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Biodegradation of ^{14}C-*tris*(2,3-Dibromopropyl) Phosphate in a Laboratory Activated Sludge System

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Tris(2,3-dibromopropyl) phosphate (TRIS) was extensively used before 1977 as a flame retardant additive in polyester fabrics, home furnishings, building materials, and in other applications. Production of TRIS was 10 million pounds per year from 1973 to 1976 (McGEEHAN & MADDOCK 1976). BLUM & AMES (1977) reported that TRIS was padded onto the surface of polyester fabrics in amounts up to 10% by weight. GUTENMANN & LISK (1975) estimated that up to 10 μg of TRIS per square inch could be removed from treated fabrics during a simulated laundering operation.

The toxic effects of TRIS have been reported by numerous workers. VAN DUUREN et al. (1978) showed that TRIS caused malignant tumors in the skin, forestomach, and oral cavity of mice treated dermally with TRIS. OSTERBERG et al. (1977) observed renal and testicular damage in male rabbits following topical application of TRIS. PRIVAL et al. (1977) implicated TRIS as a mutagen using the Ames test (AMES et al. 1975). BRUSICK et al. (1980) also reported mutagenic activity of breakdown products of TRIS found in the urine of rats exposed to TRIS. MAYLIN et al. (1977) demonstrated that immersion of TRIS-treated fabric in water containing goldfish resulted in the death of all fish within 24 h. ST. JOHN et al. (1976) showed that TRIS was metabolized to 2,3-dibromopropanol by rats. The Consumer Product Safety Commission (FEDERAL REGISTER 1977) banned from commerce the use of TRIS-treated fabrics in 1977 because of the possible harmful effects on children. BLUM & AMES (1977) were concerned not only with the hazards posed by TRIS to plant workers engaged in its production and wearers of treated fabrics but also with the environmental hazards that might be posed by its disposal in large quantities into water and soil.

The reported leaching of TRIS-impregnated fabrics during laundering indicated that TRIS could ultimately enter municipal treatment plants and septic tanks. For this reason, this laboratory investigated the fate of TRIS in a laboratory activated sludge system.

MATERIALS AND METHODS

Chemicals: Tris(2,3-dibromopropyl) phosphate, propyl-1-^{14}C, 2.3 μCi/mg, was purchased from New England

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Nuclear, Boston, MA. 2,3-Dibromopropanol and 2,3-dibromopropanoic acid were purchased from Aldrich Chemical Co., Milwaukee, WI. Precoated silica gel thin layer chromatographic (TLC) plates with fluorescent indicator were obtained from Analtech, Newark, DE.

Bis(2,3-dibromopropyl) phosphate was synthesized as follows: To a cooled mixture of 16.5 g (107 mmol) of $POCl_3$, 100 mg of $AlCl_3$, 25 mL of dry pyridine, and 50 mL of dry benzene, 46.8 g (214 mmol) of 2,3-dibromopropanol in 50 mL of dry benzene was added dropwise. The mixture was stirred at room temperature for 18 h. After evaporation of the solvent, the mixture was poured onto ice, acidified with 200 mL of 10% HCl, extracted with three 50 mL portions of ethyl ether, and the combined extracts were dried over anhydrous Na_2SO_4. After removal of the ether in a rotary evaporator, 39 g of bis(2,3-dibromopropyl) phosphate was obtained as a light yellow oil. A portion of this oil was converted to its methyl ester by reaction with diazomethane. The structure of the synthetic compound was confirmed by gas chromatography-mass spectrometry (GC/MS), nuclear magnetic resonance spectroscopy, and infrared spectrophotometry.

Instrumentation: A liquid scintillation spectrophotometer (Tri-Carb, Model 3375, Packard Instrument Co., Downers Grove, IL) was used for counting radioactivity. Radioactive spots and bands on TLC plates were detected with a two-dimensional radiochromatogram scanner (Model 940, Vangard Systems, Inc., Stamford, CT). GC/MS identification was obtained with a Model 1015 S/L mass spectrometer (Finnigan Instruments Corp., Palo Alto, CA). Oxygen uptake rates were measured with a biological oxygen monitor (Model 53, YSI Co., Yellow Springs, OH). Absorbance was measured using an ultraviolet-visible spectrophotometer (Varian Techtron, Springvale, Victoria, Australia).

Source of Microorganisms: Activated return sludge was obtained locally (Arlington County Water Pollution Treatment Plant, Arlington, VA) and was used within 1 h after collection. The sludge was diluted with a basal medium (SOAP AND DETERGENT ASSOCIATION 1965) so that the approximate concentration of suspended solids was similar to that of the municipal treatment plant sludge. The resulting mixture of sludge and basal medium is hereafter referred to as "mixed liquor". Skim milk (0.15 g/L) was added to the mixed liquor as the energy source. Activity of the microorganisms was monitored by oxygen uptake rate measurements.

Laboratory-Model Sewage Sludge System: Experiments to determine metabolic products were conducted in a 500 mL gas-washing bottle equipped with a magnetic stirrer at room temperature (21°C). Compressed air (breathing quality) was introduced through the frit, and the sidearm was connected to a CO_2 trap (250 mL gas-washing bottle) that contained 200 mL of ethanolamine and ethylene glycol monoethyl ether (1:2). Three

hundred mL of mixed liquor and 0.6 mg of labeled TRIS (0.3 mL of a 2 mg/mL solution in acetone) were added to the flask to give a concentration of 2 ppm. Two control experiments were conducted under the same conditions - one with mixed liquor containing no TRIS and the other with a sterilized mixed liquor fortified with TRIS at a concentration of 2 ppm. Experiments were conducted for 24 h. Air (28 mL/min) and stirring were provided continuously during the experiment.

Separate experiments to determine the rate of degradation of TRIS were conducted in a 2000 mL resin flask containing 2 ppm of TRIS in 1500 mL of mixed liquor. Duplicate 50 mL aliquots were withdrawn at 0, 4, 7, 24, 30, and 48 h. These samples were immediately frozen and stored frozen until analysis. The larger volume was used because many data points (hence, aliquots) were required.

Analytical Procedures: After 24 h, the mixed liquor (300 mL) was centrifuged at 2000 rpm for 20 min at 0°C. The aqueous phase was extracted with three 300 mL portions of petroleum ether. The petroleum ether extracts were combined, dried over Na_2SO_4, and concentrated. The aqueous phase was then acidified with 20 mL of concentrated HCl to produce a concentration of about 1 N. It was extracted with three 300 mL portions of ethyl ether. The ethyl ether extracts were combined, dried, and concentrated. The solids were suspended in 150 mL of distilled water and extracted with three 150 mL portions of petroleum ether. The petroleum ether extracts were combined, dried, and concentrated. The remaining suspension of solids was acidified with concentrated HCl to 1 N and was then extracted with three 300 mL portions of ethyl ether. The ethyl ether extracts were combined, dried, and concentrated. In the studies to determine the rate of degradation, 50 mL aliquots were extracted with three 50 mL portions of petroleum ether. The petroleum ether extracts were combined, dried, and concentrated. Scintillation counting and TLC analyses were performed on aliquots of all fractions.

Isolation of the Metabolite: The concentrated ethyl ether extract of the aqueous phase was streaked on preparative TLC plates which were then developed in acetic acid-methanol-benzene (5:18:77). The radioactive band was detected by radioactive scanning, isolated, and scraped off. The silica gel was extracted with 25 mL of 0.2 N sodium bicarbonate solution. The bicarbonate solution was acidified and extracted with three 25 mL portions of ethyl ether. The ethyl ether extracts were then combined, dried, and concentrated. This extract was treated with diazomethane, purified by TLC, and analyzed by GC/MS.

RESULTS AND DISCUSSION

The distribution of radioactivity from various fractions of the biodegradation studies is listed in Table 1. The total recovery of the added radioactivity was 91%. TLC of the

TABLE 1

Distribution of Radioactivity in Various Fractions

Fraction	Radioactivity Found, %[a]
Aqueous Phase	
Petroleum ether extract	21.6 ± 6.7[b]
Ethyl ether extract	18.1 ± 2.5
Remainder	3.4 ± 2.7
Solids	
Petroleum ether extract	33.7 ± 6.4
Ethyl ether extract	2.7 ± 1.3
Remainder	5.6[c]
Trap	5.8
Total	90.9

[a]Average of 4 experiments.
[b]Standard deviation.
[c]One determination.

TABLE 2

R_f Values of TRIS-Related Compounds[a]

Sample	Solvent[b]	R_f
TRIS	I	0.73
Petroleum ether extract	I	0.73
Ethyl ether extract	II	0.64
Bis(2,3-dibromopropyl) phosphate	II	0.64
2,3-Dibromopropanol	II	0.84
2,3-Dibromopropionic acid	II	0.86

[a]Spots were detected either by radioactive scanning or were visualized after spraying with 1% aqueous silver nitrate and exposed to ultraviolet light (GARDNER 1979).
[b]Solvent I - ethyl acetate-benzene (10:90).
Solvent II - acetic acid-methanol-benzene (5:18:77).

petroleum ether extracts (aqueous and solids) showed a single radioactive spot with the same R_f value (0.73) as authentic TRIS (Table 2). The recovery of undegraded TRIS was 55% (21.6% from the aqueous phase and 33.7% from the solids) after 24 h.

The mineralization of TRIS by the sludge system was indicated by the $^{14}CO_2$ in the trap, 6% of the added radioactivity. In a sterilized sludge control study, 93% of the added TRIS was recovered in the petroleum ether extracts; no metabolites were found in the ethyl ether extracts and no radioactivity was detected in the trap.

The data on the disappearance of TRIS versus time are plotted in Figure 1. The half-life of TRIS was estimated to be 19.7 h by least squares regression analysis.

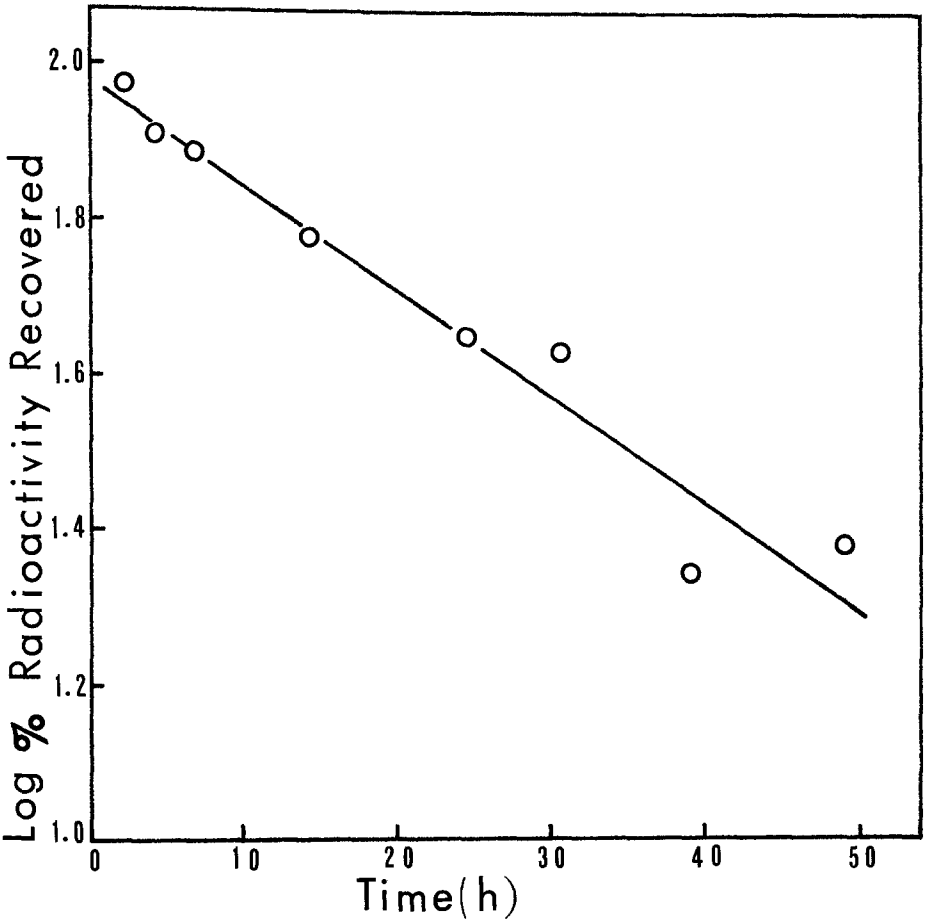

Figure 1. Biodegradation of TRIS versus time.

The ethyl ether extracts of both the solids and the aqueous phase contained 21% of the added radioactivity and the majority of this radioactivity was present in the aqueous phase (18%). TLC of this fraction showed a single radioactive spot with R_f 0.64, which is identical to the R_f of bis(2,3-dibromopropyl) phosphate (Table 2). The radioactive compound in this fraction was isolated by TLC, methylated, and analyzed by GC/MS. This compound had the same GC retention time and a mass spectrum identical to the methyl ester of bis(2,3-dibromopropyl) phosphate. Therefore, the major metabolite of TRIS in the sludge process was identified as bis(2,3-dibromopropyl) phosphate. No TRIS was detected in the ethyl ether extracts. Dibromopropanol was identified by ST. JOHN et al. (1976) as a urinary metabolite of rats treated with TRIS. However, in our experiments neither dibromopropanol nor its oxidation product, dibromopropionic acid (Table 2), was detected. The residual radioactivity in the aqueous phase and the solids is about 9% of total radioactivity after the petroleum ether and ethyl ether extractions. This radioactivity was not investigated. In summary, approximately 50% of the TRIS was degraded in 24 h, and the major metabolite was identified as bis(2,3-dibromopropyl) phosphate. The toxicity and environmental consequences of this metabolite are not known.

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