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Bioconcentration and Excretion of Phosphoric Acid Triesters by Killifish (*Oryzeas latipes*)

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In the previous paper (SASAKI et al. 1981), we reported the toxicity of several phosphoric acid triester flame retardants to killifish and goldfish. It was also recognized in a static water test system that structure differences of phosphate influence their absorption and elimination rates diversely depending upon the species of fish. To examine the biological effect of chemicals in an aquatic environment, a continuous flow test system has been recommended as better than a static water test system. However, little work has been done to compare results obtained in the two experimental systems.

This paper deals with the bioaccumulation and excretion of the phosphoric acid triesters mentioned above by killifish in a continuous flow test system, and the results are compared with those obtained in a static water test system.

MATERIALS AND METHODS

Chemicals and Fish. All chemicals and fish were the same as described in the previous paper (SASAKI et al. 1981).

Preparation of test solutions. The test chemical solutions were preparated by dilution of acetone solutions of the phosphates (TBP, TDCPP and TPP were 1 mg/ml and TCEP was 150 mg/ml) with water to suitable concentrations, namely TBP 10 ppm, TCEP 600 ppm, TDCPP 4 ppm, and TPP 1 ppm.

Analysis of chemicals. Phosphoric acid triesters in fish and water were determined by FPD-GC after extraction with suitable solvents. The methods were described in detail in the previous report (SASAKI et al. 1981).

Continuous flow test system. The design of the experimental system is shown in Fig. 1. Test chemical solution was stored in Tank F, and it was supplied to the mixing equipment D by a constant-flow micro-pump E (Nisshin Chem. Co.) at a flow speed of 3.5 - 40 ml/hr. In the mixing equipment, the test chemical solution was diluted with water which was introduced from Tank H (80 L) via a tube pump G (RP type: Tokyo Rikakikai Co., Ltd.) at a flow speed of 1000-2000 ml/hr. The diluted solution was poured into the aquarium tank A (10 L) as test breeding water.

The concentrations of phosphates in the test breeding water

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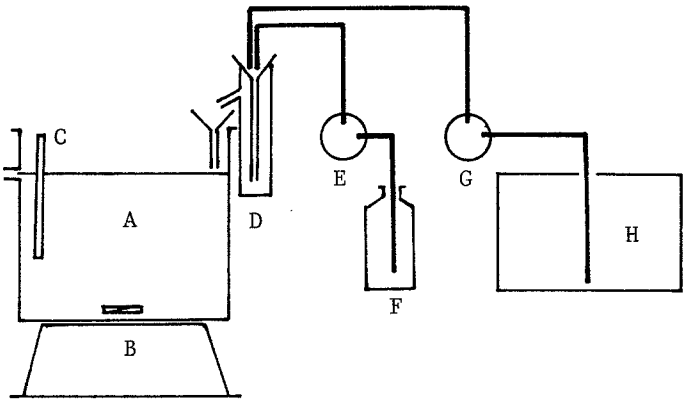

Fig.1. Continuous flow water test system.
A: aquarium tank (10L), B: magnetic stirrer, C: heater, D: mixing equipment, E: micro-pump for providing test chemical solution, F: tank for storing test chemical solution, G: tube pump for providing water, H: tank for storing water.

were roughly adjusted to the range from 1/100 to 1/10 of the LC50 value of each chemical. In the simultaneous exposure test the concentrations of chemicals in the breeding water were adjusted to 0.1 ppm (TBP), 0.09 ppm (TDCPP), and 0.02 ppm (TPP).

The test breeding water in the aquarium tank was maintained at 25°C and stirred moderately with a magnetic stirrer to keep the temperature and the concentration of chemicals homogeneous. The aquarium tank A contained 70 to 100 killifish, and groups of 3 to 4 fish were taken out at various intervals. After 11-38 days the remaining fish were transferred into another aquarium tank provided with a continuous supply of clean water, and reared for 1 or 2 days. The fish were fed TetraMin once a day throughout the experimental period. An aliquot of breeding water and a group of fish were taken out at various intervals to analyze the phosphate contents. The fish were analyzed after being stored in a freezer and water was analyzed immediately after sampling.

RESULTS AND DISCUSSION

Four phosphoric acid triesters, TBP, TCEP, TDCPP and TPP, which had been studied previously in a static water test system (SASAKI et al. 1981) were tested in a continuous flow test system.

The results are shown in Figs. 2-5. The concentrations of chemicals in water were maintained almost constant (0.093 to 0.108 ppm for TBP, 2.1 to 2.4 ppm for TCEP and 0.009 to 0.01 ppm for TPP) throughout the experimental period except in the case of TDCPP. The concentration of TDCPP in water decreased with time and at the 30th day it had fallen to 75 % of the initial level. In the simultaneous exposure test (TBP, TDCPP and TPP), the concentration of TDCPP in water also showed a decrement, but TPP (which had a larger BCR than TDCPP) did not. It is possible that TDCPP adsorp-

tion on the equipment or degradation by microorganisms in the water occurred, rather than uptake by the fish.

Accumulation. TBP was taken up rapidly by fish and the concentration in the fish reached a constant level within only one day. This level was maintained for 38 days, so that the bioconcentration ratio (BCR) remained almost constant, from 21 to 35, throughout the experimental period (Fig. 2).

TCEP and TDCPP in fish also reached maximum concentrations in the first day and these levels were maintained until the last day of exposure (Fig. 3 and 4). Consequently, the BCR of TCEP did not vary throughout the exposure period, being 1.2-1.4. In contrast the BCR of TDCPP increased about 1.7 times upon exposure for 30 days. This phenomenon is not merely due to the decrement of TDCPP concentration in water, but represents a significant elevation of BCR. The biological half-life (BH) of TDCPP was only 1.65 hours as described below, so that the TDCPP concentration in water and in the fish body should equilibrate within a few hours. Therefore, if the concentration of TDCPP were kept constant during the experiment, the concentration in the fish body would increase up to 1.7 times. This view is supported by the results in Fig. 6.

TPP concentration in the fish body increased gradually for 18 days and the value at the last day of exposure was 3 times higher than that at the first day (Fig. 5); consequently TPP gave 3 times higher BCR at the 18th day compared with the first day. TPP showed the highest BCR among the 4 phosphates, as expected from its lipophilicity.

KITAMURA (1971) concluded that in the case of periodic doses of a constant quantity of certain chemicals the bioaccumulation reached 96.8% of the maximum level after a period equivalent to 5 times the BH of the chemical. This conclusion was based on the results of oral dosages of cadmium, methyl mercury, β-BHC or γ-BHC to mice. If the above assumption is correct, the BH values of TDCPP and TPP were 1.65 and 1.2 hours as described below, so the accumulation of these chemicals should reach the limit at least within the first few days, when metabolic rates in the fish are constant. Therefore the gradual elevation of TDCPP and TPP accumulation might suggest some alteration of metabolism such as acceleration of absorption, suppression of excretion, or reduced biotransformation by killifish with increase of exposure time.

Figure 6 shows the results of simultaneous exposure of killifish to TBP, TDCPP and TPP. As mentioned previously, TDCPP concentration in the water decreased with time but the concentrations of the other chemicals remained constant. The BCR of TBP remained constant for 32 days of exposure. However, TDCPP and TPP gave 1.7 times and 2.5 times higher BCRs, respectively on the last day of exposure than at the beginning. These results are consistent with those obtained by single chemical exposure.

Elimination. As shown in Figs. 2-5, the elimination of these 4 phosphates from killifish was very fast. Therefore the BH values of these chemicals were small, namely 1.25 hr for TBP, 0.7 hr for TCEP, 1.65 hr for TDCPP and 1.2 hr for TPP, and the phosphates

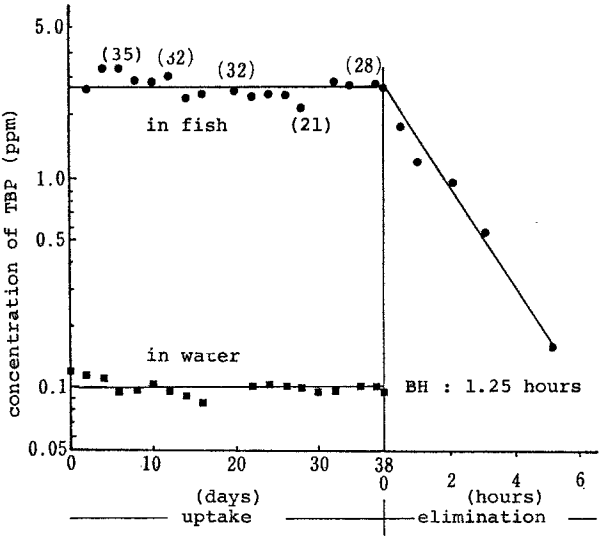

Fig.2. Uptake and elimination of TBP by killifish.
Killifish were exposed to TBP in the continuous flow system for 38 days, then transferred to clean water without TBP. At various times, 3 or 4 fish and an aliquot of breeding water were removed and analyzed for TBP. Values in parentheses are BCR. BH : biological half-life.

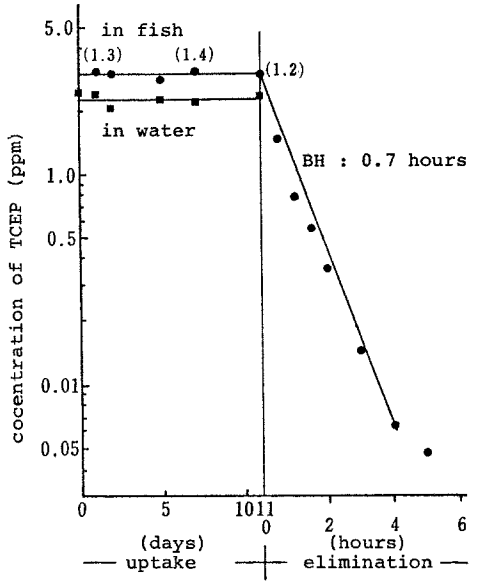

Fig.3. Uptake and elimination of TCEP by killifish.
The exposure period to TCEP was 11 days, and elimination from the fish body was observed for 24 hours. Values in parentheses are BCR. BH : biological half-life.

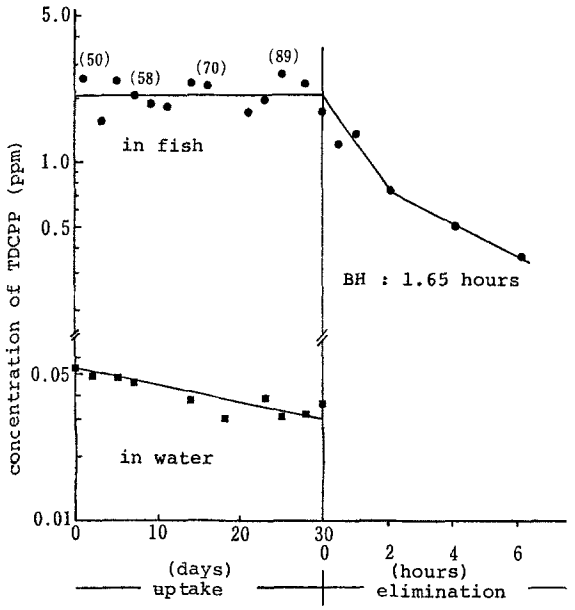

Fig.4. Uptake and elimination of TDCPP by killifish.
The exposure period to TDCPP was 30 days, and elimination from the fish body was observed for 24 hours. Values in parentheses are BCR. BH : biological half-life.

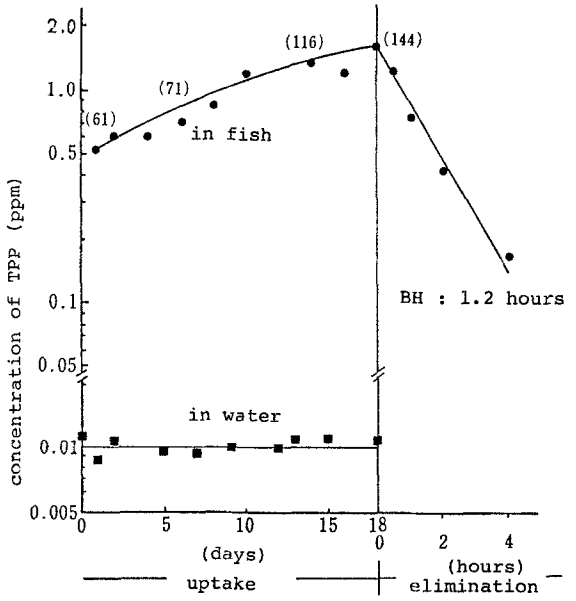

Fig.5. Uptake and elimination of TPP by killifish.
The exposure period to TPP was 18 days, and elimination from the fish body was observed for 24 hours. Values in parentheses are BCR. BH : biological half-life.

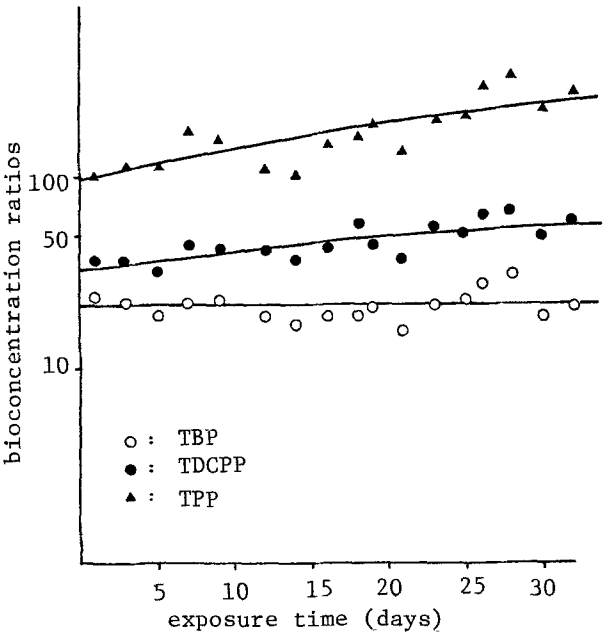

Fig.6. Simultaneous exposure of killifish to TBP,TDCPP and TPP. The concentrations of chemicals in the water were 0.093-0.113 ppm for TBP, 0.07-0.10 ppm for TDCPP and 0.016-0.020 ppm for TPP.

retained in the fish body were below the detection limit after 24 hours in clean water in all cases. Our supplemental studies on chlornitrofen (2,4,6-trichlorophenyl-4-nitrophenyl ether) and fenitrothion [O,O-dimethyl O-(3-methyl-4-nitrophenyl) thiophosphate] using killifish gave BH values of 32 hours and 0.55 hours, respectively. However, KANAZAWA (1978a, 1978b) reported higher values than ours using the topmouth gudgeon (*Pseudorasbora parva*). This may be because the dispersion of chemicals from the fish body to surrounding water can occur more efficiently in small fish than in large fish.

Table 1 shows the BCR values of the 4 phosphates, as well as chlornitrofen and fenitrothion derived from the two test systems. The BCRs of the chemicals were independent of the exposure concentration, in accord with the result of CHADWICK and BROCKSEN (1969) for dieldrin, and were also independent of the test system employed.

The values of BCR in both test systems for each chemical are plotted in Fig.7. It is apparent that BCR derived from the static water test system was slightly higher than that obtained in the continuous flow test system, but there was an excellent correlation between the results in the two systems (correlation coefficient : 1.0). Therefore it should be possible to predict the approximate BCR and the behavior of a pollutant in the aquatic environment by means of the rather simple static water test system, and the use of the continuous flow test system, which requires special equipment and a longer experimental period, may not be necessary.

Figure 8 shows the relationship between BCR and the partition coefficient of these 4 phosphates. The partition coefficients of

TABLE 1
Comparison of BCR values derived from the static water test system and continuous flow test system for killifish

Compound	Static water system exposure conc. (ppm)	BCR	Continuous flow water system exposure conc. (ppm)	period (days)	BCR
TBP	4.0	32 ± 3	0.84	4	16 ± 3 (5)
	0.2	11	0.67	5	17 ± 2 (7)
	0.06	49 ± 8	0.1	38	27 ± 4 (17)
			0.1	32	22 ± 4 (19)
TCEP	8.5	1.4	12.7	5	1.1 ± 0.15 (4)
	0.8	2.2	2.3	11	1.3 ± 0.05 (6)
	0.3	1.9			
TDCPP	1.2	100 ± 20	0.4	3	46 ± 5 (4)
	0.9	107	0.3	4	32 ± 4 (6)
	0.3	47	0.08	32	49 ± 12 (19)
			0.04	6	31 ± 6 (9)
			0.04	30	59 ± 16 (14)
TPP	0.35	612	0.03	35	189 ± 90 (17)
	0.26	157	0.02	32	193 ± 79 (17)
	0.25	390 ± 120	0.01	18	84 ± 32 (14)
Fenitrothion[a]	0.8	35	0.12	10	53 ± 11 (10)
	0.5	69			
	0.3	33			
	0.3	48			
Chlornitrofen[b]	0.4	1350 ± 86	0.01	20	3830 ± 780 (8)
	0.36	7780			

a: O,O-dimethyl O-(3-methyl-4-nitrophenyl)thiophosphate
b: 2,4,6-trichlorophenyl-4'-nitrophenyl ether
( ): number of measurements available for the calculation of BCRs.
In the static water test system, BCRs were expressed as mean values of 2 or 3 measurements during the exposure period (96 hours) and exposure concentrations were the initial ones.
In the continuous flow test system, mean values of BCRs were calculated from the number of measurements given in parentheses.

these chemicals are cited from the previous paper (SASAKI et al. 1981). BCRs from the two test systems showed a high correlation to the partition coefficients (log BCR = 0.64 log PC - 0.77, γ = 0.96). A high correlation between n-octanol/water partition coefficient and bioconcentration has been reported by many workers. METCALF et al. (1975) gave a regression equation (log BCR = 1.1587 log PC - 0.7504, γ = 0.9771) for several polychlorinated biphenyls, DDE, and so on in mosquitofish (*Gambusia affinis*). KANAZAWA (1978b) also gave a regression equation (log BCR = 1.69 log PC - 3.54, γ = 0.89) for organophosphate, organochlorine and carbamate pesticides in topmouth gudgeon. The slope of our regression equation is smaller than the others, which suggests that these 4 phosphates were metabolized more easily than the chemicals tested by METCALF et al.(1975) and KANAZAWA(1978b).

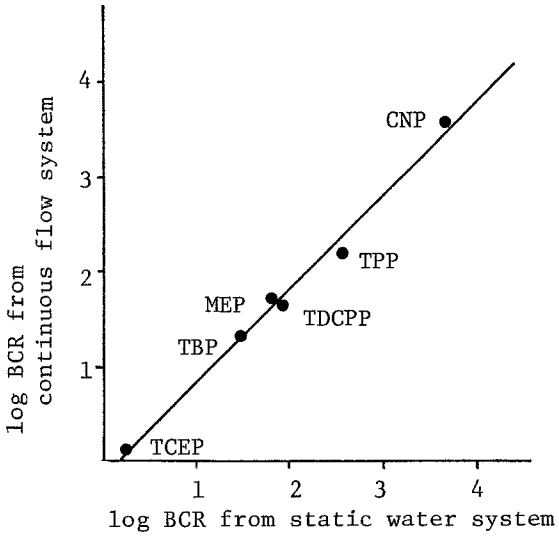

Fig.7. Relationship between BCRs derived from the two test systems (static water and continuous flow).

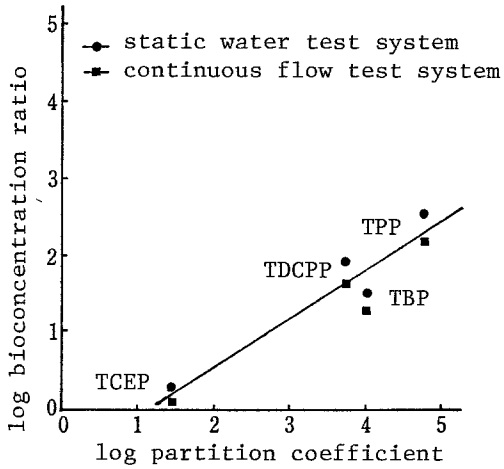

Fig.8. Relationship between partition coefficient and bioconcentration by killifish.

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