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Role of Exposure Mode in the Bioavailability of Triphenyl Phosphate to Aquatic Organisms

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Abstract. A laboratory study was conducted to investigate the role of the route of triphenyl phosphate (TPP) entry on its aquatic bioavailability and acute biological effects. Three TPP treatments were used for exposures of fish and invertebrates. These consisted of TPP dosed directly into water with and without clean sediment and TPP spiked onto sediment prior to aqueous exposures. Results of static acute toxicity tests (no sediment) were 0.78 mg/L (96-h LC50) for bluegill, 0.36 mg/L (48-h EC50) for midge, and 0.25 mg/L (96-h EC50) for scud. At 24 h, the sediment (1.1% organic carbon)/water partition coefficient (Kp) for TPP was 112. Use of this partition coefficient model to predict the sediment-mediated reduction of TPP concentration in water during toxicity tests resulted in a value that was only 10% less than the nominal value. However, the required nominal concentration of TPP to cause acute toxicity responses in test organisms was significantly higher than the predicted value by the model for both clay and soil-derived sediment. Direct spiking of TPP to soil minimized TPP bioavailability. Data from parallel experiments designed to track TPP residues in water through time suggest that sorption kinetics control residue bioavailability in the initial 24 h of exposure and may account for observed differences in LC50 and EC50 values from the sediment treatments.

Triaryl phosphate esters are used for hydraulic fluids and fire retardants (Midwest Research Institute 1979), and their losses to the environment through leakage and leaching can be substantial (Muir 1984). Most of the acute toxicity of commercial triaryl phosphate ester mixtures seems to be from the triphenyl phosphate (TPP) component (Midwest Research Institute 1979; Mayer *et al.* 1981; Cleveland *et al.* 1986). The TPP 96-h LC50 values reported by Mayer *et al.* (1981) for rainbow trout (0.40 mg/L), fathead minnow (0.66 mg/L), and sheepshead minnow (0.32-0.56 mg/L) are considerably lower than the 96-h LC50 value observed for bluegill in another study (Dawson *et al.* 1977).

Several investigators examined the bioaccumulation and environmental fate of TPP (Bengtsson *et al.* 1986; Saeger *et al.* 1979; Muir *et al.* 1982), but the role of exposure route and sediment interactions in the bioavailability of TPP has received little attention. This work examines the effect of topsoil and clay sediments on acute TPP bioavailability, which is inferred from the nominal TPP treatment levels required to elicit effects in test organisms. The mode of TPP environmental entry (*i.e.*, sorbed to particulates or in aqueous solution) is shown to be a probable factor in acute bioavailability. Also, the acute toxicity of TPP to organisms for which little previous information exists is reported.

Experimental

Materials

TPP (99% purity; see Table 1 for other physicochemical properties) was obtained from Eastman Chemical Co, Rochester, NY. Test organisms included bluegill (*Lepomis macrochirus*), scud (*Gammarus pseudolimnaeus*) and larval midge (*Chironomous riparius*). Sediment was locally obtained topsoil (wind-blown loess; 5% sand, 77% silt, 18% clay; 1.8% moisture; 1.12% organic carbon). Montmorillonite clay (17.7% moisture; 0.33% organic carbon) was obtained from the National Clay Repository, Dept. of Geology, University of Missouri. Organic carbon content of soil and clay was determined by a Coulometrics Model 150 Total Carbon Analyzer. Silver membrane filters (0.2 μm) were purchased from Selas Corp. of America, Huntington Valley, PA. and Teflon^R membrane filters (0.2, and 10 μm) were obtained from Millipore Corp., Bedford, MA. All solvents were nanograde quality from Burdick and Jackson Laboratories Inc., Muskegon, MI.

Instrumental

A Varian model 3700 gas chromatograph (GC) with a thermionic detector was used for TPP residue analysis. The GC column (2 mm id. by 183 cm long) was a 3% OV-101 on Chromosorb W-HP (80–100 mesh). Analyses were isothermal with column temperature set at 210 or 220°C; injector at 230°C; and the detector at 300°C.

Toxicity Tests

Determination of the standard aqueous toxicity of TPP to bluegill, scud, and midge was in basic accordance with procedures by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). All TPP stocks and dilutions were made in acetone.

Table 1. Physicochemical properties of triphenyl phosphate

Molecular wt[a]	326.28
Specific gravity (25°C)[a]	1.27
Melting point (°C)[a]	49–50
Boiling point (°C)[a]	245
Vapor pressure @ 25°C (mm Hg)[a]	5.3×10^{-5}
Water solubility (mg/L at 25°C)[b]	1.9
Henrys Law constant (atm × m^3/mol)	1.2×10^{-5}
Octanol-water partition[b] coefficient, K_{ow}	4.1×10^4

[a] Midwest Research Institute 1979
[b] Mayer *et al.* 1981

Ten bluegill (0.5–1.0 g each) were placed in glass jars containing 15 L of laboratory well water of known quality (Huckins *et al.* 1986) at 22°C. Following a 24-h acclimation period, fish were exposed to 0, 0.5, 1.0, 2.5, 5.0, and 10.0 mg/L of TPP. A concentration of 1 g/L of soil and clay was used for sediment interaction experiments which is within the range of values reported for soil losses in runoff (Smith *et al.* 1978). Jars receiving sediment were dosed in one of two ways: 1) A small amount of TPP stock solution (<100 μl acetone/L water) was added directly to test water immediately after addition of sediment, 2) TPP stock solution (same volume, as in "1") was spiked onto air-dried sediment, the sediment was mixed and the acetone was allowed to evaporate for 30 min, and then TPP-containing sediment was added to test water.

Tests with midge were performed with 4th-instar larvae. Ten individuals were placed in 150 mL of well water in a glass vessel at 22°C. Animals were dosed after a 4-h acclimation. Acute toxicity of TPP was determined for 0, 0.06, 0.10, 0.18, 0.32, 0.56, and 1.0 mg/L treatments of TPP. Another series of treatments (3 replicates/treatment) received separate additions of clay (1.0 g/L) and of TPP (0, 0.27, 0.54, 1.08, 2.16, and 4.32 mg/L).

Tests with scud (mid-instar, 60- to 90-d old) were conducted in 4-L aquaria with well water at 17°C. Following a 4-h acclimation period, aquaria (N = 2) were separately dosed with 0, 0.010, 0.018, 0.032, 0.056, 0.10, 0.18, 0.32, 0.56, and 1.0 mg/L of TPP.

The 96-h median lethal concentrations (LC50) for bluegill, 96-h median effective concentrations (EC50) for scud, and 48-h median effective concentrations (EC50) for midge were calculated by the method of Litchfield and Wilcoxon (1949).

Sediment/Water Partition Coefficient (Kp)

The steady state Kp value was determined using deionized water (pH ≃ 6.5) according to the methodology of Karickhoff *et al.* (1979). Both filtration and centrifugation were used for the separation of sediment (clay and topsoil) and water phases. By the filtration approach, 100 μg of TPP in acetone was spiked into amber bottles (250 mL size, Thomas Scientific) equipped with caps having teflon liners. The acetone was removed by evaporation with an air stream for 30 min. A 100-mg portion of air-dried topsoil was added along with 100-mL deionized water to each bottle. The sediment-water mixtures were then placed on a rotary shaker at 25°C and mixed for 24 h. Longer equilibration periods were not used for Kp determination because of potential TPP hydrolysis (significant factor at pH ≥ 7.8) and microbial degradation (Howard and Padmakar 1979; Saeger *et al.* 1979; Muir *et al.* 1982). After 24 h, the supernatants and sediments were decanted and filtered through 0.2-μm silver filters with a 10-μm Teflon prefilter. Filters with sediments were cut into strips <1 cm wide with clean stainless steel scissors and placed along with ≃2-g anhydrous Na_2SO_4 into 1-cm id. glass columns equipped with solvent reservoirs and Teflon stopcocks. The sediments and/or filters in the columns were extracted with 60 mL of methylene chloride at <5 mL/min to recover TPP residues. These sediment extracts were prepared for GC analysis as described for water extracts.

Two spiking levels of TPP were employed in the determination of Kp values by centrifugation. Amber glass bottles (100-mL size) were spiked separately with 3.0 and 30.2 μg of TPP, and the solvent was evaporated as described for the filtration method. Then 30 mg of sediment and 30 mL of deionized well water were added to each bottle and the mixtures were shaken for 24 h as described earlier. The sediment/water mixtures were transferred to 30-mL glass (Corex^R) centrifuge tubes and centrifuged at 12,000 rpm for 1 h and 20 min. Following centrifugation, the bulk-water phases (<1 mL water remained with each sediment pellet) were decanted into glass-separatory funnels. The water phases from both centrifugation and filtration approaches were each partitioned three times with 25-mL aliquots of methylene chloride for the recovery of TPP. The methylene chloride extracts were combined for each sample and ≃25 mL of hexane was added. The extracts were concentrated with a stream of nitrogen to ≃1-mL volume and hexane was added again to adjust the volume to 10 mL for GC analysis.

The Kp values were calculated from the relationship:

$$K_p = C_s/C_w,$$

where K_p is the sediment/water partition coefficient, C_s is the concentration of sorbate in the sediment in ng/g, and C_w is the pseudo-steady state aqueous concentration of TPP in ng/mL. Procedural losses of TPP residues, which include sorption on filters or retention of TPP micro-droplets, and non-extracted and volatilized residues were corrected by using blanks and spiked samples. This QA/QC component represented ≃20% of each sample set. Recovery of TPP from sediment and water phases averaged 85.9 ± 9.1 (N = 13) and 89.5 ± 23.4 (N = 5), respectively.

Residue Bioavailability Kinetics

Rates of TPP adsorption on sediment and desorption from sediment were examined to determine the short-term bioavailability of TPP. Desorption kinetics were studied by spiking 600 μg of TPP (acetone-solubilized) on 600 mg of air-dried soil. After a 30-min drying interval, the sediment-TPP mixture was added to a 1-L brown bottle containing 600 mL of deionized well water. Adsorption kinetics were examined by spiking 600 μg of TPP directly into 600 mL of deionized well water prior to addition of air-dried soil. Dissolution kinetics and/or solution heterogeneity were also examined by spiking 600 μg of TPP into 600 mL of deionized well water. Twenty-four bottles for each treatment were prepared and placed on a rotary shaker table at 25°C. Three replicate water samples from each treatment were removed at T = 1 min, 4 min, 16 min, 1 h, 4 h, 24 h, 48 h, and 96 h intervals and processed immediately (0.2 μm silver filters used to remove particulates) to permit monitoring of TPP concentrations. Water and sediment samples were analyzed as described earlier for Kp determinations.

Results and Discussion

Bluegill

The acute toxicity (standard method, no sediment) of TPP to bluegill (0.78 mg/L, 96-h LC50) was similar to the range of values reported by Mayer *et al.* (1981) for rainbow trout, fathead minnow, and sheepshead minnow. During toxicity tests with sediment, neither montmorillonite clay nor topsoil

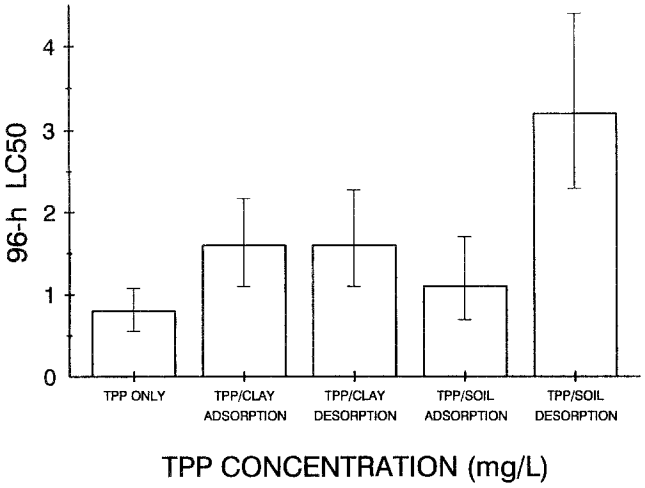

Fig. 1. Effect of substrate type and mode of addition on bioavailability of TPP to bluegill; $\bar{x} \pm 95\%$ confidence intervals are shown

adversely affected bluegill survival under the test conditions used. However, both types of sediment appeared to reduce the bioavailability of TPP to bluegill (Figure 1) as indicated by numerically higher LC50 values. Spiking TPP into water containing soil (adsorptive approach to steady state) appeared to reduce its bioavailability to bluegill less than when TPP was spiked directly on soil (desorptive approach to steady state) prior to addition to water (Figure 1). The large difference between TPP-soil adsorption and desorption LC50 values are probably significant, because the 95% confidence intervals, shown as $\pm$ of $\bar{x}$'s in Figure 1, do not overlap.

Table 2. Acute toxicity of triphenyl phosphate (mg/L). Values in parentheses represent 95% confidence interval

Species	Present study	Other studies
Bluegill	0.78[a] (.47 to 1.04)	290[b]
Scud	.25[c] (.16 to 0.39)	NC[d]
Midge	.36[e] (.25 to 0.52)	NC

[a] 96-h LC50
[b] 96-h LC50 (Dawson *et al.* 1977)
[c] 96-h EC50
[d] NC indicates no comparable data found in literature
[e] 48-h EC50

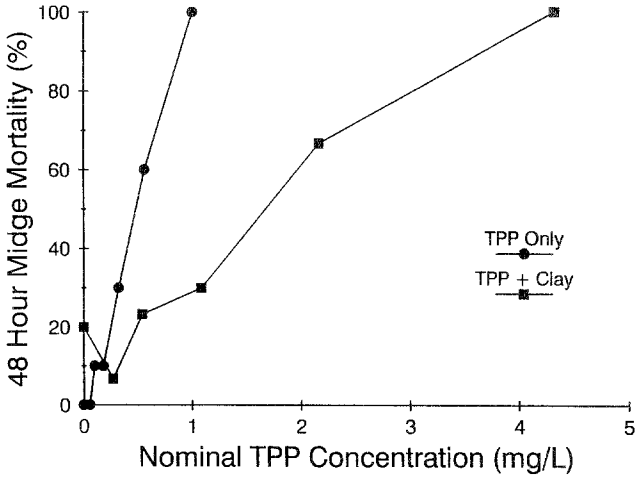

Fig. 2. Bioavailability of TPP to midge in presence and absence of montmorillonite clay

Invertebrates

Scud and midge exhibited similar sensitivities to TPP (0.25 mg/L, 96-h EC50; 0.36 mg/L, 48-h EC50, respectively; Table 2). Although no previously published information exists for these particular species, Mayer *et al.* (1981) found comparable sensitivities in *Daphnia magna* (1.0 mg/L, 48-h EC50) and mysid shrimp (0.18–0.32 mg/L, 96-h LC50). Neither clay nor topsoil had adverse effects on scud or midge at the 1.0 g/L test concentrations.

Comparison of invertebrate toxicity data for TPP to that of Sanders *et al.* (1985) for six commercial phosphate ester mixtures containing TPP ($\leq$20% w/w of each product) indicated that the mixtures were about 10-fold less toxic to midge and scud. The results of toxicity tests with variable concentrations of TPP in the presence of 1.0 g/L montmorillonite clay indicated that five-fold greater nominal concentrations of TPP were required to reach the EC50 endpoint in midges (Figure 2). Thus, the presence of clay also reduced the short term bioavailability of TPP to midge.

TPP Distribution and Kinetics

The 24-h TPP K_p value (soil-phase separation by filtration) was 112 $\pm$ 26.8 (N = 4). Filtration was used in all kinetics experiments because of the need for rapid separation of sediment and water phases. Centrifugation (12,000 rpm, 80 min) was used to validate the K_p value based on filtration and the resulting value was 96 $\pm$ 41 (N = 3) at 24 h. The measured K_p values were compared to a calculated value based on the equation by Karickhoff *et al.* (1979):

$$K_p = 0.63\ K_{ow}\ (\text{F.O.C.}),$$

where K_{ow} is the octanol-water partition coefficient of TPP and F.O.C. is the fractional organic carbon content of the sediment used. The calculated K_p value (317) for TPP using a K_{ow} of 4.2×10^4 (Mayer *et al.* 1981) differed threefold from our measured values.

The K_p for clay is not reported because of a high coefficient of variation ($\geq$50%) observed from individual determinations. Some of this variability may be explained by the lack of uniform distribution of TPP spiked on the clay surface (slurry application required for complete uniformity), assuming the clay had heterogeneous surface activity for sorbate molecules. However, the mean value clay K_p was about three-times higher than that of soil. The low organic carbon content (0.33%) of the montmorillonite clay suggests that non-organic carbon sorptive interactions, such as those ascribed to Al-, Fe-, and Mn-oxides and/or hydroxides (Terce and Calvet 1978), may have played a role in the relatively high and variable clay K_p values.

The kinetics studies with soil indicated that the initial TPP-

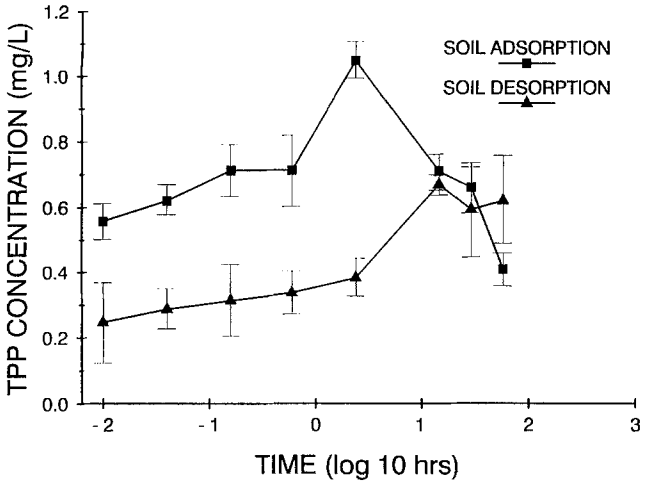

Fig. 3. Effect of soil and exposure mode on aqueous concentrations of TPP

phase distribution (0 to 24 h, water and sediment) was largely dependent upon the mode of entry into water (Figure 3). Aqueous TPP concentrations during the first 24 h were highest in the treatment receiving clean sediment first and then TPP. This treatment was designed to represent the adsorptive approach to steady-state aqueous concentrations of TPP. Examination of water residues revealed that dissolved TPP increased from 0.56 to 1.05 mg/L during the first 4 h of the adsorptive treatment and decreased during the remaining 92-h interval. The initial rise in aqueous TPP concentrations (0 to 4 h) is the opposite of normally observed rapid adsorptive (sediment) reduction of residues (Voice *et al.* 1983). This data suggests that TPP spiked into water may have precipitated out, settled to the bottom (see physicochemical properties given in Table 1), and slowly dissolved (Thomas *et al.* 1986). The volume of TPP carrier solvent (acetone) in toxicity and kinetics experiments was limited to <100 µl/L of water because of the potential for solvent effects on test organisms and changes in sediment/water distribution coefficients. Use of this low solvent volume for TPP spikes into water initially results in a very small mixing zone of TPP and water in which the aqueous solubility of TPP may be exceeded as acetone disperses into solution. Separate dissolution-mixing kinetics tests (no sediment) also revealed low initial (0–4 h) TPP concentrations in water.

The percent of the nominal concentration of TPP remaining in solution after sediment sorption was estimated using the following equation:

$$C_w = T_{cm}/G_w + (G_s * K_p),$$

where C_w = water concentration in µg/L, T_{cm} = total contaminant mass in g, G_w = water mass in g, G_s = sediment mass in g, and K_p is as defined earlier. Based on the experimental conditions of the kinetics study and the measured K_p value of 112, the concentration of TPP in water at 24 h would be reduced 10% from the nominal value or down to 0.9 mg/L. The actual measured water concentrations in kinetics experiments (adsorptive and desorptive 24-h values) were 0.71 mg/L and 0.67 mg/L, respectively. The difference between the 0.9 mg/L concentration, predicted by the previously described equation, and the measured value in the kinetics study may be due to differences in: 1) TPP spiking methods used in K_p and kinetics experiments, 2) sorptive capacity of the containers (different surface area to volume ratios) in K_p and kinetics experiments, 3) differences in TPP losses from volatilization ($H = 1.2 \times 10^{-5}$), and 4) the coefficient of variation was ≈25% for the filtration-determined K_p value of TPP.

When TPP was spiked onto soil prior to addition of water (Figure 3), dissolved concentrations of TPP rose slowly during the first 24 h, whereas the adsorptive treatment reached a water concentration maxima in only 4 h. Aqueous TPP concentrations in samples from the desorptive treatment increased from 0.25 to 0.62 mg/L ($\bar{x}$ values) during the first 24 h and remained relatively constant during the final 72 h.

Analysis of the data from the adsorptive and desorptive TPP treatments shown in Figure 3 revealed only a ≈11% difference in dissolved residue concentrations during the 96-h experiments. However, a ≈43% difference existed between the mean-aqueous TPP concentrations (adsorptive approach, 0.88 mg/L and desorptive approach, 0.50 mg/L) of the two treatments in the first 24 h. Only in the adsorptive sediment treatment did a TPP-water sample (4 h) exceed the nominal LC50 value of 0.78 mg/L for bluegill. A sharp decline in the aqueous concentration of TPP (adsorptive treatment) after 48 h suggests that biodegradation of TPP may have occurred during the last 48 h of the treatment. Also, since TPP toxicity and sorption kinetics tests were conducted at different temperatures (3°C difference excluding scud) and both molecular diffusion and sorption rates are affected by temperature, a small difference in the water concentration of bioavailable TPP would be expected in the two different tests.

Conclusions

Sorption on clay and soil reduced the initial bioavailability of TPP to aquatic organisms. The magnitude of the mitigative effect of sediment sorption on nominal LC50 values of test-organisms seems to be related to the mode of TPP-sediment exposure. This is indicated by the large difference between TPP-LC50 values for bluegill when tests with soil were conducted under adsorptive and desorptive conditions (Figure 1). Implicit in these results is that adsorption and desorption kinetics of TPP on soil are dissimilar or non-singular with the latter occurring more slowly. Also, kinetics data suggest that the relatively higher concentration of TPP in water during the first 24 h of the adsorptive exposure is the causitive factor in the lower nominal LC50 value. The standard EC or LC50's of bluegill, scud and midge were within a range of 0.1 to 0.5 of TPP-water solubility and compare well with most other published values with similar species.

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