Absorption and Elimination of Photodieldrin by *Daphnia* and Goldfish

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The absorption and accumulation of persistent chlorinated hydrocarbon insecticides DDT, [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane] and dieldrin, (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene), by aquatic organisms at low trophic levels has been shown to result in ecological hazards to the organisms at the top of the food chain especially fish and fish eating birds (RUDD 1964; WOODWELL et al. 1967). Some fresh water fishes have been shown to eliminate these insecticides on transfer to insecticide-free water. There are variations in the rate of elimination of the same insecticide by various fish or of various insecticides by the same fish. For example, transfer to clean water after a 19-hr exposure of the goldfish (Carassius auratus), bluegills (Lepomis macrochirus) and the white catfish (Ictalurus catus) to sublethal levels of radioactive DDT, dieldrin and Lindane (hexachlorocyclohexane, gamma isomer), resulted in 90 per cent elimination, of Lindane and dieldrin in, respectively, two days and two weeks. However, only 50 per cent of the accumulated DDT was eliminated in 32 days (GACKSTATOR and WISE 1967). The goldfish fed on ^{14}C-dieldrin in food for 64 days eliminated dieldrin similarly on transfer to clean water (GREZENDA et al. 1972).

Aquatic invertebrates, such as Daphnia, seem to differ from fish in that a steady state of absorption was not reached within 6-days of experimental exposure to dieldrin (KHAN and KHAN 1974). Unlike fish, they kept absorbing the insecticide with time; but the transfer of pre-exposed Daphnia to insecticide-free water, resulted in up to 96% elimination of the absorbed dieldrin (STANTON et al. 1973).

Since photodieldrin [3,4,5,6,6,7-hexachloro-12-oxahexacyclo (6.5.0.0^{2,10}. 0^{3,7}. 0^{5,9}. 0^{11,13}) tridecane], the "terminal residue" (EAGON 1969) of extensively used aldrin and dieldrin is not only more toxic to aquatic animals than aldrin and dieldrin (KHAN et al. 1973), but also extremely stable in environment (REDDY and KHAN 1974), we haye investigated the absorption and elimination of this sunlight conversion product of dieldrin in the freshwater flea, Daphnia pulex and the goldfish, Carassius auratus.

Bulletin of Environmental Contamination & Toxicology,
Vol. 13, No. 5 © 1975 by Springer-Verlag New York Inc.

Materials and Methods

The freshwater alga, Ankistrodesmus spiralis and freshwater flea, Daphnia pulex are maintained in this laboratory. The goldfish were purchased from Auburndale Goldfish Co., Chicago. They were acclimated in laboratory for 5-7 days before experimentation. ^{14}C-photodieldrin (specific activity, 4.0mci/mM) was prepared from ^{14}C-dieldrin (ROSEN and CAREY 1968); photodieldrin was 99%+ pure as checked by thin-layer and gas chromatography.

Absorption of photodieldrin (PD) by algae: Algae were exposed (21,000 cells/ml), in 1 liter of Chu-10 medium (CHU 1942), to 3.33 parts per billion (ppb) of ^{14}C-photodieldrin (total radioactivity = 94,500 DPM) for 1,2,4,8 and 24 hours. After the exposure the cells were filtered on 0.45 μ size millipore filter paper (Type HA, 0.45 μ; Millipore Corp.). The pellet was solubilized in 0.5 ml of Solvene-100 (Packard Instruments, Chicago) and then counted in 10 ml of scintillation fluid (REDDY and KHAN 1974) on a Packard Tricarb Spectrometer (Model 3390) equipped with the absolute Activity Analyzer (Model 544).

Absorption and elimination of photodieldrin by Daphnia: One hundred daphnids were exposed to 100 ml of 3.33 ppb of ^{14}C-photodieldrin for 24 hours without any food. In another experiment algae (1,000 cells/ml) were exposed for 24 hours to 3.33 ppb of ^{14}C-photodieldrin and then 100 daphnids added to the medium and the exposure continued for 24 hours. The pre-exposed daphnids were removed from the medium by filteration on a fine mesh nylon screen and then kept in clean water for 24,48 and 96 hours. At each time interval they were removed and gound in a miniature Potter-Elvehjam ground glass homogenizer using hexane-benzene (1:1). The homogenate was extracted twice with 10 ml of solvent and the radioactivity counted. In the case of the non-radioactive photodieldrin, analysis was carried out by gas chromatography (KHAN and KHAN 1974).

Absorption and elimination of photodieldrin by goldfish: These Goldfish weighing 3-5 g each were continuously exposed to 20 ppb photodieldrin (1 fish/liter) for 1,2,4, and 6 days. In another experiment, after the 24-hour exposure when the steady state was reached (KHAN and KHAN 1974), the fish were transferred to clean water and both water and the fish analyzed periodically for the insecticide.

Each fish was ground in a mortar pestle using anhydrous sodium sulfate (WILSON 1966, MUNSON 1972). The insecticide was extracted in a Soxhlet Apparatus for 24 hours using benzene-hexane (1:1). The extract was con-

centrated by evaporation in a gentle stream of air and then analyzed by gas chromatography. A Packard Model 7300 gas chromatograph outfitted with a ^{3}H-electron capture detector and a glass column (1/8' x 6', 3 per cent QF_1 on DMCS-treated Gas Chrom Q, 60/80 mesh) was used. The conditions were: temperature 0°C: inlet 210, column 200, detector 210; nitrogen flow rate: 30 ml/min (KHAN and KHAN 1974).

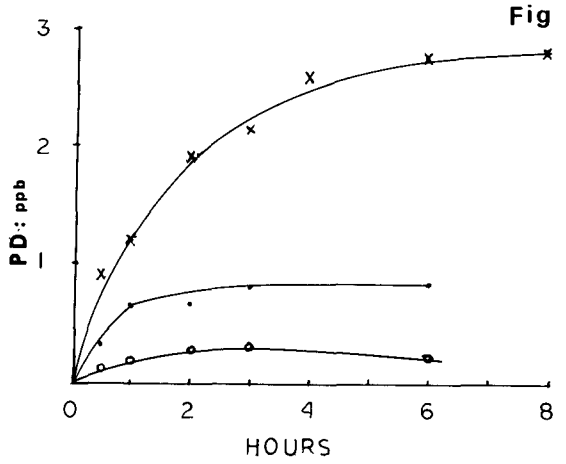
All above experiments were run in replicates of three and each experiment repeated twice. The average of values in both experiments were used. The dry weight of algae and daphnids was determined on a microbalance by desiccating them under vacuum until they weighed constant.

Results and Discussion

The rate of absorption of PD by algae, A. spiralis (21,000 cells/ml) appears to be linear up to 8 hours (Fig. 1). However, at 1,000 cells/ml the algae become saturated within 4 hr with PD and within 2 hr with dieldrin (STANTON et al. 1973). The A. amilloides (1,000 cells/ml) becomes saturated with dieldrin within 4 hr (Fig. 1). Another freshwater alga, Scenedesmus obliquus (several hundred thousand cells/ml), has been reported to become saturated with dieldrin only after 1.5 days (REINERT 1972). However, at same cell concentrations of A. spiralis, after an 8-hr exposure, only 5.3 nanogram photodieldrin/g dry wt was absorbed as compared with 12.6 nanogram of dieldrin/g dry wt. This indicates that photodieldrin is absorbed and accumulated at a slower rate than dieldrin. RICE and SIKKA (1973) using marine algae (several hundred thousand cells/ml) found that the same species absorbed dieldrin and DDT at different rates, an observation that we have confirmed with A. amilloides (NEUDORF and KHAN 1975). The differences in the accumulation of the two isomers dieldrin and photodieldrin by the same alga may be related with the differences in their water solubility. Dieldrin which is more lipophilic than photodieldrin (KHAN and KHAN 1974) may partition faster in favor of living matter than photodieldrin. However differences in rates of absorption of various insecticides by the same alga are not uncommon (RICE and SIKKA 1973).

The absorption of photodieldrin by Daphnia directly from water is less than that at same PD concentration in the presence of algae presaturated with PD (Table 1). Continuous exposure of Daphnia to photodieldrin resulted in its continuous absorption during the 6-day experiment period (Fig. 2). However, the transfer of pre-exposed (24 hr) daphnids to insecticide-free water resulted in about 50 and 70 per cent elimination of photodieldrin, respectively in 4 and 7

days. The elimination of dieldrin absorbed by daphnids in similar experiments was much faster, i.e. 95 per cent elimination in 4 days (KHAN et al. 1974, STANTON et al. 1973, RIO, unpublished). The daphnids are thus capable of eliminating dieldrin and photodieldrin if their environment is decontaminated.



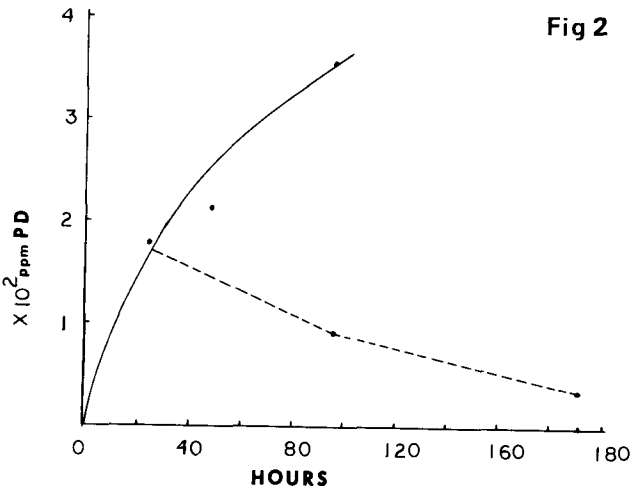


Fig. 1. Rate of absorption of photodieldrin (x--x, 21,000 cells/ml) by the freshwater alga, A. spiralis. Absorption of dieldrin by A. amilloides (o--o, 100 cells/ml; ●--●, 1,000 cells/ml) is also shown to indicate differences in rates of absorption of these insecticides.

Fig. 2. Absorption of photodieldrin by Daphnia during its continuous exposure to photodieldrin (solid lines) as well as the elimination on transfer to clean water (broken lines) after the 24-hr exposure.

Table 1. Absorption of photodieldrin by Daphnia in the presence of contaminated and uncontaminated food (algae).

Time of Exposure hr.	µg PD/g dry wt	
	uncontaminated food	contaminated food*
12	120	160
24	180	230
36	290	320

*1,000 algal cells/ml pre-exposed to 3.33 ppb photodieldrin for 24 hr before adding Daphnia

The continuous exposure of the goldfish to 20 ppb photodieldrin for 6 days showed that the steadv state equilibrium was reached within 24 hours, after which the insecticide concentration started declining slowly (Fig. 3). On transferring the fish, after the 24-hr exposure, to clean water with food resulted in initial rapid elimination of photodieldrin in 24 hours which slowed down subsequently. Similar elimination of dieldrin, DDT and Lindane has been reported for goldfish, bluegills and white catfish. About 90 per cent of the absorbed Lindane and Dieldrin were eliminated by the goldfish in 2 and 15 days, respectively, while only 50 per cent of the absorbed DDT was excreted in 32 days (GACKSTATER and WISE 1967; GREZENDA et al. 1972).

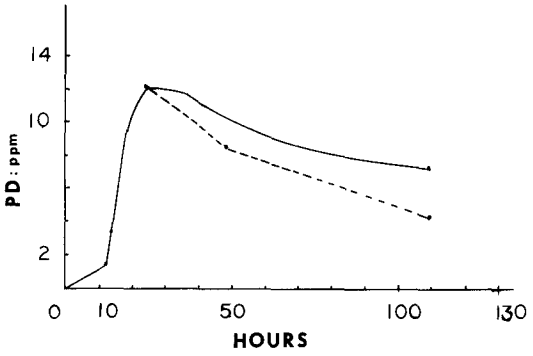


Fig. 3. Absorption and retention of photodieldrin during continuous exposure (solid lines) of goldfish to photodieldrin and its elimination on transfer of fish to clean water (broken lines) after 24 hours.

Summary

The alga, Ankistrodesmus spiralis, becomes saturated with photodieldrin in 8 hours. The Daphnia whether contaminated by feeding on the algae saturated with photodieldrin or by directly absorbing this insecticide eliminate about 50 per cent of the absorbed photodieldrin in 4 days which increases up to 70 per cent in 7 days. However, continuous exposure of daphnids to photodieldrin results in increased absorption and accumulation of this insecticide. Transfer of the goldfish contaminated with photodieldrin to clean water results in initial elimination in 24 hours whose rate is then subsequently reduced. These two organisms are thus capable of eliminating their body residues of photodieldrin, the "terminal residue: of dieldrin, if their environment is decontaminated.

Acknowledgements

This research was supported by a grant, ES-00808, from National Institute of Environmental Health Sciences.

References

CHU, S.P. J. Ecol. 30, 284 (1942).
EPIFANIO, C.E. Marine Biol. 19, 320 (1973).
GACKSTATTER, J.H. and C.M. WEISS. Trans. Amer. Fish. Soc. 96, 301 (1967).
GREZENDA, A.R., W.J. TAYLOR, and D.F. PARIS. Trans. Amer. Fish. Soc. 101, 985 (1972).
EAGON, H. J. Assoc. Off. Anal. Chem. 52, 299 (1969).
KAHN, H.M. and M.A.Q. KHAN Archiv. Environ. Contamin. Toxicol. in press (1974).
KHAN, M.A.Q., H.M. KHAN, D.J. SUTHERLAND and J.D.ROSEN. In: Survival in Toxic Environment. (M.A.Q. KHAN and J.P. BEDERKA,JR.,eds.). Acad. Press. New York, (1974).
REDDY, G. and M.A.Q. KHAN. Bull. Environ. Contam. Toxicol. (submitted for publication).
REINERT,R.E. J. Fisher. Res. Board Canada. 29, 1413 (1972).
RICE,C.P. and H.C. SIKKA. J. Agri. Food Chem. 27, 148 (1973).
RIO, D.F. Unpublished data, this laboratory.
RUDD, R.L. Pesticides and Living Landscape, Univ. of Wisconsin Press, Madison, Wis. 320 pp (1964).
STANTON, R.H., H.M. KHAN, D.F. RIO and M.A.Q. KHAN. 166th annual meeting of Amer. Chem. Soc. Chicago, Aug. 26-31 (1973).
WOODWELL, G.M., C.F. WURSTER and P.A. ISSACSON. Science 156, 821 (1967).