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FATE OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN THE ENVIRONMENT: SUMMARY AND DECONTAMINATION RECOMMENDATIONS

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Studies on the fate of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have been conducted on biodegradation plots and field test areas that have received massive quantities of Orange herbicide (a 50:50 mixture of the n-butyl esters of 2,4-dichlorophenoxyacetic acid [2,4-D] and 2,4,5-trichlorophenoxyacetic acid [2,4,5-T]). From the studies reviewed in this report, it is apparent that (1) TCDD may persist (in biotic and abiotic components) for long periods of time when initially present at extremely high concentrations on the soil surface, (2) TCDD will accumulate in the tissues of rodents, reptiles, birds, fish, and

20. Abstract (Continued)

insects when these organisms are exposed to TCDD contaminated soils (however, the levels of TCDD in the tissues apparently do not exceed the levels of TCDD found in the environment), (3) organisms tolerate, i.e., based on no observed deleterious effects, soil levels between 10-1,500 ppt TCDD, (4) TCDD is degraded by soil microorganisms, especially when in the presence of other chlorinated hydrocarbons, (5) TCDD is degraded in the presence of sunlight, (6) movement of TCDD in the abiotic portions of the environment can be by wind or water erosion of soil particles, but leaching by water alone does not appear to occur, and (7) TCDD is probably not readily released or degraded in the environment when bound to activated coconut charcoal.

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INTRODUCTION

The heterocyclic organic molecule 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) has received a great deal of attention in the last 6 years because of its highly toxic properties and the possibility of it being widespread in the environment by the use of products made from trichlorophenol, especially the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).

Although TCDD may occur as a contaminant in products made from trichlorophenol, the levels of TCDD found in any given lot of trichlorophenol is dependent upon the manufacturing process. TCDD may be produced as a by-product during an alkaline hydrolysis reaction when the temperature for making 2,4,5-trichlorophenol from tetrachlorobenzene exceeds 160°C. However, there is less likelihood of TCDD formation in the manufacturing process which starts with phenols and chlorinates them to form trichlorophenol since little or no heat is required in this reaction.

Public interest in TCDD originated in 1970 when the herbicide 2,4,5-T was implicated as a potential teratogen in pregnant rats (1). Later tests indicated that the teratogenesis may have been caused by 27 ± 8 ppm of TCDD present as a contaminant in the 2,4,5-T. As more data have been obtained (2), it has become apparent

[1] Courtney, K.D., D.W. Gaylor, M.D. Hogan, J.L. Falk, R.R. Bates, and I. Mitchell. Teratogenic evaluation of 2,4,5-T. Science 168:864-866, 1970.

[2] Schwetz, B.A., J.M. Norris, G.L. Sparschu, V.K. Rowe, P.J. Gehring, J.L. Emerson, and C.G. Gerbig. Toxicology of chlorinated dibenzo-p-dioxins. Environ. Hlth. Perspect., Experimental Issue No. 5:87-100, September 1973.

that TCDD is one of the most toxic chemicals known; the oral LD_{50} for many animal species is in the range of micrograms per kilogram. Furthermore, the known effects of TCDD include anorexia, severe weight loss, hepatotoxicity, hepatoporphyria, vascular lesions, chloracne, gastric ulcers, and teratogenicity (2). The hazard posed by the presence of even a small amount of this substance in the environment has therefore been of concern.

For a person or animal to be poisoned with TCDD, a rare set of circumstances would be required. Since present production methods are able to reduce the TCDD level to less than 0.1 ppm, it is unlikely that contaminated 2,4,5-T herbicide or even contaminated trichlorophenol would be implicated in such a poisoning. Nevertheless, two accidental poisoning episodes involving TCDD have been recently reported. In 1975, Carter et al. (3) identified TCDD as the apparent cause of an outbreak of poisoning in humans, horses, and other animals on a horse breeding farm in eastern Missouri in 1971. Exposure to TCDD followed the spraying of contaminated industrial waste oil on riding arenas for dust control. An investigation concluded that a hexachlorophene (made from trichlorophenol) factory in southwestern Missouri had accumulated distillate residues containing 306 to 356 ppm TCDD. It was this distillate residue that was subsequently disposed of via a salvage oil company and sprayed on the horse arenas.

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[3]Carter, C.D., R.D. Kimbrough, J.A. Liddle, R.E. Cline, M.M. Zack, Jr., W.F. Barthel, R.E. Koehler, and P.E. Phillips. Tetrachlorodibenzodioxin: an accidental poisoning episode in horse arenas. Science 188:738-740, 1975.

The second incident of TCDD poisoning occurred in July 1976 in Seveso, Italy (4). The source of the TCDD was a chemical factory that produced trichlorophenol through the alkaline hydrolysis of tetrachlorobenzene. When the temperature in a steam-heated reaction vessel rapidly increased, a safety disk ruptured sending a plume of trichlorophenol, TCDD and other products 30 to 50 m high above the factory. The cloud apparently rose into the air, cooled, and came down over a cone-shaped area about 2 km long and 700 m wide. An area of 110 hectares (ha) was evacuated after hundreds of animals had died and many people had reported skin disorders. Several measurements of TCDD on vegetation in an area adjacent to the factory were in the 1 to 15 ppm range, with one reading as high as 51.3 ppm. An Italian government commission (5) recommended: "removal of topsoil to a depth of 10 cm in an area of 113 ha, the dismantling of all buildings in the Seveso area, and the total disruption of all wildlife."

The need for data on the fate of TCDD in the environment is not confined to solving problems associated with the above two incidents. During the latter portion of the last decade, a program of aerial application of herbicides was conducted in Southeast Asia by the United States Air Force. In 1969, at the conclusion of this program, considerable amounts of herbicide were left unused.

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[4] Rawls, R.L., and D.A. O'Sullivan. Italy seeks answers following toxic release. Chem. Engr. News 54(35):27-35, August 23, 1976.

[5] Itay, A. Toxic cloud over Sevesco. Nature 262(5570):636-638, August 19, 1976.

One of the herbicides used extensively in this project was a herbicide designated "Orange" which was formulated as a 50:50 mixture of the n-butyl esters of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-T. In 1970, approximately 2.3 million gallons of this material was placed in storage by the Air Force. An analysis of TCDD in the Orange herbicide stocks (6) indicated that the range in concentration was 0.1 to 47 ppm TCDD. The weighted average concentration of TCDD for the 42,015 55-gallon drums of herbicide was 1.859 ppm. Because of the TCDD concentration, the herbicide could not merely be declared surplus and disposed of on the agricultural markets. Many methods have been evaluated for disposing of this material. However, regardless of the final method selected for its disposition, the storage sites where the material is currently stored (Naval Construction Battalion Center, Gulfport, Mississippi, or Johnston Island, Pacific Ocean) will need to be decontaminated.

At the request of Headquarters, Air Force Logistics Command, Wright-Patterson AFB, Ohio, in April 1972, the Department of Chemistry and Biological Sciences, United States Air Force Academy, initiated studies on herbicide Orange and TCDD. The objectives of these studies were: (1) to investigate soil incorporation/biodegradation as a disposal method for herbicide Orange; (2) to investigate the ecological effects associated with past uses of

[6]Department of the Air Force. Disposition of orange herbicide by incineration. Final Environmental Statement, November 1974, pp. 36-37.

herbicide Orange; and (3) to investigate the soil persistence and food chain accumulation of TCDD.

This report documents the available data on TCDD from these studies. Furthermore, using these data, recommendations for decontamination of an area exposed to TCDD are presented.

SOIL INCORPORATION/BIODEGRADATION STUDIES

One potential method proposed for the disposal of herbicide Orange was subsurface injection or soil incorporation of the herbicide at massive concentration rates. The premise for such studies was that high concentrations of the herbicides and TCDD would be degraded to innocuous products by the combined action of soil microorganisms and soil hydrolysis. In order to field test this concept, biodegradation plots were established in three climatically different areas of the United States; Northwest Florida (Eglin AFB), Western Kansas (Garden City), and Northwestern Utah (Air Force Logistics Command Test Range Complex). A comparison of the soils of the three sites is given in Table 1. The Utah site had a mean annual rainfall of 15 cm, while the Kansas and Florida sites had 40 and 150 cm, respectively. Table 2 describes the experimental protocol for the three sites to include when the plots were established, the method of herbicide incorporation, the experimental design and the initial calculated herbicide concentration, ppm, at the time the plots were established. Further details on the experimental protocol can be obtained from Young, Arnold and Wachinski (7).

Tables 3, 4, and 5 compare the rate of disappearance of TCDD with that of Orange herbicide for selected plots at the Utah,

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[7] Young, A.L., E.L. Arnold, and A.M. Wachinski. Field studies on the soil persistence and movement of 2,4-D, 2,4,5-T, and TCDD. Appendix G. Department of the Air Force. Disposition of orange herbicide by incineration. Final Environmental Statement, November 1974.

TABLE 1. ANALYSES OF THE TOP 15-CM LAYER FROM EACH OF THE SOIL BIODEGRADATION SITES

LOCATION	pH	ORGANIC MATTER (%)	SAND (%)	SILT (%)	CLAY (%)	SOIL DESCRIPTION
Eglin AFB, FL[a]	5.6	0.5	91.6	4.0	4.4	Sandy loam
Garden City, KS[b]	7.0	1.7	37	42	21	Silt loam
AFLC Test Range Complex, UT[c]	7.8	1.4	27	53	20	Clay loam

[a] Plots located on Test Area C-52A, Eglin AFB Reservation, Florida

[b] Plots located on the Kansas Agricultural Experiment Station, Garden City, Kansas

[c] Plots located 75 miles west of Salt Lake City, Utah

TABLE 2. DESCRIPTIONS OF THREE BIODEGRADATION STUDIES INVOLVING USE OF HERBICIDE ORANGE

LOCATION	DATE ESTABLISHED	METHOD OF INCORPORATION	TREATMENT	CALCULATED INITIAL HERBICIDE CONCENTRATION (PPM)[c]
Eglin AFB, Florida	2 Apr 1972	Simulated Subsurface Injection (30 cm band width)	4,480 kg Herbicide/ha[a]	5,000
			4,480 kg Herbicide/ha, plus soil amendments[b]	5,000
			4,480 kg Herbicide/ha plus soil amendments and activated charcoal	5,000
Garden City, Kansas	10 May 1972	Preplant Incorporate (Rototiller)	2,240 kg Herbicide/ha	1,000
			4,480 kg Herbicide/ha	2,000
AFLC Test Range Complex, Utah	2 Oct 1972	Simulated Subsurface Injection (8 cm band width)	1,120 kg Herbicide/ha	10,000
			2,240 kg Herbicide/ha	20,000
			4,480 kg Herbicide/ha	40,000

[a]Rate of herbicide calculated as active ingredient. Herbicide injected at 10-15 cm level or preplant incorporated in the 0-15 cm level. All plots duplicated.

[b]The amendments included 4.5 kg lime, 13.5 kg organic matter, and 1.4 kg fertilizer (12:4:8 for N,P,K, respectively) uniformly mixed within the top 0-30 cm of soil in the plot.

[c]Contained in the top 0-15 cm layer.

TABLE 3. CONCENTRATIONS OF HERBICIDE ORANGE AND TCDD IN PLOTS ORIGINALLY TREATED WITH 4,480 KG/HA, AFLC TEST RANGE COMPLEX, UTAH, AT VARIOUS SAMPLING DATES AFTER APPLICATION. (TCDD IN PARTS PER BILLION)

DAYS AFTER APPLICATION	TOTAL HERBICIDE[a] (PPM)	TCDD (PPMx10^{-3})
282	8,490	15.0
637	4,000	7.3
780	2,260	5.6
1,000	2,370	3.2
1,150	1,150	2.5

[a]Composite sample from replicated plots, 0-15 cm increment

TABLE 4. CONCENTRATIONS OF HERBICIDE ORANGE AND TCDD IN PLOTS ORIGINALLY TREATED WITH 4,480 KG/HA, GARDEN CITY, KANSAS, AT VARIOUS SAMPLING DATES AFTER APPLICATION. (TCDD IN PARTS PER TRILLION)

DAYS AFTER APPLICATION	TOTAL HERBICIDE[a] (PPM)	TCDD[a] (PPMx10^{-6})
8	1,950	—[b]
77	1,070	225
189	490	—[b]
362	210	—[b]
600	40	—[b]
659	<1	42

[a]Composite sampling from replicated plots, 0-15 cm increment

[b]Not determined

TABLE 5. CONCENTRATIONS OF HERBICIDE ORANGE AND TCDD IN PLOTS ORIGINALLY TREATED AT 4,480 KG/HA, EGLIN AFB, FLORIDA, AT VARIOUS SAMPLING DATES AFTER APPLICATION

DAYS AFTER APPLICATION	TOTAL HERBICIDE[a] (PPM)	TCDD[a] (PPMx10^{-6})[c]
5	4,897	375
414	1,866	250
513	824	75
707	508	46
834	438	--[b]
1,293	<10	—[b]

[a]Composite sample from the plot containing only herbicide (i.e., no lime, organic matter, or fertilizer added). Sample from the 0-15 cm increment.

[b]Analysis not completed.

[c]TCDD in parts per trillion.

Kansas, and Florida sites, respectively. Although the number of analyses have been limited, the data have indicated that TCDD (and phenoxy herbicide) degrade more rapidly in the Kansas soils (Ulysses Silt Loam) than in the Florida soils (Lakeland Sandy Loam), and least rapidly in the Utah desert soils (Lacustrine Clay Loam).

The levels of TCDD and herbicide in a soil profile from one of the Eglin AFB, Florida, biodegradation plots are shown in Table 6. Initially (e.g., day 414), the data indicate that both the herbicide and the TCDD may be leaching down into the lower soil increments. However, note that the analysis for herbicide in a soil profile obtained on day 557 shows no leaching. The method of collecting soil samples, i.e., by the use of a soil auger contaminated the lower soil increments whereas the trenching technique showed no contamination. The analysis of soil profiles at all three locations for biodegradation indicated that neither the herbicide nor the TCDD appreciably penetrated below the 15-30 cm level. Thus, we believe that the disappearance of the herbicide and the TCDD can be attributed to the action of soil microorganisms, rather than leaching.

Data for TCDD are not available at this time (analysis in progress) on the influence of soil amendments (e.g., lime, fertilizer, and organic matter) on the degradation of TCDD in the Eglin AFB, Florida, biodegradation study. However, preliminary indications are that the addition of these amendments in these Florida soils does appear to slightly enhance <u>herbicide</u> degradation. On the other hand, the presence of activated coconut charcoal in

TABLE 6. MOVEMENT OF HERBICIDE ORANGE AND TCDD IN A SOIL PROFILE, EGLIN AFB, FLORIDA. (TCDD IN PARTS PER TRILLION)

DEPTH (CM)	DAYS AFTER APPLICATION[a] 414[b] HERBICIDE (PPM)	414[b] TCDD ($PPM x 10^{-6}$)	557[c] HERBICIDE (PPM)
0-15	1,866	250	824
15-30	263	50	11
30-45	290	<25[d]	<10[d]
45-60	95	<25[d]	<10[d]
60-75	160	<25[d]	<10[d]
75-90	20	<25[d]	<10[d]

[a]Composite sample from the plot containing only herbicide

[b]Increments obtained by use of a soil auger having cup dimensions of 7.6 x 15.2 cm, diameter and length, respectively

[c]Increments obtained by use of porcelain spatula from the side of 90 cm deep trench

[d]Detection limit

the Eglin plots, at the 12 cm level, prevented degradation of the herbicide and probably also prevented degradation of the TCDD. These data are shown in Table 7.

In no instance can it be shown that TCDD levels reached a non-detectable level (less than 10 parts per trillion) within the designated time periods (see Tables 3, 4, and 5). Although biodegradation appears to reduce the level of herbicide and TCDD, the data did not follow simple exponential decay curves. For the mixture 2,4-D and 2,4,5-T herbicides, disappearance was rapid initially, but slowed substantially in the later portions of the test period. With this type of decay kinetics, meaningful half lives are difficult to calculate; however, a reasonable estimate appears to be in the range of 150-210 days. The degradation of TCDD followed a similar decay pattern. However, it appears at this time that the decreased rate of degradation of TCDD as a function of time may be even more pronounced than for the herbicides. One might speculate that the enzymes responsible for herbicide metabolism are inducible and also are involved in TCDD breakdown. If this is the case, it is not surprising that TCDD metabolism slows or ceases when the initial massive concentrations of herbicide are removed.

Microbial studies have been conducted in the biodegradation plots in both Utah and Florida (8,9). For the Utah plots,

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[8] Stark, H.E., J.K. McBride, and G.F. Orr. Soil incorporation/ biodegradation of herbicide orange. Vol I. Microbial and baseline ecological study of the U.S. Air Force Logistics Command Test Range, Hill AFB, Utah. Document No. DPG-FR-C615F, US Army Dugway Proving Ground, Dugway, Utah 84022, February 1975.

TABLE 7. COMPARISON OF HERBICIDE ORANGE DEGRADATION RATES IN PLOTS AT THE EGLIN AFB, FLORIDA, SITE, RECEIVING EITHER HERBICIDE, HERBICIDE PLUS SOIL AMENDMENTS, OR HERBICIDE PLUS AMENDMENTS AND CHARCOAL

	TREATMENT					
	HERBICIDE		HERBICIDE PLUS AMENDMENTS[a]		HERBICIDE PLUS AMENDMENTS AND CHARCOAL[b]	
	DEPTH (CM)		DEPTH (CM)		DEPTH (CM)	
DAYS AFTER APPLICATION	0-15 (PPM)	15-30 (PPM)	0-15 (PPM)	15-30 (PPM)	0-15 (PPM)	15-30 (PPM)
5	4,897	302	5,703	232	3,074	134
98	4,280	580	5,422	<50	2,767	<50
414	1,866	263	2,015	193	—[c]	—[c]
463	1,217	222	1,796	161	—[c]	—[c]
557	824	11	—[c]	—[c]	2,660	<50
707	508	<10	—[c]	—[c]	—[c]	—[c]
834	438	<10	184	<10	—[c]	—[c]
1,293	<10	<10	<10	<10	1,556	<10

[a]The amendments included 4.5 kg lime, 13.5 kg organic matter, and 1.4 kg fertilizer (12:4:8 for N,P,K, respectively) uniformly mixed within the top 0-30 cm of soil in the plot.

[b]A 1 cm layer of activated coconut charcoal was applied to the trench prior to application of the herbicide.

[c]Not determined.

samples were taken three times throughout the year (summer, winter and spring, 1973-1974), and microbial species present (bacteria, actinomycetes and fungi) were determined. Bacterial counts were higher for soils with greater concentrations of the herbicide and with greater moisture content, but the herbicide, in any concentration, had no significant effect on the mycoflora. For the Florida plots (9), soil samples were taken from all plots in June and August 1974, and in April 1975. Although bacterial and fungal levels were similar for control plots or plots receiving either herbicide or herbicide plus the soil amendments lime, fertilizer, and organic matter, the levels were significantly higher in the plots receiving the activated charcoal. Microorganisms tended to be concentrated in the level which contained the charcoal (0-15 cm), but greatly reduced in number at depths immediately below the charcoal. This effect of increasing the number of microorganisms may have been due to adsorption of growth promoting substances (e.g., nutrients and water) on the surface of the charcoal particles. Although the number of organisms were greater in these plots, the level of herbicide residue was also greatest (Table 7). Apparently, the binding of the herbicide by the charcoal prevented it from being degraded by the microorganisms.

These two microbial studies have shown that the application of 2,4-D and 2,4,5-T at massive rates (5,000-40,000 ppm) not only

[9] Cairney, W.J. Determination of soil microorganism populations in the Eglin AFB, Florida, biodegradation plots. Department of Chemistry and Biological Sciences, United States Air Force Academy, CO, 1975, unpublished.

does not sterilize the soil, but indeed stimulates the growth of certain microflora. That these bacteria, actinomycetes and fungi are proliferating to such an extent indicates that they are probably using the herbicide and TCDD as a carbon source (the exception being the charcoal plots at Eglin), and, as such, are contributing to their degradation.

FATE OF TCDD IN AN ECOSYSTEM

The biodegradation plots offered little opportunity to evaluate the ecological effects associated with the use of herbicide Orange or to investigate food chain accumulation of TCDD. Although studies were conducted on the microorganisms, plants and dominant resident vertebrate and invertebrate animals on and adjacent to these plots (8,9), they were limited to studies of less than 0.5 ha. Therefore, concurrent with the biodegradation studies, an investigation was initiated on the much larger ecosystem (terrestrial and aquatic) of a unique military test area in Northwest Florida.

In support of programs testing aerial dissemination systems, Test Area (TA) C-52A, Eglin AFB Reservation, Florida, received massive quantities of military herbicides. The purpose of these test programs was to evaluate the capabilities of the equipment systems, not the biological effectiveness of the various herbicides. Nevertheless, after several applications, test personnel began to express concern over the potential ecological and environmental hazards that might be associated with continuance of the test program. This concern led to the establishment of a research program in the fall of 1967 to measure the ecological effects produced by the various herbicides on the plant community of TA C-52A (10).

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[10] Ward, D.B. Ecological records on Eglin AFB Reservation--the first year. AFATL-TR-67-157, Air Force Armament Laboratory, Eglin AFB, Florida, 1967.

Geographical and Vegetative Features

In 1962, the Armament Development and Test Center (ADTC), Eglin AFB, Florida, established an elaborate testing installation designed to measure deposition parameters of aerially applied herbicides on the Eglin Reservation. The direct aerial application was restricted to an area approximately 2.6 km^2 within TA C-52A in the southeastern part of the reservation. The entire test area covers approximately 5 km^2 and is a grassy plain surrounded by a forest stand that is dominated by long leaf pine (Pinus palustris), sand pine (Pinus clausa), and turkey oak (Quercus laevis). The actual area of test flight paths and herbicide application is in a mechanically cleared area now occupied mainly by broomsedge (Andropogon virginicus), switchgrass (Panicum virgatum), and other low growing herbaceous vegetation.

Sampling Grids and Herbicide Deposition

Four separate test grids were established on the 2.6 km^2 test area during the 1962 through 1970 testing period. Table 8 indicates the approximate total amount of herbicides (active ingredients) applied on each test grid and the time periods of those applications.

Preliminary Ecological Studies

The first in depth animal survey was initiated on the herbicide equipment test grids and surrounding area in 1970 (11). This

[11] Pate, B.D., R.C. Voight, P.J. Lehn, and J.H. Hunter. Animal survey of test area C-52A, Eglin AFB Reservation, Florida. AFATL-TR-72-72, Air Force Armament Laboratory, Eglin AFB, Florida, April 1972.

TABLE 8. APPROXIMATE AMOUNTS OF 2,4-D AND 2,4,5-T HERBICIDES APPLIED TO TEST AREA C-52A, EGLIN AFB RESERVATION, FLORIDA

TEST GRID	GRID AREA (HECTARES)	HERBICIDES (KILOGRAMS ACTIVE INGREDIENT)	
		2,4-D	2,4,5-T
1	37.25	39,540 (1962-1964)[a]	39,540 (1962-1964)
2	37.25	15,885 (1964-1966)	15,885 (1964-1966)
3	37.25	1,263 (1967)	---
4	97.0	19,959 (1968-1970)	19,959 (1968-1970)

[a]Years when the major portion of the herbicide was applied.

survey was conducted during the time that aerial spray equipment was actively being tested. The purpose was to determine species variation and distribution patterns on the test grids and surrounding 28.5 km^2. Of the 86 species of vertebrate animals observed or collected, it was concluded that the beach mouse (Peromyscus polionotus) and the six-lined racerunner (Cnemidophorus sexlineatus) were present in sufficient numbers to conduct population studies. In the spring of 1973, analyses of random soil samples from the test area indicated that low levels (e.g., parts per billion or parts per trillion) of TCDD were persisting in areas (i.e., the flight paths) that had received repetitive aerial applications of 2,4,5-T. Based on the beach mouse populations and the residue information a study was initiated in the summer of 1973 to obtain rodents for analysis of TCDD in body tissues and for examination of TCDD in body tissues and for examination of gross and microscopic evidence of teratogenic and mutagenic effects. A trapping survey was also conducted to study habitat preference of the beach mouse to determine if population distribution was related to vegetative cover. Data from these studies (12) indicated a correlation between the levels of TCDD in rodent liver and soil; however, there was no evidence of toxic histopathology in any rodent tissue. It was also found that indeed the population distribution was related to vegetative cover.

[12] Young, A.L. Ecological studies on a herbicide-equipment test area (TA C-52A) Eglin AFB Reservation, Florida. AFATL-TR-74-12, Air Force Armament Laboratory, Eglin AFB, Florida, 1974.

In the summer of 1974 a team of military and civilian scientists undertook a more extensive investigation of the numerous components of the ecosystem of TA C-52A. Using the information from previous studies, they obtained data on the fate of TCDD in soils, rodents, reptiles, aquatic organisms, birds, and vegetation. These results have been published by Young, Thalken and Ward (13), and are summarized in the following sections of this report.

Soil Studies of TCDD Residues

Soil samples (the top 0-15 cm increment) were collected from all four of the test grids on the test area and analyzed for TCDD. With the exception of Grid I, TCDD residues were in the range of <10 (minimum detection limit) to 30 parts per trillion (ppt, $1x10^{-12}$). Soil analysis of 20 separate samples from Grid I detected levels of TCDD in the range of <10 to 1,500 ppt. This wide fluctuation in TCDD concentrations was attributable to the locations of the actual flight paths on the test grid (i.e., not all of the 37 ha received the same amount of aerially applied herbicide). It was also apparent that the massive amounts of herbicides applied to this area in 1962-1964 contained very high levels of the TCDD contaminant. Further analysis of a duplicate soil core, obtained from a site having 110 ppt TCDD, indicated that TCDD was stratified within this top 0-15 cm of soil (Table 9).

[13] Young, A.L., C.E. Thalken, and W.E. Ward. Studies of the ecological impact of repetitive aerial applications of herbicides on the ecosystem of test area C-52A, Eglin AFB, Florida. AFATL-TR-75-142, Air Force Armament Laboratory, Eglin AFB, Florida, 1975.

TABLE 9. CONCENTRATION OF TCDD IN SOIL PROFILE (1974) OF GRID I, TEST AREA C-52A, EGLIN AFB, FLORIDA

SOIL PROFILE	GRID I APPLICATIONS OF HERBICIDES (1962-1964) PARTS PER TRILLION (PPT) TCDD
0-2.5 cm	150
2.5-5 cm	160
5-10 cm	700
10-15 cm	44
Below (15-90 cm)	None detectable

TABLE 10. NUMBERS OF BEACH MICE COLLECTED DURING THE 1973 AND 1974 STUDIES OF TEST AREA C-52A

CONTROL	1973	1974	TOTAL
Male	5	12	17
Female	5	10	15
Fetuses	12	11	33
		Subtotal	65
TEST	1973	1974	TOTAL
Male	26	17	43
Female	18	13	31
Fetuses	25	9	34
		Subtotal	108
		TOTAL	173

Rodent Studies

TRAPPING DATA/HISTOPATHOLOGY. In the 8 weeks of trapping beach mice during the summer of 1973 and 6 weeks during the summer of 1974, 106 specimens were collected from Grid I. Many of the females were pregnant at the time of collection, providing 67 fetuses for examination. Table 10 indicates the numbers of males, females and fetuses collected from Grid I during 1973 and 1974.

The only significant lesions seen on histopathologic examinations of 173 adult and fetal beach mice were two instances of moderately severe, multifocal, necrotizing, hepatitis (one test, one control animal) and a single test mouse with severe venous ectasia of the renal veins in one kidney. All other lesions were of the minor or insignificant type normally observed in microscopic surveys of large numbers of field animals. The absence of liver lesions (necrosis and porphyria) in mature animals that had liver levels of TCDD from 20 ppt to 1,300 ppt (Table 11) is most significant in view of the massive quantities of both 2,4,5-T and TCDD that must have been applied to the test site. There was no evidence to indicate that TCDD was mutagenic nor carcinogenic in the field at the concentrations noted in Table 11. None of the 34 fetuses examined from animals captured on the test grid showed teratogenic defects. This leads one to the conclusion that the levels of TCDD encountered in the field failed to induce observable developmental defects. An analysis of the organ to body weight ratios of each of the control (males and females) and

TABLE 11. CONCENTRATION (PARTS PER TRILLION) OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN LIVER AND PELT SAMPLES FROM BEACH MICE, *PEROMYSCUS POLIONOTUS*, COLLECTED FROM CONTROL AND TCDD-EXPOSED FIELD SITES, 1973 AND 1974

TREATMENT	YEAR	SEX	LIVER	PELT
Control	1973	Male and Female	20[a]	ND[b]
Control	1974	Male	51	40[a]
Control	1974	Female	83	40[a]
Grid I	1973	Male and Female	540	ND[b]
Grid I	1974	Male	1,300	130
Grid I	1974	Female	960	140

[a]Minimum level of detection

[b]Not determined

test (males and females) using the Wilcoxon Rank Sum Test indicated no statistical differences between field control males and field test males nor field control females and field test females ($P \leq 0.05$).

LIVER AND PELT ANALYSIS. The presence of TCDD in the liver samples of both male and female mice collected from the control site in 1974 may have been due to high levels in one or more specimens in the pool of samples. Mice from the test area could have migrated to the periphery of the grid and wandered into the area designated as control. The closest point from the control site to the test area was 200 m. However, it is emphasized that a mouse (or mice) could have been contaminated in this way, and thus have contaminated pooled samples analyzed for TCDD. Therefore, the use of these data as truly control data must be viewed with caution.

The levels of TCDD in the liver of beach mice collected from Grid I substantiated bioaccumulation of TCDD; i.e., an accumulation of TCDD in an organism from its environment. In general, levels of TCDD in the livers were no greater than the most concentrated zones of TCDD in the soil. There are no data from these studies to support biomagnification of TCDD; i.e., an increase in concentration of TCDD in successive organisms ascending the trophic food chain.

BURROW AND DIET STUDIES. In all burrows that contained mice a consistant finding was a plug of soil pushed up into the tunnel within the first 2.5 to 25 cm of the entrance. Frequently an "escape tunnel" would extend from the nest area to within 2.5 to

15 cm of the soil surface. Although the concentration of TCDD on the pelts of beach mice from the test area was only 10-15 percent of that in their livers, Table 11, it was apparent that the mice were continually contaminating themselves as they repeatedly moved in and out of their burrows. The soil data, Table 9, substantiated the presence of a zone of TCDD within the region of the tunnel entrance. Likewise, the location of the escape tunnel suggested that even the nest itself may contain detectable levels of TCDD. An examination of the plant and insect litter within the nests indicated that the beach mouse diet was made up of about 90 percent seeds (based on caryopsis hulls) and about 10 percent insects (based on insect exoskeletons and wings). Four composite seed samples were analyzed for TCDD with no TCDD being detected in any sample (at a minimum detection limit of 1 ppt TCDD). The insect remains are currently being analyzed for TCDD.

TCDD LABORATORY UPTAKE EXPERIMENT. Twenty-two beach mice from the designated control area were brought into the laboratory and divided into a "control" group of 10 animals and dusted with 100 mg of alumina gel 10 times over a period of 28 days while the "test" group of 12 animals was dusted with 100 mg alumina gel containing 2.5 ppb TCDD 10 times over the 28 day period. Table 12 indicates control levels and test levels of TCDD in the composite liver samples and on the composite pelt samples of the alumina gel and alumina gel plus TCDD dusted animals.

These animals were given complete histopathologic examinations at the completion of the experiment with no differences being seen between control and test animals. An analysis of the organ to body weight ratios of each of the control males to test males and control females to test females using the Wilcoxon Rank Sum Test indicated a statistical difference involving only the spleens of control male and test male animals at the 95 percent confidence level. This difference was not supported by either histopathological evidence nor by morphometric data as indicated in the Hepatic Ultrastructural Study section which follows.

HEPATIC ULTRASTRUCTURAL STUDY. After the liver was removed from 30 beach mice (15 control and 15 from the test area) and weighed, a section was taken from the center of the median lobe. Representative electron micrographs were made from the liver tissue of each animal and the data obtained from each micrograph using a stereology technique. This method of quantitative analysis of the cell ultrastructure used morphometric procedures based on the techniques developed by Weibel et al. (14), as modified by Buchanan (15).

With this method, a transparent grid of intersecting lines was placed at random over the micrographs and all the line intersections (points) which were over the required cell structures were counted. All of the points lying over the mitochondria, the damaged (swollen and ruptured) mitochondria, the granular

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[14] Weibel, E.R., G.S. Kistler, and W.F. Scherle. Practical stereological methods for morphometric cytology. J. Cell. Biol. 30: 23-38, 1966.

[15] Buchanan, G.M. Effect of high dietary molybodenum on rat adrenal cortex. Unpublished thesis. University of Colorado, Boulder, CO, 1973.

TABLE 12. CONCENTRATION (PARTS PER TRILLION) OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN LIVER AND PELT SAMPLES FROM BEACH MICE, PEROMYSCUS POLIONOTUS, DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL) OR 2.5 PARTS PER BILLION TCDD (TEST)

TREATMENT	SEX	LIVER	PELT
Alumina Gel	Male[a]	ND[b]	ND[c]
	Female[a]	ND[b]	ND[c]
Alumina Gel + TCDD	Male[a]	125	45
	Female[a]	125	89

[a]Male and female livers composited for analysis

[b]Minimum detection level - 10 ppt

[c]Minimum detection level - 8 ppt

TABLE 13. CONCENTRATION (PARTS PER TRILLION) OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN COMPOSITE SAMPLES OF VISCERA OR TRUNK FROM SIX-LINED RACE-RUNNERS, CHEMIDOPHORUS SEXLINEATUS, COLLECTED FROM CONTROL AND TCDD-EXPOSED FIELD SITES

LOCATION	VISCERA	TRUNK
Control Site	ND[a]	ND[b]
Test Site	360	370

[a]Minimum detection limit - 50 ppt

[b]Minimum detection limit - 40 ppt

endoplasmic reticulum (RER) and the agranular endoplasmic reticulum (SER) were then counted. The total area of the cytoplasm was then measured in the same manner.

The volume fraction of each structure was determined to be the ratio between the point count of that structure and the total point count of the cytoplasm. In this manner the ratio of mitochondria volume to cytoplasm volume of the hepatic parenchymal cell was determined for each animal, as was the ratio of damaged mitochondria volume to total mitochondria volume, RER to cytoplasm, SER to cytoplasm, and RER to SER. Using these volume fractions or ratios as quantitative measurements of the structures in question, the hepatic parenchymal cells from treated animals were compared with those from control animals.

Similar data were collected from 22 mice brought from the field into the laboratory and exposed to 30 days of external dusting with alumina gel (with or without 2.5 ppb TCDD).

Analysis of the morphometric data using the Wilcoxon Rank Sum Test indicated no statistical differences between field control and field treatment animals, nor were there statistical differences between the control and treatment animals of the dusting study ($P \leqq 0.05$).

Reptile Studies

ANALYSIS OF REPTILE TISSUE. Chemical analysis for TCDD in body parts of the six-lined racerunner indicated significant levels of TCDD in both the visceral mass and in the trunk, Table 13.

Gross post-mortem examinations were performed on 19 racerunners collected from either a control site or from Grid I with no evidence of gross abnormalities seen in any of the specimens.

TCDD In Aquatic Organisms

Young, Lehn and Mettee (16) conducted species diversities and food chain studies in two aquatic ecosystems draining TA C-52A. Erosion of soil occurred in to a pond on the test area and in to a stream immediately adjacent to the area. TCDD levels of 10-35 ppt were found in silt of the aquatic systems, but only at the point where eroded soil entered the water. Species diversity studies of the stream were conducted in 1969, 1970, 1973 and 1974. Insect larvae, snails, diving beetles, crayfish, tadpoles, and major fish species from both aquatic systems were analyzed for TCDD. Species diversity studies indicated no significant change in the composition of ichthyofauna between these dates or a control stream. Concentrations of TCDD (12 ppt) were found in only two species of fish from the stream, sailfin shiner (*Notropis hypselopterus*) and mosquitofish (*Gambusia affinis*). The sample of mosquitofish consisted of bodies with heads and tails removed. Two samples of sailfin shiner were analyzed; one containing viscera only and the other bodies less heads, viscera, and caudal fins. Only the viscera contained TCDD. Samples of skin, muscle, gonads, and gut were obtained from spotted sunfish (*Lepomis punctatus*)

[16] Young, A.L., P.J. Lehn, and M.F. Mettee. Absence of TCDD toxicity in an aquatic ecosystem. Weed Sci. Soc. Amer. Abst. 107, p. 46, 1976.

from the test grid pond. Levels of TCDD in those body parts were 4, 5, 18, and 85 ppt, respectively. Gross pathological observations of the sunfish revealed no significant lesions or abnormalities.

TCDD In Birds of TA C-52A

Bartleson, Harrison, and Morgan (17) have conducted an extensive survey of the birds of TA C-52A. Between March 1974 and February 1975, they visited study areas twice each week at various times of day and night, and observed a total of 77 species of birds. Of this number, 44 species were observed on Grid I, the area of greatest TCDD residue. The remaining birds were seen in the surrounding clearing and bayheads projecting into the clearning. A small collection of specimens was made for species identification and for TCDD analysis. Only three species could be classified as residents which nest on the test grids. These were southern meadowlark (Sturnella magna), morning dove (Zenaidura macroura), and bobwhite quail (Colinus virginianus). TCDD residues were found in meadowlark livers (100-1,020 ppt) and in the stomachs and stomach contents (46 ppt) of these same birds. An analysis of liver and fat tissue from doves indicated concentrations of 50 ppt. An analysis of seed in the crop of the doves showed no detectable residue of TCDD. Two routes of TCDD contamination

[17] Bartleson, F.D., D.D. Harrison, and J.D. Morgan. Field studies of wildlife exposed to TCDD contaminated soils. AFATL-TR-75-49, Air Force Armament Laboratory, Eglin AFB, Florida, 1975.

were proposed for these birds. The first was through the process of dusting and subsequent ingestion of contaminated soil while preening. A second possible route was through the ingestion of soil-borne insects from the test grid; an analysis of a single composite insect sample indicated a concentration of 40 ppt TCDD.

Vegetative Succession Studies on TA C-52A

TCDD analysis of vegetation has been limited to seed samples collected in support of the rodent diet study. No TCDD was found in four samples of seeds collected from vegetation on Grids I or II. The minimum level of detection was 1 ppt. A vegetative succession study has been conducted by Young and Hunter (18) to document the re-vegetation of an area denuded first by mechanical means and then by hundreds of applications of phenoxy herbicides. Nine months (June 1971) after the last defoliant-equipment test mission, a detailed survey of the vegetation was initiated. The area was divided into a grid of 169 sections (each 122 by 122 m), and within each section the percentage vegetative coverage was visually ranked as Class 0, 0-5 percent; I, 5-20 percent; II, 20-40 percent; III, 40-60 percent; IV, 60-80 percent; and V, 80-100 percent. Three sections within each class were selected at random and surveyed for dicotyledonous plants. An unsprayed area 0.32 km northwest of the test area was also surveyed. In June 1973, each

[18] Young, A.L., and J.H. Hunter. A long-term field study of vegetative succession following repetitive application of phenoxy herbicides. Weed Sci. Soc. Amer. Abst. 1977.

of these areas was again surveyed, but in addition, a square-foot ($0.093m^2$) analysis technique was performed in 15 additional sections. These sections were randomly selected and within each section, nine areas, each $0.093m^2$, were analyzed for species composition and ground cover density. Both methods of vegetative survey were repeated in June 1976. The number of dicotyledonous species increased from 74 in 1971 to 107 in 1973, and to 123 in 1976. In 1971, 20 percent of the test area had less than 20 percent vegetative cover, while 26 percent of the test area had more than 60 percent vegetative cover. In 1976, no sections had less than 20 percent vegetative cover, but over 73 percent of the test area had a cover of more than 60 percent. The major grass species were Panicum virgatum and Panicum lanuginosum. The major dicotyledon was Diodia teres in 1971, but was replaced by Chrysopsis graminifolia in 1976. These data demonstrate the rapid invasion of dicotyledonous species despite the unusually heavy applications of phenoxy herbicides.

As a concluding remark, Test Area C-52A, Eglin AFB, Florida, has offered a unique opportunity to examine the effects of long-term, low-level exposure of biological systems to TCDD. Perhaps when the herbicide 2,4,5-T (contaminated by TCDD) was first applied to the test area (1962-1964), the levels of TCDD that accumulated on the soil may have been sufficiently high to be toxic, although there is no mention of animal deaths in the records of test missions for this area. It is of interest to note that in the

Italian TCDD episode, an estimated 0.9 to 4.5 kg TCDD were disseminated on an area of 1.4 km^2. This is approximately equal to 6.5 to 32 g/ha. Grid I on Test Area C-52A probably received between 0.07 and 1.86 kg TCDD on an area of 37 ha, or approximately 2 to 50 g TCDD/ha over a 2-year period. This range of values was arrived at using the arithmetic mean and maximum concentration of TCDD contamination of the herbicide Orange presently in the United States Air Force inventory.

LABORATORY AND GREENHOUSE EXPERIMENTS WITH TCDD

Two additional studies have been conducted in support of the previous investigations of TCDD in field ecosystems. One of these studies has been conducted by Cupello and Young (19) on the potential uptake from soil of ^{14}C-TCDD by plants. In this study, 2,240 kg active ingredient Orange herbicide/ha, containing 14 ppm ^{14}C-TCDD, was placed beneath the soil surface in specially designed growth boxes containing 100 plants of Sorghum (Sorghum vulgare) per box. The plants were grown under controlled environmental conditions for 9 weeks; 14-hour photoperiod, diurnal temperature of 35 ± 2°C and 15 ± 1°C, and a relative humidity of 60 and 85 percent, day and night, respectively. On day 64 the plants were cut at the soil surface, ground in a Wiley Mill, and extracted with hexane in a Soxhlet Extraction apparatus for 4 hours. The TCDD in the extract was then concentrated by using the Dow Chemical Company Analytical Method ML-AM 73-97.

The "TCDD concentrate" was quantitatively transferred to a scintillation vial using benzene, and 15 cc of Aquasol added to it. Analysis of the counting data from a liquid scintillation counter indicated no significant uptake of hexane extractable ^{14}C-TCDD activity in the plant material. An analysis was also performed on the plant tissue prior to hexane extraction, and after hexane extraction for 4 hours. These plant samples were combusted in a

[19] Cupello, J.M., and A.L. Young. Radiochemical bioassay of TCDD uptake in plant material. Department of Chemistry and Biological Sciences, United States Air Force Academy, CO, 1976, unpublished.

Packard Model 306 sample oxidizer, the CO_2 collected in Packard Carbo-Sorb, this solution diluted in Packard Permafluor-V, and the fluor counted in a liquid scintillation counter. These data indicated the presence of sufficient ^{14}C activity in the unextracted plant material to be equivalent to approximately 430 ppt TCDD in the plant tissue. This activity was not significantly reduced by hexane extraction.

This relatively high ^{14}C activity in the plant tissue could be explained by one of at least four hypotheses. It could represent the presence of (1) bound (non-hexane soluble) TCDD, (2) a soil biodegradation product of TCDD that was taken up and bound by the plant, (3) a metabolic breakdown product of the TCDD that was formed after incorporation of the TCDD into the plant, or (4) a contaminant in the original ^{14}C TCDD stock solution that eventually found its way into the plant either as the original contaminant or as a metabolic of it.

A second study has been conducted by Bartleson, Harrison, and Morgan (17) on the effect of tilling TCDD contaminated soil. One cubic meter of soil was collected from Grid I, TA C-52A, and removed to the laboratory. Four samples were taken from the uniformly mixed soil, analyzed and found to contain 1,100 ppt (2 samples) and 1,300 ppt (2 samples) TCDD. The contaminated soil was placed in two groups of four pots (20 cm deep and 20 cm in diameter). The four pots in each group were treated as follows: two were left outside and exposed to natural elements, and two were placed in a greenhouse and watered with a nutrient solution.

One of the two containers in each location was left undisturbed, and the other was stirred (tilled) weekly with a spatula. This stirring was not complete, and soil in the bottom of the pots was relatively undisturbed. The soil in each of the pots was emptied into a clean tray and mixed thoroughly before samples were collected and analyzed for degradation of TCDD. The data shown in Table 14 suggest that sunlight, tilling and perhaps increased temperatures (associated with the greenhouse) may promote more rapid degradation of TCDD. There also may be an additive effect from use of nutrients.

TABLE 14. DEGRADATION OF TCDD (PARTS PER TRILLION) IN A GREENHOUSE EXPERIMENT, EGLIN AFB, FLORIDA

TREATMENT	LENGTH OF EXPOSURE 0 (PPT)	9 weeks (PPT)	23 weeks (PPT)
Full Sunlight[a]			
Tilled	1,200	1,100	520
Untilled	1,200	1,000	530
Greenhouse[b]			
Tilled	1,200	640	460
Untilled	1,200	810	530

[a]Samples placed outside of greenhouse

[b]Samples watered with a nutrient solution

RECOMMENDATIONS FOR DECONTAMINATION OF TCDD EXPOSED FIELD SITES

Although there are many potential options for the decontamination of an area exposed to TCDD (see reference 4), data provided in this report would suggest that one of the most feasible options would be soil incorporation/biodegradation. The data base used in selecting this option is as follows:

1. TCDD may persist (in biotic and abiotic components) for long periods of time when initially present at extremely high concentrations on the soil surface.

2. TCDD will accumulate in the tissues of rodents, reptiles, birds, fish, and insects when these organisms are exposed to TCDD contaminated soils (however, the levels of TCDD in the tissues apparently do not exceed the levels of TCDD found in the environment).

3. Organisms tolerate, i.e., based on no observed deleterious effects, soil levels between 10-1,500 ppt TCDD.

4. TCDD is degraded by soil microorganisms, especially when in the presence of other chlorinated hydrocarbons.

5. TCDD is degraded in the presence of sunlight.

6. Movement of TCDD in the abiotic portions of the environment can be by wind or water erosion of soil particles, but leaching by water alone does not appear to occur.

7. TCDD is probably not readily released or degraded in the environment when bound to activated coconut charcoal.

Specific Recommendations

In locations where accidental TCDD contamination covers significant geographical area, e.g., many hectares, an *in situ* biodegradation program may be most effective in reducing levels of TCDD residues. Incorporation of organic material, lime, and fertilizer to enhance microbial activity may be advantageous. The biodegradation site should be tilled frequently so as to expose residue to sunlight. Watering of the site is recommended to reduce wind movement of contaminated particles and to enhance biodegradation. In locations where a limited area has been exposed to accidental contamination of TCDD, the top 0-15 cm of soil should be removed and taken to an isolated area where biodegradation procedures can be conducted. Similar treatments should be applied to these plots as would be for an *in situ* program. Protective clothing should be worn by all site personnel. The contaminated clothing should be incinerated at an approved incinerator. Following use, all equipment should be rinsed with an organic solvent (e.g., diesel fuel) to remove TCDD residue. The solvent containing TCDD residue may be collected in activated coconut charcoal and either incinerated or placed in an approved sanitary landfill, although if a sufficiently isolated land area is available, biodegradation may be feasible.

It should be noted that some TCDD residue will remain in a contaminated site. However, research on the ecosystem at Test Area C-52A, Eglin AFB, Florida, indicated that organisms do have a tolerance to low levels of TCDD. Therefore, in those areas having soil residues below 1 ppb, further efforts to decontaminate the area are not practical. These areas should, however, be fenced and posted to prevent livestock and human exposure.