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You may wish to see this in connection with your testimony in ~~the~~ the 2,4,5-T hearing. According to page 1, it should not be released without first checking with Mr. Colledge. However, it is now an exhibit in the public hearings.

FROM

M. Breenholt

Roy ALBERT

CAG Report

EXHIBIT M

FEB 28 1990

THE CARCINOGEN ASSESSMENT GROUP'S
RISK ASSESSMENTS ON

(2,4,5-TRICHLOROPHENOXY)ACETIC ACID (2,4,5-T)
(2,4,5-TRICHLOROPHENOXY)PROPIONIC ACID (SILVEX)
2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

PART 1

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PART 2

In preparation, NCI Bioassay data on TCDD if they become available, and review of epidemiological studies, concerning the exposure of 2,4,5-T, silvex, and TCDD.

PART 3

In preparation, quantitative assessment of cancer risk to human populations exposed to 2,4,5-T, silvex, and TCDD.

I. SUMMARY AND CONCLUSIONS

QUALITATIVE ASSESSMENT

(2,4,5-Trichlorophenoxy)Acetic Acid)(2,4,5-T)

(2,4,5-Trichlorophenoxy)acetic acid, widely known as 2,4,5-T is used as a vegetation growth regulator and herbicide. "Agent Orange," a defoliant used extensively by the U.S. Army in Vietnam, is a mixture of equal amounts of 2,4,5-T and (2,4-dichlorophenoxy)acetic acid. In 1970, amid growing concern about the teratogenic effects of 2,4,5-T, the EPA cancelled the registration of the compound for uses "around the home, recreation areas, and similar sites" and "in crops intended for human consumption." Before some uses were suspended in 1979, it was used primarily to clear vegetation along powerlines, highways, pipelines, and railroad rights-of-way, and on range, pasture, and forestlands.

*he means
USDA*

The commercial preparation of 2,4,5-T contains 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as an unavoidable impurity present at a concentration of 0.05 and 0.1 ppm. TCDD is considered extremely toxic.

2,4,5-T is readily absorbed by several mammalian species, including man, and is excreted unchanged - mostly in urine.

The available information about the mutagenic activity of 2,4,5-T is considered to be limited. 2,4,5-T is indicated to be a weak mutagen in Drosophila and, under acetic conditions, showed mutagenic effects in Saccharomyces cerevisiae.

Tests for the chronic carcinogenicity of 2,4,5-T were performed by several investigators. Two studies were carried out with Sprague-Dawley rats, one by the Dow Chemical Company and one by F. Leuschner (Laboratorium fur Pharmakologie und Toxikologie, Hamburg, Germany). The Dow study showed an increased incidence of carcinoma of the tongue in male rats at 30 mg/kg/day. This incidence is

marginally statistically significant ($P < 0.063$) when compared to controls. In addition, there was significant linear dose trend by the Cochran-Armitage test. In a recently completed study by F. Leuschner, there was increased incidence of interstitial cell tumors of testes. Further interpretation of these results awaits information on historical controls.

In mice, two studies by Muranyi-Kovacs et al. and two studies by Innes et al. have not provided positive evidence of oncogenic effects of 2,4,5-T. However, several deficiencies in these studies make them inadequate in assessing the lack of oncogenicity of 2,4,5-T.

In summary, one rat study on 2,4,5-T provides suggestive evidence of carcinogenicity, while another rat study showed only equivocal results. The mouse studies on 2,4,5-T were too insensitive to be considered valid negative studies.

(2,4,5-Trichlorophenoxy)Propionic Acid (silvex)

Silvex, like (to) 2,4,5-T, contains the highly toxic TCDD. Uses of silvex are similar to those of 2,4,5-T. Chronic carcinogenicity studies have been performed on mice and rats and a 2-year study has been conducted on dogs. Innes et al. (Bionetics) conducted two studies using mice, one oral and the other subcutaneous. These were found to be inadequate to assess the carcinogenicity of silvex.

Dow Chemical Company performed two feeding studies, a two year feeding study on rats and a two year feeding study on dogs. These have been found to be inadequate to rule out the carcinogenicity of silvex.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

Probably one of the most toxic chemicals known to man is 2,3,7,8-tetrachlorodibenzo-p-dioxin. The major source of its environmental contamination is from the pesticidal uses of 2,4,5-T, 2,4,5-trichlorophenol, and

silvex.

In small amounts, TCDD is a potent inducer of arylhydrocarbon hydroxylase in mammals. This is a complex enzyme system that consists of epoxidase, epoxidehydrazase, and glutathione transferase. The enzyme epoxidase is known to mediate the formation of epoxides, which are potentially active carcinogenic metabolites. TCDD does not undergo any known metabolic transformations in mammalian species. Its persistent residues of TCDD were found in liver and fat after 2-year feeding studies in rats.

Currently available studies on the mutagenicity of TCDD are inconclusive. In two bacterial systems, E. coli and Salmonella typhimurium (without metabolic activation), exhibited positive mutagenic activity. However, in another study with Salmonella typhimurium (with and without metabolic activation), the results were negative.

There are three cancer bioassay studies of TCDD: 1) a Dow Chemical Company study which used male and female Sprague-Dawley (Spartan substrain) rats dosed by dietary feeding; 2) the Van Miller et al. study which used male Sprague-Dawley dosed by dietary feeding; and 3) the Toth study which used Swiss mice administered TCDD weekly by gavage.

The study by the Dow Chemical Company of male and female Sprague-Dawley rats fed TCDD in doses of 22 ppt, 210 ppt, and 2200 ppt reveal a highly statistically significant excess incidence of hepatocellular carcinomas in female rats at the highest dose level and hepatocellular carcinomas and hepatocellular hyperplastic nodules in the female rats at the middle dose level, as compared to the controls. In addition, there was a significant increase in carcinomas of the hard palate/nasal turbinates in both high-dose males and females; of the tongue in males, and of the lung in females. The Van Miller study also showed some evidence of a carcinogenic response in the liver and lungs of male

Sprague-Dawley rats at dosages of 1000 and 5000 ppt, even though the study used a relatively small number of animals. Toth's study provides suggestive evidence that TCDD induced an increased incidence of liver tumors in male mice (females were not tested) receiving 0.7 ug/kg/week by gavage.

In summary, carcinogenic responses have been induced in mice and rats at very low doses of TCDD. These results constitute substantial evidence that TCDD is likely to be a human carcinogen. In addition, on the basis of the Dow study on TCDD, it appears that TCDD is a more potent carcinogen than aflatoxin B₁ which is one of the most potent carcinogens known. The levels of TCDD (contained as a contaminant of the 2,4,5-T) used in the 2,4,5-T studies apparently were too small to produce an observable response in those experiments. The lack of a statistically significant tumor incidence in most of the studies on the 2,4,5-T product may be attributed both to the extremely low levels of TCDD in the product relative to the levels at which it produces observable carcinogenic effects in rats and mice, as well as to the deficiencies of those studies. However, since TCDD is a carcinogen, any product containing TCDD can be considered to pose a human carcinogenic hazard.

II. INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is an extremely toxic contaminant that forms when tetrachlorobenzene is hydrolyzed in an alkaline ethylene glycol solution to produce 2,4,5-trichlorophenol. The amount of TCDD produced increases with an increase in the temperature of the reaction. The 2,4,5-trichlorophenol is used as an intermediary in the production of (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) and (2,4,5-trichlorophenoxy)propionic acid (silvex). Therefore, the TCDD contaminates both products to the same extent (see Figure 1, below). TCDD can also occur in other chlorinated phenols and in the chemicals synthesized from them. TCDD does not occur naturally in the environment, but exists only as a contaminant of other chemicals.

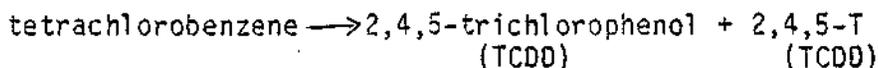


Figure 1. Formation of TCDD, 2,4,5-trichlorophenol and 2,4,5-T

In the 1960s, the TCDD content in commercial 2,4,5-T and 2,4,5-trichlorophenol ranged from 5 to 50 ppm. By the early '70s, the manufacturers had set a limit of 0.1 ppm TCDD contamination in their products.

The structure of the four compounds is shown in Figure 2 below.

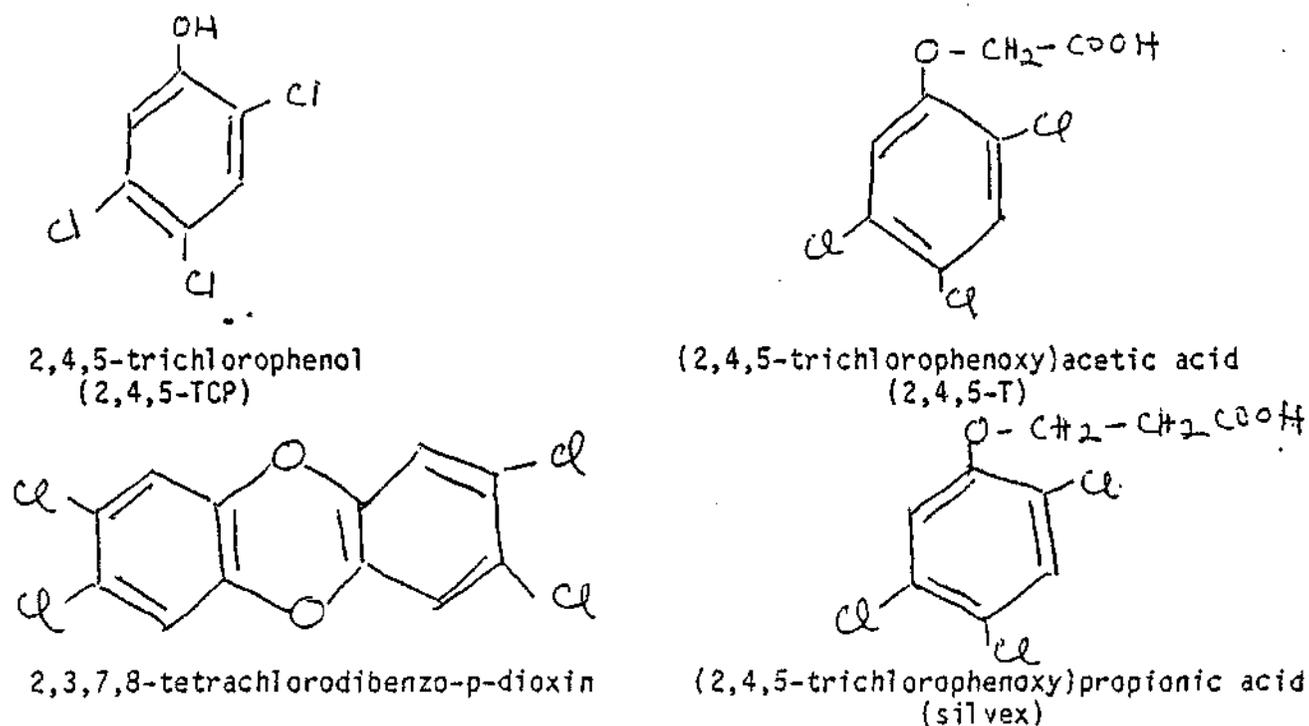


Figure 2. Structure of TCDD and TCDD-containing compounds

2,4,5-T is used as a growth regulator and herbicide. The herbicide "Agent Orange," used extensively by the U.S. Army as a defoliant in Vietnam, is a mixture of equal amounts of 2,4,5-T and (2,4-dichlorophenoxy)acetic acid. In 1970, amid growing concern about the teratogenic effects of 2,4,5-T, the EPA cancelled registration of the compound for uses "around the home, recreation areas, and similar sites" and "on crops intended for human consumption." Until EPA suspended certain uses in 1979, it was used primarily to clear vegetation along powerlines, highways, pipelines, and railroad rights-of-way, and on range, pasture, and forestlands.

III. METABOLISM

METABOLISM OF (2,4,5-TRICHLOROPHENOXY)ACETIC ACID (2,4,5-T)

The metabolic fate of 98% pure 2,4,5-T was studied in beagle dogs and adult Sprague-Dawley rats following a single oral dose of the chemical (Piper et al. 1973). The absorption of 2,4,5-T appeared to follow first order kinetics in rats and dogs. The rate at which the compounds cleared from plasma was also of the first order in rats, but in dogs, the clearance rate was much more complex than first order.

The $t_{1/2}$ values for clearance of ^{14}C -activity from the plasma of rats given doses of 5, 50, 100, or 200 mg/kg were 4.7, 4.2, 19.4, and 25.2 hours, respectively. The volume of distribution also apparently increased with dose. In dogs given 5 mg/kg, the $t_{1/2}$ values for clearance from plasma and elimination from the body were 77.0 and 86.6 hours, respectively.

Essentially all of the 2,4,5-T excreted was unchanged in the rats' urine, except for a small amount of one unidentified metabolite that was detected only in rats administered the two highest doses. Urinary excretion accounted for most of the 2,4,5-T eliminated from the body in rats; little was found in the feces.

In dogs, a greater percentage of 2,4,5-T was excreted in the feces than in the urine. Three unidentified metabolites of 2,4,5-T were detected in the urine of dogs, but there may have been fecal contamination, so the source is equivocal. The slower elimination of 2,4,5-T in the body of the dog may account for its greater metabolic alteration. The authors suggest that the kidney possesses a saturable active transport system for 2,4,5-T and that this transport system has a greater capacity in adult rats than in dogs. The longer half-life of elimination and the metabolic degradation of 2,4,5-T in dogs may

explain why 2,4,5-T is more toxic to dogs than to rats.

Five male human volunteers ingested a single 5 mg/kg dose of 99% pure 2,4,5-T containing 0.05 ppm TCDD (Gehring et al. 1973). The plasma concentration of 2,4,5-T increased rapidly and peaked at 57 ug/ml following 7 hours of administration. The subsequent clearance rates from the plasma and body were of first order, situated numerically between the rates for dogs and for rats. The 2,4,5-T was actively secreted in the urine. It was concluded that 2,4,5-T is eliminated fairly unchanged from the human body.

The volume distribution in humans was smaller than for test animals. In humans, 65% of the compound remaining after 24 hours was present in plasma, and 99% of this was reversibly bound to protein.

METABOLISM AND STORAGE OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)

In a 1976 study by Rose et al. Sprague-Dawley rats were given either a single oral dose of 1.0 ug ¹⁴C-TCDD/kg (98% pure with 2% trichlorodibenzo-p-dioxin) or repeated oral doses of 0.01, 0.1, or 1.0 ug ¹⁴C-TCDD/kg/day, 5 days per week, for 7 weeks.

The authors monitored the fate of ¹⁴C-TCDD in rats after single oral administration and found that, on the average, 83% of the dose was absorbed. Twenty-two days after the single oral dose, concentrations of ¹⁴C-activity were retained mainly in the liver (1.26% of dose) and fat (1.25% of dose). The half-life of ¹⁴C following a single oral dose was 31 ± 6 days, which followed first order kinetics. Most of the ¹⁴C-activity was detected in feces and not in urine or expired air, which indicates that TCDD and/or its metabolites are eliminated via the bile.

The authors also monitored the fate of the ¹⁴C-TCDD ingested repeatedly. Following the administration of all doses of ¹⁴C-TCDD, the average dose

absorbed was 86%. The rats were killed at 1, 3, or 7 weeks, and their liver, fat, kidney, thymus, and spleen tissues were examined for ^{14}C -activity. Activity was primarily localized in liver and fat, with radioactivity in the liver being 5 times greater than in fat. The accumulation of the isotope in both types of tissue followed first order kinetics. With continuous administration, the concentrations of ^{14}C -radioactivity in both tissues approached plateau levels by 7 weeks (73.8% of steady state values). By 13 weeks, 93% of the steady state level had been reached; the rate of accumulation of radioactivity was independent of the dose level administered over the dose range of 0.01 to 1.0 ug TCDD/kg/day.

The half-life of elimination of ^{14}C -activity in rats was 23.7 days. The ^{14}C -TCDD radioactivity was excreted primarily in the feces, with some of it in an altered chemical form, presumably having been excreted from the liver via bile. Significant amounts of ^{14}C -TCDD were also found in urine, particularly in female rats. Female rats were given 0.001, 0.01, and 0.1 ug TCDD/kg of body weight for 2 years (Kociba et al. 1978^a). After being killed, the liver and fat tissues were analyzed for TCDD. The chemical analysis of liver and fat are shown in the Table 1 below.

TABLE 1. CONCENTRATIONS OF TCDD IN RAT LIVER AND FAT AFTER 2 YEARS OF FEEDING

Dose	Concentration in liver ^a	Concentrations in fat ^a
0.001 ug/kg	540	540
0.01 ug/kg	5,100	1,700
0.1 ug/kg	24,000	8,100

^aparts per trillion

These results revealed the dose-dependent accumulation of TCDD after long-term exposure.

ARYL HYDROCARBON HYDROXYLASE (AHH) INDUCTION STUDIES WITH TCDD

TCDD causes toxic effects, which are discussed in Section V of this document. The biochemical lesions underlying the observed toxicologic effects of TCDD are not known, but certain enzyme systems have been shown to change when animals are exposed to non-lethal doses of TCDD (Hook 1975). In particular, hepatic microsomal mixed-function oxidases seem to be highly responsive to TCDD.

AHH is one of the microsomal mixed-function oxidase enzyme systems responsible for the oxidative metabolism of many exogenous and endogenous compounds, including many polycyclic aromatic hydrocarbons (Poland and Glover 1973, Kouri 1976). The metabolic oxidation of these compounds proceeds via transient chemically reactive intermediates, including epoxides (Kouri 1976).

Hepatic aryl hydrocarbon hydroxylase is a useful indicator of the inductive potential of a particular biological system, because if AHH is induced into any tissue (e.g., kidney, bowel, lung, or skin), AHH activity in liver will be induced concomitantly (Kouri 1973).

The AHH enzyme system is induced by a wide variety of drugs and polycyclic aromatic hydrocarbons, including the steroid hormones, benzo(a)pyrene and 3-methylcholanthrene, as well as TCDD and compounds that structurally resemble TCDD, i.e., polychlorinated biphenyls, 2,3,7,8-tetrachloro-dibenzofuran, 3,4,3',4'-tetrachloroazoxybenzene, and 3,4,3',4'-tetrachloroazobenzene (Poland and Glover 1976^b, Goldstein et al. 1977, Kouri et al. 1973).

Kouri et al. (1973) correlated induction of AHH by 3-methylcholanthrene (3-MC) in 14 mouse strains with a high carcinogenic index, a measure of sensitivity to chemical carcinogens which are metabolically activated to a

carcinogenic intermediate, and found that the AHH system plays a role in this activation.

TCDD is reported to produce effects on microsomal mixed function oxidases that are very similar to those produced by 3-MC (Poland and Glover 1974). Many compounds have been shown to induce certain mixed function oxidases, but enzyme-inductive plus enzyme-suppressive effects are peculiar to inducers like 3-MC and TCDD. The mechanisms involved in regulation of the mixed function oxidases vary with the organ and species (Hook 1975). The potency of TCDD in inducing hepatic AHH is 3×10^4 that of 3-MC (Poland and Glover 1974), and is 40 to 60 times that of 3-MC in inducing AHH activity in cultured human lymphocytes (Kouri et al. 1974).

There are genetic differences in AHH inducibility in humans and in mice (Poland et al. 1976^b; Kouri et al. 1974). Poland et al. (1976^b) inferred from mouse data that the hepatic cytosol species that binds TCDD is the receptor for the induction of hepatic AHH activity, and that the mutation in non-responsive mice results in an altered receptor with a diminished affinity for inducing compounds.

In conclusion, 2,4,5-T is readily absorbed by several mammalian species, including man, and excreted unchanged mostly in urine.

TCDD is a potent inducer of arylhydrocarbon hydroxylase in mammalian species. This is a complex enzyme system which consists of epoxidase, epoxidehydrase, and glutathione transferase. The enzyme epoxidase is known to mediate the formation of epoxides, which are potentially active carcinogenic metabolites. TCDD does not undergo any known metabolic transformations in mammalian species. However, its persistent residues were found in liver and fat after 2-year feeding studies in rats.

IV. MUTAGENICITY

MUTAGENICITY OF 2,4,5-T

The mutagenicity of 2,4,5-T was evaluated by Ercegovich et al. (1977), employing the procedure of Ames, using five strains of Salmonella typhimurium without activation. They concluded that 2,4,5-T is non-mutagenic.

Anderson and Styles (1978) reported that 2,4,5-T at concentration ranges from 4 to 2500 ug per plate did not cause reversions in any of the four strains of Salmonella typhimurium (TA 1535, TA 1538, TA 98, and TA 100) with or without microsomal activation. Several other investigators have reported negative responses with 2,4,5-T in bacterial test systems which have been summarized in a review by Grant (1979). Zetterberg (1978) found that 2,4,5-T increased the back mutation frequency in the histidine defective strain of Saccharomyces cerevisiae at pH values below 4.5, by approximately 300 fold at 40 mg/ml and 5000 fold at 60 mg/ml. However, the percent of survivors at the lower concentration was less than 5% and at the higher concentration less than 0.1%. The author concluded that 2,4,5-T is unlikely to cause mutations in a near neutral environment but oral administration may increase the risk of somatic mutation in the gastric tract where pH values are as low as 1.2. The 2,4,5-T used in these studies contained less than 1 ppm dioxins.

Majumdar and Golia (1974) fed Drosophila melanogaster males 1000 ppm 2,4,5-T for 15 days and found a small increase in the percentage of sex-linked recessive lethals by 0.61% over controls values of 0.05%. The herbicide was reported to contain no detectable amount of dioxin. Similar findings by Magnusson et al. (1977) also showed 2,4,5-T to be weakly mutagenic in Drosophila. In a parallel experiment, the known mutagen ethylmethanesulfonate at 250 ppm increased the incidence of sex-linked recessive lethals by 13.65%.

Fujita et al. (1975) reported chromosomal abnormalities in in vitro cytogenetics studies of human lymphocytes exposed to 10^{-7} to 10^{-4} M 2,4,5-T. Chromosome breaks, deletions, and rings were observed. Chromatid breaks increased with increasing concentrations of 2,4,5-T. It was not possible to distinguish whether this was a toxic effect or a potential genetic effect.

Yefimenko (1974) reported on an acute and chronic exposure to butyl ether 2,4,5-T in in vivo cytogenetics tests on gonadal and somatic tissue in male albino rats. Twenty-four hours after a single oral administration at doses of 1, 0.1, and 0.01 ug/kg, structural damage to bone marrow cell chromosomes was observed either as breaks or as true aberrations or rearrangements.

Chronic-exposure effects to the gonads were observed after exposure for 2-1/2 months to a dose of 0.1 ug/kg. The following effects were observed at the termination of the experiment (7 months): testicular atrophy, decreased sperm count, desquamated tubules, and aberrant cells in the germinal epithelium. These effects persisted after exposure was terminated. Chromosomal aberrations were also observed during chronic dosing. The authors' methodology appears to be inadequate, however, and thus no valid conclusions can be drawn from this study. Majumdar and Hall (1973) reported that intraperitoneal injections of 2,4,5-T (containing no measurable amount of TCDD) into gerbils at concentrations of 350 mg/kg for 5 days produced 8.2, 4.6, and 1.8 percent incidences of chromatid gaps, chromatid breaks and fragments, respectively, in bone marrow cells. Control values were given as 1.0% for gaps, 0.2% for breaks, and 0.2% for fragments. When the animals were treated at lower doses, no significant increases in chromosomal abnormalities were observed. Jenssen and Renberg (1976) performed cytogenetic tests on mice injected with 2,4,5-T at 100 mg/kg. They reported no increase over control values in incidences of micronuclei in polychromatic or normochromatic erythrocytes, or polychromatic cells 24 hours or

7 days after the injection of the chemical. They were unable to confirm the cytogenic effect reported by Majumdar and Hall (1973), but pointed out that they used extremely high doses which might cause toxic effects leading to cell death and chromosomal fractionation.

Kilian et al. (1975) examined lymphocytes for chromosomal aberrations in industrial workers exposed to 2,4,5-T in a Midland Michigan plant and compared them with a control group of workers prior to employment at the plant. They reported that there was no significant differences in the aberration rates among the control group (84 people) and those exposed for less than a year (16 people) or more than a year (17 people). However, the study did not indicate concentrations of 2,4,5-T workers might have been exposed to, and for each subject only about 20 cells were scored for chromosomal aberrations.

MUTAGENICITY OF TCDD

Hussain et al. (1972) reported positive results in three microbial test systems using a 99% pure TCDD sample obtained from FDA. Reversion to streptomycin independence in Escherichia coli Sd-4 occurred with high frequency at a concentration of 2 ug TCDD/ml. Reversion at the histidine locus of Salmonella typhimurium TA-1532 occurred at concentrations between 2 to 3 ug/ml. This indicates that TCDD produces frameshift mutations by intercalation between base-pairs of DNA. A doubling in the frequency of prophage-induction was observed in E. coli K-39 exposed to TCDD. These studies were not performed with metabolic activation, indicating that TCDD is a direct-acting mutagen. Seiler (1973) classified TCDD as a strong mutagen (where the ratio of number of revertants from treated plates per 10^8 bacteria divided by the number of spontaneous revertants per 10^8 bacteria is greater than 10) in the TA 1532 Salmonella strain which detects revertants through frameshift mutations.

However, this report did not give the source or purity of TCDD, the concentration used in the assay, the toxicity of the compound where mutagenic activity occurs, or whether microsomal activation was necessary.

However, McCann (personal communication) tested TCDD to be negative in the standard plate test with strain TA 1532, with and without microsomal activation, and Nebert et al. (1976) also reported that TCDD was not mutagenic in the *Salmonella* in vitro assay. The differences between these laboratory results and those discussed above could be due to several factors such as treatment protocols, solubility problems of TCDD, and the high toxicity of this compound. (Because of the paucity of gene mutation studies for TCDD and the inconclusiveness of the current available data, it is recommended that additional tests for gene mutations be conducted in systems such as mammalian cells in culture and sex-linked recessive tests in Drosophila).

FDA conducted somatic in vivo cytogenetics screening study on TCDD in rats and got negative results (Green 1975). Separate experiments were performed with five multiple intraperitoneal doses or a single oral dose regimen with sacrifice at 1 or 29 days. Toxicity, as indicated by slight body weight loss, was observed in the multiple dose study only at the highest dose used. This indicates that the dose levels may have been too low. Khera and Ruddick (1973) dosed male rats orally with 4 or 8 mg/kg/day TCDD for 7 days. These doses were acutely toxic and 20 survivors at the lower dose and 6 survivors at the high dose were mated after treatment seven times at 5-day intervals. Reproductive values indicated no occurrence of dominant lethal mutations. Green et al. (1977) studied the cytogenetic effects of TCDD on rat bone marrow cells. Male and female animals received 0.25, 1.00, 2.00, and 4.00 mg/kg TCDD by gavage twice a week for 13 weeks. The authors examined bone marrow cells at the end of treatment (approximately 50 cells per animal) for abnormalities. They concluded

TCDD produces chromosomal aberrations in bone marrow cells but the effect is not one of great magnitude.

Chromosome analyses on 12 hospital patients exposed to TCDD in a July, 1976 Seveso, Italy factory accident (Department of Health, Education, and Welfare, 1976) were examined for chromosomal lesions (gaps, chromatid and chromosome breaks, and rearrangements). These analyses presented at the DHEW-Subcommittee on Environmental Mutagenesis meeting, October 12, 1976 were of somatic cells from males and females ranging in age from 2 to 28. One patient had 19% cells (presumed blood cells) that were classified as having chromosomal lesions, another had 10%. The remaining patients had values comparable to control levels of 5 to 7%. Results from chromosome analyses of maternal peripheral blood, placenta, and fetal tissue in 17 women exposed to TCDD (amounts not given) who underwent spontaneous abortions were inconclusive. Reggiani (1977) reported that the frequency of spontaneous abortions in the Seveso zone did not significantly change nor did the incidence of malformations as a result of exposure to TCDD, even at the regions where the exposure was estimated through soil analysis to be greater than 10 ug/kg. Similar conclusions were reached by Tuchmann-Duplessis (1977). Reports by both Reggiani (1977) and Tuchmann-Duplessis (1977) state no increase in abnormal cytological changes in tissues of aborted fetuses or in maternal blood in the Seveso zone during the exposure incidence to TCDD. However, these findings are poorly documented and complete experimental procedures and design used to evaluate the data were not available. Furthermore, it appears from these reports that only gross macroscopic alterations were sought and not microscopic lesions which are more difficult to assess. Such lesions are very dangerous in that they may survive and be carried to future generations.

CONCLUSIONS

There is some evidence that 2,4,5-T appears to be a weak mutagen

causing point mutations. The best evidence for this is in Drosophila and Saccharomyces cerevisiae. However, evidence in Saccharomyces cerevisiae indicate the potency of the mutagenic effect may be related to the ionization of the carboxyl group of 2,4,5-T and is increased under more acidic conditions. Epidemiological evidence for mutagenicity concerning TCDD is, at the present time, inconclusive. Experimental evidence is equivocal, and until additional tests for mutations are performed, no definitive conclusions can be made.

V. TOXICOLOGY

ANIMAL TOXICITY

Toxicity of 2,4,5-T

Oral LD₅₀ levels for 2,4,5-T as referenced by NIOSH (1976), are shown in Table 2 below.

TABLE 2. ORAL LD₅₀ LEVELS FOR 2,4,5-T

<u>Species</u>	<u>LD₅₀</u>
Dog	100 mg/kg
Rat	300 mg/kg
Guinea Pig	381 mg/kg
Mouse	389 mg/kg

In a study cited by Rowe and Hymas (1954), no adverse effects were seen in dogs given 2,4,5-T by oral administration five times a week for 90 days at doses of 25 and 10 mg/kg. However, at a dose level of 20 mg/kg, all four dogs died and showed mild liver and kidney changes. A Dow Chemical Company internal report (1971a) cited by EPA's 2,4,5-T Advisory Committee summarized a study using 2,4,5-T containing 0.5 ppm TCDD. The 2,4,5-T was fed to male and female rats for 90 days at dose levels from 0 to 100 mg/kg/day. No toxic effects were observed at doses of 30 mg/kg or lower. At a dose level of 100 mg/kg/day, some hematological effects and weight loss were noted, but toxic effects were described as minor and inconsistent.

Toxicity of TCDD

TCDD is one of the most toxic chemicals known to man. Oral LD₅₀ values, shown in Table 3, range from 0.6 ug/kg orally for the male guinea pig to 275 ug/kg dermally for the rabbit. Deaths typically occur about a week or more after treatment.

Poland et al. (1971) cite a study in which rapid death in guinea pigs followed dermal application of the tarry residues from TCDD synthesis. When rabbit ears were painted with soil extracts contaminated with TCDD, hyperkeratosis and liver pathology were observed in the rabbits (Kimbrough 1973).

Kociba et al. (1978^a) conducted a 2-year chronic toxicity and oncogenicity study of TCDD in rats. In this study, the animals were maintained for 2 years on diets supplying 0.1, 0.01, and 0.001 ug TCDD/kg/day. Aside from carcinogenic effects, ingestion of 0.1 ug/kg/day caused increased mortality, decreased weight gain, slight depression of erythroid parameters, increased urinary excretion of porphyrins and delta-aminolevulinic acid, along with increased serum activities of alkaline phosphatase.

In chronic and acute oral TCDD toxicity studies on several animal species, the liver, thymus, and spleen have consistently been the target organs. Liver damage, including necrotic and degenerative changes, lipid accumulation, and increased liver weight, have been observed in mice, rats, and guinea pigs following TCDD treatment (Vos et al. 1974, Jones and Greig 1975, Gupta et al. 1973, Goldstein et al. 1973, Kimmig and Schultz 1957). Liver damage was markedly greater in rats receiving a comparable dose (Gupta et al. 1973). It has been suggested that the fatty liver observed in mice may result from the starvation and loss of body weight that occur following TCDD treatment, or may be due to the induction of mixed function oxidases (Jones and Greig 1975).

TABLE 3. LETHALITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN^{a, b}

Species and sex	Route of administration	Time of death, days post administration	LD ₅₀ ug/kg	Dose ug/kg	Number deaths/number treated
Rat, male	Oral	9-27	22	8	0/5
				16	0/5
				32	10/10
				63	5/5
Rat, female	Oral	13-43	45		
Guinea pig, male	Oral	5-34	0.6		
Guinea pig, male ^c	Oral	9-42	2.1		
Rabbit, mixed	Oral	6-39	115		
	Skin	12-22	275		
	Intraperitoneal	6-23 (all doses)	---	32	0/5
63				2/5	
126				2/5	
252				2/5	
500				3/5	
Dogs, male	Oral	9-15 (all doses)		300	0/2
				3000	2/2
Dogs, female	Oral	---		30	0/2
				100	0/2
Mice, male	Oral	---		114	---

^aResponses to individual doses are given in those cases in which an LD₅₀ could not be calculated.

^bAll values are from Schwetz et al. (1973), except that for male mice, which are from Vos et al. (1974).

^cA sample that was more than 99% pure was used. All other tests except the mice study used TCDD that was 91% pure.

Atrophy of the thymus and spleen has also consistently been found in laboratory animals (Vos et al. 1974, Kociba et al. 1975, Gupta et al. 1973). Vos et al. (1973) report that cell-mediated immunity was suppressed in guinea pigs and mice in TCDD-induced lymphoid depleted thymuses. Thigpen et al. (1975) found that mice receiving 1 ug/kg or more of TCDD by stomach tube once a week for 4 weeks had increased susceptibility to Salmonella infection. Female monkeys fed TCDD for 9 months showed hypocellularity of the bone marrow and lymph nodes as well as hypertrophy, hyperplasia, and metaplasia of the bronchial tree, epithelium, bile ducts, pancreatic ducts, and salivary gland ducts (Allen et al. 1977).

Other effects of TCDD ingestion include suppression of reproductive function in rats (Kociba et al. 1975) and disturbance of the hematopoietic system with occasional hemorrhaging in monkeys, rats, and mice (Allen et al. 1977, Kociba et al. 1975, Vos et al. 1974). TCDD interferes with the biosynthetic pathway of heme by inducing delta-aminolevulinic acid synthetase (δ -ALA), which results in hepatic porphyria in mice and rats (Goldstein et al. 1976). Increased urinary excretion of uroporphyrins has been observed in rat feeding studies (Kociba et al. 1975, Goldstein et al. 1976).

TOXICITY OF 2,4,5-T, 2,4,5-TRICHLOROPHENOL, AND TCDD IN HUMANS

The most consistently reported toxic effect of 2,4,5-T, 2,4,5-trichlorophenol, and TCDD to humans is chloracne, a disfiguring and long-term dermatitis. This has occurred in 2,4,5-T factory workers (Bauer et al. 1961, Poland et al. 1971), 2,4,5-trichlorophenol workers (Kimmig and Schulz 1957, Bauer et al. 1961, Bleiberg et al. 1964, Goldmann 1972), and laboratory workers accidentally exposed to TCDD (Oliver 1975). It has also been observed in exposed populations following the accidental production of TCDD in exothermic

reactions at chemical plants (Hay 1976, Kimmig and Schulz 1957, May 1973). In the 2,4,5-trichlorophenol plant accident in Seveso, Italy (1976), 300 to 500 grams of TCDD are believed to have been deposited in the most contaminated areas, with lesser amounts in surrounding areas (Hay 1976). In the other incidents, the level of TCDD present is not estimated.

There are scattered reports of hepatotoxic effects including abnormal liver function tests and pathological changes in the liver (Kimmig and Schulz 1957, Bauer et al. 1961, Bleiberg et al. 1964, Poland and Smith 1971). Eleven of 14 men exposed to TCDD during an exothermic reaction at a 2,4,5-trichlorophenol plant had abnormal liver function tests, but after 10 days without TCDD exposure, most tests were normal. Bleiberg et al. (1964) reported that 11 of 29 workers at a 2,4,5-trichlorophenol plant had porphyria, but in a study of the same workers 6 years later, Poland et al. (1971) found no overt clinical cases of the disease. They suggested that the change may have been due to increased attention to worker safety or to a decrease in TCDD contamination.

Bauer et al. (1961) found one case of bloody urine in exposed workers. Hemorrhagic cystitis was reported (Kimbrough et al. 1975) in a 6-year-old girl who was playing in a horse area contaminated with 2,4,5-TCP (TCDD concentration of 31 to 33 ppm). Other organ system effects that have been reported are gastrointestinal tract disturbances (Kimmig and Schulz 1957, Poland et al. 1971), neurological disturbances (Oliver 1975), and respiratory and cardiac disorders (Bauer et al. 1961). Psychological changes, including emotional instability, lethargy, diminished libido, and high manic scores on psychological tests, have also been noted (Poland et al. 1971, Oliver 1975, Bauer et al. 1961).

VI. CARCINOGENICITY

CARCINOGENICITY OF 2,4,5-T IN MICE

Muranyi-Kovacs et al. (Oral) Mouse Study (1976)

Inbred C3Hf and XVII/G strains of mice were used. They were given 100 mg/liter of (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) in drinking water for 2 months, beginning at 6 weeks of age. The 2,4,5-T product contained less than 0.05 ppm of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Thereafter, mice were given 2,4,5-T mixed with a sterile, commercial diet (UAR 1136) at concentrations of 80 ppm. It was not stated whether these levels represented maximum tolerated values. However, the author indicated that this dose was 1/40 of the LD₅₀.

The mice were examined weekly for their general health and for the presence of tumors. They were allowed to die or were killed in extremis. Complete necropsies were performed and grossly altered organs were examined histologically. The urinary bladder was distended with fixative in mice suspected of having lesions.

C3Hf control male mice survived an average of 630 days; treated male mice, 511 days ($P = 0.001$); control females, 680 days; and treated females, 620 days. Survival times for XVII/G control male mice were 521 days; for treated male mice, 583 days; control females, 569 days; and for treated females, 641 days ($P = 0.01$).

Tumor presence in C3Hf female mice ingesting 2,4,5-T is indicated in Table 4. The results show that 12 of 25 C3Hf female mice (48%) ingesting 2,4,5-T developed tumors of all types, as compared to 9 of 44 control female mice (21%) ($P = 0.03$). No other strain-sex combination yielded statistically significant values, as evidenced by the data in Tables 4 and 5. Benign and malignant tumors

TABLE 4. TUMORS IN C3Hf MALE AND FEMALE MICE INGESTING 2,4,5-T^a

Dose (ppm)	Sex	Number of Tumors				Total	No. of Mice with Tumors
		Lung	Liver	Leukemia	Other		
0	M	2	19	0	1 ^b	22	21/43 (49%)
80 ppm	M	0	10	2	1 ^c	13	12/22 (55%)
0	F	5	3	1	0	9	9/44 (21%)
80 ppm	F	0	4	3	6 ^d	13	12/25 (48%)

^aEffective number of mice surviving longer than 300 days or developing a tumor before 300 days of age.

^bPleomorphic salivary gland tumor.

^cFibrosarcoma; not included are one hyperplastic lesion of the urinary bladder and one hyperplastic lesion of the forestomach.

^dOsteogenic sarcoma, 2 sarcomas, 2 cutaneous tumors, and 1 tumor of the cervix.

TABLE 5. TUMORS IN XVII/G MALE AND FEMALE MICE INGESTING 2,4,5-T^a

Dose (ppm)	Sex	Lung	Liver	Leukemia	Other	Total	No. of Mice with Tumors
0	M	22	4	0	1 ^b	27	25/32 (78%)
80	M	14	0	1	1 ^c	16	15/20 (75%)
0	F	20	0	2	2 ^d	24	21/40 (53%)
80	F	15	0	1	0	16	16/19 (84%)

^aEffective number of mice are mice surviving longer than 300 days or developing a tumor before 300 days of age. In the XVII/G male mice, there was no significant difference between the number of tumor-bearing mice among treated animals as compared with controls, as shown in the table above.

^b1 forestomach tumor.

^c1 urinary bladder papilloma; not included are 2 hyperplastic lesions of the urinary bladders.

^d2 hemangiomas.

were considered together in this study. The authors stated that the "hepatomas" and lung tumors, which were carcinomas and alveologenic adenomas, occurred in the same proportions in control and treated mice. Treated C3Hf females had several tumors at sites not found in the controls. The authors reported a significant increase in total tumors in one strain and one sex of rats at one dose level. In reaching this conclusion, they used the Peto method and distinguished between incidental and nonincidental tumors.*

To clarify questions concerning the design, execution, and interpretation of this study, the CAG communicated with the principal author at the Curie Foundation, Marseilles, France. From this discussion and from the published account of this discussion it is concluded that: 1) the studies were very insensitive because insufficient numbers of animals were used in the treatment groups; 2) care of the animals was inadequate; 3) this study was insensitive because the dose used, 80 ppm, was only 1/40 of the LD₅₀, and appears to be less than the maximum tolerated dose; 4) histologic examination of all animal tissues as not performed; 5) only macroscopically altered tissues were examined histologically. In addition, the author recommended that more adequate studies be conducted in a greater number of species. Because of the severe deficiencies in the study, the CAG has concluded that this study does not provide significant evidence for either the carcinogenicity or non-carcinogenicity of 2,4,5-T.

Muranyi-Kovacs et al. (Subcutaneous) Mouse Study (1977)

In this study, the authors administered 2,4,5-T to two strains of mice, C3Hf and XVII/G. Subcutaneous injections were given at 10 mg/kg of body weight in an aqueous solution on days 1, 3, 6, and 10 of the animals' life. The sex, strain, number of animals, survival time, and animals with tumors are shown in Table 6.

*These results are not considered to be evidence of a oncogenic response because there is no valed basis for grouping tumors at all sites or for distinguishing between incidental and nonincidental tumors. The author did not report any increases in tumors for any specific target site.

TABLE 6. SURVIVAL TIME AND TUMOR INCIDENCE IN 2,4,5-T^a
TREATED MICE

Strain	Sex	Dose mg/kg	Number of animals	Survival time tumor-bearing	Percent of animals with tumors
XVII/G	M	0	32	25/32	78
	M	4 x 10	15	13/15	87
XVII/G	F	0	40	21/40	53
	F	4 x 10	15	4/15	25
C ₃ H/f	M	0	43	21/43	49
	M	4 x 10	11	4/11	36
C ₃ H/f	F	0	44	9/44	21
	F	4 x 10	14	3/14	21

^aTCDD content of 2,4,5-T was less than 0.05 ppm.

As indicated, there is no observed increased incidence of tumor-bearing animals as compared to the treated animals of both sexes and strains. However, this study was so incompletely reported that the details of the methodology cannot be discerned. In addition, this study was very insensitive because insufficient numbers of animals were used in the treatment groups, and only a few subcutaneous doses were administered. Therefore, these studies do not provide significant evidence of either the carcinogenicity or non-carcinogenicity of 2,4,5-T.

Innes et al. (Bionetics Laboratories) (Oral) Mouse Study (1968, 1969)

The maximum tolerated dose of 2,4,5-T* was given to two hybrid strains of mice (C57BL/6 x C3H/Anf)F₁, B6C3F₁ designated as "strain X," and (C57B/6 x AKR)F₁, B6AKF₁ designated as "strain Y." There were 18 treated mice and 18 untreated control mice of each strain and each sex. Each day, beginning at 7 days of age, 21.5 mg/kg of 2,4,5-T in 0.5% gelatin was administered by stomach tube. After weaning at 28 days of age, 60 ppm of 2,4,5-T was mixed directly in the diet and provided ad libitum. Treatment was continued for approximately 18 months.

At this time mice were killed and grossly examined both internally and externally in the areas of the neck glands and the thoracic and abdominal cavities. Histologic examination of major organs and all grossly visible lesions was performed. Thyroid glands were not examined. The postmortem results are given in Tables 7 and 8.

The results of the oral mouse study indicate that there was no significant difference between the 2,4,5-T-treated and control groups of mice with respect to tumors at specific sites, or total number of tumor bearing animals. This study, however, does not provide significant evidence for the non-carcinogenicity of 2,4,5-T because of certain defects in its design. The use of small numbers of animals and the duration of the study, which was only 18 months rather than the entire lifetime, made the study relatively insensitive for detecting an oncogenic effect.

* The Bionetics studies did not report the level of TCDD contamination in the 2,4,5-T used in those studies. The 2,4,5-T used in a reproductive study conducted at approximately the same time as the Bionetics study was reported to contain 30 ppm TCDD. It is possible that the contaminant of 2,4,5-T used in the Bionetics study was the same as that of the 2,4,5-T used in the reproductive study. However, this conclusion is far from certain without actual chemical analysis of the 2,4,5-T used in the Bionetics studies.

TABLE 7. NUMBERS OF MALE AND FEMALE MICE WITH TUMORS OF VARIOUS ORGANS
AMONG MICE INGESTING 2,4,5-T

Strain	Dose (ppm)	Reticulum Cell Sarcoma		TUMOR TYPE Pulmonary Adenoma & Carcinoma		Hepatoma	
		Male	Female	Male	Female	Male	Female
"X" matched	0	0/15	1/18	2/15	1/18	3/15	0/18
"X" pooled	0	5/79	4/87	5/79	3/87	8/79	0/87
"X"	60	1/18	0/18	1/18	1/18	4/18	0/18
"Y" matched	0	0/18	1/15	3/18	0/15	0/18	0/15
"Y" pooled	0	1/90	3/82	10/90	3/82	5/90	1/82
"Y"	60	2/18	1/18	0/18	0/18	1/18	0/18

TABLE 8. NUMBERS OF MALE AND FEMALE MICE WITH TUMORS AT ALL SITES
AMONG MICE INGESTING 2,4,5-T

		Male	Female
"X" matched	0	5/15 (33%)	2/18 (11%)
"X" pooled	0	22/79 (28%)	8/87 (9%)
"X"	60	6/18 (33%)	1/18 (6%)
"Y" matched	0	3/18 (17%)	1/15 (7%)
"Y" pooled	0	16/90 (18%)	7/82 (9%)
"Y"	60	3/18 (17%)	2/18 (11%)

Innes et al. (Bionetics Laboratories) (Subcutaneous) mouse Study (1968)

2,4,5-T in dimethylsulfoxide (DMSO) was given as a single subcutaneous injection (215 mg/kg) to two strains of male and female mice (same strains as in the oral study) at approximately 28 days of age. The mice were observed for approximately 18 months. At that time mice were killed and examined grossly, both internally and externally, in the areas of the neck, glands, and thoracic and abdominal cavities. Histologic examinations of all major organs, as well as all grossly visible lesions, were made. Thyroid glands were not examined. The authors stated that histopathologic data did not show a statistically significant difference between the 2,4,5-T-treated and control groups either with respect to tumors at specific sites, or total number of tumor-bearing animals. However, this study suffered from the same deficiencies as the Innes et al. oral study. In addition, single subcutaneous dose studies are considered to be highly insensitive for detecting an oncogenic response. Therefore, the CAG does not consider this study to provide significant evidence of the non-oncogenicity of 2,4,5-T.

CARCINOGENICITY OF 2,4,5-T IN RATS

Kociba et al. (Oral) Rat Study (1978, 1979)

The cancer bioassay study of 2,4,5-T in rats (TCDD content was not detectable in 2,4,5-T with a detection limit of 0.33 ppb) was performed by Kociba et al. In this study, one group of 86, and three groups of 50 Sprague-Dawley (Spartan) rats of each sex were administered 0, 3, 10, and 30 mg/kg body weight/day, respectively, via the diet, for periods up to 2 years.

There is some question as to the actual dosage. Adequate information is lacking to show that the amount of 2,4,5-T found in the food by chemical analysis actually remained constant in a given feed lot during the entire period of consumption of that given lot. Based on the analytical data, the actual doses given were slightly lower than the nominal doses.

There are certain aspects of this study which reduced its sensitivity for detecting a carcinogenic response. First, there was a very high early mortality among all groups of males and females (Tables 9 and 10). The mortality data show that at the termination of the study more than 50% of the females had died in the control group as well as in each of the treated groups (the mortality was 76% in the 10 mg/kg female group). Among males, mortality was approximately 92% in the controls and ranged from 78% to 92% in the treated groups. Very high mortality in both males and females was observed as early as 21 months. This early mortality is important because it reduces the number of animals at risk for late developing tumors.

The second factor which reduced the sensitivity of this study was the relatively high incidence of spontaneous tumors in some organ sites in the controls. For example, among 86 control males, three hepatocellular carcinomas and four hepatocellular neoplastic nodules were found.

The data presented in Table 11, as a whole, indicates positive suggestive evidence of the carcinogenicity of 2,4,5-T.

TABLE 9. CUMULATIVE MORTALITY DATA OF MALE RATS MAINTAINED ON DIETS CONTAINING 2,4,5-T FOR 2 YEARS

	Dose Level (mg/kg/day)			
	0	30	10	3
	No. dead (% dead)	No. dead (% dead)	No. dead (% dead)	No. dead (% dead)
Original no. in group	86	50	50	50
Days on test				
0-30	0	0	0	0
31-60	0	0	0	0
61-90	1(1.2)	0	0	0
91-120	1(1.2)	0	0	0
121-150	1(1.2)	0	0	0
151-180	1(1.2)	0	0	0
181-210	1(1.2)	0	0	0
211-240	1(1.2)	0	0	0
241-270	1(1.2)	0	1(2.0)	0
271-300	2(2.3)	0	1(2.0)	0
301-330	2(2.3)	0	1(2.0)	0
331-360	2(2.3)	0	1(2.0)	1(2.0)
361-390	2(2.3)	2(4.0)	2(4.0)	2(4.0)
391-420	5(5.8)	2(4.0)	2(4.0)	3(6.0)
421-450	6(7.0)	2(4.0)	4(8.0)	4(8.0)
451-480	9(10.5)	4(8.0)	9(18.0)	6(12.0)
481-510	10(11.6)	6(12.0)	12(24.0) ^a	10(20.0)
511-510	16(18.6)	8(16.0)	22(44.0) ^a	12(24.0)
541-570	23(26.7)	11(22.6)	24(48.0) ^a	14(28.0)
571-600	32(37.2)	16(32.0)	29(58.0) ^a	23(46.0)
601-630	47(54.6)	19(38.0)	37(74.0) ^a	30(60.0)
631-660	67(77.9)	24(48.0) ^a	38(76.0)	32(64.0) ^a
661-690	74(86.0)	27(54.0) ^a	42(84.0)	34(68.0) ^a
691-720	77(89.5)	32(64.0) ^a	45(90.0)	38(76.0) ^a
721-728	79(91.7)	39(78.0) ^a	46(92.0)	40(80.0) ^a
Total no. of rats on study	86	50	50	50

^aStatistically significant difference from control values by Fisher's Exact Probability Test, P < 0.05.

TABLE 10. CUMULATIVE MORTALITY DATA OF FEMALE RATS MAINTAINED ON DIETS CONTAINING 2,4,5-T FOR 2 YEARS

	Dose Level (mg/kg/day)			
	0	30	10	3
	No. dead (% dead)	No. dead (% dead)	No. dead (% dead)	No. dead (% dead)
Original no. in group	86	50	50	50
Days on test				
0-30	0	0	0	0
31-60	0	0	0	0
61-90	0	0	0	0
91-120	0	0	0	1(2.0)
121-150	0	0	0	1(2.0)
151-180	0	0	1(2.0)	1(2.0)
181-210	1(1.2)	0	1(2.0)	1(2.0)
211-240	1(1.2)	0	1(2.0)	1(2.0)
241-270	1(1.2)	0	1(2.0)	1(2.0)
271-300	1(1.2)	1(2.0)	1(2.0)	1(2.0)
301-330	2(2.3)	1(2.0)	3(6.0)	2(4.0)
331-360	2(2.3)	2(4.0)	3(6.0)	2(4.0)
361-390	2(2.3)	3(6.0)	3(6.0)	3(6.0)
391-420	4(4.6)	3(6.0)	4(8.0)	3(6.0)
421-450	5(5.8)	3(6.0)	5(10.0)	3(6.0)
451-480	9(10.5)	7(14.0)	8(16.0)	5(10.0)
481-510	12(14.0)	9(18.0)	13(26.0) ^a	7(14.0)
511-540	18(21.0)	11(22.5)	14(28.0)	10(20.0)
541-570	20(23.2)	12(24.0)	15(30.0)	12(24.0)
571-600	25(29.1)	15(30.0)	16(32.0)	16(32.0)
601-630	29(33.7)	18(36.0)	21(42.0)	20(40.0)
631-660	34(39.5)	24(48.0)	25(50.0)	23(46.0)
661-690	41(47.7)	26(52.0)	33(66.0) ^a	26(52.0)
691-720	46(53.5)	27(54.0)	35(70.0) ^a	29(58.0)
721-732	46(53.5)	28(56.0)	38(76.0) ^a	29(58.0)
Total no. of rats on study	86	50	50	50

^aStatistically significant difference from control values by Fisher's Exact Probability Test, P < 0.05.

TABLE 11. STRATIFIED SQUAMOUS CELL CARCINOMA OF THE TONGUE OF SPRAGUE-DAWLEY RATS FED WITH PURIFIED 2,4,5-T

	Kociba 2,4,5-T Controls	2,4,5-T dosage in mg/kg/day			Test for Trend ^b
		30 (P-value) ^a	10	3	
Males	1/83	4/49 (P = 0.063)	0/46	1/50	< 0.03
Females	0/83	1/49 (P = 0.371)	0/48	0/48	N.S.
Total	1/166	5/98 (P = 0.028)	0/94	1/98	< 0.01
%	0.60	5.1	1.0		

^aP values determined by Fisher's Exact Test (one-tailed).

^bCochran's test for trend, one-tailed, scoring = 0, 1, 2, 3.

The increase in squamous cell carcinoma of the tongue in males at the 30 mg/kg/day dose level is marginally statistically significant ($P = 0.063$). Also, the dose-related trend for the incidence of tongue tumors in males is statistically significant in the Cochran-Armitage Test ($P < 0.03$). In addition, when both sexes are combined, the observed incidence of tongue tumors in the 30 mg/kg group is significantly increased ($P = 0.028$). The sexes are not ordinarily combined for the purposes of analyzing tumor incidences because the spontaneous tumor incidence may differ in the sexes due to different hormonal or metabolic influences. However, combining the sexes in this study for the purpose of analyzing tongue tumors does not appear inappropriate because the tongue tumor incidences showed very little difference between the sexes in the controls and in the two lower dose groups.

Based on the above analysis, it is concluded that this study provides suggestive evidence of a carcinogenic effect. However, examination of the incidence of squamous cell carcinomas of the tongue in historical controls may shed further light on the biological significance of the results of this study.

Leuschner et al. (Oral) Rat Study (1979)

Leuschner et al. (1979) investigated the chronic effects of commercial grade 2,4,5-T (containing 0.05 ppm TCDD) on Sprague-Dawley (SIV50) rats. The study used four groups of 60 male and 60 female rats from the F₁ generation of a three-generation reproduction study in which the dams received 2,4,5-T at 0, 3, 10, and 30 mg/kg body weight/day in the diet. From 6 weeks (42 days) of age, rats in the four groups were placed on the same feeding regime as their mothers. The treatment continued for the duration (130 weeks) of the experiment. Three groups received 2,4,5-T dissolved in acetone, which was poured over a small quantity of feed and then mixed after the evaporation of the acetone. One group, identified in the report as the pre-mix controls, was given only acetone in the diet. A fresh diet was prepared every 7 days.

Additional groups of 60 male and 60 female Sprague-Dawley rats served as untreated controls. Rats in this group were supplied at 6 weeks of age by the same source that had supplied the F₀ generation of the three-generation study. During the experiment, clinical signs, body weights, and consumption of food and water, were monitored at regular intervals. Urinalyses were performed and hematological and clinical chemistry parameters were determined for 10 rats from each group at regular intervals. The same rats were used for measurements throughout the experiments; the authors found no effects attributable to 2,4,5-T in any of these observations. At 13 weeks, 10 rats were sacrificed from each group and examined leaving long-term exposure of groups of 50 animals each. Rats that died, were moribund, or killed during the experiment, and all surviving rats killed after 130 weeks, were necropsied. All major tissues of all animals, except for tissues of the survivors dosed at 3 mg/kg/day, were examined histopathologically.

The authors reported that they found no evidence that the test compound had

a toxic or carcinogenic effect on either male or female rats. The type and incidence of lesions observed were considered normal in old-age breeding rats of the test strain. However, a statistically significant increase in interstitial cell tumors of the testes in the high-dose group of males ($P = 0.014$), as well as a significant dose-related trend ($P < 0.01$) for these tumors were observed when comparison is made to the incidence of these tumors in the pre-mix control animals (Table 12). The significance of these results disappeared when comparison was made to the untreated control group, which had an incidence of testicular tumors higher than that in the high dose group. The incidence of testicular tumors in the untreated controls (22/50 or 44%) is very significantly higher ($P < 0.01$, used a one-tailed Fischer Exact Test) than that in the pre-mix controls (12%). Because of this difference, the two control groups cannot be pooled for comparison with the treated groups. Clearly, a compound-related increase can be shown only if the pre-mix control group alone is used as a point of comparison with the treated groups.

Because of the experimental design, the pre-mix controls are the appropriate comparison group. However, in this situation there is a question of whether the pre-mix or untreated controls manifest an atypical spontaneous rate for testicular tumors. Only more detailed information on historical controls from the same substrain, with comparable lifespans, and preferably from the same laboratory, can resolve this issue. Moreover, in the low dosage group, while 14 testicular masses were observed macroscopically, only 6 of those masses were examined microscopically. All six masses examined microscopically were testicular tumors. If the remaining eight masses were examined microscopically and were also proven to be testicular tumors, the dose-related trend would no longer be significant.

TABLE 12. INTERSTITIAL-CELL TUMORS OF TESTES IN MALE RATS

Dose	Rats with tumors	P-Value	Percent animals with tumors
untreated controls	22/50		44%
pre-mix controls	6/50		12%
10/mg/kg/day group	12/50	N.S.	24%
30 mg/kg/day group	16/50	0.014	32%

This study suffers from the following limitations: 1) the maximum tolerated dose was apparently not used; 2) the observed testicular tumors are often associated with old-age with variable incidences; 3) testicular masses were reported in 14/28 of the animals exposed at the low dose (3 mg/kg/day), but only six of these masses were diagnosed microscopically; 4) the tongue, which was a site of increase in tumor incidence in the Kociba studies, was not examined microscopically as would seem appropriate; and 5) the difference in the incidences of testicular tumors in the two control groups makes interpretation of the significance of the testicular tumor incidence in treated groups uncertain.

In conclusion, the significance of the results concerning the incidence of testicular tumors is uncertain. In addition, this test cannot be considered a valid negative study of 2,4,5-T because the highest dose used was less than the maximum tolerated dose. This reduced the sensitivity of the test for detecting the possible oncogenic effects of 2,4,5-T.

STUDIES ON (2,4,5-TRICHLOROPHENOXY)PROPRIONIC ACID (SILVEX)

Innes et al. (Bionetics Laboratories) (Oral) Mouse Study (1968, 1969)

Innes et al., under the sponsorship of the National Cancer Institute, investigated the carcinogenicity of silvex in two studies with mice, one oral and the other subcutaneous. In the oral study, groups of 18 B6C3F1 and 18 B6AKF1 mice of each sex were given the test substance daily in 0.5% gelatin by oral gavage at 46.4 mg/kg body weight beginning at 7 days of age and continuing until they reached 28 days of age. At that time, 121 ppm of silvex was administered daily in the diet. This study was carried out for approximately 18 months. Mice were housed by sex, up to six in a cage, and were given food and water ad libitum. All animals were observed daily for clinical signs and weighed weekly. The doses administered were the maximum tolerated doses, which had been selected from pre-chronic toxicity studies performed before the initiation of chronic study. The moribund mice were killed, necropsied, and selectively examined microscopically, while surviving animals were killed at approximately 18 months and necropsied. Heart, lungs, liver, spleen, kidneys, adrenals, stomach, intestines, genital organs, and tissue masses were placed in formalin. They were later sectioned, stained with hematoxylin and eosin, and examined microscopically. All but five mice, three B6C3F1 male and two B6AKF1 male or female, survived 18 months. Table 13 identifies the types of tumors and the groups in which they were found.

TABLE 13. TUMORS IN MICE EXPOSED ORALLY TO SILVEX

Type of Tumor	B6C3F1 Mice		B6AKF1 Mice	
	M	F	M	F
Reticulum-cell sarcoma, type A	1	1	0	0
Pulmonary adenoma	1	0	1	0
Hepatoma	5	0	0	0
Mammary adenocarcinoma	0	1	0	0
Angioma	1	0	0	0
Gastric papilloma	0	2	0	0
Adrenal cortical adenoma	0	0	0	1

There was no significant increase in the incidence of neoplasms in B6C3F1 or B6AKF1 male or female mice administered silvex orally. However, by current National Cancer Institute guidelines, there are a number of deficiencies in these studies: 1) only a single dose level was administered, 2) the number of animals in the treatment group (18) was too small, and 3) the experiment was terminated after only about 18 months. Because of these deficiencies, the test was relatively insensitive for detecting a possible oncogenic effect of silvex and therefore cannot be considered as significant evidence of the non-carcinogenicity of silvex.

Innes et al. (Bionetics Laboratories) (Subcutaneous) Mouse Study (1968)

In this study, groups of 18 B6C3F1 and 18 B6AKF1 mice of each sex, approximately 28 days of age, were given a single subcutaneous injection of 215 mg/kg body weight of 2-(2,4,5-trichlorophenoxy)propionic acid (silvex, supplied by M-C-B) suspended in demethylsulfoxide (DMSO), and observed for 18 months.

Procedures similar to those described above for the Bionetics oral study were followed for animal housing, care, observation, necropsies, selection of tissues, and preparation of histologic slides. All animals, except two B6C3F1 male mice, survived 18 months. Seven tumors were diagnosed in the 18 male B6C3F1 mice examined. The tumors were: two reticulum-cell sarcomas, type A; two pulmonary adenomas; one hepatoma; and two hemangiomas. Only one tumor, a gastric papilloma, was diagnosed in the 18 female B6C3F1 mice examined. A hepatoma was found in one of the male B6AKF1 mice examined. These incidences were comparable to those seen in the control animals. There was no significant increase in the incidence of neoplasms in B6C3F1 or B6AKF1 male or female mice by subcutaneous injection. However, there were a number of deficiencies in this study: 1) only one subcutaneous injection was given, 2) the number of animals in the treatment group (18) was too small, and 3) the experiment was terminated after only 18 months. Because of these deficiencies, the test was relatively insensitive for detecting an oncogenic effect of silvex.

Dow Chemical Company (Oral) Rat Study (1965), summarized in Mullison (1966) and Gehring and Betso (1978).

Groups of Dow-Wistar rats (30 males and 30 females in each group) were fed diets containing 0.0, 0.03, 0.003, and 0.001% Kurosol SL (potassium salt of silvex) for up to 24 months. Administration of the test compound began at 50 days of age. Animals were sacrificed at 12 and 18 months so that the group sizes at the end of the 2-year study could not have been more than 21 or 22 per sex; they may have been even smaller. However, the size of the groups at the end of the study cannot be exactly determined since no data were provided on the extent to which animals, other than the ones sacrificed, died before the end of the study.

There was no evidence of a toxic effect or reduced survival in female rats administered any dose compared to controls. Therefore, it does not appear that the females were administered the maximum tolerated dose. Since high-dose males exhibited a significant decrease in average body weights, it appears that they were administered an maximum tolerated dose.

No significant increase in tumors was reported. However, because small groups of animals were used and the maximum tolerated dose was apparently not used in high-dose females, this study cannot be considered as significant evidence of the non-carcinogenicity of silvex in rats.

Dow Chemical Company (Oral) Dog Study (1965), summarized in Mullison (1966) and Gehring and Betso (1978).

Groups of beagle dogs (four males and four females in each group) were fed diets containing 0.0, 0.056, 0.019, and 0.0056% Kurosol SL (potassium salt of silvex). One male and one female of each group were killed at 12 months. The remaining animals were killed at the end of the 2-year period.

No incidence of tumors was observed. However, this study does not constitute a valid cancer study because its duration was far less than life expectancy of a beagle dog and the sizes of the animal groups were exceedingly small. Therefore, this study provides no evidence of the lack of carcinogenicity of silvex in dogs.

CARCINOGENICITY OF TCDD IN RATS AND MICE

Kociba et al. (Oral) Rat Study (1977, 1978)

Although this study was reported in published form in *Toxicology and Applied Pharmacology* (1978), a fuller version was submitted in an unpublished report (Kociba et al., Dow Chemical Company, September 28, 1977).

In this study, groups of 50 Sprague-Dawley rats (Spartan) of each sex were maintained for up to 2 years on diets providing 0.1, 0.01, or 0.001 ug/kg/day TCDD. Vehicle control groups comprised 86 animals of each sex. The test was appropriately conducted with the high dose group at a level which induced signs of tissue toxicity, reduced weight increments in both sexes, and shortened lifespan in female rats. Clinical tests performed at intervals during the study monitored organ specific toxicity, particularly of the liver. Pathologic examinations included histopathologic evaluation of all major tissues in high-dose control animals, but only of selected tissues identified as possible target organs and suspect tumors in lower dose groups. This approach is suitable for the identification of a carcinogenic effect, but does not determine actual tumor incidences in all groups except in those organs identified as target organs. It, therefore, is adequate to define dose-response relationships only in these target organs. Tissues examined from most animals in all dose groups included liver, lungs, kidneys, urinary bladder, tongue, brain, testes/ovaries, and prostate/uterus. For these tissues, a quantitative analysis can be performed using the actual numbers of tissues examined histopathologically for animals at risk. For other tissues (excluding skin, mammary glands, and nasal turbinates/hard palate), actual tumor incidence cannot be evaluated for the two lower doses. For skin, mammary glands, and nasal turbinates/hard palate, the number of animals necropsied is the appropriate denominator to determine incidence, because detection of these tumors is based on observation of the tumor at necropsy.

A laboratory audit of this study by Spencer and Woodrow, Hazard Evaluation Division, Office of Pesticide Programs, did not reveal significant new information. Reviewers concluded that the study was properly conducted, adhering to the accepted procedures (memorandum from Spencer and Woodrow to Diana Reisa, and Warnick Project Manager for 2,4,5-T).

Based on data reported for food consumption, body weight, and dietary level of TCDD, the daily doses were reasonably constant for most of the study, although somewhat below the value expected in most groups during the third month.

High early mortality was observed in all groups in this study. The survival curves show progressive mortality beginning as early as the 12th month and leading to 50% mortality by 21 months.* The effects of this early mortality are a reduction in expected tumor incidence because of a truncated latency period, and a reduction in sensitivity of the study because of a reduction in number of animals at risk during the time of expected tumor manifestation. Cumulative mortality and interval mortality rates are given in Tables III-7-10 of the appendix (Clement Associates 1979).

The results of this study provide substantial evidence that TCDD is carcinogenic in rats. TCDD induced highly statistically significant increases of hepatocellular carcinomas and hepatocellular neoplastic nodules combined in female rats at doses of 0.1 and 0.01 ug/kg/day (2200 and 220 ppt in the diet, respectively). The increase of hepatocellular carcinomas, alone, in the high-dose females was also highly significant. In addition, at the highest dose level, TCDD induced a statistically significant increase in stratified

*In the 0.001 group of males, 44% mortality was as early as 18 months. The mortality patterns were analyzed by the Whitney-Wilcoxon test and Kolmogorov-Simonov test. These tests show that mortality was significantly higher in the high-dose females than in controls, and while indications of increased mortality were found in other groups, they were not part of a consistent pattern.

squamous cell carcinomas of the hard palate and/or nasal turbinates in both males and females, squamous cell carcinomas of the tongue in males, and keratinizing squamous cell carcinomas of the lungs (highly significant) in females (Tables 14-16).

TABLE 14. HEPATOCELLULAR CARCINOMAS AND HEPATOCELLULAR HYPERPLASTIC NODULES IN FEMALE SPRAGUE-DAWLEY RATS MAINTAINED ON DIETS CONTAINING TCDD

Dose level ug/kg/day	Rats with hepatocellular hyperplastic nodules	Rats with hepatocellular carcinomas	Total number of rats with both types of tumors
0	8/86 (9%)	1/86 (1%)	9/86 (10%)
0.001 (22 ppt)	3/50 (6%)	0/50 (0%)	3/50 (6%)
0.01 (210 ppt)	18/50 (36%)	2/50 (4%)	20/50 (40%) (P = 7.6×10^{-5}) ^a
0.1 (2200 ppt)	23/49 (47%)	11/49 (22%) (P = 5.6×10^{-5}) ^a	34/50 (68%) (P = 4.56×10^{-12}) ^a

^aP-values calculated according to the Fisher Exact Test (one-tailed).

TABLE 15. TUMOR INCIDENCE IN FEMALE RATS FED DIET CONTAINING TCDD

Dose level ug/kg/day	Stratified squamous cell carcinomas of hard palate or nasal turbinate	Keratinizing squamous cell carcinomas of lungs
0	1/54 (2%)	0/86 (0%)
0.001 (22 ppt)	0/30 (0%)	0/50 (0%)
0.01 (210 ppt)	1/27 (4%)	0/50 (0%)
0.1 (2200 ppt)	5/24 (21%) (P = 0.01) ^a	7/49 (14%) (P = 0.0006) ^a

^aP-values calculated according to the Fisher Exact Test (one-tailed).

TABLE 16. TUMOR INCIDENCE IN MALE RATS FED DIET CONTAINING TCDD
ALL COMPARISONS VS. CONTROLS

Dose level ug/kg/day	Stratified squamous cell carcinomas of the tongue		Hard palate/nasal turbinates stratified squamous cell carcinoma ^a	
0	0/85		0/51	
0.001 (22 ppt)	1/50	N.S. ^b	1/34	N.S.
0.01 (210 ppt)	1/50	N.S.	0/27	N.S.
0.1 (2200 ppt)	3/50	(P = 0.048)	4/30	(P = 0.016)

^aInclude examinations from both original and updated report (5/20/79).

^bNot significant at P = 0.05.

Van Miller et al. (Oral) Rat Study (1977)

Male Sprague-Dawley rats weighing approximately 60 grams each were used. There were 2 rats in each cage and 10 rats in each group. Rats ingested ground chow for only 2 weeks. They were then given 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the following concentrations: 0, 1, 5, 50, 500 parts per trillion (ppt, 10^{-12} gram TCDD/gram food); 1, 5, 50, 500, and 1000 parts per billion (ppb, 10^{-9} gram TCDD/gram-food).

Laparotomies were performed on all surviving rats at the 65th week. Biopsies were taken from all tumors observed. Rats ingested the diets with TCDD for 78 weeks, and were kept on a control diet. Surviving rats were killed at 95 weeks.

Food intake was significantly lower in rats ingesting 50, 500, or 1000 ppb TCDD than in the controls, and they lost weight. All of the rats in the dose groups died between the second and fourth weeks of treatment. The food intake for rats receiving the other dose levels was similar to that of the controls. Weight gain was significantly less for rats given 5 ppb TCDD. TCDD intake and mortality of rats are shown in Table 17.

TABLE 17. TCDD INTAKE AND MORTALITY IN RATS

Dose ^a	Weekly dose per rat (ug/kg body weight)	Week of first death	Number of rats dead at 95th week
0 ppt	----	68	6/10 (60%)
1 ppt	0.0003	86	2/10 (20%)
5 ppt	0.001	33	4/10 (40%)
50 ppt	0.01	69	4/10 (40%)
500 ppt	0.1	17	5/10 (50%)
1 ppb	0.4	31	10/10 (100%)
5 ppb	2.0	31	10/10 (100%)

^a Rats at 50, 500, and 1000 ppb dose levels were all dead within four weeks.

Complete necropsies were done and samples of tissues were taken for microscopic examination in controls and each treatment group (Laboratory audit and personal communication with author). Special staining methods were used as an aid in the diagnosis of neoplasms. Various benign and malignant tumors were found in each treatment group. No tumors were observed in the controls (Table 18).

Statistically significant increases of squamous cell tumors of the lungs and neoplastic nodules of the liver were observed in rats ingesting 5 ppb TCDD (See Table 19). In addition, two animals in the 5 ppb dose group and one animal in the 1 ppb dose group had liver cholangiocarcinomas, which are rare in Sprague-Dawley rats. These tumors were not found in any of the lower dose groups or in the controls. These results provide evidence of a carcinogenic effect.*

The observation of no tumors of any kind in the controls is unusual for Sprague-Dawley rats. In addition, the reporting of the study was not extensive. These factors may tend to lessen the reliance which can be placed on the positive results of this study. However, this study does provide independent confirmation of the findings of the Kociba study that TCDD causes observable carcinogenic effects in rats at very low doses.

*The audit of this study brought out the fact that it was intended only to be a rangefinding study. Therefore, only a small number of animals was used. This may have made the study relatively insensitive for detecting carcinogenic effects at doses lower than 1 ppb.

TABLE 18. BENIGN AND MALIGNANT TUMORS IN RATS INGESTING TCDD

Dose ^a	Benign	Malignant	Number of tumors	Number of rats with tumors
0	0	0	0	0/10 (0%) ^b
1 ppt	0	0	0	0/10 (0%)
5 ppt	1	5	6 ^c	5/10 (50%) ^d
40 ppt	2	1	3 ^e	3/10 (30%)
400 ppt	2	2	4 ^f	4/10 (40%) ^g
1 ppb	0	4	5 ^h	4/10 (40%)
5 ppb	8	2	10 ⁱ	7/10 (70%)

^aRats at dose levels 50, 500, and 1000 ppb were all dead within four weeks.

^b40 male rats used as controls for another study, received at the same time and kept under identical conditions, did not have neoplasms when killed at 18 months.

^c1 rat had ear duct carcinoma and lymphocytic leukemia
 1 adenocarcinoma (kidney)
 1 malignant histiocytoma (retroperitoneal)
 1 angiosarcoma (skin)
 1 Leydig cell adenoma (testis)

^dThree rats died with aplastic anemia.

^e1 fibrosarcoma (muscle)
 1 squamous cell tumor (skin)
 1 astrocytoma (brain)

^f1 fibroma (striated muscle)
 1 carcinoma (skin)
 1 adenocarcinoma (kidney)
 1 sclerosing seminoma (testis)

^gOne rat had a severe liver infarction.

^h1 rat cholangiocarcinoma and malignant histiocytomas (retroperitoneal)
 1 angiosarcoma (skin)
 1 glioblastoma (brain)
 1 malignant histiocytoma (retroperitoneal)

ⁱ1 rat had squamous cell tumor (lung) and neoplastic nodule (liver)
 2 rats cholangiocarcinoma and neoplastic nodule (liver)
 3 squamous cell tumors (lung)
 1 neoplastic nodule

TABLE 19. LIVER TUMORS IN RATS INGESTING TCDD

Dose(ppb)	Neoplastic nodules	Cholangio-carcinomas	Squamous cell tumors of the lungs
0	0/10 (0%)	0/10 (0%)	0/10
1	0/10 (0%)	1/10 (10%)	0/10
5	4/10 (20%) P = 0.043	2/10 (20%) ^a	4/10 (40%) P = 0.043

^a The two animals had both neoplastic nodules of the liver and cholangiocarcinomas.

Toth et al. (Oral) Mouse Study (1979)

This study investigated the carcinogenicity of TCDD in Swiss mice. Ten-week-old outbred Swiss /H/Riop mice were used. TCDD was administered in a sunflower oil vehicle by gavage to groups of 45 male mice once a week at doses of 7.0, 0.7, and 0.007 ug/kg body weight for a year (groups 9, 10, and 11, respectively, in Table 20). Matched male vehicle controls were administered sunflower oil once a week. Matched controls to a companion study investigating the carcinogenicity of (2,4,5-trichlorophenoxy)ethanol (TCPE) contaminated with low levels of TCDD, were administered carboxymethylcellulose (the vehicle used in that study) once a week. Two untreated controls were also maintained.

This study appears to be generally well-conducted. However, the administration of TCDD over a period of only one year, which is far short of the life expectancy of the mice used, made the study relatively insensitive. Animals were followed for the entire period of their lifespans. Autopsies were performed after spontaneous death or when the mice were moribund, and all organs were examined histologically. Sections were stained with hematoxylin and eosin for light microscopy. Pathological findings were evaluated and analyzed statistically. The findings of the TCDD study and the comparison study on TCPE are given in Table 20 reproduced from the journal in which this study is reported.

Analysis of the results of this study focused on the incidence of liver tumors in the groups treated with TCDD and the incidence of these tumors in the matched controls (group 12) and in the males in the three other control groups. Males in groups 3 and 8, the two untreated control groups, had 26% and 33% liver tumors, respectively ($P > 0.20$). The carboxymethylcellulose male controls (group 7) had 33% (32/96) liver tumors. No significant differences in liver

tumors were observed when males in all four control groups were compared to each other by $P > 0.05$. Nevertheless, there was evidence that the incidence of liver tumors in the control groups was associated with the average lifespan in the respective groups. The two groups that had less than 600 days average survival (groups 3 and 12) had the fewest liver tumors (26% and 18%, respectively). On the other hand, the two groups that had an average survival of greater than 600 days (groups 7 and 8), had 33% liver tumors each. The test for linear trend (tumors vs. days of average survival) was not quite significant ($P = 0.065$).

Among the three treatment groups (groups 9, 10, and 11), the middle dose (0.7 ug/kg) showed the highest incidence of liver tumors (21/48 = 48%). This incidence was significantly higher than the incidence of liver tumors in either the sunflower oil controls ($P < 0.01$) or the pooled controls (all four control groups combined) ($P < 0.025$).

The highest dose group (7.0 ug/kg) had an increased incidence of liver tumors compared to the matched sunflower oil controls (13/43 = 30%) but this increase was not statistically significant ($P = 0.11$). The incidence of liver tumors in the high-dose group was comparable to that of the pooled controls. The highest dose group, however, had a much reduced average survival in comparison to any of the control groups (only 424 days compared to 577, 588, 615, and 651 days in the four control groups). This poor survival may have accounted for the lack of a statistically significant increase in liver tumors in the high dose group. Furthermore, if time-to-tumor data had been available, it is highly likely that the high-dose group would have shown a significant decrease in time-to-tumor compared to the controls. Therefore, the increase in

liver tumors that was observed in the high-dose group in comparison to the matched control group, although not statistically significant, is considered to be consistent with an oncogenic effect.

In conclusion, the results of this study provide suggestive evidence of an oncogenic effect.

TABLE 20. CUMULATIVE DATA ON TUMOUR INCIDENCE
(Taken from Toth et al. 1979)

Group	Treatment		Sex	Effective no. of mice	No. of tumour bearing mice	Animals with tumours of				Average life-span (d)	
	TCPE (mg/kg)	TCDD (ug/kg)				Vehicle ^a (mg/kg)	liver no. (%)	lung no.	lymphomas no.		other organs no.
1	67.0	0.112 (1.6 ppm)	50	M	88	69	42 ^b (18)	50	7	16	595
				F	83	61	7 (8)	52	15	25	652
2	70.0	0.007 (0.1 ppm)	50	M	98	78	57 ^c (58)	18	11	16	571
				F	96	59	9 (9)	39	15	23	582
3		control	50	M	93	63	24 (26)	44	8	17	577
				F	84	67	4 (5)	41	23	13	639
4	7.0	0.07 (10 ppm)	50	M	93	79	25 (27)	38	18	22	641
				F	96	60	10 (10)	38	19	19	589
5	7.0	0.0007 (0.1 ppm)	50	M	94	77	23 (24)	50	23	17	660
				F	93	71	8 (9)	42	36	21	590
6	0.7	0.00007 (0.1 ppm)	50	M	97	78	24 (25)	51	20	17	643
				F	94	64	5 (5)	38	22	21	566
7	--	--	50	M	96	74	32 (33)	44	14	22	615
				F	84	55	4 (5)	38	18	17	565
8		control	50	M	96	78	32 (33)	38	22	15	651
				F	91	57	4 (4)	31	24	19	549
9		7.0	10	M	43	27	13 (30)	11	6	7	424
10		0.7	10	M	44	36	21 (48)	18	12	4	633
11		0.007	10	M	44	39	13 (29)	27	10	6	649
12	--	--	10	M	38	27	7 (18)	15	6	7	588

^aCarboxymethylcellulose in groups 1-8, sunflower oil in groups 9-12

^bp < 1%

^cp < 0.1%

ESTIMATION OF TCDD LEVELS IN 2,4,5-T STUDIES

As discussed above, all of the 2,4,5-T studies which either did not demonstrate an oncogenic effect or had ambiguous results (i.e., all 2,4,5-T studies except the one conducted by Kociba), failed to provide significant evidence of either the oncogenicity or lack of oncogenicity of 2,4,5-T because of deficiencies in their design. Nevertheless, it is useful to calculate the highest doses of TCDD which were administered in these studies as a contaminant of 2,4,5-T and compare these doses to the doses of TCDD which induced an oncogenic response in the TCDD studies. The calculated doses are based on the reported contamination levels of the 2,4,5-T used in the studies and the dose of 2,4,5-T administered. Tables 21 and 22 show that the doses of TCDD administered as a contaminant in each of the 2,4,5-T studies, with the possible exception of the Bionetics oral mouse study, were below those doses which produced an observable oncogenic response in the TCDD studies on the same species. Thus, especially in view of the deficiencies and insensitivity of the 2,4,5-T studies, it is not surprising that the TCDD contamination did not induce an observable oncogenic effect. In the Bionetics oral mouse study, if it is assumed that the 2,4,5-T administered was contaminated with 30 ppm TCDD, then the TCDD dose was 0.27 ug/kg/day. This is higher than the dose which induced an oncogenic response in the Toth mouse study (0.07 ug/kg/day) and which apparently induced an oncogenic effect according to the preliminary results of the on-going NCI oral mouse study (0.19 ug/kg/day, or possibly lower). The absence of an oncogenic effect in the Bionetics study may be explained by several factors. First, the study was much more insensitive than either the Toth or NCI studies because the group sizes were much smaller. Second, different strains or substrains of mice were used. Third, as explained earlier, the TCDD contamination may not have been as high as 30 ppm.

TABLE 21. COMPARISON OF DOSE LEVELS OF TCDD IN 2,4,5-T^a STUDIES WITH RESPECT TO THE TCDD STUDY IN MICE WHERE POSITIVE TUMOR INCIDENCE WAS OBSERVED

Study	Strain of Mouse	Route	Dose-level		Tumors observed
			2,4,5-T mg/kg/day	TCDD ug/kg/day	
Bionetics (Innes)	F ₁ hybrid of C57Bl/6 and C3H/AWf (Strain "A") or "X"	diet	9	0.27	-
	F ₁ hybrid of C57Bl/6 and AKR (Strain "Y" or "B")	diet	9	0.27	-
Muranyi- Kovacs	XVIIG	diet	12	6.0 x 10 ⁻⁴	-
	C3Hf	diet	12	6.0 x 10 ⁻⁴	-
NCI (in progress)	86C3F1 Male ^b	gavage	--	9.6 x 10 ⁻⁴	+
				4.8 x 10 ⁻³	
				4.8 x 10 ⁻²	
86C3F1 Female ^b	gavage	--	3.8 x 10 ⁻³	+	
			1.9 x 10 ⁻²		
			1.9 x 10 ⁻¹		
Toth	Swiss male	gavage	--	1.0	+
				0.1	+
				.001	
Bionetics (Innes)	"A or Y"	subcutaneous	215 mg/kg (one dose only)	6.4 (one dose only)	-
	"Y or B"		--	--	-
Muranyi- Kovacs	XVIIG ₁	subcutaneous	10(4 doses only)	5 x 10 ⁻⁴ (4 doses only)	-
	C3Hf		10(4 doses only)	5 x 10 ⁻⁴ (4 doses only)	-

^aTCDD contaminant in 2,4,5-T
 30 ppm--Innes et al. Study (assumed in this analysis, see pages 34-35)
 0.05 ppm--Muranyi-Kovacs et al. Study
 0.05 ppm--Leuschner et al. (German Study)
 0.33 ppb--Dow Chemical Company Study

^bCarcinogenic in male and/or female.

TABLE 22. COMPARISON OF DOSE LEVELS OF TCDD IN 2,4,5-T STUDIES WITH RESPECT TO THE TCDD STUDY IN RATS WHERE POSITIVE TUMOR INCIDENCE WAS OBSERVED

Study	Strain	Route	Dose-level		Tumor Observed
			2,4,5-T mg/kg/day	TCDD ug/kg/day	
Kociba 2,4,5-T ^a	Sprague-Dawley (Spartan)	diet	3	1.0×10^{-6}	-
			10	3.3×10^{-6}	-
			30	9.9×10^{-6}	±
Leuschner 2-4-5-T ^b	Sprague-Dawley (SIV50)	"	3	1.5×10^{-4}	-
			10	5×10^{-4}	-
			30	1.5×10^{-3}	?
Kociba TCDD	Sprague-Dawley (Spartan)	"	--	1×10^{-3}	-
			--	1×10^{-2}	+
			--	1×10^{-1}	+
NCI TCDD	Osborne-Mendel	gavage	--	8.0×10^{-4}	
			--	4.0×10^{-3}	
			--	4.1×10^{-2}	+
Van Miller TCDD ^c	Sprague-Dawley	diet	--	5.0×10^{-2}	+ (?)
			--	2.50×10^{-1}	+ (?)

^aMarginally positive response ($P = 0.063$) for carcinoma of tongue at the 30 mg/kg/day group. Although no detectable TCDD was present, it is assumed for this analysis that TCDD is present at the level of detection (0.33 ppb).

^bSignificance of increase of interstitial cell tumors of testes at the 30 mg/kg/day group is unclear because of great disparity in incidences in two different control groups.

^cCertain aspects of this study tend to lessen the reliance which may be placed on its results.

Potency of TCDD

The carcinogenic potency of TCDD is greater than that of aflatoxin B₁, which is one of the most potent carcinogens known. This conclusion comes from a comparison of the tumor incidence in male Fischer rats (Wogan et al. 1974), which were fed 1 ppb of aflatoxin B₁, with the incidence of the same tumor type in female Sprague-Dawley rats (Kociba et al. 1977) fed 0.01 ug/kg/day (0.21 ppb). The potency of each of these compounds was estimated by calculating the slope of the linear one-hit model for these compounds. The slope (B) is calculated according to the following formula:

$$B = \frac{1}{d} \ln \frac{1 - P_c}{1 - P_t}$$

d = dose inducing carcinogenic effect in the respective studies on TCDD and aflatoxin.

P_c = tumor incidence in control animals in the respective studies.

P_t = tumor incidence in treated animals in the respective studies at dose d.

The specific type of tumor whose incidence provides the greatest "B" value was selected for calculating B. On this basis, in both the TCDD and aflatoxin studies, hepatocellular carcinomas and hepatocellular neoplastic nodules (combined) were selected for calculating B.

Table 25 shows that TCDD is more potent than aflatoxin by a factor of $1.906/0.394 = 4.84$. On this basis, it is estimated that TCDD is a more potent carcinogen than aflatoxin B₁ roughly by a factor of five.

TABLE 23. COMPARISON OF CARCINOGENIC POTENCY OF TCDD WITH AFLATOXIN

	<u>TCDD</u>	<u>Aflatoxin</u>
Author	Kociba et al.	Wogan et al.
Species	Sprague-Dawley rats	Fischer rats
Sex	Female	Male
Tumor incidence in controls (Pc)	9/86	1/18
Dose (d) Tumor incidence in treated animals (Pt)	0.21 ppb, 20/50	1 ppb, 8/22
Carcinogenic Potency (8)	1.906 (ppb) ⁻¹	0.394 (ppb) ⁻¹

SUMMARY OF LABORATORY ANIMAL STUDIES ON 2,4,5-T, SILVEX, AND TCDD

There is suggestive evidence that 2,4,5-T is carcinogenic in rats. The chronic mouse studies on 2,4,5-T suffered from deficiencies in design or conduct which made them insensitive for detecting an oncogenic response. Therefore, these studies do not provide significant evidence of either the carcinogenicity or non-carcinogenicity of 2,4,5-T in mice.

All the chronic animal studies on silvex suffered from deficiencies in design or conduct which made them insensitive for the purpose of detecting an oncogenic effect. Therefore, these studies do not provide significant evidence of either the carcinogenicity or non-carcinogenicity of silvex.

Studies on rats provide substantial evidence that TCDD is carcinogenic in rats. In addition, there is highly suggestive evidence that TCDD is oncogenic in mice. On the basis of the best conducted rat study (Kociba, et al. 1977, 1978), it appears that TCDD is of the most potent carcinogen known.

VIII. REFERENCES

- Allen, J.R., D.A. Barsotti, J.P. Van Miller, L.J. Abrahamson, and J.J. Lalich. 1977. Morphological changes in monkeys consuming a diet containing 500 ppt of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Food and Cosmetic Toxicology* 15:401-410.
- Anderson, D. and J. Styles. 1978. The bacterial mutation test. *British Journal of Cancer* 37:924-930.
- Bauer, H., K.H. Schulz, and V. Spiegelberg. 1961. Occupational intoxication in the production of chlorinated phenol compounds. *Archives for Industrial Hygiene* 18:538-555.
- Bionetics Research Laboratories, Inc. 1968. Evaluation of carcinogenic, teratogenic, and mutagenic activities of selected pesticides and industrial chemicals. I. Carcinogenic study. Prepared for the National Cancer Institute.
- Bleiberg, J.M. Wallen, R. Brodtkin, and I Applebaum. 1964. Industrially acquired porphyria. *Archives for Dermatology* 89:793-97.
- Carter, C.D., R.D. Kimbrough, J.A. Liddle, R.E. Cline, M.M. Zack, W.R. Barthel, R.E. Koehler, and P.E. Philips. 1975. Tetrachlorodibenzodioxin: an accidental poisoning episode in horse arenas. *Science* 188: 738-740.
- Clement Associates. 5/15/79. Exposure, toxicity, and risk assessment of 2,4,5-T/TCDD. Prepared for EPA under contract no. 68-01-5095.
- Department of Health, Education, and Welfare - Subcommittee on Environmental Mutagenesis. Meeting report October 12, 1976.
- Dow Chemical Company. 1971. Internal report submitted to the EPA's Advisory Committee.
- Ercegovich, C.D., and K.A. Rashid. 1977. Mutagenesis induced in mutant strains of Salmonella typhimurium by pesticides. 174th American Chemical Society National Meeting. Division of Pesticide Chemistry 43.
- Fujita, K., H. Fujita, and Z. Funazaki. 1975. Chromospheric abnormalities brought about by the use of 2,4,5-T. *J. Jpn Assoc. Rural Med.* 24:77-79.
- Gehring, P.J., Kramer, C.G., Schwetz, B.A., Rose, J.Q. and Rowe, V.K. (1973). The fate of 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) following oral administration to man. *Toxicol Appl. Pharmacol.* 26:352.
- Goldman, P.J. 1972. Extremely severe acute chloracne due to trichlorophenol decomposition products. *Industrial Medicine, Social Medicine, Industrial Hygiene.* 7:12-18.

- Goldstein, J.A., P. Hickman, H. Bergman, and J. Vos. 1973. Hepatic porphyria induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin in the mouse. *Research Communications in Chem. Path. and Pharmacol.* 6(3):919-928.
- Goldstein, J.A., P. Hickman, H. Bergman, J.D. McKinney, and M.P. Walker. 1977. Separation of pure polychlorinated biphenyl isomers into two types of inducers on the basis of induction of cytochrome P-450 or P-448. *Chemical Biological Interaction* 17:69-87.
- Grant, W. 1979. The genotoxic effects of 2,4,5-T. *Mutation Research* 65:83-119
- Green, S. March 9-13, 1975. "Cytogenetic evaluation of several dioxins in the rat." 14th Annual Meeting Society of Toxicology.
- Green, S., F. Moreland, and C. Sheu. May 1977. Cytogenetic effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on rat bone marrow cells. *FDA By-Lines.* No. 6:292-294.
- Gupta, B.N., J.G. Vos, J.A. Moore, J.G. Zinkl, and B.C. Bullock. 1973. Pathological effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in laboratory animals. *Env. Health Perspect.* 5:125-140.
- Hay, A. 1976. Seveso: the aftermath. *Nature* 263:538-540.
- Hook, G.E., J.R. Haseman, and G.W. Lucier. 1975. 2,3,7,8-Tetrachlorodibenzo-p-dioxin, induced changes in the hydroxylation of biphenyl by rat liver microsomes. *Pharmacol.* 24:335-340.
- Hussian, S., L. Ehrenberg, G. Lofroth, and T. Gejvall. 1972. Mutagenic effects of TCDD on bacterial systems. *Ambio.* 1:32-33.
- Innes, J.R.M., B.M. Ulland, Valerio, Marion G., L. Petrocelli, L. Fishbein, E.R. Hart, A.J. Pallota, R.R. Bates, A.L. Falk, J.J. Gart, M. Klein, I. Mitchell, and J. Peters. 1969. Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: A preliminary note. *J. Nat'l Cancer Institute* 42:1101-1114.
- Jenssen, D., and L. Renberg. 1976. Distribution and cytogenetic test of 2,4-D and 2,4,5-T phenoxyacetic acids in mouse blood tissues. *Chem. Biol. Interactions* 14:291-299.
- Jones, G., and J.B. Greig. 1975. Pathological changes in the liver of mice given 2,3,7,8-tetrachlorodibenzo-p-dioxin experimentally. *Basel.* 1(11):1315-1317.
- Khera, K., and J. Ruddick. 1973. Polychlorodibenzo-p-dioxins: perinatal effects and the dominant lethal test in Wistar rats. *Advances in Chemistry Series* 120:70-84.

- Kilian, D., M. Benge, R. Johnston, and E. Whorton, Jr. March 28-29, 1975. Cytogenetic studies of personnel who manufacture 2,4,5-T. New York Academy of sciences workshop on occupational monitoring and genetic hazards.
- Kimmig, T. and K.H. Schulz. 1957. Chlorierte aromatische zyklische Aether als Ursache der sogenannten Chlorakne. Kurze Originale Mitteilungen. 11:337-338.
- Kociba, R.J., P.A. Keller, C.N. Park, and P.J. Gehring. 1975. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD): Results of a 13-week oral toxicity study in rats. Toxicol. and Applied Pharmacol. 34(2): 001-022.
- Kociba, R.J., D.G. Keys, J.E. Beyer, R.M. Carreon, C.E. Wade, D.A. Dittenber, R.P. Kalnins, L.E. Frauson, C.N. Park, S.D. Bernard, R.A. Hummel, and C.G. Humiston. 1978^a. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats. Toxicol. and Appl. Pharmacol. 46:279-303.
- Kociba, R.J., D.G. Keyes, R.W. Lisowe, R.P. Kalnins, D.D. Dittenber, C.E. Wade, S.J. Gorzinski, N.H. Mahle, and B.A. Schwetz. 1978^b. Results of a two-year chronic toxicity and oncogenic study of rats ingesting diets containing 2,4,5-trichlorophenoxy acetic acid (2,4,5-T). A report submitted to EPA September 27, 1978. (Published 1979. *Fd. Cosmet. Toxicol.* 17:205 to 221.)
- Kouri, R.E. 1974. Aryl hydrocarbon hydroxylase induction in human lymphocyte cultures by 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Life Science* 5:1585-1595.
- Kouri, R.E. 1973. Relationship between levels of aryl hydrocarbon hydroxylase inducibility and sensitivity to chemically induced subcutaneous sarcomas in various strains of mice. *Nat'l Cancer Institute.* 50:363-368.
- Kouri, R.E. 1976. Relationship between levels of aryl hydrocarbon hydroxylase activity and susceptibility to 3-methylcholanthrene and benzo [a] pyrene-induced cancers in inbred strains of mice. *Carcinogenesis* 1:139-151.
- Leuschner, F., A. Leuschner, F. Hubscher, W. Dantenwill, and P.V. Rogulja. Apr. 9, 1979. Chronic oral toxicity of 2,4,5-T. Batch No. 503, control No. 153574 b. called for short - 2,4,5-T in Sprague-Dawley (SIV 50) rats. *Laboratorium Fur Pharmakologie und Toxikologie.*
- Magnussen, J., C. Ramel., and A. Eriksson. 1977. Mutagenic effects of chlorinated phenoxyacetic acids in *Drosophila melanogaster*. *Hereditas* 87:121-123.
- Majumdar, S., and J. Golia. 1974. Mutation test of 2,4,5-trichlorophenoxyacetic acid on *drosophila melanogaster*. *Can. J. Genet. Cytol.* 16:465-466.
- Majumdar, W. and R. Hall. 1973. Cytogenetic effects of 2,4,5-T on in vivo bone marrow cells of mangoliann gerbils. *Journal of Heredity* 64:213-216.
- May, G. 1973. Chloracne from the accidental production of tetrachlorodibenzodioxin. *Brit. J. Indus. Med.* 30:276-283.

- Muranyi-Kovacs, I., G.R. Rudali, and J. Imbert. 1976. Bioassay of 2,4,5-trichlorophenoxy acetic acid for carcinogenicity in mice. *Brit. J. Cancer.* 33:626-633.
- Muranyi-Kovacs, I., G. Rudali, and J. Imbert. 1977. Study on the carcinogenicity of 2,4,5-T in mice (meeting abstracts), Fourth Meeting of the European Association for Cancer Research held at Universite de Lyon, September 13-15, 1977. European Association for Cancer Research, Lyon, France.
- National Institute of Occupational Safety and Health. 1976. Registry of Toxic Effects of Chemical Substances.
- Nebert, D., S. Thorgeirsson, and J. Felton. 1976. Genetic differences in mutagenesis, carcinogenesis, and drug toxicity, in: F. de Serres, J. Fouts, J. Bend, and R. Philpot (Eds.), *In Vitro Metabolic Activation in Mutagenesis.*
- Oliver, R.M. 1975. Toxic effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin in laboratory workers. *Brit. J. of Indus. Med.* 32:49-53.
- Piper, W.N., J.Q. Rose, M.L. Leng, and P.J. Gehring. 1973. The fate of 2,4,5-trichlorophenoxy acetic acid following oral administration to rats and dogs. *Env. Health Perspect.* 5:241-244.
- Poland, A., D. Smith, G. Metter, and P. Possick. 1971. A healthy survey of workers in a 2,4-D and 2,4,5-T plant. *Arch. Environ. Health.* 22:316-327.
- Poland, A., and E. Glover. 1974. Comparison of 2,3,7,8-tetrachlorodibenzo-p-dioxin, a potent inducer of aryl hydrocarbon hydroxylase, with 3-methylcholanthrene. *Mol. Pharmacol.* 10:349-359.
- Poland, A., and E. Glover. 1976a. Stereospecific high affinity binding of 2,3,7,8-tetrachlorodibenzo-p-dioxin by hepatic cytosol. *J. Biol. Chem.* 51:4936-4946.
- Poland, A., and E. Glover. 1976b. 3,4,3',4'-tetrachloroazoxybenzene and azobenzene: Potent inducer of aryl hydrocarbon hydroxylase. *Science.* 194:627-630.
- Reggiani, G. Sept. 5-10, 1977. Medical problems raised by the TCDD contamination in Seveso, Italy. Presented at the 5th International Conference on Occupational Health in the Chemical Industry.
- Rose, J.Q., T.H. Ramsey, R.A. Wentzler, R.A. Hummel, and P.J. Gehring. 1976. The fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) following single or repeated oral doses to rats. *Toxicol. Appl. Pharmacol.* 36:209-226.
- Rowe, V.K., and T.A. Hymas. 1954. Summary of toxicological information on 2,4-D and 2,4,5-T type herbicides and an evaluation of the hazards to livestock associated with their use. *Am. J. Vet. Research.* 132:2165-219.

- Schwetz, B.A., J.M. Norris, G.L. Sparscho, V.K. Rowe, P.J. Gehring, J.L. Emerson, and C.G. Gerrbig. 1973. Toxicity of chlorinated dibenzo-p-dioxins. *Env. Health Perspect.* 5:87-98.
- Seiler, J. 1973. A survey on the mutagenicity of various pesticides. *Experientia* 29:622-623.
- Thigpen, J.E., R.E. Fatt, E.E. McConnell, and J.A. Moore. 1975. Increased susceptibility to bacterial infections as a sequelae of exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Infection and Immunity.* 12:119-1324.
- Tuchmann - Duplessis, H. Nov. 26, 1977. Embryo problems posed by the Seveso accident. *Le Concours Medical.* No. 44.
- Van Miller, J.P., J.J. Lalich, and J.R. Allen. 1977. Incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Chemosphere* 6(9):537-544.
- Vos, J.G., J.A. Moore, and J.G. Zinkl. 1973. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. *Environ. Health Perspec.* 5:149-162.
- Vos, J.G., J.A. Moore, and J.G. Zinkl. 1974. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on C57BL/6 mice. *Toxicol. Appl. Pharmacol.* 229-241.
- Wogen, G., S. Palialunga, and P. Newburne. 1974. Carcinogenic effects of low dietary levels of aflatoxin B₁ in rats. *Food Cosmet. Toxicol.* 12:281.
- Yefimenko, L.P. 1974. Data for assessing the Gonadotropic and mutagenic effect of the herbicide butylether 2,4,5-T. *Gigiena Truda Professional Nye Zabolovan.* 18:24-27.
- Zetterberg, G. 1978. Genetic effects of phenoxy acids on microorganisms in: C. Ramel (Ed) Chlorinated phenoxy acids and their dioxins, mode of action, health risks, and environmental effects.

TABLE III-7. CUMULATIVE MORTALITY OF MALE RATS IN STUDY OF KOCIBA ET AL.
(1977a)

Time (end of 30-day period) N=	Controls (86)	ug/kg/day TCDD		
		0.1 (50)	0.01 (50)	0.001 (50)
1-7,	0.0	0.0	0.0	2.0
8	0.0	2.0	0.0	2.0
9	0.0	4.0	0.0	2.0
10	0.0	4.0	0.0	2.0
11	2.3	4.0	0.0	2.0
12	5.8	8.0	0.0	2.0
13	7.0	12.0	0.0	2.0
14	10.5	18.0	4.0	4.0
15	12.8	18.0	14.0	14.0
16	16.3	20.0	22.0	14.0
17	18.6	28.0	28.0	24.0
18	24.4	34.0	34.0	44.0*
19	31.4	44.0	46.0	50.0
20	41.9	46.0	54.0	56.0
21	48.8	62.0	68.0	60.0
22	58.1	74.0*	76.0*	68.0
23	69.8	78.0	84.0	74.0
24	77.9	84.0	88.0	76.0
25	82.6	90.0	92.0	78.0

*Interval of greatest difference, D, in cumulative mortality curves of controls and treatment group. None of the differences were statistically significant (Kolmogorov-Smirnov test, $P > 0.05$).

TABLE III-8. CUMULATIVE MORTALITY OF FEMALE RATS IN STUDY OF KOCIBA ET AL.
(1977a)

Time (end of 30-day period) N=	Controls (86)	ug/kg/day TCDD		
		0.1 (50)	0.01 (50)	0.001 (50)
0-5	0.0	0.0	0.0	0.0
6-8	1.2	0.0	0.0	0.0
9	1.2	2.0	0.0	0.0
10	1.2	4.0	2.0	0.0
11	1.2	8.0	2.0	0.0
12	1.2	16.0	4.0	4.0
13	3.5	20.0	4.0	4.0
14	3.5	26.0	8.0	6.0
15	7.0	28.0	12.0	10.0
16	12.8	32.0	18.0	12.0
17	15.1	38.0	18.0	18.0
18	18.6	44.0	20.0	22.0
19	25.6	56.0*	30.0	34.0*
20	34.9	60.0	36.0	36.0
21	40.7	66.0	46.0*	44.0
22	58.1	82.0	60.0	52.0
23	64.0	86.0	66.0	58.0
24	70.9	88.0	72.0	66.0
25	70.9	92.0	72.0	68.0

*Interval of greatest difference, D, in cumulative mortality curves of controls and treatment group. The mortality curve for the rats fed 0.1 ug/kg/day differed significantly from that for controls (D = 30.4, P < 0.01, Kolmogorov-Smirnov test). The other two groups did not differ significantly from controls (P > 0.05).

TABLE III-9. MALES: INTERVAL MORTALITY RATES

Days	Control		0.1 ug/kg/day		0.01 ug/kg/day		0.001 ug/kg/day	
	d/1	rate	d/1	rate	d/1	rate	d/1	rate
40-30	0/86	0.000	0/50	0.000	0/50	0.000	1/50	0.020
31-210	0/86	0.000	0/50	0.000	0/50	0.000	0/49	0.000
211-240	0/86	0.000	1/50	0.020	0/50	0.000	0/49	0.000
241-270	0/86	0.000	1/49	0.020	0/50	0.000	0/49	0.000
271-300	0/86	0.000	0/48	0.000	0/50	0.000	0/49	0.000
301-330	2/86	0.023	0/48	0.000	0/50	0.000	0/49	0.000
331-360	3/84	0.036	2/48	0.042	0/50	0.000	0/49	0.000
391-420	3/80	0.038	3/44	0.068	2/50	0.040	1/49	0.020
421-450	2/77	0.026	0/41	0.000	5/48	0.104	5/48	0.104
451-480	3/75	0.040	1/41	0.024	4/43	0.093	0/43	0.000
481-510	2/72	0.028	4/40	0.100	3/39	0.077	5/43	0.116

(Continued on following page)

TABLE III-9 (continued)

Days	Control		0.1 ug/kg/day		0.01 ug/kg/day		0.001 ug/kg/day	
	d/1	rate	d/1	rate	d/1	rate	d/1	rate
511-540	5/70	0.071	3/36	0.083	3/36	0.083	10/38	0.263
541-570	6/65	0.092	5/33	0.152	6/33	0.182	3/28	0.107
571-600	9/59	0.153	1/28	0.036	4/27	0.148	3/25	0.120
601-630	6/50	0.120	8/27	0.296	7/23	0.304	2/22	0.091
631-660	8/44	0.182	6/19	0.316	4/16	0.250	4/20	0.200
661-690	10/36	0.278	2/13	0.154	4/12	0.333	3/16	0.188
691-720	7/26	0.269	3/11	0.273	2/8	0.250	1/13	0.077
721-726	4/19	0.211	3/8	0.375	2/6	0.333	1/12	0.083

Terminal Kill 15

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 χ^2 corrected for continuity for combined interval:

421-510	7/77	vs	5/41 ($\chi^2 = 0.04$, n.s.)	12/48 ($\chi^2 = 4.63$, $p < 0.05$)	10/48 ($\chi^2 = 2.54$, n.s.)
451-540	10/72	vs	8/41 ($\chi^2 = 0.37$, n.s.)	10/43 ($\chi^2 = 1.27$, n.s.)	15/43 ($\chi^2 = 6.37$, $p < 0.025$)
481-570	13/72	vs	12/40 ($\chi^2 = 1.48$, n.s.)	12/39 ($\chi^2 = 1.67$, n.s.)	18/43 ($\chi^2 = 6.59$, $p < 0.025$)
511-600	20/70	vs	9/36 ($\chi^2 = 0.03$, n.s.)	13/36 ($\chi^2 = 0.32$, n.s.)	16/38 ($\chi^2 = 1/47$, n.s.)

TABLE III-10. FEMALES: INTERVAL MORTALITY RATES

Days	Control		0.1 ug/kg/day		0.01 ug/kg/day		0.001 ug/kg/day	
	d/1	rate	d/1	rate	d/1	rate	d/1	rate
0-150	0/86	0.000	0/50	0.000	0/50	0.000	0/50	0.000
151-180	1/86	0.012	0/50	0.000	0/50	0.000	0/50	0.000
181-240	0/85	0.000	0/50	0.000	0/50	0.000	0/50	0.000
241-270	0/85	0.000	1/50	0.020	0/50	0.000	0/50	0.000
271-300	0/85	0.000	1/49	0.020	1/50	0.020	0/50	0.000
301-330	0/85	0.000	2/48	0.042	0/49	0.000	0/50	0.000
331-360	0/85	0.000	4/46	0.087	1/49	0.020	2/50	0.040
361-390	2/85	0.024	2/42	0.048	0/48	0.000	0/48	0.000
391-420	0/83	0.000	3/40	0.075	2/48	0.042	1/48	0.021
421-450	3/83	0.036	1/37	0.027	2/46	0.044	2/47	0.043
451-480	5/80	0.063	2/36	0.056	3/44	0.068	1/45	0.022
481-510	2/75	0.027	3/34	0.088	0/41	0.000	3/44	0.068
511-540	3/73	0.041	3/31	0.097	1/41	0.024	2/41	0.049

Continued on following page

TABLE III-10. (continued)

Days	Control		0.1 ug/kg/day		0.01 ug/kg/day		0.001 ug/kg/day	
	d/1	rate	d/1	rate	d/1	rate	d/1	rate
541-570	6/70	0.086	6/28	0.214	5/40	0.125	6/39	0.154
571-600	8/64	0.125	2/22	0.091	3/35	0.086	1/33	0.030
601-630	5/56	0.089	3/20	0.150	5/32	0.156	4/32	0.125
631-660	15/51	0.294	8/17	0.471	7/27	0.259	4/28	0.143
661-690	5/36	0.139	2/9	0.222	3/20	0.150	3/24	0.125
691-720	6/31	0.194	1/7	0.143	3/17	0.177	4/21	0.191
721-726	0/25	0.000	2/6	0.333	0/14	0.000	1/17	0.059
Terminal Kill	25		4		14		16	
χ^2 corrected for continuity for combined interval:								
421-510	10/83	vs	6/37 ($\chi^2 = 0.11$, n.s.)	5/46 ($\chi^2 = 0.0$, n.s.)	6/47 ($\chi^2 = 0.0$, n.s.)			
451-540	10/80	vs	8/36 ($\chi^2 = 1.13$, n.s.)	4/44 ($\chi^2 = 0.8$, n.s.)	6/45 ($\chi^2 = 0.01$, n.s.)			
481-570	11/75	vs	12/34 ($\chi^2 = 4.80$, $p < 0.05$)	6/41 ($\chi^2 = 0.0$, n.s.)	11/44 ($\chi^2 = 1.34$, n.s.)			
510-600	17/73	vs	11/31 ($\chi^2 = 1.08$, n.s.)	9/41 ($\chi^2 = 0.0$, n.s.)	9/41 ($\chi^2 = 0.0$, n.s.)			

08/02/78	OK	1 human received medical attention	2,4-D In a home incident, an adult male developed symptoms of chest pain, vomiting and dizziness after oral and dermal exposure to an undetermined amount of 2,4-D.
08/04/78	IA	1 human affected	2,4-D (ester) 2,4,5-TP (ester) Piperonyl butoxide Pyrethrins Petroleum distillate N-Octyl bicycloheptane dicarboximide A 12-year-old boy sprayed pesticide onto his fingers; the skin became white. He washed his hands several times. The subject remained asymptomatic.
08/06/78	NM	1 human affected	2,4-D Nineteen hours after a neighbor sprayed his yard, an individual over 65 years of age developed nausea. The subject had been undergoing radiation therapy for cancer.
08/08/78	CA	2 humans received medical attention	2,4-D A male and female, 24 to 30 years of age, developed diarrhea, burning throat, abdominal swelling, cramps, muscle tenderness and stiffness of their backs and extremities after eating blackberries that had been sprayed with the chemical. They visited a physician.
08/08/78	OH	Soil contamination	2,4-D 2,4-D (amine) M CPP (amine) A 2.5-ton truck overturned at the junction of an interstate expressway and a road, demolishing the tractor and spilling 10 gallons of a 300-gallon mixture of water containing 9 pints of M CPP and 6 pints of 2,4-D - 2,4-D (amine). An EPA agency supervised decontamination by spreading cement over the contaminated area.