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Biological and Chemical Degradation of Tetraethyl Lead in Soil

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Tetraethyl lead (TEL) was first introduced by General Motors for use as an antiknock agent in gasoline in 1923 (Noden, 1980; Rhue *et al.*, 1992). Its analogous chemical tetramethyl lead (TML) was subsequently introduced in 1960. The two chemicals were added to gasoline either singly or as mixtures to achieve desired octane numbers. Production of leaded gasoline in the US peaked in early 1970 and steadily declined after 1975 (Grandjean and Nielsen, 1979). Cars of 1975 and later models use only unleaded gasoline. Higher levels of lead in the urban environment have been attributed to extensive use of leaded gasoline in automobiles for over 50 yrs (Mielke *et al.*, 1989).

With the advent of more sensitive analytical techniques in the late 1970 and early 1980, mainly through the improvement of the hyphenated GC-AAS techniques, alkyl lead compounds were found to be ubiquitously present in the environment. Alkyl lead compounds were detected in aerosol dusts (Laxen and Harrison, 1977), rainwater (Radojevic and Harrison, 1987a) surface water (Chau *et al.*, 1979) snow (Van Cleuvenbergen *et al.*, 1986), sediments (Chau *et al.*, 1984), soils (Blais and Marshall, 1986), fish (Chau *et al.*, 1984), and leaves (Van Cleuvenbergen *et al.*, 1990). Ionic trialkyl lead (Triethyl lead (TREL) and trimethyl lead (TRML)) were the major species found in the environment. It is expected that with the discontinuation of the use of leaded gasoline in automobiles the levels of alkyl lead in the environment should decline.

Degradation pathways of tetraalkyl lead compounds in soil, including TEL, are not known. However, degradation pathways for tetraalkyl lead in aqueous solutions were proposed to proceed through a series of sequential dealkylation, and eventually to inorganic lead (Rhue *et al.*, 1992). For example, TEL was first degraded to monoionic TREL which was in turn degraded to diionic diethyl lead (DEL), and finally to Pb^{2+}. The fate of tetraalkyl lead in subsurface soils underneath old leaking underground storage tanks, previously used for storage of leaded gasoline, is not known.

Microorganisms capable of degrading tetraalkyl lead, including TEL, have not

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been reported. However, microbial degradation could be partially responsible for the disappearance of TRML in soil. Blais *et al.* (1990) reported that the levels of TRML in nonsterile soil were lower than that in corresponding autoclaved soil after 24 hr of incubation.

This investigation was initiated to determine biological and chemical transformation rates of TEL to ionic organolead forms (TREL and DEL), and mineralization rates of TEL in surface and subsurface soils. In order to achieve these objectives, ^{14}C-labeled TEL was used so that conversion from nonionic form to ionic form could easily be determined and evolution of CO_2 from the mineralization of TEL could be measured.

MATERIALS AND METHODS

Ethyl-1 labeled ^{14}C-tetraethyl lead (TEL) was purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO). Analytical grade TEL was obtained from All-Chemie LTD (Ft. Lee, NJ). Both ^{14}C-TEL and TEL were dissolved in n-hexane in glass bottles wrapped with aluminum foil at a radio dosage of 1 μCi/mL and a concentration of 2000 μg/mL, respectively.

Arredondo fine sand (Grossarenic Paleudult) was used for this study. Soil samples at 15-cm increments were collected from a virgin forest site on the campus of the University of Florida, Gainesville FL. Selected soil properties are shown in Table 1.

Table 1. Selected properties of soil samples used for this study.

Soil depth (cm)	pH	Soil-water content	Organic C	Sand	Silt	Clay
		—— (g/kg soil) ——		—— (%) ——		
0-15	5.5	55	11.8	92	7	1
15-30	4.6	46	4.7	93	5	2
30-45	5.6	28	3.9	93	4	3

To avoid volatilization loss of TEL, 50 g of soil (oven-dry weight basis) were transferred to a 250 mL Erlenmeyer flask with a teflon-lined screw cap. After adding 0.1 μCi of ^{14}C-TEL and 1000 μg/g of TEL, an additional 50 g of the soil were immediately added to the top of the soil in the flask. After a brief but thorough mixing, the flask was closed tightly with a Teflon-lined screw cap under which a stainless steel vial (3 mL capacity) containing 0.5 mL of 8 N KOH was hung, supported by a stainless steel wire. At predetermined intervals, all vials were replaced with new vials containing fresh KOH. The KOH in the removed vials was diluted with deionized H_2O to 5 mL. 0.5 mL of the diluted KOH was used for determination of total ^{14}C-activity in the KOH traps by liquid scintillation counting. In addition, 2 mL of the KOH were transferred to a plastic centrifuge tube which had contained 0.5 mL of $BaCl_2$ (200 g/L) resulting in the formation of insoluble $BaCO_3$, including $Ba^{14}CO_3$. After centrifugation, ^{14}C in the supernatants was quantified by liquid scintillation counting. The difference between ^{14}C in the KOH and ^{14}C in the supernatant was considered to be $^{14}CO_2$ (Ou, 1989). To reduce photodecomposition, all flasks were wrapped with aluminum foil and the experiments were carried out under semi-dark conditions. The flasks were incubated in the dark at 25°C. At the end of 28 d of incubation, 10 g of soil from each flask were transferred to a 50 mL glass tube for solvent extraction as described below. ^{14}C remaining in the solvent-extracted soil samples was combusted to $^{14}CO_2$ by a sample oxidizer and quantified by liquid scintillation counting. Identical experimental procedures were carried out for autoclaved Arredondo soil.

For determination of transformation rates of TEL to ionic species (TREL and DEL), a series of 30 mL glass centrifuge tubes wrapped with aluminum foil were prepared and 5 g of the Arredondo soil were initially added to each tube. Then, 0.05 μCi of ^{14}C-TEL and 100 μg of TEL were applied to the surface of the soil, and another 5 g of the soil were immediately added. After closing with Teflon-lined screw caps, the tubes were mixed by shaking in a reciprocal shaker for 5 min. The tubes were incubated in the dark at 25 °C. At predetermined intervals, two tubes from each depth were extracted with 10 mL of n-hexane and 10 mL of EDTA (2 g of disodium EDTA/100 mL of deionized H_2O, pH 9.0). The tubes were shaken for 1 hr in a reciprocal shaker and were then centrifuged at 2000 rpm for 20 min. After centrifugation, hexane and EDTA were separated, the hexane phase being on the top and the EDTA phase being under the hexane. 0.5 mL from each phase was carefully removed for determination of ^{14}C activity by liquid scintillation counting. Identical experimental procedures were carried out for autoclaved Arredondo soil. All experiments were duplicated.

RESULTS AND DISCUSSION

TEL is a hydrophobic compound with a very low water solubility (Feldhake and Stevens, 1963), but is highly soluble in hydrophobic solvents such as gasoline, benzene, and hexane. On the other hand, ionic ethyllead species (TREL and DEL) are highly water soluble, but not soluble in hydrophobic solvents. This study took advantage of these two contrasting properties: hydrophobic TEL would be fractionated into hexane, while ionic ethyllead species would remain in the aqueous phase. With the employment of ^{14}C-TEL, thus, transformation rates of TEL to ionic forms in soil could easily be determined. This is analogous to hexane being successfully used for separation of the organophosphorus insecticide methyl parathion from their polar hydrolysis product *p*-nitrophenol in bacterial culture fluids (Ou and Sharma, 1989).

It was consistently observed that ^{14}C in hexane (^{14}C-TEL) initially disappeared in nonsterile soil samples more rapidly than in the corresponding autoclaved soil samples (Figure 1), especially for surface samples. Disappearance of ^{14}C-TEL in the nonsterile samples leveled off earlier than in the corresponding autoclaved

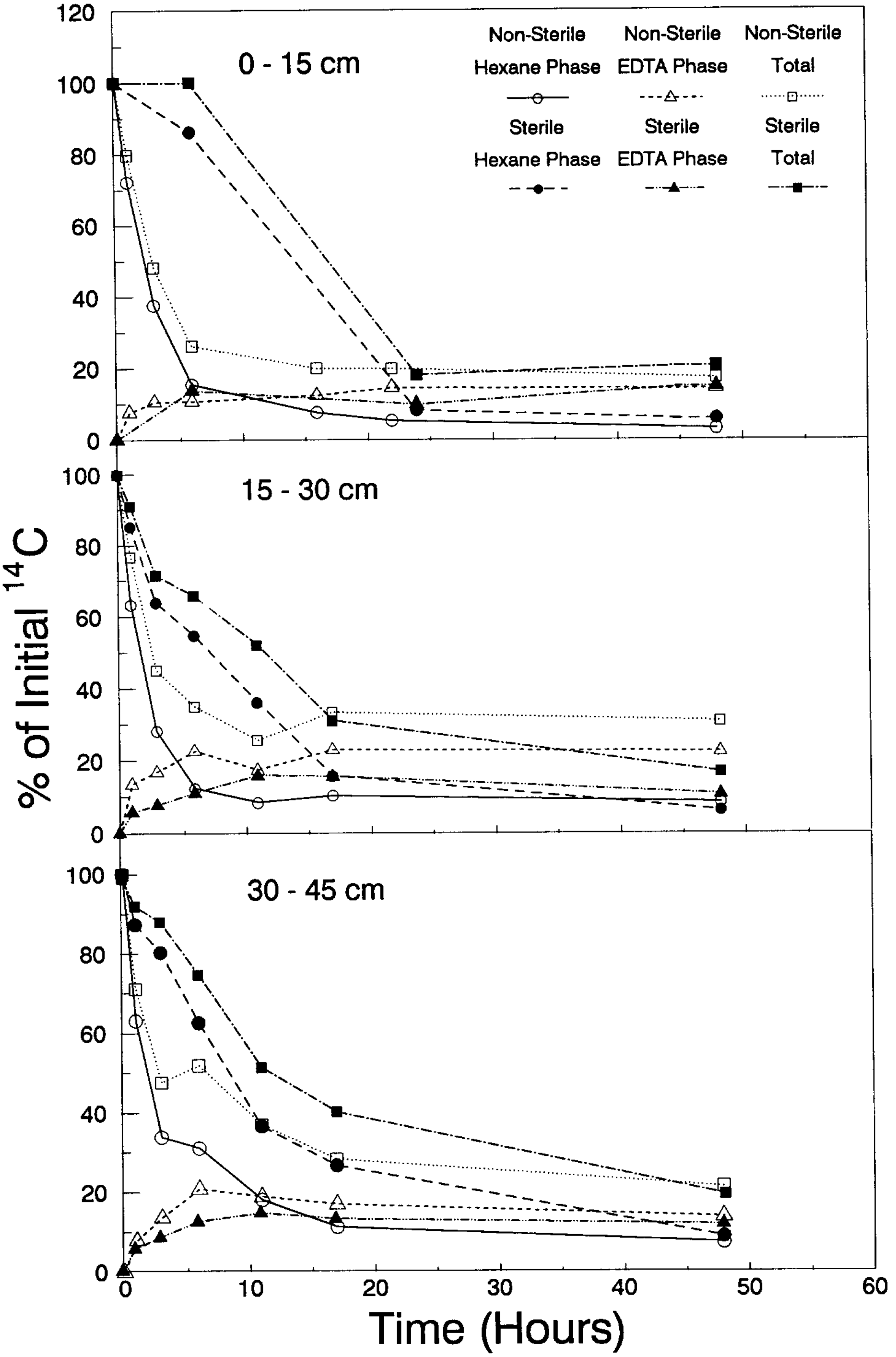

Figure 1. Distribution of ^{14}C in hexane and ^{14}C in EDTA in nonsterile and autoclaved Arredondo soil.

samples. The time required for 50% disappearance of ^{14}C-TEL in the three layers of nonsterile samples (0 to 15, 15 to 30, and 30 to 35 cm depth) was 2, 1.5 and 2 hr, respectively. Whereas time required for 50% disappearance of ^{14}C-TEL in the corresponding sterile samples was 14, 7 and 8 hr, respectively (Figure 1).

Ionic ^{14}C (^{14}C-TREL and ^{14}C-DEL) slowly but steadily appeared in the EDTA phase in nonsterile samples, but leveled off after 3 to 6 hr of incubation (Figure 1). More ionic ethyllead species were formed in subsurface samples than in surface sample and up to 23% of the applied ^{14}C was associated with the ionic forms. Similar patterns for the formation of ionic ^{14}C were observed for the corresponding autoclaved samples. But the levels of ionic ^{14}C in the autoclaved samples were generally lower than in the corresponding nonsterile samples. Time required for 50% disappearance of nonionic ^{14}C plus ionic ^{14}C in the three layers of nonsterile samples (0 to 15, 15 to 30, and 30 to 45 cm depth) was 14, 7 and 8 hr, respectively. Whereas, time required for 50% disappearance of nonionic ^{14}C and ionic ^{14}C in the corresponding layers of the autoclaved samples was 17, 12 and 12 hr, respectively (Figure 1).

Figure 2 shows the distribution of the ratio of ^{14}C activity fractionated into aqueous EDTA solution to ^{14}C activity fractionated into hexane phase (^{14}C in EDTA/^{14}C in hexane) in nonsterile and autoclaved Arredondo soil. It should be noted that if ^{14}C in EDTA/^{14}C in hexane is smaller than 1, this indicates that more TEL than ionic TREL and DEL is present in the soil; and, if ^{14}C in EDTA/^{14}C in hexane is larger than 1, less TEL is present than the ionic forms. Less than 1% of ^{14}C-TEL standard could be fractionated into the EDTA phase. This indicated that radio purity of ^{14}C-TEL was better than 99%. Once ^{14}C-TEL was applied, ^{14}C in EDTA/^{14}C in hexane increased in all nonsterile and sterile soil samples. Since disappearance of TEL and formation of ionic forms were more rapid in nonsterile soil than in the corresponding autoclaved soil (Figure 1), it would be expected that ^{14}C in EDTA/^{14}C in hexane in the nonsterile samples would be larger than in the corresponding autoclaved samples. In fact, values of ^{14}C in EDTA/^{14}C in hexane in the three layers of nonsterile soil were consistently larger than in the corresponding autoclaved samples during 48 hr of incubation. This suggests that, in addition to chemical degradation, biological degradation also plays a role in the transformation of TEL to ionic TREL and DEL.

$^{14}CO_2$ was initially rapidly evolved from ^{14}C-TEL treated surface and subsurface soil (Figure 3), and leveled off after 3 d of incubation. Total amounts of $^{14}CO_2$ evolved from these samples were progressively smaller with soil depth. No $^{14}CO_2$ was evolved from ^{14}C-TEL treated autoclaved soil (data not shown). Thus, mineralization of TEL in soil is a microbially mediated process. Total ^{14}C recoveries from the three layers of soil after 28 d of incubation were poor (Table 2), ranging from 17.6 to 28.0% of the applied ^{14}C. Since the majority of ^{14}C trapped in KOH traps was $^{14}CO_2$ and only 0.2% or less of the applied ^{14}C

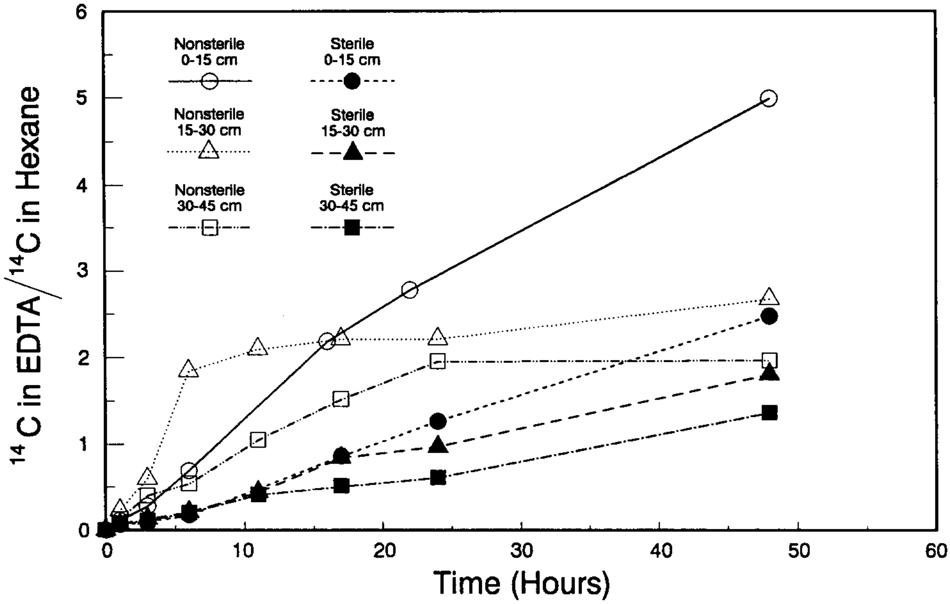

Figure 2. Distribution of ^{14}C in EDTA/^{14}C in Hexane extracted from nonsterile and autoclaved Arredondo soil treated with ^{14}C-TEL.

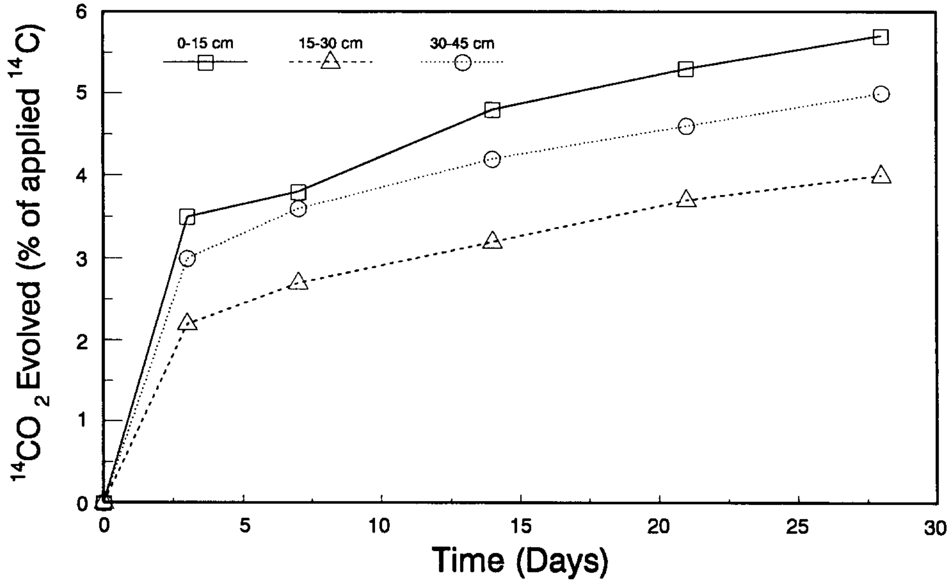

Figure 3. $^{14}CO_2$ evolution from Arredondo soil treated with ^{14}C-TEL.

trapped in the KOH traps was associated with ^{14}C organics, possibly ^{14}C-TEL, it was likely that ^{14}C not accounted for was associated with volatile ^{14}C organics (possibly volatile degradation products) which had little capacity to be trapped in KOH. No ^{14}C was found in hexane extracts, and 3.9% to 6.2% of the applied ^{14}C was found in EDTA phase (Table 2). This indicated that after 28 d, TEL in nonsterile soil was completely degraded to ionic forms and other unknown volatile products.

Table 2. Distribution of ^{14}C in nonsterile Arredondo soil after 28 d incubation with ^{14}C-TEL.

Depth (cm)	$^{14}CO_2$	^{14}C-organics	^{14}C in hexane	^{14}C in EDTA	Nonextractable ^{14}C	Recovery
	(% of applied ^{14}C)					
0-15	5.7	0.1	0	6.2	16.0	28.0
15-30	4.0	0.2	0	3.9	9.5	17.6
30-45	5.0	0.2	0	5.7	13.0	23.9

Our results indicate that transformation of TEL to ionic forms in soil occurred both biologically and chemically. It is well known that use of autoclaving for soil sterilization alters soil physical and chemical characteristics (Skipper and Westermann, 1973). Therefore, chemical degradation rates of TEL in nonsterile and autoclaved soils may not be the same. Nevertheless, biological degradation certainly contributes to the disappearance of TEL in soil. Mineralization of TEL did occur in nonsterile soil.

The fact that, after 28 d of incubation, no TEL could be detected in the Arredondo soil and considerable amounts of ionic ethyllead species were present suggests that TEL is less persistent in soil than its degradation products, ionic ethyllead species. This finding is in agreement with the fate of tetraalkyllead compounds and ionic alkyllead species in water, where ionic lead species are considerably more stable than tetraalkyllead compounds (Radojevic and Harrison, 1987b). This study did not determine the distribution of individual ionic ethyllead species and, thus, whether triethyllead or diethyllead is the dominant species remains to be determined.

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