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Toxicity, Absorption and Elimination of Phosphoric Acid Triesters by Killifish and Goldfish

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Phosphoric acid triesters (phosphates) have been widely utilized as flame retardant plasticizers, hydraulic fluids and so on. Some of them are considerably lipophilic and tend to be bioconcentrated in adipose tissues. Indeed, TBP (tributylphosphate), TCEP (trichloroethyl phosphate) and TDCPP [tris(1,3-dichloroisopropyl) phosphate] have frequently been detected in water, sludge or fish in Japan (Japan Environment Agency 1977, 1978, 1979). Therefore phosphate in general must be regarded as potential, if not actual, environmental pollutants.

There have been several studies on the chronic toxicity and the mutagenicity of phosphate. Tri-*o*-cresyl phosphate has been shown to possess neurotoxicity (HINE et al. 1956). Tris(2,3-dibromopropyl) phosphate and TDCPP were shown to be mutagenic after they had been activated by mouse or rat liver homogenate (BLUM and AMES 1977, GOLD et al. 1978).

Based on the octanol/water partition coefficients of these phosphates, SAEGER (1979) concluded that the phosphates have moderate potential to accumulate in aquatic organisms. WAGEMANN et al. (1974) reported on the fish toxicity of triarylphosphates, and LOMBARDO and EGRY (1979) reported the accumulation of TPP (triphenylphosphate) in rainbow trout.

However, at present, much uncertainty remains about the environmental consequences and biological effects of phosphate. This paper deals with studies on the toxicity, absorption and elimination of 4 flame retardants (TBP, TCEP, TDCPP and TPP) with killifish and goldfish as test freshwater fish in a static water test system.

MATERIALS AND METHODS

Chemicals. TBP(tributylphosphate) was purchased from Wako Pure Chem. Co., and TCEP (trichloroethyl phosphate), TDCPP [tris(1,3-dichloroisopropyl) phosphate] and TPP (triphenylphosphate) were purchased from Tokyo Kasei Industry Co. Solvents were distilled before use in an all-glass Widmar distillation unit. Tap water was used after dechlorination by passage through an activated charcoal column.

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Fish. Goldfish, *Carassius auratus*, weighing 0.8-2.8 g and killifish, *Oryzias latipes*, weighing 0.1-0.2 g were purchased from a market and acclimated to the laboratory conditions for at least 10 days at 25°C.

GLC determination. A gas liquid chromatograph, model 4BM-PF (Shimadzu Seisakusho Ltd.), with a flame photometric detector was used for the determination of phosphates. Samples were injected directly into a 2 m x 4 mm id glass column packed with 7% OV-101 on Gas Chrom Q (80—100 mesh). The column temperature was set 250 to 290°C depending on the phosphate being analyzed.

Estimation of partition coefficient. A 50 mg portion of phosphate was accurately weighed and added to a separatory funnel containing 50 ml each of *n*-octanol saturated with water and distilled water saturated with *n*-octanol. The funnel was shaken mechanically for 2 hours and then allowed to stand overnight. Aliquots of the aqueous phase were extracted three times with *n*-hexane (dichloromethane in the case of TCEP) and analyzed for phosphates by GLC. The concentration in *n*-octanol was calculated by subtracting the amount in the aqueous phase from the original quantity. The partition coefficient was obtained as the phosphate concentration in *n*-octanol divided by that in water.

Estimation of LC50. Groups of 7-9 killifish or goldfish were held for 96 hours without feeding in 1 L (7 L for goldfish) of test solution. The water temperature was maintained at 25°C and the beakers were not aerated. The LC50 value was estimated from the number of survivals after 96 hours.

Static water test system. Water containing phosphate was prepared in 3-4 beakers (2 L). One of them was used as a control (no fish) to examine the stability of phosphates in water and the others were each used to rear 10-20 killifish or 3-5 goldfish. They were kept at 25°C without aeration. The original concentration of the test chemical were roughly adjusted to 3-4 ppm (TBP), 1-3 ppm (TCEP), 1 ppm (TDCPP) or 0.25 ppm (TPP). The fish were not fed for 48 hours before and throughout the experiment. The concentrations of the phosphates in water as well as in the fish were determined at various time intervals according to the method described below. At each sampling time, all the fish in one beaker were taken out and frozen until required for analysis.

Analysis of chemicals. For the determination of the chemicals in water, a 5 ml aliquot of the water was extracted twice with 5 ml of *n*-hexane (for extraction of TCEP, 5 ml of dichloromethane was used). For the analysis of fish, 1 to 4 fish were ground with 20 g of anhydrous sodium sulfate and extracted with 30 ml of ethyl acetate. The extracts were dried and analyzed by GLC.

RESULTS AND DISCUSSION

Partition coefficient. It is well known that the *n*-octanol/water

partition coefficient (O/W-PC) is closely related to water solubility (CHIOU 1977) and is a predictive factor in assessing whether a chemical will tend to be bioconcentrated or not. The O/W-PC of phosphate given in TABLE 1 varied from 27 for TCEP to 57260 for TPP and showed a good correlation with solubility data in the literature (SAEGER et al. 1979, ELDEFRAWI et al. 1977).

TABLE 1
Partition coefficients and solubilities of phosphoric acid triesters

Compound	Partition coefficient	Aqueous solubility
TBP	9720	280[a]
TCEP	27	7000[b]
TDCPP	5720	100[b]
TPP	57260	1.9[a]

a. SAEGER 1979
b. ELDEFRAWI 1977

Acute toxicity. The LC50 value of TCEP for killifish was a little higher than for goldfish but the other compounds gave almost the same values in the two species (TABLE 2). The acute toxicities of these phosphates seemed to be similar to those of organophosphorus insecticides such as malathion, dimethoate, dichlorvos and salithion, but much lower than those of organochlorine insecticides such as *pp'*-DDT, lindane and chlorinated cyclodienes. There was a good negative correlation (correlation coefficient : -0.94) between O/W-PC and LC50 for both species.

A characteristic manifestation of toxicity, the deformation of the spine, was caused by three phosphates (but not TBP) (TABLE 3) and protrusion of the eyes was caused by TCEP when fish were exposed for 24-72 hours to concentrations near the LC50 (the morbidity rate was 20-70 %).

Deformation of the spine caused by organophosphorus and carbamate insecticides has been reported by many workers (MEYER 1966, EATON 1970, NISHIUCHI 1971). IMADA (1979) concluded that deformation of the spine resulted from convulsive muscle contraction caused by inhibition of cholinesterase. TCEP and TDCPP are weak inhibitors of acetylcholinesterase (ELDEFRAWI et al. 1977), and so the deformation of the spine might be caused at least partly by that mechanism. Protrusion of the eyes was observed in carps exposed to Cartap [1,3-bis(carbamoylthio)-2-(N,N-dimethylamino) propane hydrochloride] (OHUCHI and KAWABE 1979), but the cause of this phenomenon is still obscure.

Bioconcentration. The behavior of phosphates in the static water test system is illustrated in Fig.1. The exposure doses (μg chemical in water/g body weight) for both species were set at similar levels and the concentrations of TBP, TDCPP and TPP in the water at the start were about 20-40% of LC50 (in the case of TCEP, 1% of LC50).

TABLE 2
50%-lethal concentrations of phosphoric acid triesters

Compound	LC50 (ppm) killifish	goldfish
TBP	9.6	8.8
TCEP	210	90
TDCPP	3.6	5.1
TPP	1.2	0.70

Duration of contact : 96 hours
Temperature : 25 C

TABLE 3
Deformation of the spine in killifish caused by phosphoric acid triesters

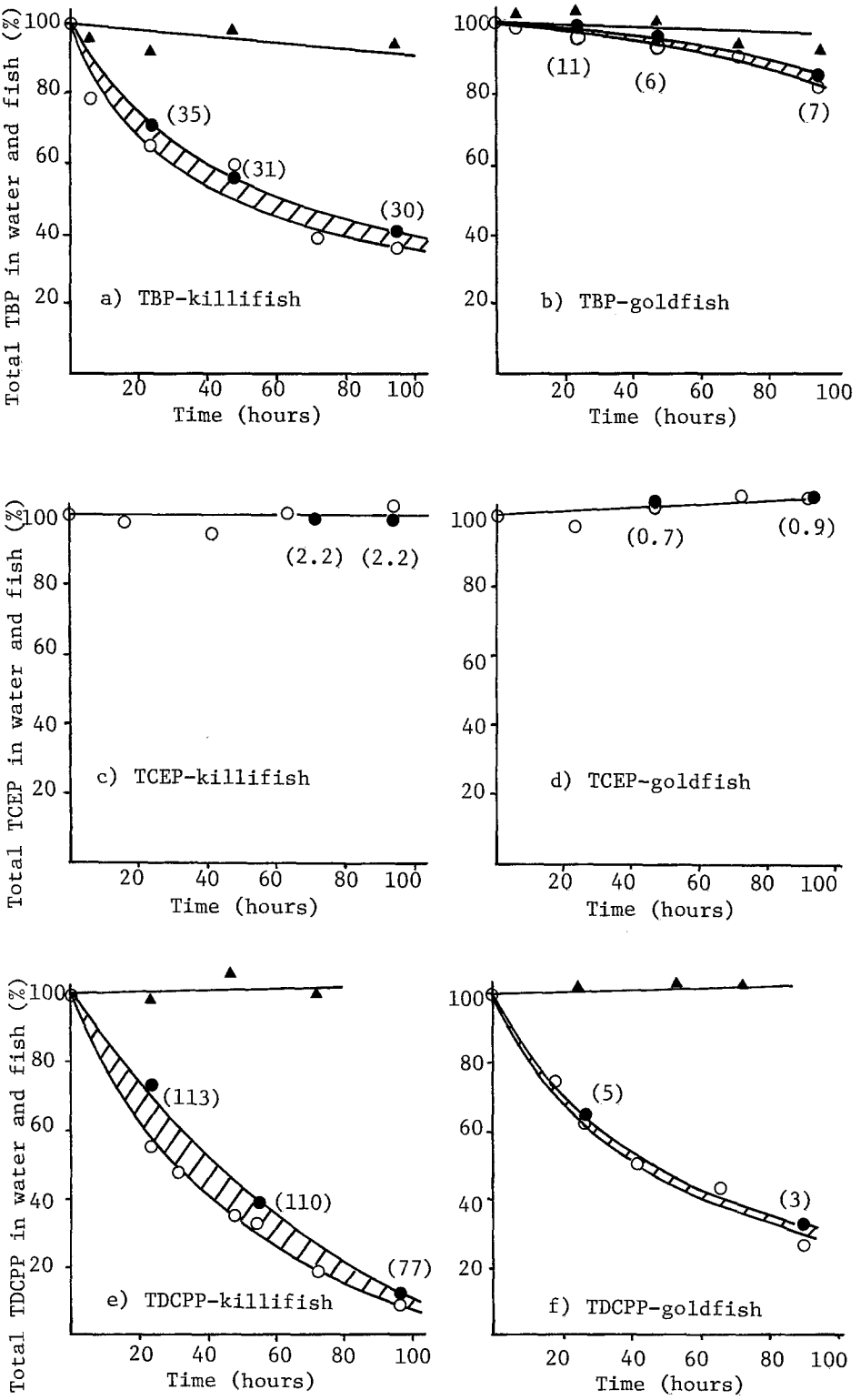
Compound	Concentration (ppm)	Exposure time (hours)	Result[a]
TCEP	200	72	3/12
TDCPP	3.5	24	7/10
TPP	1.1	24	2/10
TPP	1.1	72	4/10

a. morbidity/survival

As shown in Fig.1, the test chemicals were very stable in the control breeding water at 25°C for 96 hours in the absence of fish. TBP in the water containing killifish decreased rapidly compared with that in the water containing goldfish (Fig.1-a,b). The half-lives of TBP in the water containing killifish and goldfish were 58 hours and far more than 100 hours, respectively. Furthermore, the amount of TBP in the fish body varied with species as shown in Fig.1-a,b. It appears that killifish absorb and metabolize TBP more actively than goldfish. The bioconcentration of TBP in killifish was about 3 times greater than in goldfish. In spite of the variation of TBP concentration in the water, the bioconcentration ratio (BCR) was almost constant throughout the experimental period as indicated in parentheses in the figure, and a similar tendency was recognized in all other cases.

Fig.1-c,d shows that TCEP remained quantitatively in the water in both experiments and was not accumulated in the fish bodies ; consequently BCR was very low.

TDCPP in the water and in the whole system decreased with time (Fig.1-e,f). The half-lives of TDCPP in water were 31 hours for killifish and 42 hours for goldfish, so that the absorption of TDCPP occurred at similar rates in both fish. As regards the concentration of TDCPP in the fish body, however, species difference was observed, namely killifish accumulated 79 ppm and


Total TBP in water and fish (%)
100
80
60
40
20
(35)
(31)
(30)
a) TBP-killifish
20 40 60 80 100
Time (hours)
(11)
(6)
(7)
b) TBP-goldfish
20 40 60 80 100
Time (hours)
Total TCEP in water and fish (%)
(2.2) (2.2)
c) TCEP-killifish
20 40 60 80 100
Time (hours)
(0.7)
(0.9)
d) TCEP-goldfish
20 40 60 80 100
Time (hours)
Total TDCPP in water and fish (%)
(113)
(110)
(77)
e) TDCPP-killifish
20 40 60 80 100
Time (hours)
(5)
(3)
f) TDCPP-goldfish
20 40 60 80 100
Time (hours)

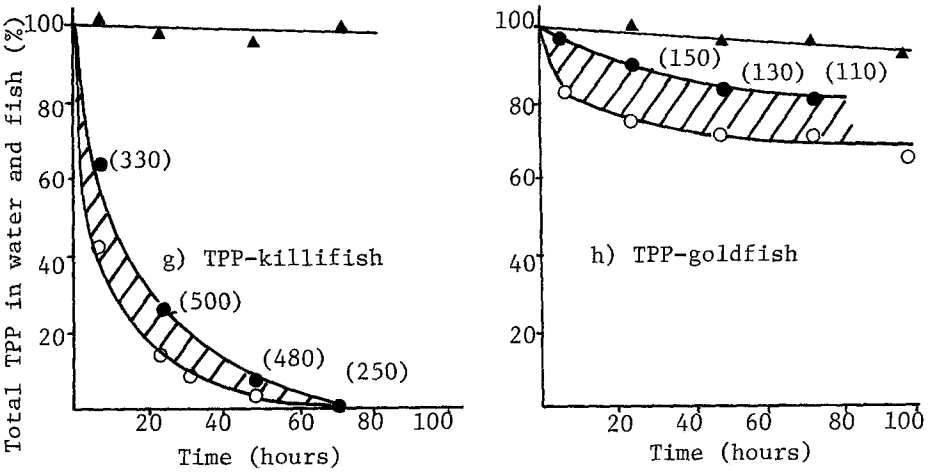


Figure 1. The behavior of phosphate in the static water test system with killifish and goldfish.
The vertical axis indicates the total amount of phosphate in the breeding water and in the fish bodies expressed as a percentage of the amount in the water at the start. (▲) shows the amount of phosphate in control breeding water without fish and (○) shows that in the test tank water. ▨ indicates the amount of phosphate in the fish bodies. (●) represents the total amount of phosphate retained in the whole system. Values in parentheses are bioconcentration ratios at the mesurement points.

18 ppm TDCPP but goldfish only 3.5 ppm and 0.8 ppm after 24 and 96 hours, respectively. Thus, BCR of TDCPP for killifish was much larger than that for goldfish. The difference in BCR suggests that there may be a difference in metabolic activity for TDCPP in these two species.

Fig.1-g,h shows the results for TPP. In the case of killifish, the decrease of TPP in water was the fastest among the chemicals tested, and the half-life was only 5 hours. In contrast, goldfish took up TPP rapidly in the first 5 hours but more slowly thereafter, so that the half-life in water was over 100 hours. The BCR of TPP was large compared with those of other phosphates in both species, as expected from its large O/W-PC. TPP originally present in the water was absorbed and metabolized completely by killifish within 72 hours, while goldfish absorbed TPP at a very slow rate, but accumulated TPP for a longer period. Thus, the ability to metabolize TPP is high in killifish while it is very low in goldfish.

In the 4 experiments discussed above, the original concentration of chemicals in water was set at levels from 0.25 ppm (TPP) to 4 ppm (TBP) depending on the LC50. To confirm that the above results were derived from differences of chemical nature rather than of test concentration, experiments at approximately the same

concentration (0.6-1.0 μmol/L) were also carried out.
The time courses of disappearance of the phosphates from water containing them at approximately the same concentrations were similar to those described above (Fig.2).

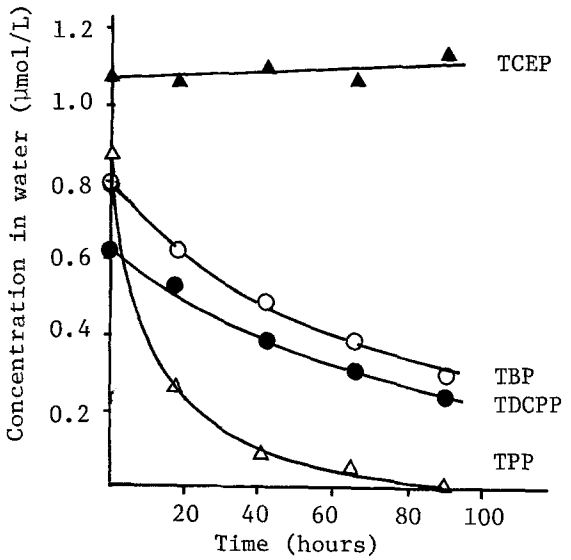


Figure 2. Persistence of phosphoric acid triesters in water in the presence of killifish.

From these results it would appear that the bioconcentration of chemicals should be assessed on the basis of not only O/W-PC or water solubility but also the capacity for absorption and the metabolic behavior. For such a purpose the graphical presentation of data as proposed in this paper is very convenient, since it makes visual judgement rather straightforward. The chemical and species differences in the absorption and elimination of these phosphates probably reflect variations in the metabolic enzyme activities of the fish and in the metabolic pathways for the phosphates.

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