# Formation of "Photodieldrin" by Microorganisms

Abstract. *Photodieldrin, previously reported as the major conversion product of dieldrin by sunlight, was found among the metabolic products of dieldrin among microorganisms isolated from various environments including soil, water (Lake Michigan), rat intestines, and rumen stomach contents of a cow.*

One of the major concerns of environmental toxicologists has been the effects of persistent pesticides on our environment. Perhaps among the most important aspects of pesticidal pollution is the formation of stable "terminal residues" (*1*) of the original pesticide. Such metabolic formation has been known to take place mainly by the action of microorganisms and sunlight (*2*). Studies on stable terminal residues are important since such work should uncover the actual nature of pesticidal pollution. An excellent example was the discovery (*3*) that TDE (DDD, 1,1-dichloro-2,2-bis[*p*-chlorophenyl]ethane) forms in various environments as the result of DDT (1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane) applications, mainly through the action of microorganisms.

Photodieldrin (II) has been reported to be the major conversion product of dieldrin (I) by the action of sunlight (*4*, *5*). Photodieldrin appears to be more toxic (*6*) to many forms of biological systems than the parent compound. Although photodieldrin can be degraded in the housefly (*7*), the toxicological implication of the presence of photodieldrin in environments remains largely unknown.

Of a survey of dieldrin-degrading capacities among 650 isolated soil microorganisms (*8*), 12 degraded this compound. One of the most active isolates produced hitherto unreported ketones, an aldehyde and an alcohol derivative of dieldrin (*9*). We have extended the study to cover microbial isolates secured from water (Lake Michigan), rat intestines, and rumen stomach contents. One metabolic product is common to all active microorganisms tested. The identity of this product of dieldrin is reported herein.

Table 1. Occurrence of photodieldrin in various microbial media incubated with [$^{14}$C]dieldrin.

| Sources of microorganisms | Microbial isolates tested (No.) | Microbial isolates producing photodieldrin (No.) | Microbial isolates producing additional metabolites (No.) |
|---|---|---|---|
| Dieldrin-contaminated soils | 500 | 42 | 30 |
| Lake Michigan water | 110 | 10 | None |
| Lake-bottom silt | 190 | 93 | 5 |
| Rat intestine contents | 24 | 3 | 4 |
| Rumen stomach contents | 30 | 10 | None |

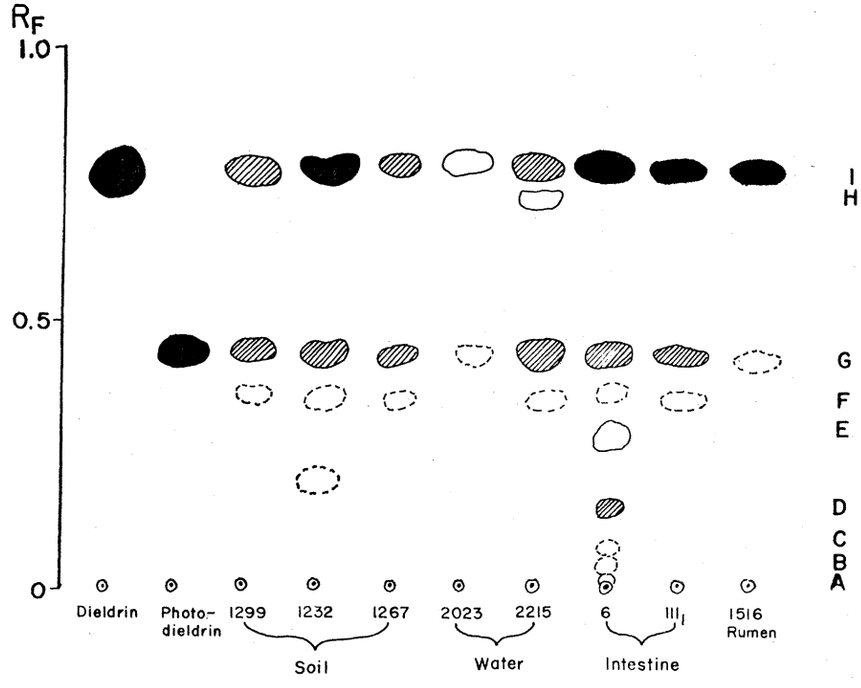
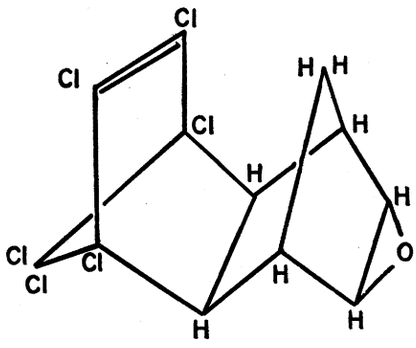



Fig. 1. Thin-layer chromatograms of [$^{14}$C]dieldrin and its metabolites produced by selected microorganisms. Spot *I* represents dieldrin, and spot *G* represents photodieldrin. Black spots show strongest radioactivity; shaded spots, medium radioactivity; and open circles with solid line, weak radioactivity.

The methods utilized to survey the metabolic activities of microorganisms were identical to those reported previously (*8*). The aquatic microorganisms were sampled both from the lake bottom silt and from water samples from Lake Michigan and its tributaries including Green Bay, Sturgeon Bay, and Fox River. Intestinal microbes were isolated from two male albino rats (fed on dog-food biscuits and water) obtained from Rolfsmeyer Co., Madison, Wis. The rumen microorganisms were isolated from a Holstein cow fed on alfalfa-hay diet in the experimental farm, Department of Meat and Animal Science, University of Wisconsin. The methods of isolation and incubation of aquatic and rat intestinal microorganisms were similar to those reported earlier (*8*). The rumen microorganisms were isolated under anaerobic conditions on brain heart infusion agar at 37°C. Each isolate was first inoculated in 10 ml of a solution of yeast extract and mannitol as described by Fred and Waksman (*10*) and maintained at 30°C for 57 hours (37°C in the case of rumen organisms). This mixture was then incubated with 10 $\mu$l of $10^{-3}M$ [$^{14}$C]dieldrin (universally labeled, specific activity 9.4 mc/mmole) in a screw-capped 20-ml test tube for 30 days without shaking. The extraction as well as the remainder of the procedure was similar to that previously described (*8*).

Inasmuch as photodieldrin itself is always present in commercially available [$^{14}$C]dieldrin, the insecticide was always purified on a thin-layer chromatographic system (on silica gel G with a 1 : 1 ether-hexane mixture as the mobile phase) before use. In all cases the same amount of [$^{14}$C]dieldrin (1 nmole in 10 $\mu$l of ethanol per milli-



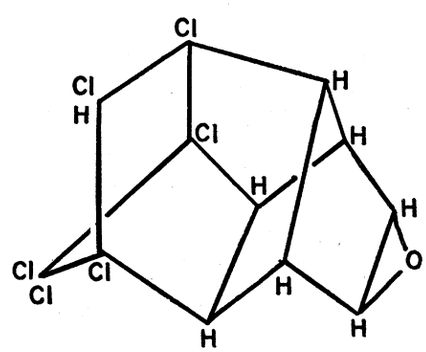
I

liter of medium) was added to samples of the medium and incubated for 30 days to avoid the possibility of contaminants being confused as the metabolic products by microbes. Under such treatment neither photodieldrin nor any other metabolites were formed.

Photodieldrin was the major metabolic product in the microbes from dieldrin-contaminated soils and lake-bottom silt (Table 1).

To identify the major metabolic product, photodieldrin was prepared by the method of Rosen *et al.* (*5*). In addition, an authentic sample was obtained from Rosen. The major microbial metabolic product was first isolated by thin-layer chromatography and then compared to the authentic photodieldrin with four different solvent systems: ether-hexane (1 : 1) (Fig. 1); acetone-hexane (1 : 4); methylene chloride–carbon tetrachloride (1 : 1; and benzene–ethyl acetate (3 : 1). The $R_F$ values of photodieldrin under the above conditions were 0.47, 0.46, 0.46, and 0.82, respectively.

Also two different gas-liquid chromatography systems were employed to identify the major metabolic product. The columns used were a 3 percent SE30 and a 6 percent QF 1 (on Chromosorb W, 1.5 m by 0.3 cm) at 180°C with a nitrogen flow rate of 30 ml/mm. A Varian-Aerograph gas chromatographic system (model 1848) and an electron-capture detector were used for this purpose. All the chromatographic matching tests indicated that the major microbial metabolic product was identical to photodieldrin.



II

The presence of photodieldrin in environments has been shown (*4*, *5*) although the formation of the same end product by environmental microorganisms has not been suspected. More information on the toxicological properties of photodieldrin is needed, since the chances are great that this compound can form from dieldrin in various water and soil systems.

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**References and Notes**

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## Immunoglobulin Structure: Amino Terminal Sequences of Kappa Chains from Genetically Similar Mice (BALB/c)

Abstract. *The amino terminal portion of 20 kappa chains from the highly inbred BALB/c mouse has been examined on an automatic protein sequencer. These proteins can be divided into at least nine groups (subgroups) based on sequence patterns which are so distinct that each subgroup is probably encoded by at least one germ-line gene. The subgroups of mouse kappa chains are generally quite different from those of human kappa chains.*

Remarkable advances have been made toward an understanding of the genetic basis of antibody diversity and evolution through the amino acid sequence analysis of homogeneous immunoglobulins produced by plasmacytomas in man (*1*, *2*). These studies have shown that the immunoglobulin molecule is composed of light (~ 23,000 daltons) and heavy (~ 55,000 daltons) chains which are disulfide-linked. Man produces two types of light chains (λ and κ). The human κ chain has been most thoroughly studied and serves here as a model for all immunoglobulin chains (*2*). The amino terminal half of the κ chain (~ residues 1 to 107) is designated the variable (V) region (*3*) because it is different for each myeloma protein studied (> 50). The carboxy terminal half of this chain (~ residues 108 to 214) is designated the common (C) region and is invariant in all human κ chains apart from a single genetic polymorphism (*4*). The V region sequence diversity in human light chains is impressive. More than 50 human κ and 50 human λ chains have been examined, and each protein appears to be different from all the others (*5*). This diversity, however, must be viewed with caution as the human and most other species whose immunoglobulins have been studied are outbred populations and as such have substantial genetic polymorphism (*6*).

For this reason, we have examined the κ chains from genetically similar individuals of the BALB/c strain of mice that have been consecutively brother-sister mated through more than 110 generations (*7*). Some information has been published on BALB/c κ chains. The nearly complete amino acid sequences from two κ chains, M-41 and M-70, are available (*8*) as well as fragmentary sequences from a number of others (*9*)

We report here our efforts to further characterize the nature and extent of V region variability in this inbred strain by examining the amino terminal sequences of 20 myeloma κ chains and the κ light chains prepared from a pool of mineral oil–induced ascites (Fig. 1) (*10*). The G, H, and F (IgG, IgH, and IgF) proteins were chosen because of