric Hazards of Drugs of Abuse, 1969.

Congressional Testimony:

On "Cancer and Mutation-Producing Chemicals in Polluted Urban Air", at Hearings before the *Subcommittee on Air and Water Pollution of the Committee on Public Works*. July 29-31, 1968, presided by Senator Edmund S. Muskie.

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APPENDIX II

SUMMARY AND CONCLUSIONS

Teratology deals with the etiology and development of congenital malformations. Congenital malformations are generally defined as gross structural abnormalities of prenatal origin, present at birth or manifesting shortly after, which kill or disable. In a broader sense, teratogenesis is considered to include histological, biochemical, and functional abnormalities of prenatal origin.

Congenital malformations present obvious personal, medical, and social stresses. Additionally, it has been recently estimated that the costs to society of one severely malformed child, in terms of medical and other care and deprivation of potential earnings, amount to several hundred thousand dollars.

There are now well over 400 substances that, in various forms and combinations, are currently used as pesticides. Pesticides may represent an important potential teratogenic hazard. Therefore any teratogenic pesticide to which the population is exposed should be promptly identified so that appropriate precautions can be taken to prevent risk of human exposure. It is feasible to test these substances for teratogenic effects in test animals so that potential hazards to human health can be evaluated.

For these and other reasons detailed in the report, we conclude that:

a. All currently used pesticides should be tested for tetratogenicity in the near future in 2 or more mammalian species chosen on the basis of the closest metabolic and pharmacologic similarity to human beings possible. Pesticides should be tested at various concentrations including levels substantially higher than those to which the human population are likely to be exposed. Test procedures should also reflect routes related to human exposure. Apart from the obvious route of ingestion, attention should be directed to other routes of exposure, including inhalation exposures from pesticide aerosols and vaporizing pesticide strips used domestically and exposures from skin absorption. Parenteral administration is an appropriate test route for pesticides to which humans are exposed by inhalation, or for pesticides which are systemically absorbed following ingestion.

b. The use of currently registered pesticides to which humans are exposed and which are found to be teratogenic by suitable test procedures in one or more mammalian species should be immediately

restricted to prevent risk of human exposure. Such pesticides, in current use, include Captan; Carbaryl; the butyl, isopropyl, and isooctyl esters of 2,4-D Folpet; mercurials; PCNB; and 2,4,5-T. The teratogenicity of 2,4-D, the other salts and esters of both 2,4-D and 2,4,5-T, and that of IPC should be investigated further.

c. Pesticides found to be inactive after appropriate testing can be considered as provisionally safe, unless other evidence of teratogenicity develops.

d. No new pesticide should be registered until tested for teratogenicity by suitable procedures. Any pesticide found to be teratogenic should only be used in circumstances where risk of human exposure is minimal.

e. Efforts should be made to improve and standardize procedures for teratogenicity testing and population monitoring.

A scientific group or commission should be charged with responsibility for continued surveillance of the whole problem of pesticide teratogenesis.

Methodologies for Teratogenicity Testing

Introduction

Prior to 1963, the Food and Drug Administration did not require evaluation of teratogenicity. As a result of the thalidomide disaster, the need for data on teratogenicity became evident. In 1963, the President's Science Advisory Committee on "Use of Pesticides" recommended that toxicity studies on pesticides include effects on reproduction through at least 2 generations in at least 2 species of warmblooded animals. Observations to be included were effects on fertility, size and weight of litters, fetal mortality, teratogenicity, and growth and development of sucklings and weanlings. Such toxicity studies including the three-generation procedure were not designed primarily to detect teratogenicity and thus may not be appropriate.

The potential teratogenicity of chemicals may be detected by two complementary approaches. First, chemicals or other agents may be administered to experimental animals to determine whether they induce prenatal damage. Secondly, and on a *post hoc* basis, human populations may be epidemiologically surveyed to detect geographical or temporal clusters of unusual types or frequencies of congenital malformations. Combinations of these approaches are likely to ensure early detection and identification of teratogenic hazards.

Experimentally, a complex of factors are needed to elicit teratogenic effects. These relate to gestation period, genotype of the pregnant animals, dosage, mode of administration and metabolic transformation of teratogen. For example, teratogens may be effective only at a certain dose range, whether high or low, narrow or wide, below which development is apparently undisturbed, and above which death *in utero* results.

Most agents are teratogenic only in the developmentally labile early period of gestation, during which active organogenesis occurs. In humans, this sensitive period extends aproximately from the end of the first week of pregnancy to the 12th week. Other circumstances may also influence the effectiveness of human teratogens, such as maternal nutritional, demographic, socioeconomic, and cultural factors, physiological states, and temporal and seasonal situations. Thus a potential teratogen may manifest its effect only when particular conditions conjoin.

The relationship between human exposure to a teratogen and subsequent induction of congenital abnormalities is generally not obvious. Any one teratogen may produce a multiplicity of effects and any specific effect may be produced by various teratogens. In test animals, the teratogenetic response may differ from species to species. In humans, differences in genetic, metabolic, and environmental influences may contribute to a variety of specific effects from exposure to a particular teratogenic agent. Induced and spontaneous effects may be difficult to distinguish. The teratogenicity of thalidomide might have been missed had it not produced malformations rarely encountered; additionally, only a fraction of the pregnant women who took thalidomide had defective children.

Consequently, further data on the possible teratogenic effects of pesticides in experimental animals are urgently needed to provide a basis for evaluating potential hazards to human health.

Ancillary methods

Preliminary screening can be accomplished by the use of nonmammalian species, particularly the chick embryo. These tests may give useful ancillary data prior to further testing in mammals. However, negative results in these systems alone should not be considered proof of safety.

Use of lower mammalian species

a. Purity, composition, stability, and source of compounds under test should be determined.

b. At least two mammalian species should be tested. These should be chosen on the basis of metabolic and pharmacokinetic similarity to humans. If possible, commercially available inbred strains should be used; if not, intra-species variability must be recognized. Species commonly used include mice, rats, hamsters, rabbits, dogs, cats; sheep and swine have also been used.

c. Preliminary mammalian experiments should determine the amounts of the compound and its appropriate metabolites necessary

to produce serum levels comparable to ranges likely to be found in humans after high level accidental exposure as well as potential exposures assuming extensive use of that pesticide. Multiples of these dosages, up to the mammalian maternal LD_{50} should be administered to determine the lowest dosage causing a significant increase in fetal death, or resorption. Dosage in this critical range should be tested for teratogenic effects with care to distinguish these effects from other embryotoxicity and to determine dose-response relationships.

d. Compounds should be administered, by appropriate routes, within the critical dose range determined by preliminary tests. Parenteral administration is an appropriate test route for pesticides to which humans are exposed by inhalation, or for pesticides which are systemically absorbed following ingestion. Compounds should first be tested by single administrations of a range of doses at various times during the phases of active organogenesis. The substance should be administered at discrete times throughout the period of organogenesis as various organs are developing, since some substances have specific effects on the development of particular organs. By this technique, the possibility of inducing hepatic microsomal or other enzymes facilitating metabolic detoxification or activation of the substance is also minimized. If no teratogenic effects are detected by this technique, subsequent testing should be based on repeated administrations of the substance at daily intervals or if feasible, intervals of less than 24 hours during the entire period of organogenesis.

e. When appropriate, metabolites should also be tested for teratogenic effects.

f. Additional investigations should include—

i. Determination of appropriate plasma and fetal levels of compounds;

ii. Determination of the biological half-life of the compound in test animals;

iii. Metabolic studies to identify mechanisms of detoxification or activation of compounds when appropriate; and

iv. Determination, when appropriate, of the possible potentiating effects of protein deprivation or concomitant exposure to other pesticides or other environmental agents.

g. All procedures, including those relating to animal breeding, housing, handling, feeding, husbandry, methods for examining fetuses for congenital malformations, defining the onset of pregnancy, and classifying congenital malformations should be rigorously standardized. Numbers of pregnant animals and offspring must be adequate for statistical significance. All tests must be replicated on independent occasions and with contemporaneous controls.

Nonhuman primates

Results from lower mammalian species may warrant subsequent testing in nonhuman primates The following considerations should be noted:

a. Records of menstrual cycles are essential. Primates whose reproductive history is known and have previously delivered normal young should be selected for testing. Timing of ovulation, and therefore gestation, should be accurately determined by allowing the males and the females to be together for no more than 3 consecutive days. Vaginal smearing, to determine the presence of spermatozoa should be avoided; the use of Tullner's method for determining chronic gonadotropin levels and rectal palpation is preferable.

b. Compounds should be carefully administered in controlled dosages.

c. Pregnant animals should be handled only minimally.

d. Compounds should be administered during the various phases of organogenesis. Embryos can be obtained by laparotomy any time after the first 100 days of gestation; the mother may be subsequently used for other experimental procedures. Additionally, some young should be allowed to go to term to identify possible teratogenic effects detectable only in the neonatal period.

Population monitoring

It has been shown (see Literature Review) that some pesticides induce congenital malformations in experimental animals providing a critical dose is appropriately administered at critical times. When animal experiments indicate that a pesticide is teratogenic, human effects should be retrospectively evaluated, when possible, by study of pregnancies during which the mothers were inadvertently exposed to the pesticide, such as a result of farm work, accidental ingestion, or industrial exposure. Prospective epidemiologic approaches may involve follow-up of large numbers of people over long periods of time, and be slow, tedious, expensive, or difficult to implement. It is not appropriate to conduct prospective epidemiological studies on human populations with pesticides previously shown to be teratogenic by experimental animal studies or retrospective human data. Human exposure to such compounds must be minimized by appropriate regulatory preventive action.

Prospective epidemiological approaches for pesticides in current use may provide important information, however, it should be realized that no major teratogen has yet been recognized in this way. The malformations induced by X-ray, German measles, thalidomide, and mercury—Minamata disease, were each recognized by an alert medical

practitioner who observed a cluster of cases and then traced the cause to its source.

What can be done to enhance prompt recognition of such clusters should they occur from previously unsuspected teratogens in the future? A variety of existing data resources can be used for this purpose. In each, the occurrence of congenital malformations in substantial segments of the population is being recorded in a standard fashion. The best of these resources are local, rather than statewide or national. The prepaid medical program of the Kaiser-Permanente Hospitals and Clinics in the San Francisco Bay Area are of particular interest. A detailed study there of the occurrence of malformations among 16,000 births represents a good model for additional investigations. A similar study has been made by the Health Insurance Plan of Greater New York, but its 30 or more cooperating clinics are less easily coordinated than the Kaiser system.

A citywide surveillance, known as the Metropolitan Atlanta Congenital Defects Program (jointly directed by Emory University School of Medicine, the Georgia Department of Public Health, and the National Communicable Disease Center, USPHS), involves reports on all children with congenital malformations born to residents of the five-county Atlanta area. As yet, no cluster of cases has suggested an environmental influence since the program began in October, 1967.

In a substantial number of States, birth certificates contain an item concerning congenital malformations. The completeness and accuracy of such reporting varies considerably and depends on the physician's interest and diligence and on the conspicuousness of the abnormality. Birth-certificate data on malformations in New York State are more extensive than those of many other States and have been effectively used in several research studies. Nationally, however, no attempt has been made to collect and evaluate all data on malformations that are available on birth certificates.

A select committee convened by the National Center for Health Statistics (NCHS), has recommended, in an excellent but little known report, that efforts be made to improve and use information on congenital malformations recorded on birth certificates (Vital and Health Statistics, Documents and Committee Reports, NCHS Series 4, Number 7, March 1968). Implementation of this recommendation would be of great value, for monitoring to detect the teratogenic effects of newly introduced or geographically localized environmental chemicals or other agents.

To enhance our ability to recognize significant changes in congenital malformation rates, a systematic collection of data from concentration points should be established. Specifically, a surveillance should be made of claims submitted to private, State, or local agencies for the medical care of children with birth defects. Because the Children's Bureau, DHEW, has so much experience with these agencies, its assistance should be sought in planning the surveillance network.

Data from foreign countries should also be evaluated as part of a national effort to study possible relationships between pesticides and congenital malfunctions.

In studying the possible relationships between exposure to pesticides and the occurrence of diseases, statistical associations, if present, will provide important information. However, when possible it is important to secure additional information concerning the following:

a. Dose-response relationships.
b. Absence of alternative explanations.
c. Biological plausibility.
d. Consistency with other knowledge from clinical, laboratory, and epidemiologic research.
e. Disappearance of the effect when the presumed cause is removed.

In particular, as clusters of specific anomalies are recognized, through whatever resources that presently exist or may be developed, any possible relationships to pesticides would be clarified by the use of laboratory techniques to measure the maternal, fetal, or neonatal body burden of suspect chemicals.

There are national units engaged in teratologic research, but each is following a set method. There is a critical and immediate need to establish a national or international center to study congenital malformations in man not by a single method but by whatever techniques are most appropriate for testing or generating hypotheses. The center should be diversified and fast moving, ready to use local, national, or international resources in order to determine the significance of laboratory or clinical data.

Literature Review

Animal studies

For convenience, detailed results of the Bionetics study are presented in a subsequent section.

Much of the total available literature and data reviewed by this Panel were methodologically inadequate to support definitive conclusions. Additionally, the authors of many reports tended to confuse or equate embryotoxicity and other adverse effects on reproduction with teratogenicity. It is also apparent from the literature that insufficient attention has been directed towards problems of interactions in testing for teratogenesis.

The Panel considered the following information to be of significance:

a. Captan and Folpet.—These pesticides have been shown to be teratogenic in chicken embryos (Verrett et al., 1969). Captan was also shown to be teratogenic in rabbits (McLaughlin, 1969), although other rabbit studies yielded negative results (Kennedy et al., 1968; Fabro et al., 1965). The enhancement by protein deprivation of the acute toxicity of captan to rats (Boyd, 1968), was noted with particular interest. The teratogenicity of captan and Folpet in mice was demonstrated in Bionetics studies. Unpublished data on captan in monkeys were evaluated and found inadequate; in these studies, the duration of organogenesis was not entirely covered and controls were not appropriate. However, the 3/7 abortions observed at the highest dosage given, 25 mg./kg., may be indicative of an embryotoxic hazard due to captan.

b. Carbaryl.—This was tested at 66.7 and 200 p.p.m. in the diet of pregnant mice (FAO/WHO, 1967). In two litters at the 200 p.p.m. level, a total of seven instances of skeletal malalignment, nonfusion, incomplete ossification, and one case of cleft palate and gross facial malformation were noted, as opposed to no malformations in the low-level group and two cases of cleft palate in controls. Teratogenetic findings for carbaryl are also reported in the Bionetics study. In a study in beagle dogs fed carbaryl during gestational periods at levels of 50, 25, 12.5, 6.25, and 3.125 mg./kg. body weight daily, teratogenic effects were found at all but the lowest dose level (Smalley, 1968).

c. Mercurials.—Organomercury compounds: Various mercury containing pesticides were evaluated under the heading "phenylmercury acetate (and other organomercury compounds)" by the 1966 Joint Meeting of the FAO Working Party and the WHO Expert Committee on Pesticide Residues (FAO/WHO, 1967). The results of additional experimental work have been reported in the 1967 Evaluations of Some Pesticide Residues in Food. Additional information on "Methylmercury" was published by the Ecological Research Committee, the Swedish Natural Science Research Council (1969) Bulletin no. 4. by Goran Lofroth, where embryotoxic effects in mice (reported by Frölén and Ramel) were discussed along with other data. When given subcutaneously, in doses of 0.11 mg. on day 7 of gestation, phenylmercuric acetate was reported to cause fetal malformations in mice. Eye, tail, and central nervous system defects were noted (Murakami *et al.*, 1956).

d. Organochlorine.—Embryotoxicity in rats and dogs has been reported for organochlorines including dieldrin, chlordane, and kepone. In the absence of convincing data, kelthane has been claimed to be teratogenic in mice (An Der Lan, 1964); see also Bionetics studies.

e. Organophosphates.—The cholinesterase-inhibiting organophosphate insecticides, guthion, parathion, diazinon, Bidrin, Trithion, and EPN, have been shown to be teratogenic when injected directly in the yolk sac of chick embryos. The malformations were nonspecific or common to all organophosphates (Fish, 1966). It was also claimed that these compounds are teratogenic in mice. The data reported, however, suggested that organophosphates, like the organochlorines, act by reducing litter size and producing embryotoxicity rather than by producing specific teratogenic effects. See also Bionetics studies.

f. Thiram.—Thiram was reported to be teratogenic in hamsters at 250 mg./kg. (Robens, 1969). In the Bionetics study it was not found to be teratogenic. In a study of three generations of rats, no toxicological effects were observed at a dietary level of 48 p.p.m. (FAO/WHO, 1967). However, Thiram should be further investigated for possible teratogenic effects.

g. Miscellaneous reproductive effects.—Placental transfer of dieldrin and incidence of stillbirths have been studied in cows (Braund, 1968); increased stillbirth rates have been claimed in cows fed with DDT (Labon, 1965). The estrogenic activity of o,p'DDT has been related to reproductive effects in chicken, quail, and rats (Bateman, 1968, Wurster, 1968; Porter and Weimeyer, 1969). Diminished population size and reproductive failure have been produced in sparrow hawks by DDT and dieldrin (Porter and Weimeyer, 1969). These resulted from a decreased eggshell thickness, increased breakage of eggs, and increased egg eating by parent birds. Other studies of interest include the following: Finnegan, 1949; Tauber, 1950; Fisher, 1952; Narpozzi, 1956; Swann, 1958; Cottrell, 1959; Marliac, 1964; Backstrom, 1965; Hathaway, 1967; Ware, 1967; Weike, 1967; Carlton, 1968; Keplinger, 1968; Khera, 1968; Verrett, 1969; Legator, 1969.

Bionetics animal studies

Bionetics Research Laboratories of Litton Industries, during 1965–68 under a contract for the National Cancer Institute (NCI Contracts PH 43–64–57 and PH 43–67–735), tested various pesticides and related compounds for teratogenic effects. These studies were designed as large-scale screening tests. The Bionetics data were reanalyzed statistically to account for litter effects. The results of this statistical re-evaluation are presented in this section. More detailed material on these pesticides will be published in the future.

a. Summary of findings from Bionetic animal studies.—Tested more extensively than other pesticides, 2,4,5-T was clearly teratogenic as evidenced by production of statistically increased proportions of

litters affected, and increased proportions of abnormal fetuses within litters in both DMSO and Honey for both C57BL/6 and AKR mice. In particular, cleft palate and cystic kidneys were significantly more prevalent. In addition, a hybrid strain resulting from a C57BL/6 female and AKR male showed significant increases in anomalies, in particular cystic kidney, when administered at 113 mg./kg. of body weight in DMSO.

Additionally, 2,4,5-T was tested in Sprague-Dawley rats. When given orally at dosages of 4.6, 10.0 and 46.4 mg./kg. on days 10 through 15 of gestation, an excessive fetal mortality, up to 60 percent at the highest dose, and high incidence of abnormalities in the survivors was obtained. The incidence of fetuses with kidney anomalies was three-fold that of the controls, even with the smallest dosage tested.

PCNB produced an increase in renal agenesis between litters, and within litters, when administered orally from days 6–14 or days 6–10 of pregnancy. However, renal agenesis was not produced when PCNB was administered only from days 10–14 of pregnancy. These effects were produced in only the C57BL/6 strain of mice.

Other pesticides producing a statistically significant increase in the proportion of litters containing abnormal fetuses and in the increased incidence of abnormal fetuses within litters were: Captan, Folpet, 2,4-D isooctyl ester, 2,4-D butyl ester, 2,4-D isopropyl ester, carbaryl (Sevin), and IPC. These pesticides produced elevated inci- and in one solvent only. The results for carbaryl and for IPC were less consistent than for other compounds. (The pesticides 2,4,5-T, PCNB, captan, Folpet, carbaryl, IPC, and the butyl and isopropyl esters of 2,4-D were statistically significant at the .01 level, for one or more tests. This criterion is similar to that adopted by the Technical Panel on Carcinogenesis, Chapter 5, to identify "positive" compounds. The isooctyl ester of 2,4-D was significant at the 0.05 level.)

Compounds inducing only an increase in the proportion of abnormal fetuses within litters were: a-naphthol, and 2,4-D methyl ester. The statistical significance of these results was relatively weak; further study is required before any conclusions can be reached. Similarly, 2,4-D produced only an increase in the proportion of abnormal litters during 1965 in AKR mice. Due to the teratogenic activity of certain of its esters, 2,4-D should be studied further.

Carbaryl plus piperonyl butoxide did not show an overall increase in nonspecific anomalies, but resulted in significantly more cystic kidneys for doses above 10 mg./kg. carbaryl plus 100 μl./kg. piperonyl butoxide.

It must be emphasized that failure to detect statistically significant increases of anomalies may be due to insensitivity resulting from experimental variation and small numbers of litters tested. In addition, higher fetal mortality among some of the "negative" compounds may be selectively eliminating abnormal fetuses.

b. Methods.—Four strains of mice were used: C57BL/6, AKR, C3H, and A/Ha. Most of the studies were performed with the C57BL/6 strain. A hybrid fetus resulting from mating a C57BL/6 female with an AKR male was used to study a few compounds. More restricted studies were also made on Sprague Dawley rats; results of these with reference to 2,4,5-T are considered separately.

Most compounds were administered subcutaneously in 0.1 ml. solutions of dimethylsulfoxide (DMSO). Water soluble compounds were administered in saline, and some times also in DMSO. Compounds administered orally were given by gavage in 0.1 ml. in a 50-percent honey solution. Groups of positive controls and untreated controls were included, as well as controls receiving only DMSO, saline, or honey. While controls were run periodically throughout the duration of the study, compounds and controls were not matched with respect to either route or date of administration.

Virgin females were used in these studies. The onset of pregnancy was determined by detection of vaginal plugs. Compounds were administered daily from the sixth to the 14th day of pregnancy (15th day for AKR mice). Mice were sacrificed on the 18th day (19th day for AKR mice) of gestation. On sacrifice, fetuses were examined for anomalies. Approximately two-thirds of the fetuses were then stored in Bouin's solution until necropsy. Remaining fetuses were stained with alizarin red S after proper processing. Numbers of resorption sites and dead fetuses were also scored.

c. Statistical analysis.—All analyses were performed on a *per* litter basis rather than a *per* fetus basis, since initial investigations indicated that the occurrences of anomalies among fetuses within litters were correlated. The large litter-to-litter variation may reflect some maternal effect, an indication of the effective dose level of the compound actually reaching the fetuses, experimental variation, or, as is most likely, some combination of the three factors.

While there were no statistically significant time trends within the various control groups in terms of the onset of fetal anomalies in the C57BL/6 mice, the incidence of fetal mortality was certainly time-dependent in this strain, with 1965 being characterized by a low incidence of prenatal deaths. Furthermore, there was a period of approximately 6 months, extending from the latter part of 1965 into early 1966, during which no control animals were tested. During this period a change in the substrain of C57BL/6 mice used in the study took place. Finally, among abnormal litters, as defined by litters con-

taining at least one abnormal fetus, there was some suggestion that the distribution of abnormal fetuses *per* litter was stochastically larger in the DMSO controls than it was in the untreated controls. Thus, the possibility exists of a time/strain/solvent interaction that is undetectable in the controls because the level of background teratologic activity is relatively low. This potential interaction effect could either enhance or dissipate the effect of any given compound, depending on the conditions under which it was administered. Thus, the data were necessarily separated by both time period and solvent for the purposes of analysis. Similarly, an increase in fetal anomalies in the DMSO controls of the AKR mice was noted after November 1966. Thus, the AKR data were analyzed separately in two time periods.

It should be noted that not all compounds were administered on more than one occasion or in more than one solvent or strain. Thus, in general the compounds in the study cannot be compared for teratogenic potential, since those that were tested extensively were more likely to show some adverse effect and, perhaps, less likely to appear consistent over time, solvent, and/or strain.

As noted, approximately two-thirds of the fetuses were stored in Bouin's solution until necropsied; the remainder being stained with alizarin red. However, in many instances the proportion of necropsied fetuses was slightly higher for the compound under investigation than for the corresponding controls. It is doubtful if this discrepancy could have any appreciable effect on the conclusions since the incidence of anomalies detectable only by necropsy among control animals was relatively low. Furthermore, if all of the control and test mice had been necropsied, the significance of the differences observed in this study would be intensified. Thus, no effort was made to correct for inequalities in the necropsy/stain ratio in the present analysis. Additionally, no attempt was made to correct for differences in litter sizes or sex-ratios within litters, since both of these factors may, at least in part, reflect effects of the compound under test.

d. Results.—Data for pesticides yielding a statistically increased level of anomalies in C57BL/6 and AKR mice are listed in tables 1 and 2, respectively. The proportion of abnormal litters gives the proportion of litters containing one or more abnormal fetuses, as a measure of the prevalence of anomalies across litters. The proportion of abnormal fetuses *per* litter gives a measure of the prevalence of anomalies within litters. The proportion of abnormal fetuses *per* litter for litters containing abnormal fetuses gives a measure of the prevalence of anomalies within effected litters. A significant increase of dead fetuses and resorptions is also listed. Some tests were conducted on only one particular day or on adjacent days as listed. Eye anomalies, mainly microphthalmia and anophthalmia, accounted for approximately 50 percent of the individual anomalies in C57BL/6 mice. To a large extent, results in table 1 reflect changes in the incidence of eye anomalies. Yet, when the data were analyzed excluding fetuses with microphthalmia only, there were no striking changes in the results. In the last column of table 1, statistically significant increase in various types of anomalies other than eye anomalies are listed. The positive controls, trypan blue and ethyleneimine, table 1, and 6-aminonicotinamide, table 2, showed elevated levels of anomalies, although the latter control did not yield consistent results over all dose levels.

Only those test conditions which resulted in statistically elevated incidences of anomalies are listed in tables 1 and 2. Some compounds gave no increase in anomalies (based on the overall incidence if tested in both time periods) when tested in other solvents, strains, or dose levels (table 3). It must be emphasized that failure to detect a statistically significant increase in anomalies may only be a reflection of experimental insensitivity due to experimental and biological variaton and insufficient number of litters. Thus, compounds showing no increases cannot be considered nonteratogenic. For example, trypan blue in DMSO at the highest dose level tested, 37.5 mg./kg., did not show an increase in anomalies, possibly due to higher fetal mortality. Standard corrected 2 × 2 chi-square tests (*1*) were used to compare the proportion of abnormal litters for the compound with the controls in the same solvent. In the cases where tests were conducted in two time periods, the results from the two chi-squares were combined (*1*). The levels of statistical significance for the combined tests are listed under the total column for proportion of abnormal litters.

The distribution of the proportion of abnormal fetuses per litter (tables 1 and 2) for compounds were compared with the appropriate control distribution by use of the nonparametric Mann-Whitney U-test (*2*). This test requires that the proportion of abnormal fetuses per litter is independent from litter to litter, but requires no assumption about the frequency distribution of these proportions. Again, where litters were run in both time periods, the significance level for the combined tests is given under the total column. Bracketed data include groups which were combined before statistical tests were conducted.

STATISTICAL REFERENCES

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TABLE 1.—*Tests which displayed significant increases of anomalies (C57BL/6 mice)*

Compound	Solvent	Dose per kg of body weight	Proportion of abnormal litters			Proportion of abnormal fetuses per litter			Proportion of abnormal fetuses per litter in abnormal litters			Increased mortality	Tests repeated over time	No. of live litters		Increased anomalies other than eye
			1965	1966–68	Total	1965	1966–68	Total	1965	1966–68	Total			1965	1966–68	
Negative controls:																
Untreated	None		.42	.39	.40	.08	.11	.10	.18	.28	.25			26	69	
Controls	DMSO		.53	.41	.46	.16	.12	.13	.33	.28	.29			70	112	
Do	Saline		.52	.37	.43	.13	.10	.11	.24	.28	.26			31	46	
Do	Honey			.47	.47		.15	.15		.32	.32				32	
Positive controls:																
Trypan blue	DMSO	5.0 mg	.60		.60	.32		.32	.54		.54	Yes		5		Hydrocephaly.
Do	DMSO	12.5 mg	.86		.86	.44***		.44***	.52**		.52**	Yes		7		Hydrocephaly.
Do	DMSO	37.5 mg	.60		.60	.36		.36	.60		.60	Yes		5		Hydrocephaly.
Do	Saline	5.0 mg	1.00		1.00	.61***		.61***	.61**		.61**			5		
Do	do	12.5 mg	.71		.71	.49**		.49**	.69***		.69***	Yes		7		
Do	do	37.5 mg	.71		.71	.33*		.33*	.46**		.46**	Yes		7		Hydrocephaly.
Ethyleneimine	do	4.64 μl	1.00*		1.00*	.49***		.49***	.49***		.49***	Yes	No	7		
Experimental:																
2,4,5-T	DMSO	113 mg		.79**	.79**		.56***	.56***		.71***	.71***	Yes		14		Cleft palate cystic kidney.
2,4,5-T	Honey	46.4 mg		1.00*	1.00*		.37**	.37**		.37	.37		No	6		
2,4,5-T	do	113 mg		1.00**	1.00**		.70***	.70***		.70***	.70***	Yes		9		Cleft palate cystic kidney.
PCNB (days 6–14)	do	215 mg		.88*	.88*		.25**	.25**		.29	.29		No	8		
PCNB (days 6–14)	do	464 mg		.67	.67		.25	.25		.38	.38			12		Renal agenesis.
PCNB (days 6–10)	do	464 mg		1.00	1.00		.38	.38		.37	.37		No	10		Renal agenesis.
Captan	DMSO	100 mg	1.00*	.61	.71***	.58***	.27	.35***	.58**	.44	.49 **	Yes		6	18	
Folpet	DMSO	100 mg		.77 **	.77 **		.29 ***	.29 ***		.38 *	.38 *				13	
2,4-Isooctyl ester	DMSO	48 μl	1.00 *		1.00 *	.24		.24	.24		.24			6		
2,4-D Isooctyl ester	DMSO	130 μl		.67	.67		.28 **	.28 **		.41 *	.41 *				15	
2,4-D Butyl ester	DMSO	100 μl		.75 **	.75 **		.25 ***	.25 ***		.34	.34				20	Agnathia.
2,4-D Isopropyl ester.	DMSO	94 μl		.70 **	.70 **		.26 ***	.26 ***		.37 *	.37 *				20	
Carbaryl	DMSO	100 mg	1.00 *	.54	.71 **	.46 ***	.16	.26 **	.46 *	.29	.37			6	11	Hydrocephaly, skeletal
IPC	DMSO	850 mg	1.00 **	.43	.71 *	.46 ***	.09	.27 **	.46 *		.46 *			7	7	
α-Napthol	DMSO	10 mg	.86		.86	.33 *		.39 *	.38		.38			7		
2,4-D Methyl ester	DMSO	106 mg		.83	.83		.30 *	.30 *		.36	.36				6	
Carbaryl+Piperonyl Butoxide.	DMSO	10 mg+100 μl		.50	.50		.13	.13		.26	.26		No		6	Cystic kidney
Do	DMSO	46.4 mg+464 μl		.50	.50		.16	.10		.21	.21				12	Cystic kidney

Significance level: *(.10). **(.05). ***(.01).

TABLE 2.—*Tests which displayed significant increases of anomalies (AKR mice)*

Compound	Solvent	Dose per kg of body weight	Proportion of abnormal litters			Proportion of abnormal fetuses per litter			Proportion of abnormal fetuses per litter in abnormal litters			Increased mortality	Tests repeated over time	No. of live litters		Special anomalies
			†11/66	12/66††	Total	†11/66	12/66††	Total	†11/66	12/66††	Total			†11/66	12/66††	
Negative controls:																
Control	DMSO		.05	.37	.21	.01	.06	.03	.11	.16	.15			37	35	
Do	Honey			.00	.00		.00	.00							12	
Positive controls:																
6-amino-nicotinamide.	DMSO	.34 mg	.56 ***		.56 ***			.31 **	.55		.55			9		Cleft palate.
6-amino-nicotinamide (1).	DMSO	.68 mg	.00		.00	.00		.00						7		
Experimental:																
2,4,5-T	DMSO	113 mg	.50 ***	1.00 **	.71 ***	.20 **	.40 ***	.29 ***	.40 *	.40 **	.40 *			8		6 Cleft palate
2,4,5-T	Honey	113 mg		1.00 ***	1.00 ***		.54 ***	.54 ***		.54	.54	yes			6	Cleft palate
2,4-D	DMSO	98 mg	.43 **	.29	.36 *	.12	.05	.06	.28	.16	.22			7	7	

*Significance Level .10. **Significance Level .05. ***Significance Level .01.
†Through 11/66 ††After 11/66
Note: (1) With the .66 mg/kg dose, as compared to the .34 mg/kg dose, fewer implantations and a higher fetal mortality were encountered, resulting in fewer live fetuses per litter.

TABLE 3.—*Tests which showed no significant increase of anomalies (with particular doses, solvents, or test strains)*

Compound	Strains	Solvent	Dose per kg. body wt.	Increased mortality (C57BL/6)	Total number of litters
2,4,5-T	C57	DMSO	21.5 mg.		6
PCNB (days 10–14)	C57	Honey	464 mg.		9
PCNB	AKR	Honey	464 mg.		9
Captan	C57	Honey	100 mg.		12
Do	AKR	DMSO	100 mg.		13
Folpet	C57	Honey	100 mg.		5
Do	AKR	DMSO	100 mg.		13
2,4-D Isooctyl ester	C3H	DMSO	48 µl.		6
Do	A/Ha	DMSO	24 µl.		5
Do	AKR	DMSO	130 µl.		8
2,4-D Butyl Ester	C57	DMSO	46 µl.		6
Do	AKR	DMSO	100 µl.		10
2,4-D Isopropyl Ester	C57	DMSO	46 µl.		6
Do	AKR	DMSO	94 µl.		6
Carbaryl	C3H	DMSO	100 mg.		8
Do	C57×AKR	DMSO	100 mg.		6
Do	AKR	DMSO	464 mg.		13
IPC	C3H	DMSO	850 mg.		11
IPC	AKR	DMSO	850 mg.		13
2,4-D Methyl Ester	AKR	DMSO	106 mg.		7
Do	C57×AKR	DMSO	106 mg.		5
o,p'-DDD	C57	DMSO	100 mg.		13
Do	AKR	DMSO	100 mg.	Yes	12
2,4-D	C57	DMSO	100 mg.		16
Do	C57	Honey	100 mg.		12
Do	C3H	DMSO	100 mg.		6
Do	C57×AKR	DMSO	98 mg.		11
Zectran	C57	DMSO	10 mg.		7
Do	AKR	DMSO	10 mg.		7
Thiram	C57	DMSO	10 mg.		8
Do	AKR	DMSO	115 mg.		7
Ferbam	C3H	DMSO	4.64 mg.		6
Do	C57	DMSO	4.64 mg.		6
Monuron	C3H	DMSO	215 mg.		7
Do	C57	DMSO	215 mg.		13
Do	C57	Honey	215 mg.		9
Do	AKR	DMSO	215 mg.		13
Diuron	C3H	DMSO	215 mg.		6
Do	C57	DMSO	215 mg.		6
2,4-D Ethyl Ester	C57	DMSO	86 µl		7
Do	AKR	DMSO	86 µl		7
Atrazine	C3H	DMSO	46.4 mg.		6
Do	C57	DMSO	46.4 mg.		13
Do	AKR	DMSO	46.4 mg.		15

TABLE 3.—*Tests which showed no significant increase of anomalies (with particular doses, solvents, or test strains)*—Continued

Compound	Strains	Solvent	Dose per kg. body wt.	Increased mortality (C57BL/6)	Total number of litters
Piperonyl Butoxide	C3H	DMSO	1000 µl	----	6
Do	C57	DMSO	1000 µl	----	6
Do	C57	DMSO	21.5 µl	----	6
p,p'-DDD	C57	DMSO	46.4 mg.	----	6
p,p'-DDT	C57	DMSO	46.4 mg.	----	6
Carbaryl + Nicotinamide	C57	DMSO	100+61 mg.	----	10
Nicotinamide	C57	DMSO	61 mg.	Yes	6
CIPC	C57	DMSO	1000 mg.	----	6
Nabam	C3H	DMSO	21.5 mg.	----	6
Do	C57	DMSO	46.4 mg.	----	6
Do	C57	Saline	46.4 mg.	----	14
Do	AKR	DMSO	46.4 mg.	----	5
Do	AKR	Saline	46.4 mg.	----	14
Propazine	C3H	DMSO	464 mg.	----	6
Dieryl	C57	DMSO	21.5 mg.	----	6
Perthane	C57	DMSO	100 mg.	----	6
Ovex	AKR	DMSO	185 mg.	----	7
Tedion	AKR	DMSO	217 mg.	----	6
Amitrol	C57	Saline	464 mg.	----	13
Do	C57	Honey	215 mg.	Yes	8
Do	AKR	Saline	464 mg.	----	14

Human studies

Epidemiologic data on possible effects of pesticides on human reproduction and teratology are grossly inadequate. Prospective studies on this subject are difficult to design and almost nonexistent, except for the community pesticide program of the Food and Drug Administration.

Chlorinated hydrocarbons.—In a recent review (Khera and Clegg, 1969), no adverse human reproductive effects were attributed to DDT and other chlorinated hydrocarbons. Studies on 240 pregnant women indicated that 21 percent had significant first trimester pesticide exposure, and that 52 percent were exposed during their entire pregnancy. No statistical difference in numbers of patients with anomalies existed between these exposed groups (Nora et al., 1967). Low values of DDT residues have been found in a small number of human placentas (Rappolt et al., 1969). Sharply reduced tissue levels were also found in 68 newborn infants (Zavon, 1969). Pesticide levels in human milk have not shown any relation to perinatal toxicity (Laug et al., 1951; Lofroth, 1969; Curley and Kimbrough, 1969). Studies on 152 mothers showed transplacental passage of DDT and DDE (O'Leary, 1969). Low placental and high vernix levels were noted; fetal blood levels were one-half maternal levels. In a similar study on premature infants (O'Leary, 1969), high fetal levels were noted; no relationship between maternal blood levels of DDE and DDT and the incidence of first trimester spontaneous abortion were found, although the number of pregnant women reported on was inadequate for firm conclusions.

Organophosphates.—Evidence of teratogenic potential of organophosphates in humans has been reviewed and found inconclusive (Khera and Clegg, 1969).

Mercurials.—Consumption by Japanese pregnant women of fish and shellfish contaminated by methylmercury produced a high incidence of infantile cerebral palsy (Matsumoto *et al.*, 1965). This condition has been termed fetal Minamata disease.

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Senator HART. Before adjourning, I should add for the record [illegible] had been scheduled Dr. DuBridge of the Office of Science and Technology, but Mr. Bickwit advises that in the face of a 5:30 appointment, he has asked not to be heard, but instead submits his statement for the record.

(The statement follows:)

STATEMENT OF DR. LEE A. DUBRIDGE, DIRECTOR, OFFICE OF SCIENCE AND TECHNOLOGY

SENATE COMMERCE COMMITTEE,
APRIL 15, 1970.

Mr. Chairman, Members of the Subcommittee, Let me say at the outset that I am pleased to have an opportunity to discuss with this Subcommittee certain aspects of the herbicide, 2,4,5-T. An examination of the subject illustrates a number of important issues relating to the Federal Government's involvement with pesticides. I believe that these deserve some discussion and I am glad to have the privilege of exploring them with you.

The herbicide, 2,4,5-trichlorophenoxyacetic acid is a member of a family of pesticides which have served mankind very well for a long period of time. This group of compounds, known as phenoxy or auxin herbicides, have been used since the late 1940's and resulted from research work performed during the 1940's on herbicides and defoliants for military as well as civilian use.

The toxicity of 2,4,5-T was studied in line with the requirements for its registration by the Department of Agriculture. The toxicology required for this registration of 2,4,5-T was aimed primarily at determining its *acute* toxicity. In this regard, it is now quite clear that the experiments performed for this purpose (almost all of which were done by or for the manufacturing industry seeking the registration) revealed that 2,4,5-T was relatively non-toxic

This herbicide demonstrated a persistence in soil and water which was very short (on the order of three months for total disappearance). It is true also, as you heard last week, that only rare instances of 2,4,5-T residues have been discovered in the food surveys performed by the Department of Health, Education and Welfare.

As a result of these findings, plus its proved utility as an herbicide and as a defoliant, 2,4,5-T was considered a very beneficial and safe herbicide and with good reason. As evidence for this, it is plain that the demand for 2,4,5-T has risen, especially in the last several years. The production of 2,4,5-T in the United States increased from 7.0 million to 42.5 million pounds between 1960 and 1968. Domestically, it has proved its worth as a valuable adjunct in the clearing of range and pasture lands of brush, in the clearing of roadsides and rights-of-way, in the suppression of aquatic weeds, in the limited use for control of weeds in croplands, and for altering physiological responses of crops. The increase in production apparently has reflected the demand for 2,4,5-T both domestically and as a defoliant for military operations in southeast Asia. In fact, the domestically used quantities actually decreased between 1964 and 1966.

In 1964, the National Cancer Institute of the National Institutes of Health undertook on contract with the Bionetics Research Laboratories, Incorporated, a screening study of a large number of economic poisons. As you have heard, the general purpose of this study was to ascertain the potential for cancer, for genetic alteration, and the potential of producing birth defects for this long list of pesticides. All who have been concerned with this subject recognize the value of the Bionetics study as a screening mechanism for these potential hazards. 2,4,5-T was among the list of materials screened. One of the results of this study was that a particular lot of commercial grade 2,4,5-T provoked birth

defects in mice and rats if administered in sufficiently l[illegible] doses at an appropriate stage of pregnancy in these animals. These res[illegible] were available in 1968 and they were subsequently further analyzed statistically by the National Institute of Environmental Health Sciences.

No further action was taken on the findings of the Bionetics study after August 1968 nor was the information on teratogenesis publicly available. However, copies of the study reports did find their way to members of Congress, to journalists, and to some members of the scientific community. Coincidentally, in May or June of 1969, a number of anecdotal articles appeared in the Vietnamese press which reported an unusual incidence of congenital abnormalities and abnormalities of pregnancy in certain parts of Vietnam. In some cases, these reports were linked to defoliation operations. The evidence for this is, however, extremely doubtful.

It was with this background that I met in October of last year, in my capacity of Executive Secretary of the Environmental Quality Council, with representatives of the several Federal agencies which were most concerned with the use of this herbicide. It was the consensus of these representatives that this research information from the Bionetics study warranted serious consideration including certain restrictions on the use of 2,4,5-T. The announcement of these intended actions occurred on October 29.

One of these actions was a limitation of defoliation operations in Vietnam. The limitation, which did occur subsequently, took the form of restricting defoliation to non-populated areas. Another announced action was aimed at the Government's own use of 2,4,5-T domestically in programs of brush and weed control. These programs were mainly pursued by the Department of Agriculture and the Department of the Interior. Here the Government did restrict the application of this herbicide so as to reduce possible exposure to man. The State Department, which to some extent had been a party to the use of 2,4,5-T along our border with Canada, took steps to reduce human exposure here and make available to foreign countries technical data about the subject.

The Department of Agriculture agreed to cancel the registration of 2,4,5-T for use on food crops by the first of the year unless the Food and Drug Administration could, by that time, satisfy itself that it had enough evidence to establish a negligible tolerance limit for human exposure. The food crop uses of 2,4,5-T incidentally represented a minority of its total domestic use.

At the time of the announcement of concerted Government actions, I also assembled a panel of experts within the Office of Science and Technology to review all that is known about of 2,4,5-T. This Panel has prepared a report on the subject which I expect to make available within a few weeks. During the course of this review, it became known that an impurity of 2,4,5-T was of potential importance. The impurity, a polychlorinated dioxin, was apparently very toxic and had been identified in batches of 2,4,5-T as early as 1957. It arose partly as an impurity of the chlorphenol starting material and partly as a result of the temperatures and pressures of certain of the reactions in the manufacturing process. It had provoked severe skin irritations among workers in 2,4,5-T plants in Germany and in the United States. The discovery of this industrial hazard had led one U.S. manufacturer to curtail his process until he was able to reduce the dioxin content to less than 1.0 ppm in the 2,4,5-T product. This eliminated the skin irritation problem.

Within weeks after my announcement, some additional animal experiments were begun in two laboratories simultaneously. These experiments were directed towards confirming and extending the results of the Bionetics studies. In addition, they were aimed at finding out whether the apparent teratogenic agent was 2,4,5-T itself, or a potent impurity.

Fortunately, the experiments needed to test for teratogenesis are essentially acute, short-term studies. With an expectation of meaningful results from these experiments in a fairly short period of time and in view of the potential

of the dioxin impurity, the Department of Agriculture in consultation with Interior, HEW, and my office delayed any action toward cancellation of the food crop registrations for 2,4,5-T as you heard last week.

One of the sets of confirmatory studies was undertaken by the Government, itself. The results of these experiments, pursued at the National Institute of Environmental Health Sciences, were made known to you by Dr. Steinfeld this morning. Essentially, these results implicated both 2,4,5-T (in the purest form available) and its dioxin impurity as potential teratogens. This story, I think you will agree, represents an example of some appropriate Government actions. As you have heard and as Dr. Steinfeld pointed out, the results of these confirmatory studies were translated into immediate actions in the form of a series of announced Government restrictions on uses of 2,4,5-T.

Let me now turn to what I believe are some important lessons to be learned from this fascinating case study. First, let me review. We have here an example of a chemical substance intentionally placed into the environment by man for the betterment of his welfare. Where the aim has been to exchange capital for labor in land and waterway management, there can be no doubt that this herbicide has proved its worth.

In view of what we have now learned, I am persuaded that we must consider some changes in our procedures and we must be willing to submit our regulatory systems for pesticides, as for other chemicals in the environment, to examination. The very excellent report of the Secretary's Commission on Pesticides, headed by Dr. Emil Mrak, will, of course, serve to point up this issue.

From a number of indications it is quite apparent that we, as a society, have relatively recently begun to ask more sophisticated questions about adverse effects on health of a variety of chemical substances. In part, this has come about because of some additional scientific knowledge and investigative tools. In part, it has arisen simply by virtue of some increased concern about the safety of environmental chemicals. For example, the realization that environmental agents may be major contributors to the incidence of cancers in the epidemiological sense is a fairly recent observation.

In brief, then, we have set our sights higher in terms of the questions we would like the scientific community to ask about pesticides. As I reminded you at the outset, the total amount of background toxicology performed on 2,4,5-T had been limited to studies of acute toxicity—performed essentially by industry as directed by the Federal Government. No one had seriously suggested that the hazards of birth defects, genetic change, or cancer be tested for in the case of 2,4,5-T, nor were there tools to screen for these diseases. The Bionetics study represented a step up in degree of sophistication of research.

Certainly this evolutionary process is a highly commendable situation and is one which is to be encouraged. There do exist some dilemmas, however. The major dilemma accrues from the fact that there is no real end point to this questioning process. The more research that is performed, the more new questions will be raised about the chemical under investigation. That is, it is quite obvious that decisions virtually always will have to be made on admittedly incomplete information. Perhaps the goal we should seek is a sufficiently flexible system to allow us to change our minds (when confronted with new information), coupled with an explicit acknowledgement of the perpetually interim state of our scientific knowledge. Again, the Bionetics study is illustrative. The

Bionetics' results were new and unexpected findings—albeit tentative findings. It is the nature of science that experimental results are always subject to further confirmation and refinement. The discovery of teratogenesis in experimental animals required confirmation and further investigation to make that finding meaningful. Fortunately, the experiments to do this were begun almost immediately, as you know, and the results have just now become available. What these results have done is to sustain our earlier concern about this herbicide. At the same time, scientific logic would dictate that we should continue to apply more research effort to better understand these findings.

Then too, the more sophisticated the scientific investigations become, the more expensive they are. The cost of the Bionetics study was approximately two and one-half million dollars. Remember, this was only a screening study. Much more extensive studies would surely be desirable.

A related question that is raised concerns the distribution of these costs. As I have noted, in the case of pesticides, the tradition has been for the Government to impose on industry the obligation of proving that a material is safe and of performing the toxicology necessary for that proof. As the cost and the time required for this background research rises, the manufacturers may be less and less inclined to pursue the development of new products of limited or uncertain marketability. Since we depend on the manufacturing industry for this development, we may be discouraging innovative and improved products. Hence, I submit that additional public investment may have to be made in the future in background research relating to health and other effects of environmental agents—including pesticides. The President's Science Advisory Committee is studying these issues presently.

Let me touch now on the subject of the translation of research findings into policy decisions and regulations. I have made the point that the heart of the Federal Government's control over pesticides resides in the process of registration with the Department of Agriculture. This registration is based, in part, on toxicological information supplied by the manufacturer. There has been relatively little thought given to the subject of how to incorporate new, unexpected information which is collected outside the registration process into the regulatory process. This was clearly demonstrated after the Bionetics study.

Finally, let me raise the question of the latitude available for regulation of pesticides. Under the existing Federal Insecticide, Fungicide and Rodenticide Act (which law regulates pesticides), the burden of proof of safety resides with the manufacturer. In the case of an existing registration, the options for action available to the Government, however, are relatively few. These are cancellation or suspension of the registration. Both of these are relatively drastic actions and are not supposed to be entered into capriciously. If a registration is cancelled (which was the suggestion made for 2,4,5-T), the decision may be appealed by the manufacturer and it then befalls the *Government* to prove that a hazard exists, rather than the industry to establish its safety. In short, there does not exist a mechanism whereby the Government may exercise prudent and unequivocally effective restraint temporarily on the receipt of new, unexpected information, and while awaiting more definitive results.

There are now under discussion a series of proposed amendments to the Federal Insecticide, Fungicide, and Rodenticide Act. These matters are seriously being considered in these discussions before your Committee.

Watershed Studies with 2,4-D, 2,4,5-T, and Picloram[1]

Description of Watersheds

The two experimental watersheds are located in the southern Appalachian near Waynesville, North Carolina. Watershed 1 contains 4.64 acres, and watershed 2 contains 3.66 acres. The slopes of both watersheds averaged about 35 to 40 percent. The predominant soil is Halewood clay loam. The watersheds are delineated and enclosed so that no surface or subsurface flow can enter. Each is equipped with a weir installed to bedrock, and total flow from the watersheds is measured. Three 0.05-acre plots with catchment devices for surface runoff determination are superimposed on watershed 2. The vegetative cover was a mixed grass sward containing discontinuous infestations of herbaceous weeds and small woody plants.

Experimental Procedure

The map of watershed 1 (Figure 1) shows the nine 0.05-acre plots sprayed in 1967 and the three large plots (1.16 acres) sprayed in 1968 and 1969. There are three replications of three treatments in 1967. Large plots A, C, and D were sprayed with the same herbicides in 1968 and 1969. The application rate was 2 lb/A. When large plots were used, there was one replication per watershed. The map of watershed 2 (Figure 2) shows the small plots sprayed in 1967 and 1968. The plots were sprayed as shown in 1967. In 1968 herbicide treatments were rotated so that each plot received a different herbicide. The application rate was 2 lb/A in 1967 and 1968. In 1969 the large plots were sprayed at a rate of 4 lb/A. The treatments were adjusted so that the herbicide assigned to each surface runoff plot had not been applied to that plot in 1967 or 1968. There were three replications of treatments when small plots were used. Treatments on large plots were unreplicated within a watershed.

The chemical and common names of the herbicides were 3,6-dichloro-o-anisic acid [dicamba], 2,4-dichlorophenoxyacetic acid [2,4-D], 2,4,5-trichlorophenoxyacetic acid [2,4,5-T], and 4-amino-3,5,6-trichloropicolinic acid [picloram].

The herbicides were applied in September, 1967 and in August, 1968 and 1969. All applications were made with a Knap-sac sprayer. The herbicidal formulations were as follows:

dicamba (dimethylamine salt)
2,4-D (alkanolamine salts)
picloram (potassium salt)
2,4,5-T (propylene glycol butyl ether ester in 1968)
2,4,5-T (triethylamine salt in 1969).

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[1] A contribution of the North Carolina State University Agricultural Experiment Station. This research was supported by the U. S. Department of Agriculture under Contract No. 12-14-100-8938(34).

For several months after spraying, grab samples of water were collected at the flumes during storms, and runoff samples were removed from the surface-runoff tanks at the end of each rain storm. Water and soil samples were shipped to Raleigh for analysis. Soil samples were frozen before shipment. Water samples were shipped as soon as possible after collection and were stored at 4°C on arrival in the laboratory. Usually, analysis of water samples began within 3 to 4 days after collection.

An electron-capture gas chromatographic method was developed for simultaneously measuring residues of the four herbicides. Low limits of detection of 2,4-D in water was 0.002 to 0.003 ppm; for picloram, 2,4,5-T, and dicamba the limit was 0.0005 to 0.001 ppm.

Results

Water samples collected from flumes at the base of each watershed during and after rain storms in 1967 contained 2,4-D, but concentrations of picloram and dicamba were below the limits of detection. The highest concentration of 2,4-D (0.028 ppm) occurred shortly after peak runoff of the first storm after application. The level decreased with each subsequent storm and was below the limit of detection in samples taken between September 27, 1967 and June 17, 1968 when sampling was discontinued until the 1968 application.

Although one-fourth of watershed 1 was sprayed with each of three herbicides in 1968, neither 2,4,5-T nor picloram was detected in flume water, and only low concentrations of apparent 2,4-D (0.003 to 0.005 ppm) occurred sporadically (Table 1). A small interference peak with a retention time in the gas chromatograph equal to that of 2,4-D raises some doubt about the authenticity of 2,4-D values in the 0.002 to 0.004 ppm range (Tables 1 and 2).

Concentrations of the herbicides in flume water samples collected in 1969 from watershed 1 were below the detection limit in all cases (Table 3).

After the 1969 applications, 2,4,5-T was detected in water samples taken at the base of watershed 2 during the first and second storms (Table 4). The highest concentration was 0.048 ppm in a sample collected while runoff was increasing during the second storm. The concentration was less in other samples and decreased to less than 0.001 ppm when flow returned to normal. Low concentrations of picloram were detected in flume samples during the second storm also. The maximum concentration was 0.003 ppm in a sample collected while flow rate was decreasing. Picloram was detected at 0.002 ppm in the first base-flow sample taken after the storm, but levels were less than 0.001 ppm in all samples thereafter.

Residues of 2,4-D, 2,4,5-T, and picloram in soil at several times after application are shown in tables 5, 6, and 7, respectively. The 2,4-D disappeared rapidly from soil. Although picloram persisted for several months, none was detected 1 year after application. A very small amount of 2,4,5-T was present in the 0 to 6-inch soil depth at 3 and 7 months after application, but none was found 12 months after.

Table 1. Concentrations (ppm) of 2,4-D, 2,4,5-T, and picloram in water from the flume of watershed 1 over a 4-month period after application of 2 lb/A of each herbicide to 25% of the watershed area on August 21, 1968.

Date	Flow condition	2,4-D (ppm)	2,4,5-T (ppm)	Picloram (ppm)
8-25-68	Peak	<0.002	<0.0005	<0.0005
	Base	<0.002	<0.0005	<0.0005
8-31-68	Peak 1	<0.002	<0.0005	<0.0005
9- 1-68	Half-down	<0.002	<0.0005	<0.0005
	Peak 2	<0.002	<0.0005	<0.0005
	Peak 3	<0.002	<0.0005	<0.0005
	Base	<0.002	<0.0005	<0.0005
9- 6-68	Base	<0.002	<0.0005	<0.0005
9-13-68	Base	<0.002	<0.0005	<0.0005
10- 3-68	Half-down	0.005	<0.0005	<0.0005
	Base	0.002	<0.0005	<0.0005
10- 6-68	Half-down	0.004	<0.0005	<0.0005
	Base	0.003	<0.0005	<0.0005
10-16-68	Base	<0.002	<0.0005	<0.0005
10-24-68	Base	<0.002	<0.0005	<0.0005
11- 6-68	Base	0.002	<0.0005	<0.0005
12- 1-68	Peak	<0.002	<0.0005	<0.0005
12-13-68	Peak 1	<0.002	<0.0005	<0.0005
	Peak 2	<0.002	<0.0005	<0.0005
	Peak 3	<0.002	<0.0005	<0.0005
12-14-68	Base	<0.002	<0.0005	<0.0005
12-20-68	Base	<0.002	<0.0005	<0.0005
12-22-68	Peak 1	<0.002	<0.0005	<0.0005
	Peak 2	<0.002	<0.0005	<0.0005
12-24-68	Half-down	<0.002	<0.0005	<0.0005
12-26-68	Base	<0.002	<0.0005	<0.0005

Table 2. Concentrations (ppm) of 2,4-D, 2,4,5-T, and picloram in water from the flume of watershed 2 over a 4-month period after application of 2 lb/A of each herbicide to 4% of the watershed area on August 20, 1968.

Date	Flow condition	2,4-D (ppm)	2,4,5-T (ppm)	Picloram (ppm)
8-25-68	Half-up	<0.002	<0.0005	<0.0005
	Peak	0.002	<0.0005	<0.0005
	Half-down	<0.002	<0.0005	<0.0005
8-31-68	Peak 1	<0.002	<0.0005	<0.0005
9- 1-68	Half-down	<0.002	<0.0005	<0.0005
	Peak 2	<0.002	<0.0005	<0.0005
	Peak 3	<0.002	<0.0005	<0.0005
	Base	<0.002	<0.0005	<0.0005
9- 6-68	Base	<0.002	<0.0005	<0.0005
10- 3-68	Half-down	<0.002	<0.0005	<0.0005
	Base	<0.002	<0.0005	<0.0005
10- 6-68	Half-down	0.003	<0.0005	<0.0005
	Base	<0.002	<0.0005	<0.0005
10-16-68	Base	<0.002	<0.0005	<0.0005
10-24-68	Base	<0.002	<0.0005	<0.0005
11- 6-68	Base	0.003	<0.0005	<0.0005
12- 1-68	Half-up	<0.002	<0.0005	<0.0005
12-13-68	Peak 1	<0.002	<0.0005	<0.0005
	Peak 2	<0.002	<0.0005	<0.0005
	Peak 3	<0.002	<0.0005	<0.0005
12-14-68	Base	<0.002	<0.0005	<0.0005
12-20-68	Base	<0.002	<0.0005	<0.0005
12-22-68	Peak 1	<0.002	<0.0005	<0.0005
	Peak 2	<0.002	<0.0005	<0.0005
12-24-68	Half-down	<0.002	<0.0005	<0.0005
12-26-68	Base	<0.002	<0.0005	<0.0005

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Table 3. Concentrations (ppm) of 2,4-D, 2,4,5-T, and picloram in water from the flume of watershed 1 over a 4-month period after application of 2 lb/A of each herbicide to 25% of the watershed area on August 13, 1969.

Date	Flow condition	2,4-D (ppm)	2,4,5-T (ppm)	Picloram (ppm)
8-16-69	Half-down	<0.003	<0.001	<0.001
	Base	<0.003	<0.001	<0.001
8-22-69	Peak	<0.003	<0.001	<0.001
	Half-down	<0.003	<0.001	<0.001
	Base	<0.003	<0.001	<0.001
8-29-69	Base	<0.003	<0.001	<0.001
9- 5-69	Half-down	<0.003	<0.001	<0.001
	Base	<0.003	<0.001	<0.001
9-12-69	Base	<0.003	<0.001	<0.001
9-19-69	Half-down	<0.003	<0.001	<0.001
	Base	<0.003	<0.001	<0.001
9-23-69	Peak 1	<0.003	<0.001	<0.001
	Peak 2	<0.003	<0.001	<0.001
	Half-down	<0.003	<0.001	<0.001
9-24-69	Base	<0.003	<0.001	<0.001
10- 1-69	Base	<0.003	<0.001	<0.001
10- 2-69	Base	<0.003	<0.001	<0.001
10- 8-69	Base	<0.003	<0.001	<0.001
11-28-69	Base	<0.003	<0.001	<0.001
12- 7-69	Peak	<0.003	<0.001	<0.001
	Half-down	<0.003	<0.001	<0.001
	Base	<0.003	<0.001	<0.001
12-10-69	Peak	<0.003	<0.001	<0.001
	Half-down	<0.003	<0.001	<0.001
	Base	<0.003	<0.001	<0.001
12-17-69	Base	<0.003	<0.001	<0.001
12-29-69	Base	<0.003	<0.001	<0.001

Table 4. Concentrations (ppm) of 2,4-D, 2,4,5-T, and picloram in water from the flume of watershed 2 over a 2-month period after application of 4 lb/A of each herbicide to 25% of the watershed area on August 14, 1969.

Date	Flow condition	2,4-D (ppm)	2,4,5-T (ppm)	Picloram (ppm)
8-16-69	Half-down	<0.003	<0.001	<0.001
	Base	<0.003	0.019	<0.001
8-22-69	Half-up	<0.003	0.048	<0.001
	Peak 1	<0.003	0.031	<0.001
	Peak 2	<0.003	0.006	0.002
	Half-down	<0.003	0.003	0.003
	Base	<0.003	<0.001	0.002
8-29-69	Base	<0.003	<0.001	<0.001
9- 5-69	Half-down	<0.003	<0.001	<0.001
	Base	<0.003	<0.001	<0.001
9-12-69	Base	<0.003	<0.001	<0.001
9-19-69	Peak	<0.003	<0.001	<0.001
	Half-down	<0.003	<0.001	<0.001
	Base	<0.003	<0.001	<0.001
9-23-69	Half-up	<0.003	<0.001	<0.001
	Peak 1	<0.003	<0.001	<0.001
	Half-down	<0.003	<0.001	<0.001
	Peak 2	<0.003	<0.001	<0.001
	Peak 3	<0.003	<0.001	<0.001
9-24-69	Base	<0.003	<0.001	<0.001
10- 1-69	Base	<0.003	<0.001	<0.001
10- 2-69	Base	<0.003	<0.001	<0.001
10- 8-69	Base	<0.003	<0.001	<0.001

Table 5. Residues of 2,4-D (lb/A) in soil at 0 to 3 months after application of 2 lb/A August 20, 1968 to Watershed 2.

Soil depth (inches)	Months after application		
	0	1.5	3.0
0-3	0.79	0.03	0.02
3-6	-	<0.02	<0.02
6-12	-	<0.04	<0.04
12-18	-	<0.04	<0.04
18-24	-	<0.04	0.04
Total	0.79	0.03	0.06

Table 6. Residues of 2,4,5-T (lb/A) in soil at 0 to 12 months after application of 2 lb/A August 20, 1968, to Watershed 2.

Soil depth (inches)	Months after application				
	0	1.5	3.0	7.0	12.0
0-3	1.14	0.15	0.03	0.04	<0.01
3-6	-	0.01	<0.01	<0.01	<0.01
6-12	-	0.04	<0.02	<0.02	<0.02
12-18	-	0.08	<0.02	-	-
18-24	-	0.02	<0.02	-	-
Total	1.14	0.30	0.03	0.04	<0.04

Table 7. Residues of picloram (lb/A) in soil 0 to 12 months after application of 2 lb/A August 20, 1968 to Watershed 2.

Soil depth (inches)	Months after application				
	0	1.5	3.0	7.0	12.0
0-3	1.27	0.52	0.37	0.08	<0.02
3-6	-	0.11	0.08	0.06	<0.02
6-12	-	0.18	0.04	0.06	<0.04
12-18	-	0.10	0.06	-	-
18-24	-	0.04	0.06	-	-
Total	1.27	0.95	0.61	0.20	<0.08

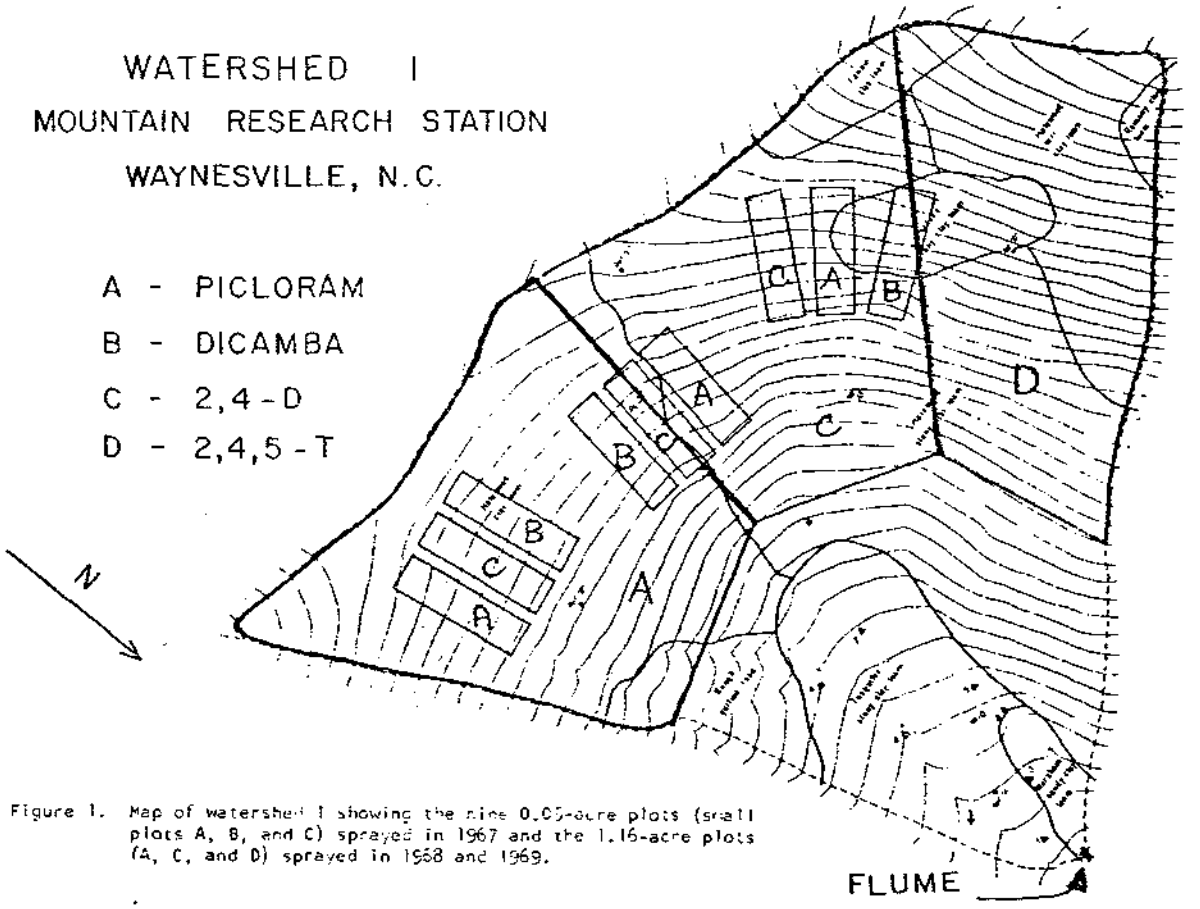

Figure 1. Map of watershed 1 showing the nine 0.05-acre plots (small plots A, B, and C) sprayed in 1967 and the 1.16-acre plots (A, C, and D) sprayed in 1968 and 1969.

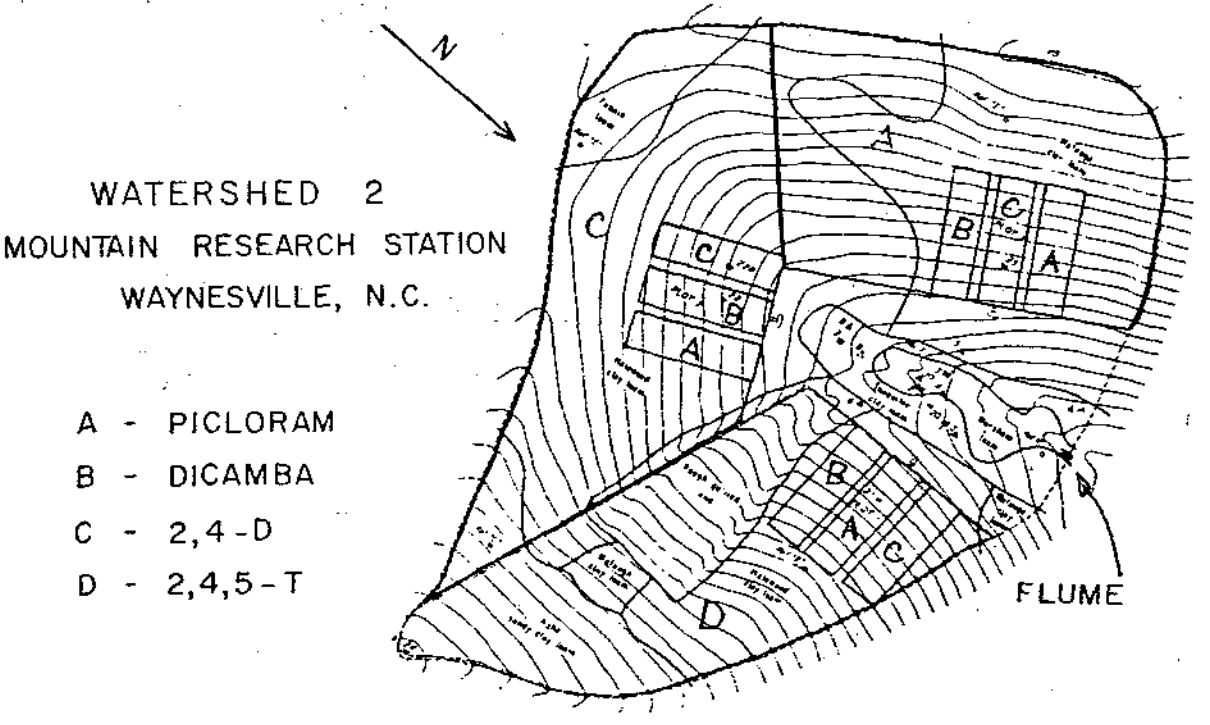

Figure 2. Map of watershed 2 showing the nine 0.05-acre plots sprayed in 1967 and 1968 and the 1-acre plots sprayed in 1969.

Senator HART. May I thank all who assisted in the development of this very informative hearing. We stand adjourned.

(Whereupon, at 5:15 p.m., the subcommittee was adjourned.)

(The following material was subsequently received for the record:)

STATEMENT OF HON. LEE METCALF, U.S. SENATOR FROM MONTANA

Mr. Chairman, I appreciate the opportunity to outline briefly a few of the special problems which arise from the use of herbicides such as 2,4,5,-T in Montana.

I will not go into the potential health dangers involved with the widespread use of 2,4,5,-T and other insufficiently tested herbicides. Others, far better qualified than I to assess these dangers, have already presented their professional opinions to the Subcommittee. What I would like to bring to the Subcommittee's attention, however, is the particular impact the use of herbicides has upon a vast agricultural State such as Montana.

Montana, as you know, is in the unique situation of lying at the head of three major international drainages which carry waters into the Gulf of Mexico and the Pacific and Arctic Oceans. The river systems whose headwaters lie in Montana, the Columbia, the Missouri-Mississippi, and the Saskatchewan, pass through over half of the land area of our nation and part of that of Canada.

Into these drainages enormous quantities of herbicides and pesticides are sprayed each year. Forests, grazing lands, grain fields, power line rights of way, and highway shoulders are sprayed with a wide variety of chemical poisons. Herbicides are used by Federal agencies, State agencies, private companies, farmers, and home gardeners. The State undergoes a veritable deluge every year.

I hardy need to point out the potentially dangerous effects of such chemicals, if they are found to persist and collect in water supplies and in the food chain. Ecologists have pointed out that the little understood processes of accumulation, biological magnification, and synergism can bring about completely unexpected results from commonplace chemicals.

One would assume because of the pervasive use of 2,4,5,-T and other herbicides and because of the harmful side effects which have already been discovered to result from some group of these chemicals, that truly exhaustive tests would be required to be run on any new candidate for herbicidal use. Unfortunately, this is not the case. To our astonishment we have been told in effect that the burden of proof is on the public to prove that a proposed substance is dangerous rather than on the supplier to prove that it is not.

The result of this convoluted notion of regulation is that enormous amounts of potentially dangerous herbicides are spread about our land, they eventually find their way into water systems and merge with others as they travel their way to the oceans. Once the Department of Agriculture places its seal of approval on a chemical, the substance can be sprayed with impunity. Whatever detrimental effects may result are thereafter extremely hard to ascertain. The unfortunate results may in fact only become visible years from the time they are used.

The Montana State Legislature is now considering a new Pesticide Commission which would provide much better regulatory machinery at the State level. However, due to the problem of human and financial resources, such State commissions will always have to take their cue from the Federal regulatory bodies. And as it now stands, the Federal regulating process is a very confused and weak one.

What is vitally needed, Mr. Chairman, is a clearly delineated Federal regulatory policy in the field of pesticides, herbicides, and defoliants which would:

1. Place the burden of proof of the safety of a product upon the manufacturer;
2. Provide for thorough, independent testing in government laboratories;
3. Place the sole responsibility for the approval of the health dangers of a product with the Secretary of the Department of Health, Education and Welfare;
4. Substantially strengthen the enforcement machinery in the agencies which control pesticides; and
5. Create a national policy for the use of pesticides by Federal agencies.

Concerning the final point, I think it is extremely important that the use of these chemicals in national parks be critically examined. Our national parks and primitive areas were set up specifically in order to sector off the few small, unspoiled areas which remain in our industrialized country to be preserved as natural regions. I was dismayed to learn recently that a 20-foot wide strip through the middle of one of our most beautiful national parks has been defoliated with another herbicide, picloram.

Mr. Chairman, it is incredible to me that Glacier-Waterton International Peace Park should be subjected to defoliants for the creation of a "North American DMZ" through its center. It is an unpleasant irony that a park which was established to commemorate the peaceful relationship between two nations and to preserve a unique glacial wilderness must be divided in two by a senseless and ugly defoliated strip. Moreover, even less is known about the possible teratogenic and carcinogenic effects of this chemical picloram than about 2,4,5-T, the agent under immediate suspicion.

Our lack of a national policy in the area of herbicides and pesticides reaches its ultimate absurdity in this defoliated strip in Glacier Park. I urge the Subcommittee to consider the far-reaching measures which are necessary to control all aspects of the use of pesticides. Only a comprehensive approach to the problem will enable us to avoid mistakes such as DDT and 2,4,5,-t.

OFFICE OF THE SECRETARY OF DEFENSE,
Washington, D.C., April 21, 1970.

HON. PHILIP A. HART,
Chairman, Subcommittee on Energy, Natural Resources, and the Environment, Committee on Commerce, U.S. Senate, Washington, D.C.

DEAR MR. CHAIRMAN: The following information is provided for the record in response to a staff request from your Subcommittee:

Deputy Secretary of Defense David Packard on April 15 ordered the immediate suspension of the use of 2,4,5-T within the Defense establishment pending a more thorough evaluation of the system.

Sincerely,

J. F. LAWRENCE,
Brigadier General, USMC,
Deputy Assistant to the Secretary for Legislative Affairs.

DEPARTMENT OF AGRICULTURE,
OFFICE OF THE SECRETARY,
Washington, D.C., April 21, 1970.

MR. LEONARD BICKWIT,
Staff Counsel, Subcommittee on Energy, Natural Resources, and the Environment, U.S. Senate, Washington, D.C.

DEAR MR. BICKWIT: The following comments on the April 15 joint U.S. Department of Agriculture, U.S. Department of Health, Education, and Welfare, and U.S. Department of the Interior release "Home Uses of 2,4,5-T Suspended" are offered for the record of the Hearings of the Committee.

The release is not clear on the cancellation of registered uses of 2,4,5-T on food crops. The action to be taken is the cancellation of all registered uses of 2,4,5-T on apples, blueberries, barley, corn, oats, rice, rye, and sugarcane.

The term "cancellation" as here used means that each registrant will be notified that his registered products subject to this action shall cease to move in interstate commerce 30 days after notification of cancellation. During this 30-day period, the registrant may correct the labels on his cancelled products to delete the cancelled uses. The registrant may appeal the cancellation action and ask for an advisory committee or public hearing. In such case, the registrant's cancelled products may move in interstate commerce until action on the appeal is completed.

The term "suspension" as used in the release with reference to action to suspend all registered uses of liquid formulations around the home and all registered uses of 2,4,5-T on lakes, ponds, and ditch banks, means that such suspended products shall cease to move in interstate commerce upon receipt of notice of the order of suspension by the registrant. The registrant may appeal the suspension action but in such case the suspended products cannot move in interstate commerce while action on the appeal is in process.

Sincerely,

T. C. BYERLY,
Assistant Director, Science and Education.

CENTER FOR STUDY OF RESPONSIVE LAW,
Washington, D.C., April 30, 1970.

Senator PHILIP HART,
U.S. Senate,
Washington, D.C.

DEAR SENATOR HART: May we commend you and the Senate Commerce Committee for investigating so thoroughly the hazards presented by weed-killer 2,4,5-T. It was encouraging to see USDA relent and prohibit certain uses of the chemical. However, after this brief commendation, we would like to make clear our feeling that the bans on 2,4,5-T were inadequate, and the press release announcing the ban misleading to the public.

According to the official press release, "In exercising its responsibility to safeguard public health and safety, the regulatory agencies of the Federal Government will move immediately to *minimize human exposure* to 2,4,5-T *and its impurities*. The measures being taken are designed to provide *maximum protection* to women in the child-bearing years by eliminating formulation of 2,4,5-T use in household, aquatic and recreational areas (emphases added)." We contend that the measure taken reflect an utter disregard on the part of the USDA for public health and safety and represent *minimal* rather than *maximal* protection.

1. The use of Silvex (also called 2,4,5-TP; 2-(2,4,5-trichlorophenoxy) propionic acid) has not been suspended. Silvex is as closely related to 2,4,5-T as pancake are to waffles. Because of this similarity one can predict with confidence that Silvex will prove to be about as teratogenic as 2,4,5-T. Furthermore, the series of chemical reactions by which Silvex and 2,4,5-T are made are almost identical. These reactions lead unavoidably to the formation of the dreaded tetra-dioxin contaminant. If any serious attempt were being made to "minimize human exposure" to hazardous weed-killers and the impurities contained therein, the home use of Silvex would certainly be banned. Silvex, incidently, is a more common ingredient in garden products than is 2,4,5-T.

2. "Minimizing human exposure to impurities in 2,4,5-T and its impurities" would demand an immediate, total recall of 2,4,5-T and related compounds from retail stores and homes (with the manufacturers reimbursing stores and consumers). Although the USDA has called for recall in a private communication to manufacturers, no recall will be complete unless the announcement is published in the Federal Register and a list of products containing the dangerous substances is published in newspapers so that consumers may return what they had previously purchased.

3. "Minimizing human exposure" to impurities in 2,4,5-T would necessitate suspending the use of 2,4-D (the most widely used weedkiller), 2,4-DP (found in Scott's Turf Builder Plus 4), and pentachlorophenol (present in Ortho Triox Liquid Vegetation Killer). The processes by which all three of these compounds are made lead unavoidably to formation of dioxins.

4. "Minimizing exposure to 2,4,5-T and its impurities" would necessitate suspending *all uses and formulations* of all chlorophenoxy and chlorophenol weedkillers:

(*a*) The possible persistence of dioxins represents a very real danger, as pointed out by Drs. Verrett and Epstein, Mr. Bickwit and ourselves at the Commerce Committee hearings.

(*b*) Accidental exposure to the weed-killers or their contaminants due to direct spraying, drift or residues on food is possible, indeed inevitable, when these weed-killers are used on crops, ranges, rights-of-way, neighbors' yards, etc. In testimony before the House Subcommittee on National Security and Scientific Developments on December 2, 1969, Dr. Arthur Galston of Yale University said, "I suggest that its (2,4,5-T) teratogenicity is such that even its use in such apparently innocuous domestic manners as clearing brush near powerlines is undesirable. Such chemicals could find their ways into water supplies and could be ingested in teratogenic doses."

If the regulatory agencies truly wish to exercise their responsibility to safeguard public health and safety, particularly that of women of childbearing age, by minimizing human exposure to 2,4,5-T and its impurities they will certainly have to go far beyond the recently announced minimal actions.

If possible we would like this letter made part of the hearing record.

Thank you once again for your interest in this important matter.

Sincerely yours

HARRISON WELLFORD.
JAMES TURNER.

STATEMENT OF R. L. CUSHING ON BEHALF OF THE HAWAIIAN SUGAR PLANTERS' ASSOCIATION, HONOLULU, HAWAII

The Hawaiian Sugar Planters' Association, an association representing all of Hawaii's 24 sugar-producing companies, wishes to present its views on the proposed cancellation of registration for the herbicide 2,4,5-T. By implication, the fate of other phenolic-derived pesticides, including the important herbicide 2,4-D which is chemically related to 2,4,I-T, is also at stake.

2,4,5-T and 2,4-D have been used widely in Hawaii since about 1948 for control of weeds in sugarcane fields. They are valuable for this purpose and there are no other equally effective herbicides registered by the U.S. Department of Agriculture for use in sugarcane. Cancellation of registration of 2,4,5-T and possibly that of other related pesticides, as originally proposed by Dr. Lee DuBridge, the President's Science Adviser, on October 26, 1969, would cause serious economic hardship to sugarcane growers. A letter explaining our position was sent to Dr. DuBridge on February 5, 1970, and he gave us a very thoughtful, thorough response on March 3.

We believe adverse publicity about all pesticides, such as the unconfirmed reports of birth defects in Vietnam attributed to 2,4,5-T, is based largely on emotion and on inadequate scientific investigation. At the same time we recognize and emphasize that massive aircraft treatments of crops, water sources, jungle, and possibly villages in Vietnam differs almost totally from the moderate, controlled agricultural spraying in the U.S.

We recognize the possible hazards from poorly controlled pesticides and the need for adequate protection of the public. This is the purpose of the federal regulatory statutes governing pesticides; we believe these laws and regulations are sufficient to insure food crops free of harmful pesticide residues and to protect the public from contact with the chemicals. In support of this statement we quote from Dr. DuBridge's reply to our letter.

"Among 5,300 samples of foodstuffs tested for this material by the Food and Drug Administration during the past five years, only two were found to contain more than trace quantities of 2,4,5-T; only 25 contained detectable amounts. Thus occurrence of 2,4,5-T in foodstuffs is indeed rare."

Laws of the State of Hawaii permit use of 2,4-D and 2,4,5-T herbicides when applied in such a manner that detectable amounts of spray do not drift to sensitive crops, home gardens, or ornamental plants. Hawaii's semi-tropical climate and year-round conditions favorable for plant growth make the control of all weed species an imperative, constant necessity if crops are to be grown successfully and economically. Application of herbicides, such as 2,4-D and 2,4,5-T, and other pesticides are moderate, as dictated by economic factors and by state and federal laws. Moreover, sugarcane in Hawaii is a two-year crop. Control of weeds by application of herbicides is necessary only in the first few months of growth. Even then the amounts are small and the applications are made with care and precision. Because of these circumstances, no residues of 2,4-D or of 2,4,5-T have ever been found in the harvested crop. In the many years of using 2,4,5-T and related herbicides there have been no known cases of illness or physical defects either in the men who applied them, their families or the public. Neither is there any record of injury or hazard to wildlife or recreation.

It now seems probable that the birth defects ascribed to 2,4,5-T in the Bionetics Laboratories study were caused by a contaminant present in the single sample of the herbicide apparently used in the investigation. We understand this fact was pointed out to the Food and Drug Administration by the Dow Chemical Company who had been aware of the contaminant for several years and had modified its manufacturing plant so as to avoid producing it. It is difficult to understand how a carefully controlled scientific study of such importance could be conducted without first determining the purity of the 2,4,5-T used by Bionetics.

It is unfortunate that formulated products from several commercial sources were not included as well as highly purified 2,4,5-T. We feel the subsequent publicity given the Bionetics work in the press, in *Science* magazine, and in the Whiteside article in the *New Yorker*, was hasty, poorly informed, emotional, and calculated to gain public attention at the sacrifice of scientific verity.

We believe the regulatory personnel of the U.S. Department of Agriculture and the Food and Drug Administration acted with discretion and sound judgment, in the face of considerable pressure, to suspend final action against 2,4,5-T until all relevant data were evaluated from other studies to be completed this spring. It is

our understanding at this time that the "no residue" or zero tolerance registrations for 2,4,5-T have been extended for the remainder of 1970 to establish whether finite tolerances can be assigned. We concur in these decisions and believe much of the credit for new, more accurate scientific data should go to the laboratories of Dow Chemical Company and of the Food and Drug Administration.

It is not our intent to use pesticides whose application can be shown to be detrimental to the field workers or to the public. Our main concern is the unfortunate consequence for ourselves and all agriculture if cancellations of registrations of these and other useful pesticides can take place suddenly and without adequate notice. It is probable that pressure for more cancellations is likely to come, as this one seems to have, from emotion rather than from careful consideration of sufficient scientific evidence.

The collection of information necessary to obtain registration may take many years. There is now no satisfactory alternative for 2,4,5-T. One alternative is known and field testing on it was first begun in Hawaii in 1963. However, field evaluations, residue analyses, and soil- and water-movement studies in a two-year crop to provide data to meet registration requirements take so much time that the chemical company has not yet been able to file for registration of this alternative compound.

It is our hope that the investigation of the safety of 2,4,5-T and of other phenolic-derived pesticides will continue, but that the results will be carefully and scientifically evaluated. We fear that decisions based on emotion instead of on fact and reason could deprive American farmers of essential pesticides. We are confident that your committee will provide such reasoned judgment.

(The following is referred to on p. 376.

ABSTRACT

Teratogenic Study of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in the Rat. G. L. Sparschu, F. L. Dunn and V. K. Rowe, The Dow Chemical Company, Midland, Michigan.

2,3,7,8-Tetrachlorodibenzo-p-dioxin has been found to occur in small amounts as a contaminant in some commercially manufactured samples of 2,4,5-trichlorophenoxy acetic acid. The purpose of this study was to learn whether the presence of this impurity possibly could account for the fetal abnormalities in test animals reported in a recent study (unpublished data: Bionetics Research Laboratories, Bethesda, Maryland).

The dioxin was administered by gavage in 9:1 corn oil-acetone solution in doses of 0 (control), 0.03, 0.125, 0.5, 2.0 and 8.0 micrograms per kilogram body weight per day to groups of 24 (control) and 12 (treatment) pregnant female Sprague-Dawley derived rats on days 6 through 15 of gestation.

On day 20 of gestation, each dam was sacrificed and a cesarean section performed. The number of viable and dead fetuses and early and late resorptions was recorded. Each fetus was examined for any gross abnormalities. Two-thirds of each litter were fixed in Bouin's solution, Wilson sections were examined under the dissection microscope, and tissues were studied for histopathology. One-third of each litter were fixed in alcohol and examined for skeletal abnormalities by alizarin red-S staining.

Presented at the meeting of the Society of Toxicology, Atlanta, Georgia, March 17, 1970.

No differences were observed in the fetuses taken from dams treated at the dosage of 0.03 μg/kg/day and those taken from dams that received the solvent vehicle only. At the 0.125 μg/kg/day dosage, all parameters studies were within normal limits except for a very slight decrease in average weight, and the occurrence of intestinal hemorrhage (18/127) and subcutaneous edema (22/80) in the fetuses from dams that received this treatment. At the 0.5 μg/kg/day level, the number of fetuses was reduced and the number of resorptions and fetal deaths was increased. The average weight of the viable fetuses was very slightly decreased. The incidence of intestinal hemorrhage (36/99) and subcutaneous edema (31/65) was markedly increased over that seen in the 0.125 μg/kg/day treatment.

At the 2.0 μg/kg/day level, only 7 viable fetuses were obtained. These were from 4 of the 11 litters examined. Resorptions were numerous, intestinal hemorrhage was frequent (4/7), and subcutaneous edema was present in all of the 4 fetuses examined by Wilson section. One fetus from this treatment level was found to have a kinked tail and two of its feet were somewhat misshapen. Skeletal examination, however, revealed no evidence of bone abnormalities.

The 8.0 μg/kg/day dosage level proved to be toxic to the dams. There were no viable fetuses in the dams which were examined on day 20 of gestation. All resorptions occurred early and no evidence of fetal tissue was found.

Skeletal examinations revealed delayed ossification of some sternebrae and skull bones. This occurred generally throughout the various groups, including controls, and is not considered to be of practical significance.

The results of this study indicate a high level of maternal and fetal toxicity to be associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. Its presence in the sample tested in the Bionetics Laboratories study could well have accounted for the observations reported and attributed to 2,4,5-trichlorophenoxy acetic acid.

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