CHLORINATED DIOXIN RESEARCH

United States Department of Agriculture

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PCB-General

INTRODUCTION

The most recent class of chlorinated industrial compounds coming under scrutiny as a possible threat to our environment are the chlorinated dibenzo para dioxins which appear as impurities in a number of industrial chemicals. What are the chlorinated dioxins, how do they arise, and what are some of their properties?

In the production of the herbicide 2,4,5-T, the starting material for the aromatic portion of the compound is tetrachlorobenzene. Tetrachlorobenzene is hydrolyzed to trichlorophenol which is subsequently reacted with chloroacetic acid to form 2,4,5-T (Figure 1). In the production of the trichlorophenol if the temperature of the reaction exceeds 160°, there are formed substantial amounts of several impurities, one of which has been identified as the 2,3,7,8-tetrachlorodibeno para dioxin (TCDD). From a chemical standpoint, these are highly insoluble lipophilic materials that generally result from high temperature reactions involving chlorinated phenols. They are extremely poisonous substances. For example, TCDD has an LD_{50} of 0.0006 in guinea pigs. Consequently, extreme care must be exercised in handling and manipulating this compound under laboratory conditions.

In addition to its extreme toxicity, these compounds are known to produce chloracne. Chloracne or follicular hyperkeratosis was first described by Herxheimer in Germany in 1899 and is an industrial disease which causes skin eruptions near the sebaceous glands, giving rise to pimples, blackheads, comedones, and general skin irritations on the face, arms, and shoulder regions. There were three major outbreaks of chloracne in industrial plants in Germany prbducing chlorinated phenols in the 1950's. In 1964 when the Dow Chemical Company engaged in producing large quantities of 2,4,5-T for the Department of Defense, 60 workers at the Midland plant contracted chloracne. Two or three years were required for recovery of some of these workers after the initial exposure. A third problem caused by the chlorinated

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dioxins is chick edema or a disease of chicks that occurs periodically in the United States and kills millions of young chicks. The disease is characterized by hydropericardium and is manifested by a buildup of fluid in the heart sac and lungs. The hexachlorodibenzo-p-dioxin was first identified as one of the constituents of toxic fat in feeds responsible for chick edema disease. TCDD also causes chick edema, but other higher chlorinated dioxins are the factors recognized as contaminants of toxic fats in chicken feeds. The chemical screening method for chick edema factor involves only the recognition of these higher dioxins (i.e., the hexa, hepta, and octa isomer). Studies by Higginbotham and his associates of the Food and Drug Administration have shown that the tetrachlorodioxin is more toxic than the hexa isomer. Finally, it is known that the tetrachlorodioxin is a powerful teratogen and was probably responsible for some of the biological effects observed in the test animals in the Bionetic study, when 2,4,5-T was examined for teratogenicity.

Chlorodioxins in Commercial Samples of Formulated Pesticides

When it became apparent that chlorinated dioxins might be implicated in the teratogenic effects observed in samples of 2,4,5-T used in the Bionetics study, the Agricultural Research Service, USDA, initiated a program to assess the significance of chlorinated dioxins in currently registered pesticides and the fate of these contaminants in the environment. The first phase of this program was to identify those pesticides that could conceivably be contaminated with chlorodioxins and then devise an adequate method and survey system for determining their significance in these samples. In cooperation with the Food and Drug Administration, at least 18 compounds were identified as chlorophenols or as being derived from chlorinated phenol precursors. The pesticides include:

2,4-D	nemacide
2,4-DB	ronnel
2,4-DP	PCP
2,4,5-T	nitrofen
silvex	chloroneb
sesone	animert
falone	tetradifon
dicamba	zytron
tricamba	erbon

In cooperation with the Pesticide Registration Division, we were able to collect approximately 110 samples containing 18 phenolic pesticides from various regional laboratories.

Our first step was to develop a sensitive and reliable method for the detection of the tetrachlorodioxin and other dioxin impurities in these technical grade materials. Dr. E. A. Woolson and Mr. R. Thomas, analytical chemists in the Crops Research Division and Pesticide Registration Division, have devised a method that is generally applicable for the phenoxy herbicides and other pesticides and with an additional step for the chlorinated phenols as well. A copy of this method is available for those wishing to undertake dioxin research. The results of our dioxin survey are shown in Table 1. This survey includes 105 pesticide samples including 79 phenoxy herbicides, 14 chlorophenols, and 10 other compounds in the group of 18 collected materials. This table shows the number of pesticides that contain tetrachlorodioxin residues in the range of less than 0.1 ppm, those that contain 0.1-1.0 ppm, those that contain 1-10 ppm, and those that contain more than 10 ppm of the dioxin impurities. In the phenoxy family, 53 of the 79 compounds or approximately 67% contain less than 0.1 ppm or were below our level of detection; 5 or 6.3% contained concentrations in the range of 0.1-1.0 ppm; 9 or 11.4% in the range of 1-10 ppm; and 12 or 15.2% in the range of greater than 10 ppm. None of the chlorinated phenols contained any measurable amounts of tetrachlorodioxin and only one of the samples in the other class contained this impurity in the range of 0.1-1.0 ppm. From the labels on these samples of phenoxy herbicides, we were able to identify a single manufacturer whose product had a majority of the measurable residues. A survey of the history of production by this company indicated that samples prior to 1969 contained greater than 10 ppm in most of the 2,4,5-T samples, and there was a subsequent drop after this date to substantially smaller amounts. This particular company has since ceased all production of 2,4,5-T; and consequently, commercial samples of 2,4,5-T presently being manufactured normally contain less than 0.5 ppm of the chlorinated dioxins.

The chlorinated phenols present a considerably more complex problem and contain a number of impurities including higher members of the dioxin family. Table 2 shows a different type of survey in which we measured the amounts tetra-, hexa-, hepta-, and octa-dioxin present in current and past samples of chlorinated phenols. Unfortunately, we were unable to get a complete picture of the dioxin content since, theoretically, there are 75 possible isomers of the dioxins and we have only 6 or 7 of the synthetic isomers available for comparison. For example, the hepta-isomer is not available for quantitative purposes; and therefore, some of our values are estimates based on extrapolations. In addition, we know that there may be more than one isomer of the hexachlorodioxin appearing in these samples. Consequently, we have to sum our estimates under one heading. Measurement and detection of dioxins in the chlorinated phenols requires an additional step in the analytical process, and we have found that mild treatment with a 1:1 mixture of sulfuric acid and nitric acid at low temperatures will remove a substantial number of the

impurities in these chlorinated samples. Nitration does not remove the tetrachlorodioxin or higher dioxins; and therefore, we are able to eliminate a large number of interfering peaks by the nitration step. This method has not been submitted to collaborative study, and we do not know how it will compare with other methods presently being used for measuring dioxins. Examination of Table 2 suggests that chlorinated phenols contain substantial amounts of higher chlorinated dioxins and that five samples contained more than 100 ppm of the hepta-dioxin and five contained more than 100 ppm of the octa-dioxin. We must admit at this point that these are estimates based on measurements of peak heights. The exact quantitation of these peaks is confounded by lack of some standards and by the fact that we are unable to resolve some of the materials from impurities as evidenced by GC-Mass Spectrometry. We know that other impurities exist in the same region; namely, chlorinated furans, chlorinated hydroxyphenyl ethers, and related methoxylated compounds in which one or more chlorine atoms are replaced by OCH_3, including methoxy-chlorinated dioxins. The contribution that these materials are making to the observed peaks is difficult to resolve at this time; and consequently, we would prefer to call these values estimates rather than precise analytical figures. Each of the neutral fractions from the chlorophenols has been examined by GC-Mass Spec combination to identify the impurities in these materials; and although our analysis of parent peaks and fragmentation patterns suggests the presence of these other neutral compounds (especially the dimethoxychlorinated dioxins) in the cleaned up extracts, their exact contribution to each of the major peaks is uncertain at this time.

Environmental Studies on the Chlorinated Dioxins

In order to overcome some of the inherent problems of detecting minute amounts of chlorinated compounds in biological systems, we needed an alternative method for detecting tetrachlorodioxin residues in plant and soil samples. The Dow Chemical Company at Midland, Michigan, generously supplied us with ^{14}C-labeled TCDD uniformly labeled in both rings, uniformly labeled 2,7-dichlorodibenzo para dioxin, and uniformly labeled 2,4-dichlorophenol. With the analytical standards and labeled materials, we initiated a series of environmental-related research projects to determine the fate of the dioxins in plants and soils.

Plant Uptake Studies. Dr. A. R. Isensee, plant physiologist, has conducted this phase of the dioxin program. The uptake of labeled TCDD by soybeans and oats from a Lakeland sand receiving 0.06 ppm TCDD is shown in Figures 2 and 3. Lakeland sand is found in the coastal regions of the United States and would represent an ideal medium for maximum uptake of any pesticide, since it has a very low adsorptive capacity, i.e., its organic matter content is less than 0.5%. An examination of Figures 2 and 3 shows that the same phenomenon occurs with both plant species; that is, a rapid uptake of radioactive material during the first two sampling periods and then a gradual decline in concentration with time. The amount of TCDD in the aerial portion of the plant was determined

by harvesting the leaves and stems, taking them to dryness, grinding them, and then combusting and trapping the labeled CO_2 in monoethanolamine. The ^{14}C-content was determined by liquid scintillation counting. The plant data suggest that uptake of TCDD from soils is not a significant phenomenon, due to the following reasons. First, in order to obtain a statistically significant number of counts in the plant tissues, assuming that 0.1 of 1% of the material could conceivably be taken up, we applied 0.06 ppm TCDD to the soils. Although this would appear to be a reasonably low amount of dioxin, it represents the amount equivalent to approximately 40,000 times the amount of TCDD that would appear in soils from a 2-lb/acre application of 2,4,5-T containing 1 ppm of TCDD as an impurity. Since our past experience with the chlorinated hydrocarbon insecticides suggests that uptake is a function of concentration in soils, the amount of radioactivity taken up would be substantially higher than that ever likely to be experienced under actual field conditions. Second, the decline in concentration of radioactivity is greater than can be accounted for by normal dilution of plant growth and therefore must be attributed to translocation or volatilization from the leaf surface or metabolism. We are investigating the metabolism of this material in plant tissues at the present time. Finally, we know that the limit of detection by the use of radioactively-labeled materials and liquid scintillation counting is approximately 0.01 ppm; and you notice that at the termination of the experiment, 40 days, the plant samples are approximately twice the level of detection or in the neighborhood of 0.02 ppm. In other words, the amount of radioactivity in these plants is of questionable significance. When oats and soybeans were taken to maturity, no ^{14}C could be detected in the harvested grain or bean.

Soil Persistence. The persistence of TCDD in two soils at three rates of application at 4 dates is shown in Figures 4 and 5. The rates of application were 1, 10, and 100 ppm; the dates of sampling were 20, 40, 80, and 160 days; and the soils were Lakeland sand and a Hagerstown silty clay loam. The rates of application were selected based on the fact that we did not know how persistent this dioxin would be in soils. Therefore, if there was a rapid decrease of TCDD, one could perhaps get some accurate measurements from the higher rates of application and determine its biological half-life in soils. The two soils selected represent extremes in regard to biological activity. The Lakeland sand is low in organic matter content and consequently does not support a large microbial population. In contrast, the Hagerstown soil is biologically quite active; and if microbial metabolism were a significant route of dissipation in soils, the Hagerstown soil should exhibit fairly rapid degradation. The soils were extracted with a 1:1 hexane/acetone solution and injected into the GC for analysis by ECD or FID. Recovery studies with the radioactive material suggest we were able to recover about 85% of the applied material. Although the data from the Hagerstown soil are fairly erratic, it is fairly consistent with the data collected from the Lakeland sand; that is, TCDD is a persistent compound. If we are able to recover about 80-85% of the applied material on each of the sampling dates and if the data are corrected

for percent extraction, we would be able to account for about 95% of the material applied on day 0. The persistence of the tetrachlorodioxin is not surprising since it is an insoluble, nonpolar chlorinated substance and from past experience these factors would imply a recalcitrant molecule. We anticipate continuing these studies with further sampling at 320 and 640 days.

Photolysis. Dr. J. R. Plimmer, organic chemist, is responsible for photochemical studies on the dioxin project. The photochemical stability of TCDD has been examined in natural sunlight and under laboratory conditions. Many organic molecules decompose in sunlight. We have examined the breakdown of TCDD in methanol with a sunlamp under laboratory conditions with a peak emission of 310 mμ. These results are shown in Figure 6. Under laboratory conditions, the half-life of TCDD is about 3 1/2 hours. We have also conducted similar experiments under natural sunlight and we find that the molecule is rapidly decomposed in sealed ampoules under natural sunlight. At the bottom of Figure 5 we note the appearance and then slow decline of a small amount of material which has been identified as the trichlorodioxin. Subsequent analysis and investigation of the products resulting from photochemical decomposition suggests that the reaction proceeds by successive dechlorination of the TCDD to give rise to the trichloro compound and presumably the di, mono, and completely dechlorinated dioxin. We have isolated and identified the trichlorodioxin by mass spectral analysis. We are still trying to assess the significance of photochemical degradation under natural conditions in which water is the solvent and our preliminary data suggest that the process is probably extremely slow in the natural environment.

Soil Mobility. Dr. C. S. Helling, soil scientist, has been measuring the leaching of TCDD, 2,4-D and 2,4,5-T in soils. Mobility was examined in five soils; Norfolk sandy loam, Lakeland sand, Hagerstown silty clay loam, Barnes clay loam, and Celeryville muck. The TCDD did not move in any of the five soils and remained on the surface. The phenoxy herbicides moved extensively in the two sandy soils (Norfolk and Lakeland), was intermediate in the two clay soils (Hagerstown and Barnes), and moved very slightly in the muck soil.

Additional Research Underway or Contemplated. We have reviewed some of the highlights of the work undertaken on the dioxins in our laboratory at Beltsville. In addition to the studies I have discussed, there is other work underway which is still in progress or being summarized at the present time. For example, we have applied small amounts of radioactive dioxin to a leaf surface and tried to determine whether it is translocated to the rest of the plant parts. Our evidence indicates that translocation does not occur and most of the material can still be found on the leaf at harvest time. We have applied radioactive TCDD to the leaf surface and then by simulating rainfall with an eye dropper attempted to measure the amount of dioxin that would be

removed. Our preliminary work with washoff of dioxin from the leaf surface indicates that about 40% of the dioxin is removed with the first 10 ml washing which would be equivalent to about 1/5 inch of rainfall. The second washing of 10 ml does not remove any further dioxin. This experiment is difficult to analyze, however, since the dioxin has to be put on with a surfactant; and consequently, we believe that the surfactant is somehow enhancing the washoff process. We are trying to determine whether it is possible to biosynthesize dioxins from chlorinated phenols in soils, and we have recently completed an experiment in which we treated soils with 10, 100, and 1000 ppm of 2,4-dichlorophenol, 2,4,5-trichlorophenol. We are now in the process of analyzing these soils for dioxin content. Our preliminary survey suggests that no dioxins are produced biosynthetically in soils. We are also examining the nature of the radioactive metabolites that may be found in soils incubated with labeled TCDD for 90 days. These experiments and several others are still in progress, and we hope to complete our work in this area by the end of this calendar year.

Conclusion

The results of our research thus far suggests that the environmental insult from the tetrachlorodioxin has probably not been substantial from pesticide usage patterns in the past. Therefore, the magnitude of the problem in no way approaches that encountered with DDT or some of the chlorinated cyclodiene insecticides. This is indeed fortunate since these materials are substantially more hazardous than the chlorinated insecticides. The environmental work suggests that these materials are immobile in soils; that is, they do not tend to leach down into the soil profile, they are persistent but not readily taken up by plant roots and translocated to the aerial portion of the plant, that they are not translocated from leaf applications and that some of the material can be washed off of the leaf surface. We know that they are photodecomposed in the laboratory but this is probably a slow reaction under natural conditions. We know that they do not appear to be volatile materials but will codistill by a phenomena similar to other insoluble lipophilic materials such as DDT. We would be glad to share this information we have collected on the chlorinated dioxin at the termination of the existing experiments and would be glad to collaborate on methodology with any country presently anticipating dioxin research.

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2,4,5-T Manufacture : Dioxin Formation

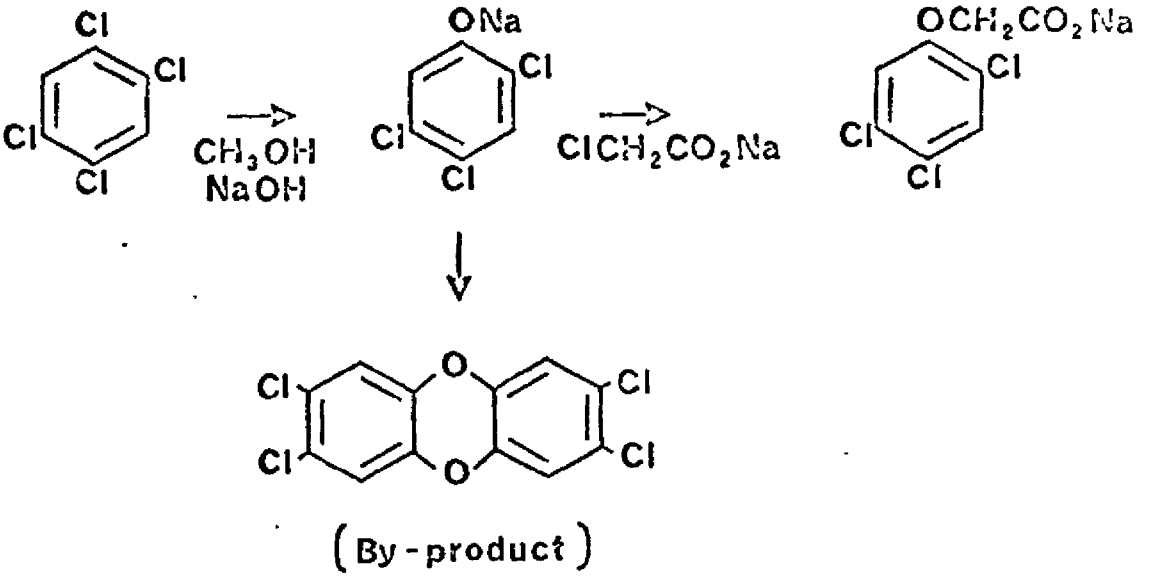

Figure 1.

DIOXIN SURVEY

PESTICIDE FAMILY	SAMPLES ANALYZED	CONCENTRATION RANGE PPM < .1	.1-1.0	1-10	> 10
PHENOXY	79	53	5	9	12
CHLOROPHENOLS	14	14	0	0	0
OTHER	10	9	1	0	0

Table 1.

CHLOROPHENOL SURVEY

DIOXIN	CONCENTRATION RANGE PPM			
	< .1	.1-10	10-100	> 100
TETRA	14	0	0	0
HEXA	2	5	6	0
HEPTA	2	3	4	5
OCTA	3	3	3	5

Table 2.

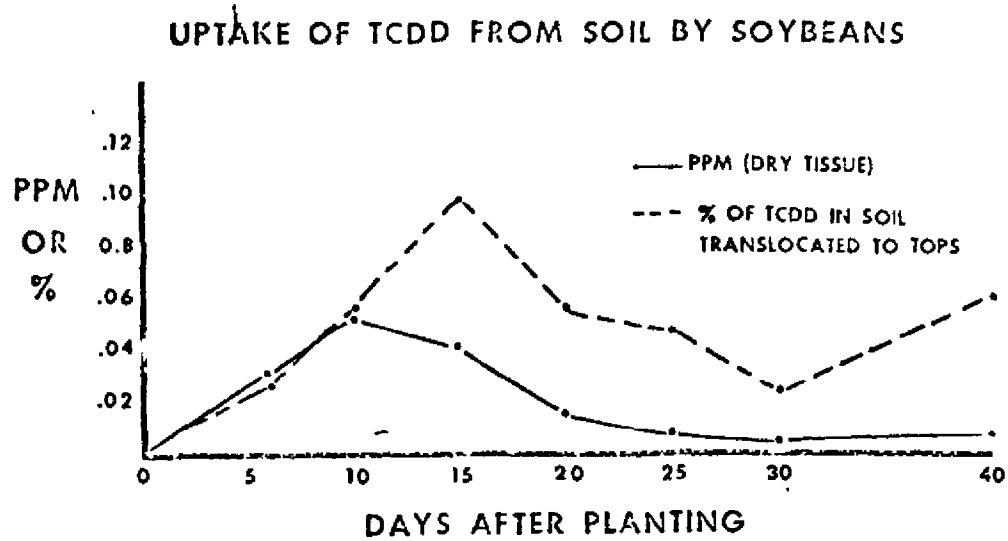
UPTAKE OF TCDD FROM SOIL BY SOYBEANS
PPM OR %
.12
.10
0.8
.06
.04
.02
PPM (DRY TISSUE)
% OF TCDD IN SOIL TRANSLOCATED TO TOPS
0
5
10
15
20
25
30
40
DAYS AFTER PLANTING

Figure 5.

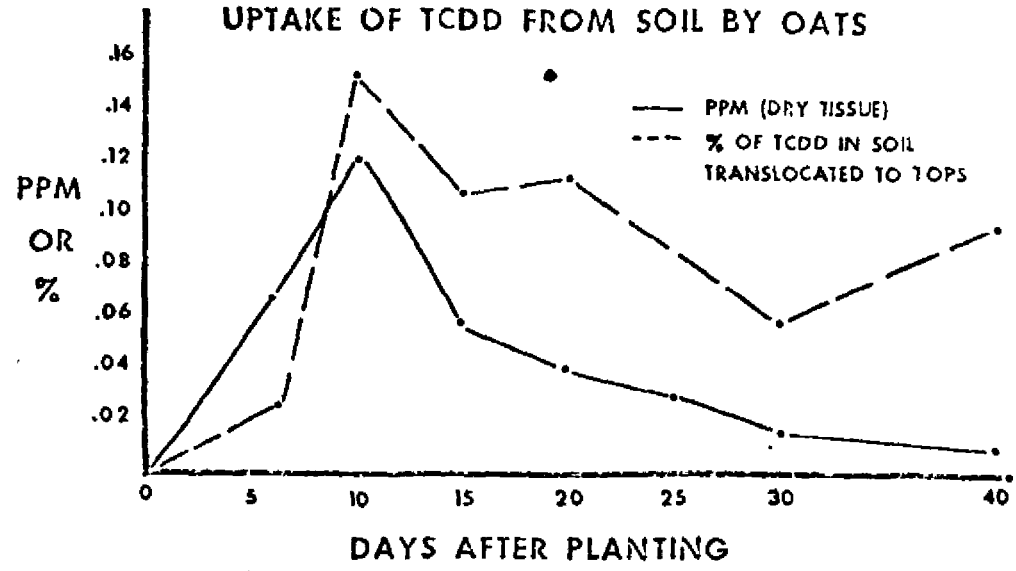
UPTAKE OF TCDD FROM SOIL BY OATS
PPM (DRY TISSUE)
% OF TCDD IN SOIL TRANSLOCATED TO TOPS
PPM OR %
.16
.14
.12
.10
.08
.06
.04
.02
0
5
10
15
20
25
30
40
DAYS AFTER PLANTING

Figure 3.

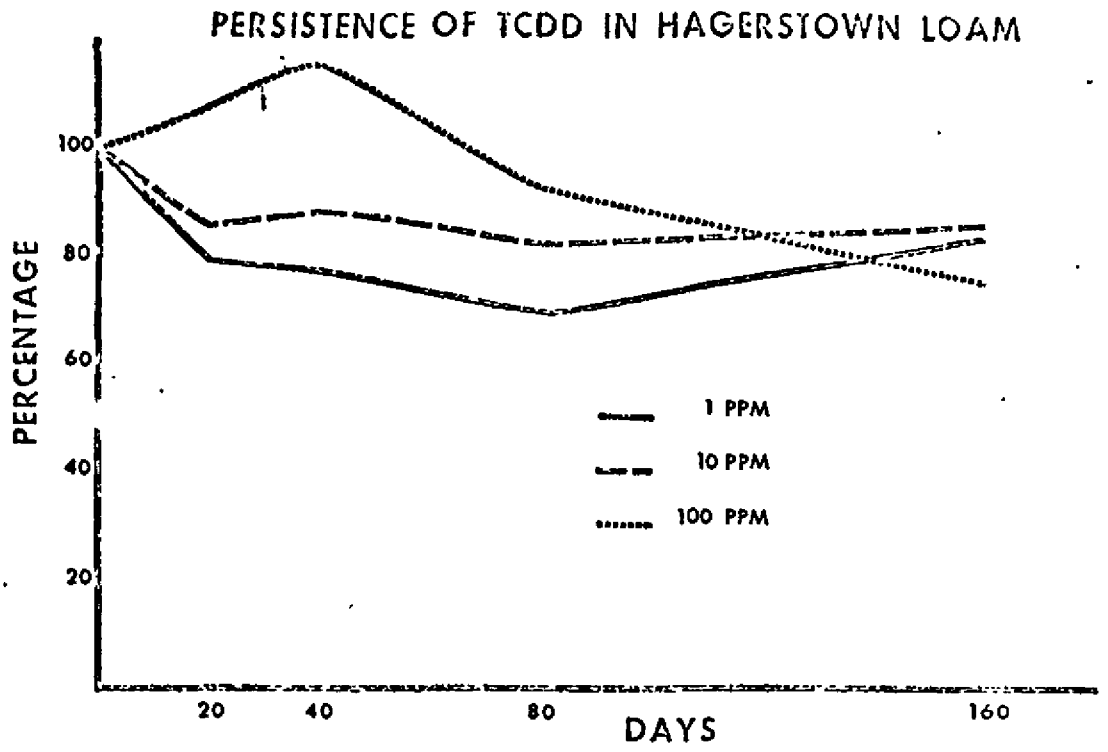
PERSISTENCE OF TCDD IN HAGERSTOWN LOAM
PERCENTAGE
100
80
60
40
20
1 PPM
10 PPM
100 PPM
20
40
80
160
DAYS

Figure 4.

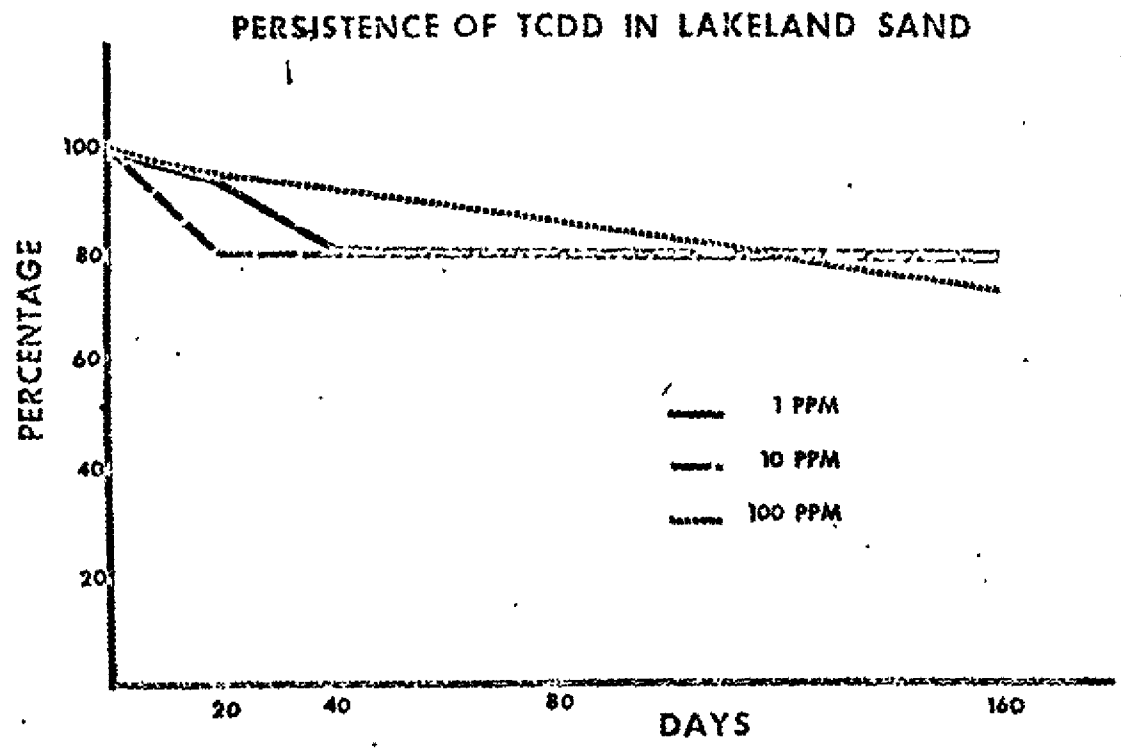

Figure 5.

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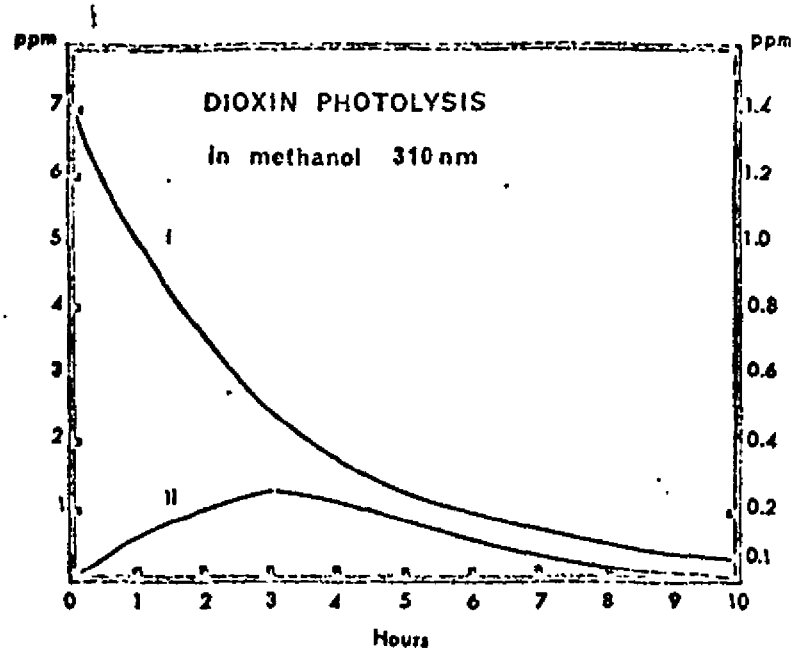

Left I 2,3,7,8 - Tetrachloro
Right II 2,3,7 - Trichloro

Figure 6.

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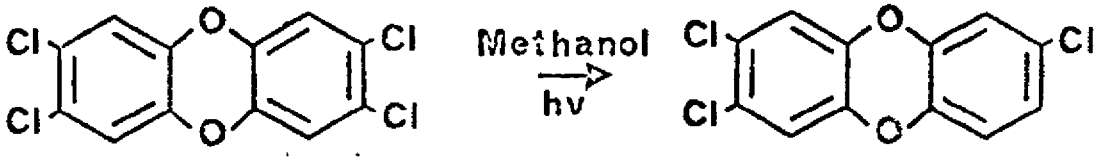
Dioxin Photolysis



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