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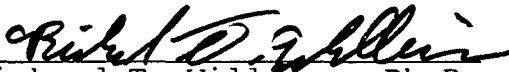
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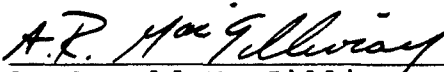
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LITERATURE REVIEW OF BIODEGRADATION
IN SOIL OF SELECTED ROCKY MOUNTAIN
ARSENAL CONTAMINANTS: ISODRIN,
DIELDRIN, DIISOPROPYLMETHYL-
PHOSPHONATE, 1,2-DIBROMO-3-
CHLOROPROPANE, AND p-CHLORO-
PHENYLMETHYLSULFOXIDE

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1. INTRODUCTION

Soils and groundwater at the Rocky Mountain Arsenal (RMA), Colorado have been contaminated with a variety of organic chemicals. Among these chemicals are 1,2-dibromo-3-chloropropane (DBCP), diisopropylmethylphosphonate (DIMP), p-chlorophenylmethysulfoxide (PCPMSO), isodrin, and dieldrin. The U.S. Army Toxic and Hazardous Materials Agency (USATHAMA) is investigating the possibility that microorganisms in soil may transform these five compounds to intermediates or innocuous end products. WESTON is conducting a laboratory study to evaluate the biodegradability of these compounds in soil under RMA relevant conditions. As part of this study, WESTON is conducting a literature review to determine the current state of knowledge regarding these subject areas.

1.1 Purpose. The purpose of this literature review is to provide USATHAMA with a brief summary regarding the biodegradability of DIMP, DBCP, PCPMSO, isodrin, and dieldrin.

2. LITERATURE SUMMARY

2.1 General. Preliminary indications regarding the biodegradability of these compounds can be obtained from directly and indirectly applicable studies. Unfortunately, very few of the former exist. Not all of the compounds have been investigated in biodegradation or other fate studies.

In general, the available literature indicates that these compounds are relatively resistant to biodegradation. The major line of indirect evidence indicating that these compounds are relatively resistant to biodegradation is simply their persistence in natural environments for extended periods of time. At the RMA these compounds have been a long-term contamination problem. Biodegradation, or other fate and transport processes, has not removed them. Little information exists in the open literature regarding the environmental persistence of PCPMSO and isodrin in geographical regions outside the RMA. DBCP and dieldrin, however, have received a significant amount of attention.

Each of the five chemicals is discussed in detail in a separate subsection. The structural formulas for all five compounds are presented in Figure 1.

2.2 DBCP. DBCP is an agricultural nematocide, and is used as a soil fumigant. Persistence is a critical property for pesticide effectiveness, and this property has enhanced the agricultural utility of DBCP. Under field conditions, however, a number of factors may combine to reduce DBCP to below detection levels. These factors include volatilization, diffusion, adsorption, and physical and/or biological degradation. Volatilization of DBCP is the most important loss process from soil. For this reason, persistence was found to be much greater when DBCP was applied by soil injection rather than with irrigation water (Hodges and Lear, 1974). This was true for sandy clay loam, silt loam, and loamy sand soils.

The persistence of DBCP has been studied in a few agricultural areas treated with DBCP. For example, contamination of groundwater and subsurface soil was investigated in California's San Joaquin Valley (Nelson et al., 1981). The significant findings from this study are that:

- (a) DBCP was found in subsoils between the ground surface and water table in parts per billion amounts.
- (b) Clays and silts adsorbed higher amounts of DBCP than sand but still did not act as barriers to downward migration of DBCP.

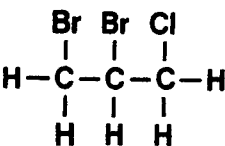
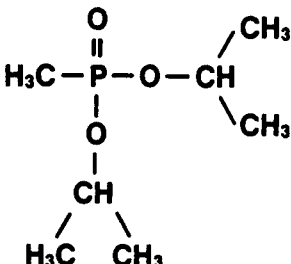
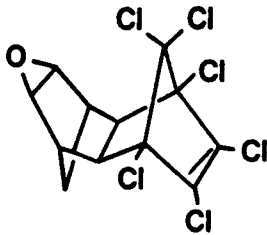
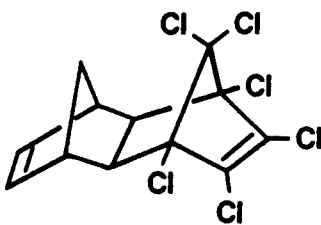
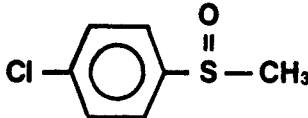
Name	Abbreviation	Structural Formula
Dibromochloropropane	DBCP	
Diisopropyl Methyl Phosphonate	DIMP	
Dieldrin	—	
Isodrin	—	
P-Chlorophenyl Methyl Sulfoxide	PCPMSO	

FIGURE 1 STRUCTURAL FORMULAS OF DBCP, DIMP, DIELDRIN, ISODRIN AND PCPMSO

DBCP remained in the topsoil of one site 6 to 7 years after a single application. DBCP also reached groundwater below South Carolina peach orchards fumigated for nematocide control (Carter et al., 1984).

DBCP can influence both microbial activity and numbers (Berkowitz et al., 1978). At a concentration of one percent in soil DBCP acted as follows:

- (a) Somewhat reduced the microflora population and totally suppressed CO₂ release, suggesting that DBCP inhibited the activity of, but did not eliminate, the microflora.
- (b) Reduced the number of proteolytic bacteria by 53 percent, cellulytic bacteria by 97 percent, and the nitrifiers by 100 percent.
- (c) Reduced nitrogen mineralization by almost 50 percent.

Although quantitative measurements were not made, DBCP reduced the growth rate of the bacterium Pseudomonas solanocearum (Libman and Leach, 1962). In contrast, DBCP increased microbial numbers and oxygen uptake within a sample of tropical soil (Elliot and Donawa, 1977).

Dehalogenation (removal of bromine and/or chlorine) from DBCP was observed in soil (Castro and Belser, 1968). This halide release occurred only under microbial growth supporting conditions and only when the substrate was present during the growth phase. The inclusion of a carbon source (glycerol) was also required, indicating that the observed transformation of DBCP is likely cometabolic. Biotin and thiamine were also added to the test soil. The maximum transformation was 63 percent in 4 weeks. Transformation was most rapid at pH 8.

Approximately 100 soils from orchards and fields throughout southern California were screened for their ability to dehalogenate DBCP. Approximately 75 percent of the samples examined showed some capacity to bring about the release of bromine. In addition to bromine and chlorine, the other biotransformation product from DBCP was n-propanol. Despite intensive efforts over a 4-year period, all attempts to isolate a single organism or mixed population that could degrade DBCP when incubated with sterile soil-water or any other media failed (Castro and Belser, 1968).

2.3 Dieldrin. Dieldrin is an insecticide that has been used extensively in the past to protect corn and control termites. However, it is no longer registered for general use. Dieldrin is part of the cyclodiene group of insecticides along with isodrin, aldrin, and endrin.

Dieldrin was not biodegraded in standard screening tests (Tabak, 1981), and has been found to be extremely persistent in soils (Sanborn et al., 1977) under aerobic and anaerobic conditions (Castro and Yoshida, 1971). The half-life of dieldrin in soil was projected to be greater than 7 years (Nash and Woolson, 1967). No biodegradation of dieldrin has been noted in river waters (Eichelberger and Lichtenberg, 1971; Sharom, 1980). Although the biotransformation of dieldrin is extremely slow, there is some evidence that microorganisms can form photodieldrin (Figure 2) from dieldrin (Nash and Woolson, 1967; Matsumura et al., 1979).

A field study by Nash and Woolson (1967), which found the half-life of dieldrin to be approximately 7 years, was conducted in Congaree sandy loam soil. The soils received dieldrin equivalent to 0 to 448 kg of insecticide per hectare. At initiation of the experiment, the soil and insecticide were mixed uniformly. Treatment and maintenance of the soil was such that leaching, volatilization, photodecomposition, and mechanical removal were minimized. The higher dosage rates utilized were thought to have had toxic effects on the soil microflora, thus inhibiting biodegradation. At the conclusion of the study 31 percent of the original technical grade dieldrin remained for both high (100 ppm) and low (25 ppm) doses after 15 years.

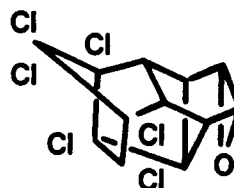
Castro and Yoshida (1971) also evaluated the fate of dieldrin in soil, comparing degradation under aerobic and anaerobic conditions by using tropical upland and flooded soils, respectively. In the upland soil, water was provided to maintain 80 percent of the maximum water-holding capacity of the soil. The flooded soil was maintained with a water level 5 cm above the soil surface. Dieldrin persisted in both the flooded and upland soil.

Twelve of 650 soil microorganisms, isolated from a site heavily contaminated with pesticides, including six Pseudomonas cultures and two Trichoderma cultures, degraded dieldrin (Matsumura and Boush, 1967). Ketone, aldehyde, and alcohol derivatives of dieldrin were produced by one of the most active isolates (Matsumura and Boush, 1968). Other studies (Vockel and Korte, 1974; Matsumura et al., 1970) indicate that a major product of dieldrin transformation by microorganisms is photodieldrin. It has been reported that Trichoderma koningii converted dieldrin to carbon dioxide, however, only 3 percent of the initial dieldrin was evolved as carbon dioxide (Bixby et al., 1971).

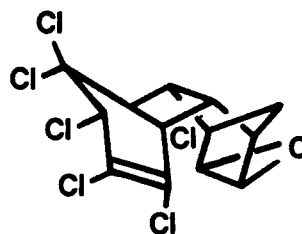
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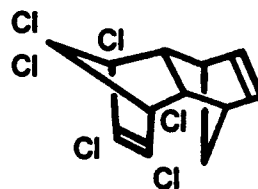
Photodieldrin



Endrin



Aldrin



**FIGURE 2 STRUCTURAL FORMULAS OF
PHOTODIELDRIN, ENDRIN AND ALDRIN**

Dieldrin and photodieldrin were not found to be susceptible to biodegradation in several other studies. No degradation of dieldrin was observed in sewage lagoon sediment (Halvorsen et al., 1971). When 100 samples of estuarine and oceanic surface films, marine plankton, and algae were incubated with cyclo-dienes (including dieldrin), 35 of 100 estuarine cultures were able to transform dieldrin to photodieldrin (Matsumura and Boush, 1972). Photodieldrin has been reported to be recalcitrant to degradation by algae (Reddy and Khan, 1975). Microbial degradation of dieldrin by waste composting was attempted. After 3 weeks, 97 percent of the dieldrin remained unaltered (Mueller and Korte, 1975).

Dieldrin has low solubility in water and strong adsorption to soil, which makes leaching into groundwater unlikely. Small amounts of dieldrin may volatilize from soil or be carried into the air by dust particles. Soil runoff can carry particle-associated dieldrin to water systems (Schooner et al., 1983). Hydrolysis is not an important degradation process for dieldrin (Callahan, 1979; Eichelberger and Lichtenberg, 1971). The half-life of dieldrin in water is greater than 4 years (Callahan, 1979). When irradiated with sunlight, dieldrin degrades to photodieldrin in distilled water with a half-life of 2 months (Callahan, 1979).

2.4 DIMP. DIMP is a by-product formed during synthesis of the nerve agent isopropylmethylphosphorofluoridate (Sarin). Unfortunately, few studies exist that directly address the biodegradation of DIMP. The most significant study (Spanggord et al., 1979) evaluated the rate of microbial and photochemical transformation of DIMP in RMA soil and water samples. Biotransformation of DIMP was not observed in natural waters despite attempts to acclimate the microorganisms utilized. In acclimated soil, approximately 5 percent of the added ^{14}C -DIMP was converted to $^{14}\text{CO}_2$ after 17 weeks of incubation at 25°C . At 10°C biotransformation was essentially nonexistent. A half-life of more than 2 years was predicted for the biotransformation of DIMP to CO_2 in soil.

In soil lysimeter studies using soils from non-RMA locations, downward movement of DIMP was demonstrated (O'Donovan and Woodward, 1977). DIMP was applied to soil by two methods. The first method used chronic application of a 20-ppm solution of DIMP in distilled water. The solution then percolated downward through the soil. This method resulted in a thin layer of soil at the top of the column that had a relatively high concentration of DIMP (18 to 33 ppm). A more dilute distribution (3 to 9 ppm) of DIMP was present throughout the remaining soil profile. The second method consisted of preparing 20 ppm mixtures of DIMP in soil in the top 1 foot of a soil column, which was then leached with distilled water. This resulted in a

slightly broadened band of DIMP moving downward through the soil column. DIMP, originally at 20 ppm in the 1-foot thick surface layer, was not detectable upon termination of the irrigation experiments within 2 feet of the surface in several cases. In the remaining cases, DIMP was also undetectable at greater depths. These results indicate that DIMP applied to soil can move through soil with irrigation water flow (O'Donovan and Woodward, 1977).

A series of radioactive tracer experiments was performed to provide estimates regarding the volatility of DIMP from soil mixtures (O'Donovan and Woodward, 1977). Radioactive DIMP and soil mixtures at 20 ppm DIMP were placed in columns. Half the columns were 25 mm wide by 4 inches deep and contained dry soil. The other columns were 55 mm wide by 6 inches deep and contained moist soil. The contaminated soil columns were subjected to an air flow of 100 ml per minute across their surfaces for 250 hours. At the completion of the experiment, both sets of soil columns were analyzed for radioactivity content. The dry soil retained over 95 percent of the initial radioactivity. The moist soil retained approximately 78 percent of the DIMP.

DIMP and related compounds are environmentally persistent because of a relatively unique structural property, a nonreactive C-P bond (Daughton et al., 1979; Verweij et al., 1976). Organophosphorus pesticides are generally considered nonpersistent because of the susceptibility of phosphoesters to hydrolysis (Daughton et al., 1979). Compounds with a C-P bond, however, are known to be resistant to chemical hydrolysis, biodegradation, thermal degradation, and photolysis (Vasu and Roy, 1985).

There is no evidence for biological breakdown of C-P bonds under environmental conditions where an abundance of phosphate is present in comparison with the amount of phosphorus contained in C-P bonds (Verweij et al., 1979a). Among microorganisms, only a few species have been cited as capable of utilizing the C-P containing methyl phosphonic acid (MPA). Zeleznick et al. (1963) determined that Escherichia coli Crookes strain was able to utilize MPA (6.1 g/L) and ethyl phosphonic acid (EPA) (6.8 g/L) when they were present as the sole phosphorus source. The total phosphorus content of the bacteria pellets after 24-hours incubation with MPA as the sole phosphorus source increased approximately 10-fold (Zeleznick et al., 1963).

Daughton et al. (1979a, 1979b) detected the cleavage of C-P bonds during the catabolism of O-alkyl alkylphosphonates by Pseudomonas testosteroni. Under aerobic conditions with O-alkyl alkyl phosphonate as the sole phosphorus source (concentrations of 0.008 to 0.10 mM), this bacteria cleaved the C-P bonds and converted the organophosphorus toxicants to innocuous natural products (Daughton et al., 1979a, 1979b). Pseudomonas testosteroni was capable of growth with ionic MPA, O-IMP, O-PMP, EPA, or O-EPA as the sole phosphorus source. Alkanes were produced from the C-P bond cleavage. This work by Daughton et al. (1976) was the first to establish the following:

- (a) Biodegradation of a simple alkylphosphonate.
- (b) Complete conversion of an organophosphorus toxicant to natural products.
- (c) The catabolic pathway for an alkylphosphonate.

This pathway is presented in Figure 3.

2.5 Isodrin In the past, isodrin had been marketed as an insecticide under the trade name Telodrin. Isodrin is part of the cyclodiene group of insecticides along with aldrin, dieldrin, and endrin. Isodrin is also an intermediate in the formation of endrin (endrin is its epoxide), and is an isomer of aldrin. Isodrin is made by the slow reaction of cyclopentadiene with the condensation product of vinyl chloride and hexachlorocyclopentadiene (Brooks, 1974).

Because isodrin was not widely used as an insecticide, data on its biodegradation and environmental fate are limited and usually inferred indirectly from studies of other cyclodienes. A review of the available literature revealed no direct laboratory or field studies on the microbial degradation of isodrin. However, some degradation of other cyclodiene insecticides by soil microorganisms is well documented. Because of the close relationship between aldrin, endrin, and isodrin, a brief summary of studies investigating the biodegradation of these compounds is presented.

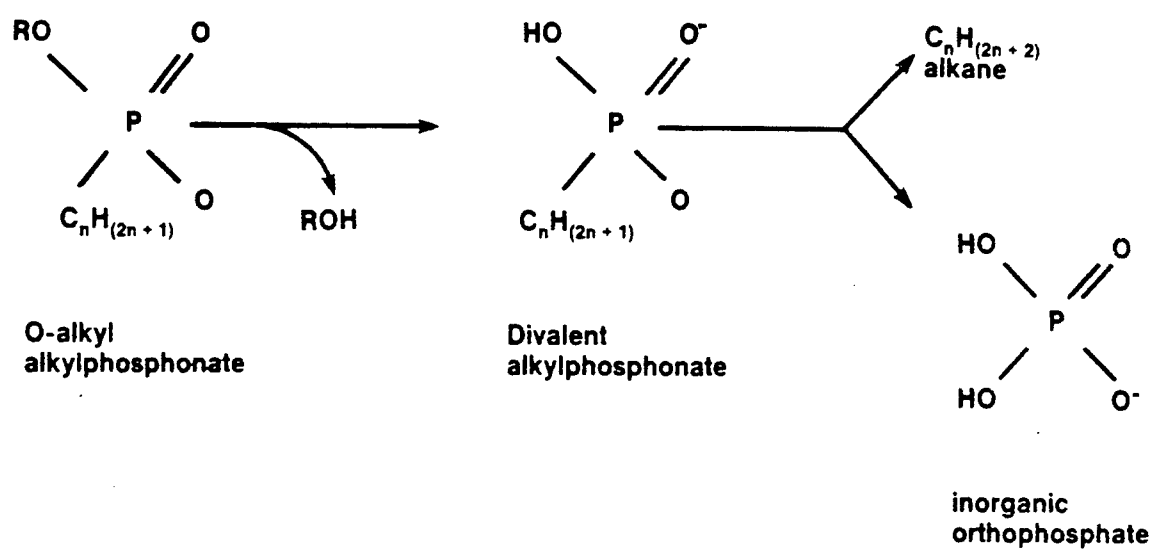


Figure 3 Pathway of O-alkyl alkylphosphonate catabolism
by *Pseudomonas testosteroni*

The biodegradation of aldrin has been reported in numerous studies. Aldrin was degraded to unidentified products by Chlorella pyrenoidosa (Elsner et al., 1972). Dieldrin and keto-aldrin were produced from the degradation of aldrin by Penicillium funiculosum (Murado-Garcia, 1973). Ninety-two cultures were isolated from soil that degraded aldrin to dieldrin and other unidentified metabolites (Tu et al., 1968). There were 13 out of 20 dieldrin-degrading organisms that also transformed aldrin, with the major product being 6,7-dihydroxyaldrin (Patil and Matsumura, 1970). The epoxidation of aldrin to dieldrin in soil is reportedly more effectively carried out by fungi and actinomycetes than by bacilli (Tu et al., 1968).

In a series of 150 soil cultures, 25 were able to convert endrin to ketoendrin (Matsumura et al., 1971). All 20 dieldrin-degrading cultures tested also transformed endrin to ketoendrin (Patil and Matsumura, 1970).

Despite the observation of cyclodiene conversion in the laboratory, extrapolation to field conditions is difficult (Cowley and Lichtenstein, 1970). Partial degradation of cyclodienes has been observed under some field conditions. When simulated flooded and upland soil conditions were tested, aldrin disappeared more rapidly under the upland conditions (Castro and Yoshida, 1971).

Aldrin was thought to be more stable in flooded than in upland soil because of a lack of molecular oxygen, which was required for the epoxidation of aldrin. Endrin was also persistent in flooded soils, with the exception of Casiguan soil (Castro and Yoshida, 1971).

When 100 samples of estuarine and oceanic surface films, marine plankton, and algae were incubated with cyclodienes, 35 of 100 estuarine cultures were able to degrade aldrin, dieldrin, and endrin to trans-aldrindiol, photodieldrin, and keto-endrin, respectively (Matsumura and Bousch, 1972). No open ocean water samples were found to be that active. Sea bottom sediments were ineffective at transforming cyclodienes; algae were found to be necessary for activity (Patil et al., 1972).

Studies have been conducted on the overall persistence of cyclodienes, including isodrin, in soil. Extreme functional persistence of the cyclodiene insecticides has been observed, and was attributed to the highly stable ring structure and the toxicity of the few known transformation products. Isodrin has a half-life in soil of 4 to 8 years with degradation products that include endrin (TOXNET, 1987). In a long-term persistence study of isodrin and endrin in soil, Nash and Harris (1973) found that 16 percent of the applied isodrin and 39 percent of the applied endrin remained after 16 years.

Despite their relative immobility, considerable movement of the cyclodiene insecticides eventually occurs under field conditions because of the unusually great persistence of the parent compounds and transformation products. The relative mobilities of chlorinated hydrocarbons, including the cyclodienes, as determined by Nash and Woolson (1968) in sandy loam were BHC (lindane) > isodrin > heptachlor > endrin > toxaphene > dieldrin > aldrin > dilan > chlordane. Of the aldrin and dieldrin (but not the isodrin), 85 percent remained in the upper 23 cm of soil, with the greatest concentration from 7 to 23 cm. Isodrin was distributed more evenly than aldrin, dieldrin, toxaphene, or chlordane after application of 73 kg/ha of soil.

2.6 PCPMSO. The compound p-chlorophenylmethylsulfoxide (PCPMSO) is related to some insecticides and other pesticides (Blair et al., 1984). PCPMSO is found in trace amounts as an intermediate of the herbicide Planavin. The PCPMSO arises from the incomplete tungstate-catalyzed oxidation of the corresponding p-chlorophenylmethylsulfide (PCPMS) with hydrogen peroxide (Sanderson and Swift, 1973). The authors postulate that another possible environmental source of PCPMSO may be oxidation of discharged PCPMS in the air, although no direct evidence of this is cited (Miller et al., 1976). PCPMSO and PCPMS should also exhibit interconvertibility in the environment by oxidation by hydroperoxides in soil (Bateman and Hardgrove, 1954a, b; Overberger and Cummins, 1953a, b) and biochemical pathways (Kresze et al., 1965).

The degradation in soil of PCPMSO was evaluated in a laboratory study during a 160-day period (Guensi et al., 1979). Total CO₂ evolution from soil was used as an indicator of the broad effects of the PCPMSO on soil microbial populations. Concentrations of 0.5 and 5.0 ppm PCPMSO had no effect on total CO₂ evolution. A 50-ppm concentration, however, did lower total CO₂ evolution by 8.17 percent (Table 1).

Evolved ¹⁴CO₂ as a percent of the initial dpm of ¹⁴C-PCPMSO added was used to measure ring cleavage and subsequent oxidation of PCPMSO. A 50-ppm treatment had only 6.14 percent ¹⁴CO₂ evolved compared to 16.11 and 10.67 percent ¹⁴CO₂ evolved in the respective 0.5- and 5.0-ppm treatments (Table 1). The rate of compound destruction (ng/day) increased with higher contaminant concentrations. Ranges reported were 9 to 16 ng/day for the 0.5-ppm treatment, 52 to 108 ng/day for the 5.0-ppm treatment, and 238 to 711 ng/day for the 50-ppm treatment (Table 1).

TABLE 1. DEGRADATION OF PCPMSO IN SOIL

PCPMSO in soil (ppm)	Total CO ₂ evolved (ug/g)	Evolved ¹⁴ CO ₂ (% of ¹⁴ C)	PCPMSO distillation (ng/day)	Recovery in soil only (% of ¹⁴ C)	Recovery in soil and evolved gases (% of ¹⁴ C)
0.0	20.75	---	---	---	---
0.5	21.70	16.11	9 - 16	80.6	96.71
5.0	19.01	10.67	52 - 108	84.5	95.17
50.0	12.58	6.14	238 - 711	76.1	82.24

Modified from: Guenzi et al., 1979

Recovery of ^{14}C in soil and evolved gases was 96.71, 95.17, and 82.24 percent of the total added for the 0.5-, 5.0-, and 50.0-ppm treatments, respectively. See Table 1 for a recovery breakdown. The rubber tubing in the experimental apparatus may have caused some losses of ^{14}C from volatile ^{14}C -PCPMSO. Since a decrease in the rate of $^{14}\text{CO}_2$ produced with time was observed, there appears to be no indication of microbial adaptation to degrade this compound. The authors report a significant correlation between total CO_2 (mg C) and ng of chemical lost as $^{14}\text{CO}_2$ for the 0.5- and 5.0-ppm treatments. No correlation existed for the 50-ppm treatment. The association of total CO_2 and compound degradation implies a microbial role in the degradation of PCPMSO at low soil concentration levels (Guensi et al., 1979).

3. SUMMARY

The five compounds included in this report are: 1,2-dibromo-3-chloro-propane (DBCP), an agricultural nematocide; dieldrin, an insecticide; diisopropylmethylphosphonate (DIMP), a by-product of nerve agent production; isodrin, an insecticide; and p-chlorophenylmethylsulfoxide (PCPMSO), a compound formed in pesticide manufacture. These compounds are among the contaminants detected at Rocky Mountain Arsenal (RMA), Colorado.

The half-life in soil of the compounds under investigation is reported as greater than 7 years, 2 years, and 4 to 8 years for dieldrin, DIMP, and isodrin, respectively. The half-life in soil of DBCP and PCPMSO has not been determined, but slow degradation has been reported for both.

DBCP at 1 percent in soil reduced microflora activity. However, dehalogenation of DBCP to n-propanol has been reported in soil under growth supporting conditions and in the presence of a cometabolic carbon source. Dieldrin was not biodegraded in standard screening tests, and additional studies have demonstrated extremely slow or no biodegradation. However, transformation to photodieldrin by a limited number of soil isolates has been reported. For example, the fungal culture Trichoderma koningii converted 3 percent of the original dieldrin added to carbon dioxide.

DIMP is resistant to chemical and biological degradation because of the stable C-P bond. Only a few microorganisms are able to cleave the C-P bond, and this occurs only when C-P is the sole phosphorus source. No data could be found on isodrin biodegradation under laboratory or field conditions. Because of the close relationship between isodrin and other cyclodiene pesticides that have been studied, it can be inferred that limited biodegradation of isodrin occurs. PCPMSO inhibited soil microflora at 50 ppm. Slow biodegradation of the compound has been reported at 50 ppm in soil, with slightly higher rates observed at 5.0 and 0.5 ppm.

The evidence that exists for the biodegradation and transformation of dieldrin, DBCP, DIMP, isodrin, and PCPMSO indicates that limited transformation of the investigated compounds occurs. However, the available information is limited, especially as it relates to RMA-specific environmental conditions.

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