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Model Ecosystem Determination of the Metabolic and Environmental Fate of Tetrachloro-DDT

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A potential hazardous waste site investigation was conducted by the Environmental Protection Agency (U.S. EPA 1980) to determine whether ground water, surface water, or area soils and sediments were contaminated as a result of waste water discharges or improper solid waste disposal practices of a pesticide manufacturer. One of the compounds disharged into the environment was 1,1,1,2-tetrachloro-2,2-bis(p-chlorophenyl)ethane, commonly referred to as tetrachloro-DDT. Unlike a great many of the DDT analogs, tetrachloro-DDT has come under only limited scrutiny, mainly because it was dismissed as having poor insecticidal properties (Browning et al. 1948; Lord 1948; Metcalf 1948; Rogers et al. 1953; Tahori 1955) relative to DDT and other analogs. Its metabolism in ingesting organisms, and degradative pathways in the environment have consequently been left uncertain.

This model ecosystem study was undertaken to examine the unanswered questions concerning the metabolic and environmental fate of tetrachloro-DDT. The relevance of this study pertains to disposal practices of pesticide manufacturers who use tetrachloro-DDT as a product precursor (e.g., in chlorobenzilate fabrication).

MATERIALS AND METHODS

Tetrachloro-DDT was synthesized via a photo-induced chlorination of DDT (Grummitt 1945). Radiolabeled (^{14}C) 1,1,1,2-tetrachloro-2,2-bis(p-chlorophenyl)ethane had a specific activity of 1.3047×10^3 dpm/µg, as determined by liquid scintillation counting. Radiopurity was determined by thin-layer chromatography (TLC) to be approximately 95%, with an unknown impurity of lesser polarity comprising the bulk of the remaining 5%. Non-radiolabeled tetrachloro-DDT had a purity of

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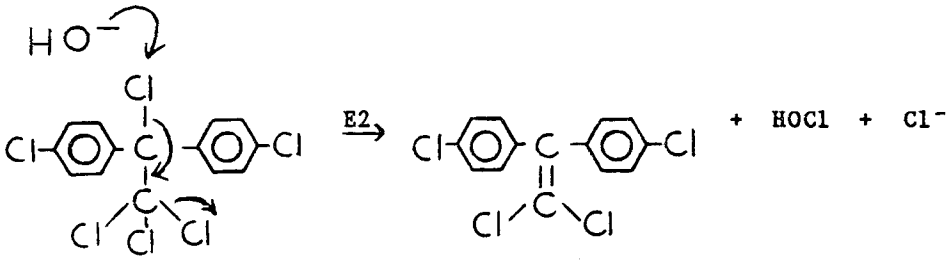
Table 1. ^{14}C-radiolabeled tetrachloro-DDT ecosystem analysis (5 mg starting material; 1.3047×10^3 dpm/µg): R_f values and amounts of parent compound and degradation products found in water and organisms.

		Parts per million (ppm) of tetrachloro-DDT equivalents				
	R_f	water	Oedogonium alga	Physa snail	Culex mosquito	Gambusia fish
total ^{14}C extractable		0.02311	26.65	6.41	9.97	3.95
Unknown1	0.60	ND	2.11	0.224	0.614	0.221
Unknown2	0.55	ND	5.93	1.03	1.05	0.830
DDE	0.50	0.00486	10.6	4.43	6.75	2.45
tetra-chloro-DDT	0.50	ND <0.00446	ND <1.76	ND <0.490	ND <0.895	ND <0.181
DDT	0.35	ND	0.192	0.141	0.428	ND
	0.28 smear		1.18			
DDD	0.19	0.00005	0.56	ND	0.141	ND
polar, (includ. dicofol)	0.04 to 0.00	0.0182	6.08	0.589	0.991	0.448
unextractable		0.0472	1.32	1.02	1.12	0.482

ND = not detected, GLC detection limits listed for tetrachloro-DDT

approximately 92% with impurities identified by gas-liquid chromatography (GLC) as DDE (approx. 3.5%), DDD (approx. 2.6%), and DDT (approx. 1.9%).

The Metcalf model ecosystem (Metcalf 1971, 1976) was used to monitor the transport of tetrachloro-DDT from the terrestrial to the aquatic environment. This methodology demonstrates pathways of biological accumulation through a variety of food chain organisms, while allowing qualitative and quantitative estimation of uptake levels, and metabolic products. The model ecosystem further provides information concerning the environmental fate and ecological magnification of



Tetrachloro-DDT DDE

Figure 1. Proposed mechanism of DDE formation from tetrachloro-DDT precursor.

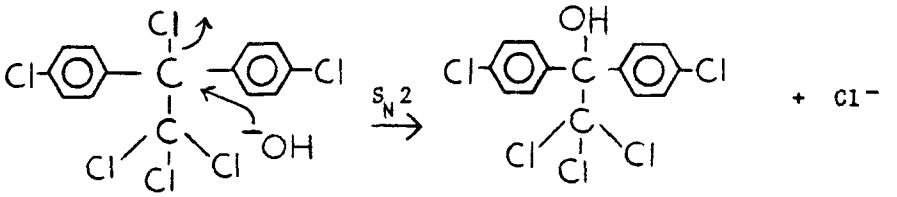
parent compound and products, while affording a quantitative indication of persistence and biodegradability.

Concentrated extracts derived from ecosystem organism homogenates were applied to fluorescent silica gel (Analtech Silicar 7GF, 250 μm) TLC plates; petroleum ether (Skelly-solve B) was used as the solvent. Tetrachloro-DDT could not, however, be separated from DDE with any of a number of TLC solvent systems tested. Instead, GLC on a 5% DC-11 (silicone high vacuum grease) stationary phase, with an 80/100 mesh, DMCS, acid-washed, Chromosorb W support (Abou-Donia 1974) was performed to distinguish between tetrachloro-DDT and DDE. The gas chromatograph (Hewlitt-Packard 5730A, electron capture detection, 3380A plotter-integrator) temperatures (degrees C) utilized for all runs were 250 for the detector and injector, and 190-250 at 2 degrees/min for the column oven. Nitrogen carrier gas flowed at 30 ml/min prior to the start of temperature programming, on a 6' x 0.25" silylated glass column.

Extract products from the ecosystem containing non-radiolabeled starting material taken through the standard work-up procedure, were identified and quantified via GLC using conditions identical to those described above. Lastly, a kinetic study to determine the hydroxide-induced rate of dechlorination (Metcalf and Fukuto 1968) of tetrachloro-DDT was also performed.

RESULTS AND DISCUSSION

The results of the model ecosystem evaluation using the ^{14}C-radiolabeled tetrachloro-DDT are given in Table 1. DDE is clearly the major metabolite and the ultimate environmentally recalcitrant degradative product of tetrachloro-DDT. Parent tetrachloro-DDT was not detected in any of the organism or water extracts. The maximum possible undetected levels, calculated from GLC



Tetrachloro-DDT Dicofol

Figure 2. Proposed mechanism of dicofol formation from tetrachloro-DDT precursor.

detection limits, are listed in Table 1. Formation of DDE from parent tetrachloro-DDT is postulated to occur via a biologically catalyzed E2 mechanism (Fig. 1), wherein, hydroxide ion initiates nucleophilic attack upon the 2-chlorine atom of tetrachloro-DDT. A combined inductive effect created by the two chlorophenyl groups and the trichlorocarbon moiety, produces an electron shift towards the carbon atom at the 2-chloro juncture, thus increasing the susceptibility of the 2-chlorine atom to nucleophilic attack. This chlorine atom is believed to leave in the form of hypochlorous acid, initiating a concerted mechanism whereby the remaining surplus electrons participate in double bond formation between the two ethane carbon atoms, and a 1-chloro atom is ejected as chloride ion, thus forming DDE.

Polar metabolites, most likely to be comprised largely of dicofol, also appear to be significant products of tetrachloro-DDT degradation. Dicofol is postulated to be formed via S_N2 replacement of the 2-position chloride ion with hydroxide ion, occurring by classical backside nucleophilic attack on the 2-carbon atom (Fig. 2). A major limitation to this pathway is realized, however, when considering the steric hindrance created by three vicinal bulky chlorine atoms. This factor appears to be quite inhibitory, keeping dicofol production well below that of DDE.

The unidentified non-polar impurity detected in the original synthesized radiolabeled tetrachloro-DDT (approximately 5%), hypothesized to be a previously denoted rearrangement product $(p\text{-(chlorophenyl)}CCl_2)_2$ (Tomar et al. 1974), appeared in all organism extracts (R_f=0.55), along with another low polarity compound (R_f=0.60) suspected to be a metabolite of the former, possibly $(p\text{-(chlorophenyl)}CCl)_2$. Two distinct peaks believed to represent these two compounds appeared on the GLC tracings of all radiolabeled organism extracts.

Table 2. Biodegradative index (BI) and ecological magnification (EM) for the tetrachloro-DDT ecosystems.

$$BI = \frac{[\text{polar metabolites}]}{[\text{non-polar metabolites}]}, \qquad EM = \frac{[\text{DDE}] \text{ in organism}}{[\text{DDE}] \text{ in water}}$$

	water	Oedogonium (alga)	Physa (snail)	Culex (mosquito)	Gambusia (fish)
^{14}C-Radiolabeled ecosystem:					
BI	3.65	0.295	0.101	0.110	0.128
EM	-	2181	912	1389	504
Non-radiolabeled ecosystem:					
EM	-	4284	4754	6776	1974

Table 3. Total radioactivity of ^{14}C-radiolabeled model ecosystem water, measured in parts per million (ppm) of tetrachloro-DDT equivalents.

Day of model ecosystem operation						
1	2	3	5	7	14	21
0.0304	0.0367	0.0429	0.0499	0.0438	0.0633	0.0440

ppm of tetrachloro-DDT equivalents in water

These same two peaks were conspicuously absent from a second set of ecosystem extracts acquired from non-radiolabeled starting material, leading to the conclusion that the compound exhibiting the highest R_f by TLC, was a metabolite of the original radiolabeled impurity. Finally, DDT and DDD were detected in a few extracts at very low levels.

Calculation of the ecological magnification indices based upon data obtained for the DDE metabolite, along with the biodegradability indices, are listed in Table 2. The total radioactivity measurements obtained from ecosystem water at the indicated intervals, are listed in Table 3.

Results from the model ecosystem evaluation using non-radiolabeled starting material are given in Table 4.

Table 4. Non-radiolabeled tetrachloro-DDT ecosystem analysis (50 mg starting material): GLC retention times and amounts of parent compound and degradation products identified in water and organisms.

	Retention time (min)	Parts per million (ppm) of tetrachloro-DDT equivalents				
		water	Oedogonium alga	Physa snail	Culex mosquito	Gambusia fish
identified total		0.0322	39.53	27.67	47.54	13.31
dicofol	5.09	0.0137	1.01	ND <1.18	2.08	0.89
DDE	8.39	0.0050	21.42	23.77	33.88	9.87
DDD	10.05	masked	7.01	3.90	7.11	2.55
DDT	12.14	0.0025	1.85	ND <2.22	4.47	ND <1.11
tetra-chloro-DDT	16.36	0.011	8.24	ND <4.56	ND <7.75	ND <2.28

ND = not detected, GLC detection limits listed beneath

The preponderance of DDE among the metabolites was even more evident than in the study using radiolabeled material. This increase in DDE, as well as other detectable metabolite levels, is probably a reflection of the ten-fold increase in initial parent material weight (50 mg vs. 5 mg) used in the non-radiolabeled study. Parent tetrachloro-DDT was detected this time in both algae and water extracts. Maximum possible levels of tetrachloro-DDT are given for the other organism extracts, based upon GLC detection limits. Dicofol was found at low levels in all but the snail extracts. Quantification of the ecological magnification indices for the non-radiolabeled ecosystem were again based upon data obtained for the DDE metabolite; they are listed in Table 2.

The model ecosystem determination that dechlorination to produce DDE represents the major route of tetrachloro-DDT decomposition was also arrived at in a microorganism-induced anaerobic degradation study (Beland et al. 1974). This supportive finding was achieved using simulated flooded soil conditions which showed a steady increase in DDE production from tetrachloro-DDT over a four week period.

Table 5. Second-order rate constants for dechlorination (k).

Compound	Temperature	k (liters/mole/sec)
tetrachloro-DDT	28 ± 0.5 degrees C	1.2×10^{-4}
tetrachloro-DDT	31 ± 0.5 degrees C	2.7×10^{-4}
DDT	31 ± 0.5 degrees C	7.0×10^{-2}

The kinetics study to determine the hydroxide-induced rate of dechlorination revealed that chloride ion liberation followed second-order kinetics for both tetrachloro-DDT and DDT; results are listed in Table 5. The rate obtained for DDT ($k = 7.0 \times 10^{-2}$ l/mole/s at 31 degrees C) agrees well with the literature value of 7.110×10^{-2} l/mole/s at 30.37 degrees C (Cristol 1945). Comparison of the results obtained for the two compounds at 31 degrees C, indicates that hydroxide induced dechlorination occurs approximately 260 times slower for tetrachloro-DDT than for DDT. This finding is consistent with the previously suggested E2 mechanism of hydroxide attack on the 2-position chlorine atom. As would be expected, attack at the electronegative chlorine atom is kinetically less favorable than attack at a hydrogen atom located in the same 2-position (i.e., DDT). Thin-layer chromatography of the hydrolysis product revealed that dicofol formation was not an important degradative pathway.

The results presented here indicate that the widely documented conclusions revealing the insidious nature of DDT environmental contamination also apply to the tetrachloro-DDT analog, since the major environmentally persistent product of degradation for the two compounds is the same.

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