# Fate of Photodieldrin[1] Under Various Environmental Conditions

by GUNDA REDDY and M. A. Q. KHAN
*Department of Biological Sciences*
*University of Illinois at Chicago Circle*
*Chicago, Ill. 60680*

There is a great concern about the persistence of some widely used pesticides, especially the chlorinated hydrocarbons such as DDT and dieldrin whose residues are commonly present in soils, water, plants, and terresterial and aquatic animals (cf. EDWARDS 1970, MATSUMURA 1972 and MENZIE 1972). The residues of aldrin and dieldrin under sunlight, form photoaldrin and photodieldrin, respectively. Photoaldrin is further converted to photodieldrin (cf. KHAN et al. 1974). Photodieldrin (PD), the metabolic product of photoaldrin or dieldrin is considered as their "terminal residue" (EAGON 1969). However, the fate and the persistence of photodieldrin in environment has not been reported. Therefore we conducted some preliminary experiments to learn more about the persistence of photodieldrin under various environmental conditions.

## Materials and Methods

$^{14}$C-photodieldrin was prepared by ultraviolet irradiation of $^{14}$C-dieldrin (Amersham/Searle Corporation, specific activity 79 mCi/mM). The final purified product (specific activity 4.3 mCi/mM) was essentially free of interferring compounds as tested by electron capture gas liquid chromatography (GLC) and thin layer chromatography (TLC) followed by X-ray autoradiography (REDDY and KHAN 1974).

### Treatment and Extraction of Bean Leaves

Bush Red Kidney beans (*Phaseolus vulgaris*) grown in the greenhouse for 10 to 15 days were used. Primary leaves weighing about 700 to 800 mg were treated with 2 $\mu$g $^{14}$C-photodieldrin (12,800 dpm) in 50 $\mu$l methanol by spreading on their upper surface with the help of a Hamilton microsyringe (IVIE and CASIDA 1971). Two primary leaves were used in duplicates. Immediately after the application of the insecticide, the

[1] For chemical formulae see KHAN et al. (1973)



Bulletin of Environmental Contamination & Toxicology,
Vol. 13, No. 1 © 1975 by Springer-Verlag New York Inc.

plants were placed 12" distance under light (110-125 volts, 60 cycles) in the laboratory (temp 24 ± 1°C) for different lengths of times. In another set of experiments, treated plants were kept in sunlight in the month of July 1973. They were watered every alternate day. Control plants were treated with methanol only.

Chlorinated hydrocarbon insecticides are quite insoluble in water and they form residues on or in a variety of crops, with a limited degree of penetration into the plant (CASIDA and LYKKEN 1969). To extract PD from leaves, two methods, 1) leaf washings and 2) leaf extraction, were employed. The former was employed to find out how much PD or its metabolite(s) were present on leaf surfaces. The later method was used to determine the amount of absorbed PD or its metabolite(s) in the tissue of the leaf.

After different time intervals, the leaves were cut at the base of the petiole and immediately soaked in a beaker containing 10 ml ether to extract photodieldrin (IVIE and CASIDA 1971). The leaves were again washed two times with 5 ml ether. The ether washes were pooled and evaporated to dryness in a gentle stream of air. The residue was re-dissolved in one ml of acetone, which was used for analyses for liquid scintillation and TLC autoradiography (REDDY and KHAN 1974). The solvent system benzene:ethyl acetate (3:1) was used to develop the TLC plates. In order to check the absorbed photodieldrin or its metabolites(s) in the tissue of the leaf; the leaves were extracted, after ether washings, as follows. The leaves were homogenized with sand and anhydrous sodium sulphate in a morter-pestle with about 10 ml of methanol. The homogenate was filtered over Whatman No. 1 filter paper by suction. The residue was again extracted with 10 ml of methanol and filtered. The combined extracts were filtered over anhydrous sodium sulphate to remove traces of water. The filtrate was used for liquid scintillation and for TLC autoradiography.

Bean leaves treated with $^{14}$C-PD as described earlier, were also stored in clean plastic bags in the freezer at -20°C, soon after the treatment. At different times 0 hr and 10, 20, 30 and 45 days the $^{14}$C-PD present in leaves was extracted and analyzed as described earlier. All experiments were carried out in duplicate.

Treatment and Extraction of Algae

Freshwater algae, Ankistrodesmus spiralis (27,800 cells/ml) were taken in 250 ml of Chu-10 medium (CHU

1942) in one liter conical flasks. $^{14}$C-photodieldrin (16,400 dpm/0.66 µg/200 µl acetone) was added to these cultures with the help of a Hamilton microsyringe. Controls were run similarly without algal cells. During the exposure period, the cultures were continuously bubbled with air and mixed thorougly by shaking the flasks at least twice a day to avoid sedimentation or aggregation of algal cells. The level of water was maintained by adding required amount from time to time. After 7, 15 and 30 days the cells were harvested by centrifuging at 17,600 xg for 15 minutes. The algae and liquid culture medium were extracted for analyses.

Algal cells were homogenized with sand in a morter with a pestle using about 10 ml of acetonitrile and 5 ml of water. The homogenate was filtered through suction. The residue was re-extracted with acetonitrile and water and then filtered. The combined filtrate was transferred to a clean separatory funnel using about 50 ml of chloroform. About 10 ml of distilled water was added to separatory funnel and mixed thoroughly by shaking. The chloroform layer was drained into a clean separatory funnel and washed again with 10 ml of distilled water. The water phase was reextracted with 20 ml of chloroform to ensure complete extraction. The combined chloroform extract was filtered through anhydrous sodium sulfate. The filtrate was evaporated to dryness and the residue transferred to a test tube with acetone and used for liquid scintillation counting and TLC analysis.

The liquid culture medium was extracted two times with 100 ml benzene followed by 100 ml ethyl ether. The benzene and ether extracts were pooled and evaporated to dryness on a rotary flash evaporator. The residue was taken in acetone which was used for liquid scintillation counting and TLC analysis to find out if any metabolic products were formed in the culture medium during one month experimental period.

After extraction of the liquid culture medium, its aliquots were analyzed by liquid scintillation using emulsifier Insta-Gel (Packard Instrument, Inc.) in order to determine the amount of PD or its metabolites dissolved in water. No attempt was made to analyse, if any, hydrophilic metabolites present in the aquous phase by TLC.

Exposure of Silica-Gel TLC Plates

Pre-coated silica gel F-254 (0.25 mm thick) plates (E. Merck of Darmstadt-Germany) were employed to study the persistance of $^{14}$C-PD under various conditions. $^{14}$C-PD (12,800 dpm/2 µg/100 µl acetone)

was spotted on plates. The plates were kept under U.V. light (Ultraviolet-products, Inc., 115 volts, 60 cycles), at 12" distance, temp. 24 ± 1°C; room light (24 ± 1°C); and in the dark, in a refrigerator (4°C) for different periods of time. The plates were developed in solvent system, benzene:ethyl acetate (3:1), and exposed for 25 to 30 days to No-Screen X-Ray film. After developing the film, the corresponding darkened areas were scrapped and the radioactivity counted. All experiments were carried out in duplicates.

## Results and Discussion

### Persistance of PD on Bean Leaves in Sunlight and Laboratory Light

The results of the persistance of $^{14}$C-PD on bean leaves under laboratory light and sunlight are presented in Fig. 1. The total recovery of $^{14}$C-PD at 0 hour was about 100 percent. However, as the duration increased, the $^{14}$C-PD slowly but steadily dissipiated on leaf surface under both conditions. The analysis of ether washings of the leaf showed that loss of PD from the bean leaf surface in laboratory and in sunlight was about 50% in one day and 85 to 90% in 8 days. The loss of PD on the surface of the leaf could be due to its volatilization, as well as its absorption, translocation or degradation by the plant.

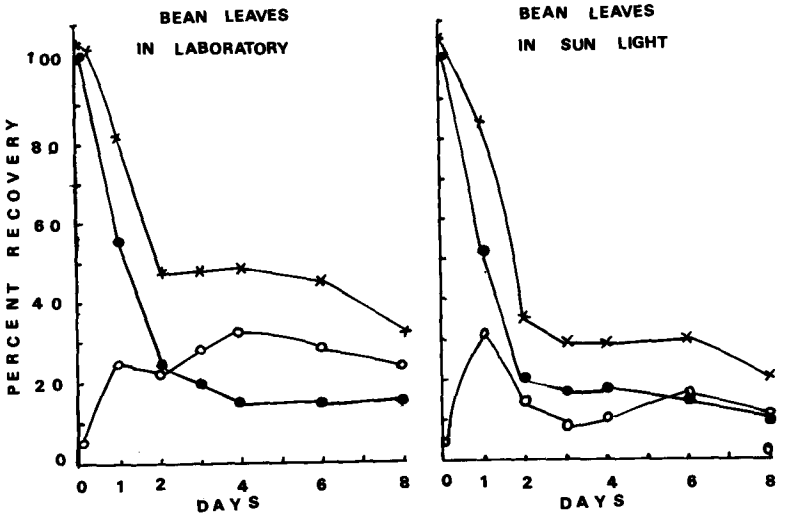


Fig. 1. Behavior of $^{14}$C-photodieldrin on bean leaves in the laboratory and sunlight. Percent radioactivity recovered in leaf washings (●—●), leaf extracts (○—○), total of both (x—x).

However, the analysis of the leaf extract showed (Fig. I) that PD was indeed absorbed into the leaf which could not be extracted by simple rinsing with ether. The recovery of absorbed PD in the leaf (i.e. in leaf extractions) kept in the laboratory at 0 hour, 4 days and 8 days, was about 5%, 33% and 17%, respectively. Similar trend of recovery of PD in the bean leaves kept in sunlight was observed but however with a low degree of recovery. The decrease in the recovery of $^{14}$C-PD during the 8-day period suggests that PD is either metabolized by plant or volatilized from the surface. The volatilization or metabolism of PD seems to be more on bean leaves exposed to sunlight as compared with the leaves exposed to light in the laboratory. This was further confirmed by the analyses of the leaf washings, and leaf extracts by TLC followed by autoradiography. No metabolic products were detected in ether washings of bean leaves kept in laboratory or sunlight. However, a PD metabolite was observed in leaf extracts kept in sunlight for 2 to 4 days. The Rf values of PD and its metabolites were 0.38 and 0.48, respectively. No metabolite could be detected in the leaves exposed to light in the laboratory. This suggests that the metabolite recovered in the leaf extracts might have formed either due to sunlight energy (or ultraviolet rays) or due to metabolic conversion by the plant. No metabolic product was detected in the extracts of leaves exposed to sunlight for 6 or 8 days. This could be due to further metabolism or volatilization. The above results show that about 35% and 20% of the applied $^{14}$C-PD was present in bean leaves exposed in week days to laboratory light and sunlight, respectively.

Stability of $^{14}$C-PD on Bean Leaves in the Freezer

$^{14}$C-PD was considerably stable on the bean leaves when stored for 45 days. The analysis of leaf washings and leaf extractions (organic phase) by liquid scintillation counting showed that about 80% of the total PD could be recovered after freezing for 10 to 45 days (Fig. 2). The 20% loss in 1 1/2 month could be due to volatilization and water solubility of PD. The loss of photodieldrin due to volatilization seem to be very slow when compared with that of aldrin and dieldrin. LICHTENSTEIN et al. (1968) showed that the applied $^{14}$C-aldrin and $^{14}$C-dieldrin disappeared rapidly from the sterile nutrient agar covered with petri dishes. Half of the applied aldrin volatilized from the agar during the first day of incubation, while dieldrin volatilized more slowly and at constant rate. No detectable metabolites were found in the leaf washings or leaf extractions (organic phase) when analyzed by TLC followed by autoradiography.

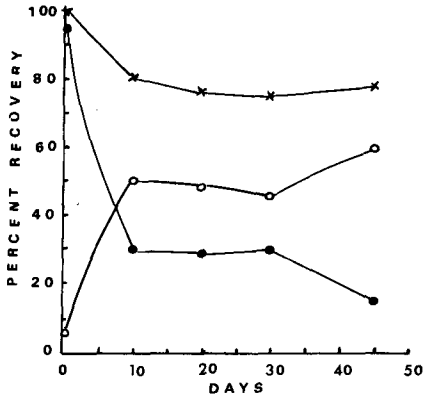


Fig. 2. Stability of $^{14}$C-photodieldrin on bean leaves stored in the freezer. Percent radioactivity recovered in leaf washings (●—●), leaf extracts (o—o); total of both (x—x).

Persistance of PD in Algae

$^{14}$C-PD, under laboratory conditions, was persistent up to 30 days in the water (100% recovery) with and without freshwater algae, Ankistrodesmus spiralis (Table 1). About 40% of the added PD was adsorbed or absorbed by the algal cells during the 30-day exposure. There was no change in accumulation of PD in algal cells with time, probably this might be due to their saturation, which occurs within 4 hrs (KHAN and KHAN 1974). The total recovery of PD from algae, and water was between 90 to 100% as determined by liquid scintillation counting. Analysis of organic phase of algae and water extract by TLC showed no detectable metabolites. On the basis of the total recovery of PD and the analysis of extracts by TLC, it can be said that PD appears to be highly persistent or stable in water under these environmental conditions. Although in our preliminary experiments we did not attempt to analyze metabolic products in aquous phase.KARTE (1970) found 2 hydrophilic metabolites (unidentified) of $^{14}$C-photodieldrin in the presence of microorganisms, Aspergillus flavus and Penicillium notatum.

Stability of $^{14}$C-PD on Silica Gel Plates Under U.V. Light, Artificial Light and in Dark

Results of the stability of PD under U.V. light, laboratory light and in dark (refrigerator) are shown in figure 3. About 90 to 95% of the applied PD was recovered from TLC plates stored in the refrigerator or kept in laboratory light for one month. No metabolic products were detected under these con-

TABLE I

Persistance of $^{14}$C-Photodieldrin in Water and Algae

| Experiment | Percent Recovery of $^{14}$C-Photodieldrin* | | | |
|---|---|---|---|---|
| | Algae cell Extract | Water Extract | Water | Total Recovery |
| 7 days | | | | |
| control | --- | 100 (164) | 5 (8) | 105 |
| sample | 37 (61)** | 54 (88) | 5 (8) | 96 |
| 15 days | | | | |
| control | --- | 97 (159) | 3 (4) | 100 |
| sample | 38 (62) | 46 (75) | 5 (8) | 89 |
| 30 days | | | | |
| control | --- | 103 (169) | 8 (13) | 111 |
| sample | 43 (71) | 45 (74) | 4 (9) | 92 |

* Average values of two replicates.

**Values in parenthesis show the x10$^2$ dpm recovered from total 16,400 dpm.

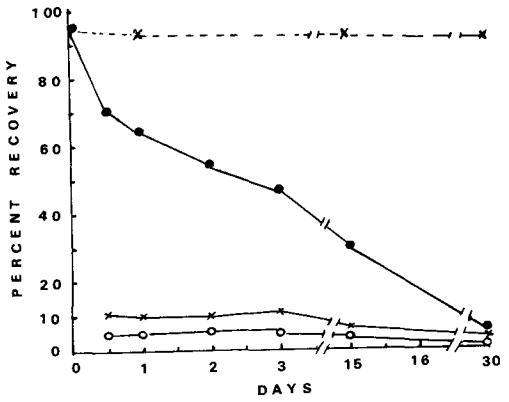


Fig. 3. Recovery of $^{14}$C-photodieldrin from silica gel F-254 plates exposed to laboratory light or kept in dark (x--x), and U.V. light (●—● photodieldrin; x—x metabolite I, ○—○ metabolite II).

ditions. However, under U.V. light PD formed two metabolic products. Metabolite I which stayed at origin (Rf = 0.00) and Metabolite II (Rf = 0.40) in the solvent system used. The Rf value of PD was 0.48. The above metabolites were formed within 12 hours of exposure to U.V. light. Our results are in agreement with BENSON (1971) who showed the formation of two metabolic products from PD under U.V. light-irradiation in 68 hours. The percent recovery of PD after 12 hours of exposure to U.V. light was about 70 which gradually decreased to 5 in 30 days. The percent decrease in the recovery of Metabolite I was from 11 to 4 and Metabolite II from 5 to 0.4.

The above results clearly show that as in case of other insecticides the persistance of PD varies with the type of substrate to which chemical is applied, the amount, formulation, method of application, and with various extrinsic factors like temp, light, etc. HARRISON et al. (1967), working with various insecticides, showed that application of emulsifiable concentrates were more persistant than those applied as dispersible powders on foliage. Insecticides, DDT and dieldrin being more persistant, their residues took 11 weeks to fall to below 5% of the initial deposit.

Photodieldrin seems to be highly persistent in algal cultures while on bean leaves, its residues were only 20 to 30% of the initial deposit. This may be due to difference between the rate of penetration and metabolism. BEARD and WARE (1969) working with endosulfan found more residues in beets than in bean, however, the penetration into bean leaves was greater than in beets. Our continued interest in identification and evaluation of metabolic products of "terminal residue"--photodieldrin, will help to shed more light on its possible ecological hazards.

## Acknowledgement

This study was supported by a grant ES00808 from National Institute of Environmental Health Sciences. We are thankful to Mr. James McCorkle, for providing bean plants.

## References

BEARD, J.E. and G.W. WARE. J. Agri. Food Chem. 17, 216 (1969).
BENSON, W.R. J. Agri. Food Chem. 19, 66 (1971).
CASIDA, J.E. and L. LYKKEN. Ann. Rev. Plant Physiol. 20, 607 (1969).
CHU, S.P. J. Ecol. 30, 284 (1942).
EAGON, H. J. Assoc. Off. Anal. Chem. 52, 299 (1969).

EDWARDS, C.A. Persistent pesticides in the environment CRC press. The Chemical Rubber Co. (1970).
HARRISON, R.B., D.C. HOLMES, J. ROBURN and J.O.G. TATTON. J. Sci. Fd. Agri. 18, 10 (1967).
IVIE, G.W. and CASIDA, J.E. J. Agri. Food Chem 18, 410 (1971).
KARTE, F. J. Assoc. Off. Anal. Chem. 53, 187 (1970).
KHAN H.M. and M.A.Q. KHAN. Archiv. Environ. Cont. Toxicol. in press (1974).
KHAN, M.A.Q., R.H. STANTON, D.J. SUTHERLAND, J.D. ROSEN and N. MITRA. Archives Environ. Cont. Toxicol. 1: 159 (1973).
KHAN, M.A.Q., H.M. KHAN, D.J. SUTHERLAND and D.J. ROSEN. Survival in toxic environment. M.A.Q. KHAN and J.P. BEDERKA, Eds. New York, Academic Press 1974. In press.
LICHTENSTEIN, E.P., J.P. ANDERSON, T.W. FUHREMANN, and R.K. SCHULZ. Science, 159, 1110 (1968).
MATSUMURA, F. Environmental toxicology of pesticides F. MATSUMURA, G.M. BOUSH and T. MISTO, eds. New York, Academic Press 1972. p. 33.
MENZIE, C.M. Ann. Rev. Entomol. 17, 199 (1972).
REDDY, G. and M.A.Q. KHAN. Submitted for publication. 1974.