ワキンにおける各種クロロフェノールの毒性と蓄積性との関係

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Relation between Toxicity and Accumulation of Various Chlorophenols in Goldfish*1

Kunio KOBAYASHI*2, Hiroshi AKITAKE*3, and Kazuyoshi MANABE*4

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A study has been made of the relation between the toxicity and the accumulation in goldfish, *Carassius auratus*, of seven chlorophenols: 2-chlorophenol; 4-chlorophenol; 2,4-dichlorophenol; 2,4,5-trichlorophenol; 2,4,6-trichlorophenol; 2,3,4,6-tetrachlorophenol; and pentachlorophenol. An increase of the Cl-atom number in the chlorophenols caused an abrupt increase in toxicity to the fish and also increased the concentration ratios in their media to lethal or sublethal concentrations.

On the other hand, the concentrations of the chlorophenols found in the dead fish in the media varied within the range of 75 to 268 μg/g body weight, as compared with the LC_{50} values.

The results suggest that the increase of toxicity from polychlorinated phenols is mostly due to their accumulation in the fish. Their concentrations in the fish eventually achieve a certain lethal level (roughly 100–200 μg/g body weight), although the chemical form, locality, and physiological activity of the chlorophenols in the tissues of the fish must also be involved.

In our previous papers,[1,2] it has been shown that toxicity of pentachlorophenol (PCP) to goldfish, *Carassius auratus*, is approximately 200 times high as that of phenol on the basis of 24-h LC_{50} value (ppm).

However, a very small difference was observed between the amounts of phenol and PCP which had been accumulated in the fish found dead in media during exposure, while the concentration ratios of phenol and PCP in goldfish at 5-day exposure were approximately 2 and 1000, respectively.[1,2]

The present study was undertaken to ascertain the relation between toxicity and accumulation of various chlorophenols in goldfish.

Materials and Methods

Materials

The study has been made of the toxicity and accumulation of seven chlorophenols, *i.e.*, 2-chlorophenol (2-CP); 4-chlorophenol (4-CP); 2,4-dichlorophenol(2,4-CP); 2,4,5-trichlorophenol(2,4,5-CP); 2,4,6-trichlorophenol(2,4,6-CP); 2,3,4,6-tetrachlorophenol(2,3,4,6-CP) and pentachlorophenol, and phenol in goldfish having an average body weight of 2 g each.

Toxicity Test

In a long time exposure, the fish may accumulate considerable amounts of chlorophenols in some organs such as gall bladder in a conjugated-form, as previously reported.[3,4] Therefore, the experiment on the toxicity and accumulation of the chlorophenols in goldfish was carried out in a 12-h or 24-h period.

Ten fish were placed in each 10 *l* of media containing each chlorophenol in several concentrations across the 24-h LC_{50} value. The media were kept at 20°C and renewed at 8-h intervals, throughout the experiment. The mortality of fish was observed, and the dead fish during the test were taken out from media and assayed for chlorophenols contents.

Accumulation of Chlorophenols by Fish

Thirty goldfish were placed in 60 *l* of each chlorophenol medium containing in a sublethal concentration, *i.e.*, 20, 10, 3, 2, 2, 1, 0.25 and 0.1 ppm for phenol, 2-CP, 4-CP, 2,4-CP, 2,4,6-CP, 2,4,5-CP, 2,3,4,6-CP and PCP, respectively. At selected times during the course of the experiment (0.5 to 12 h), 5 fish were removed and subjected to the determination of chlorophenols accumulated.

Determination of Chlorophenols in Fish

Mono- and di-chlorophenols accumulated in fish

*1 Studies on the Metabolism of Chlorophenols in Fish——X.

*2 Lab. Fish. Chem., Fac. Agr., Kyushu Univ., Fukuoka, Japan (小林邦男: 九州大学農学部水産化学教室).

*3 Fisheries Section of Hyogo Prefecture, Kobe, Japan (秋武 宏: 兵庫県農林部水産課).

*4 Sewage Works Bureau of Fukuoka City, Fukuoka, Japan (真鍋和義: 福岡市下水道局水質試験所).

were determined colorimetrically by using the 4-aminoantipyrine method combined with chloroform extraction after distillation,[5] as previously used for phenol accumulated by fish.[2] The absorbance was determined at 460 nm for phenol and 4-CP and at 470 nm for 2-CP and 2,4-CP.

Tri- and tetra-chlorophenols accumulated in fish were also determined by using the 4-aminoantipyrine method combined with xylene extraction after steam-distillation, as usually used for the determination of PCP contained in animal tissues.[6] The absorbance was determined at 475, 480, 485 and 547 nm for 2,4,5-CP, 2,4,6-CP, 2,3,4,6-CP and PCP, respectively.

Results and Discussion

Fig. 1 shows the toxicity of the tested chlorophenols to goldfish. In most of the chlorophenols, the mortality of fish was enhanced with increasing their concentrations in media, although some exceptional mortality which rather reduced with increasing the concentration was observed in the ranges of 20 to 80 ppm 2-CP and of 10 to 40 ppm 4-CP media.

The survival rates at 24-h exposure to the media containing the chlorophenols in various concentrations were summarized in Fig. 2. An increase of the Cl-atom number in the chlorophenols caused an abrupt increase in toxicity, and the

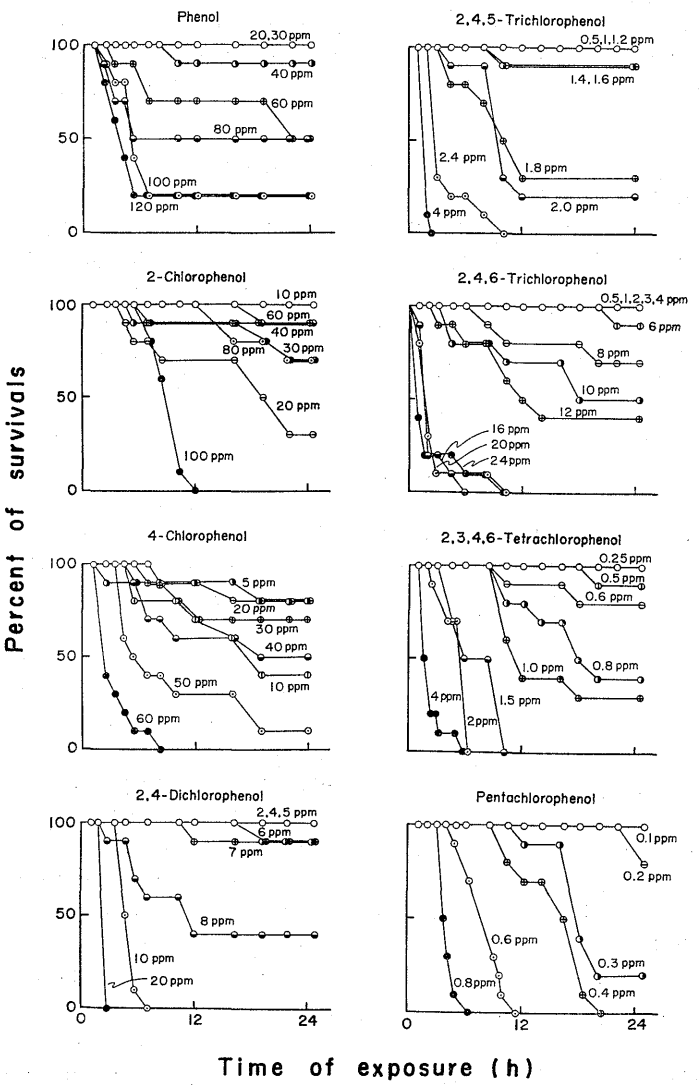

Fig. 1. Survival time of goldfish exposed to various chlorophenols media.

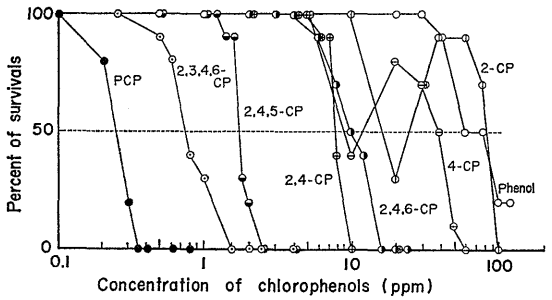

Fig. 2. Survival rates of goldfish at 24-h exposure to various chlorophenols media.

Table 1. The toxicity and accumulation of chlorophenols in goldfish

Chlorophenols	25-h LC_{50} (ppm)	Amount found in dead fish (μg/g)	Concentration ratio*	
PCP	0.27	95	475	(0.2 ppm)
2,3,4,6-CP	0.75	75	93	(0.8 ppm)
2,4,5-CP	1.7	112	62	(1.8 ppm)
2,4,6-CP	10.0	200	20	(10 ppm)
2,4-CP	7.8	268	34	(8 ppm)
4-CP	9.0	101	10.1	(10 ppm)
2-CP	16	128	6.4	(20 ppm)
Phenol	60	114	1.9	(60 ppm)

* Values were obtained in fish which died in the concentration of each chlorophenol shown in parentheses most close to the 24-h LC_{50} value among the tested media.

position of Cl-atom also affected on the toxicity as observed between 2-CP and 4-CP or 2,4,5-CP and 2,4,6-CP.

Table 1 shows the 24-h LC_{50} values of chlorophenols, which were calculated from the data shown in Fig. 2, and the amount of each chlorophenol found in the dead fish in the medium containing the chlorophenol at the concentration most close to the 24-h LC_{50} value among the test media. PCP shows the lowest 24-h LC_{50} value (0.27 ppm) among the tested chlorophenols, while the highest value was 60 ppm of phenol.

It may be thought that even when very small amounts of polychlorinated phenols were accumulated by the fish as compared with phenol, the reactive Cl-atoms of the chlorophenols remarkably inhibit some vital functions such as oxidative phosphorylation[7] in the fish, resulting in the death of fish. As compared with the LC_{50} values, however, much small variations in the concentrations of chlorophenols found in the dead fish were observed, ranging from 75 to 268 μg/g body weight. The concentration ratios of chlorophenols found in the dead fish increased with an increase

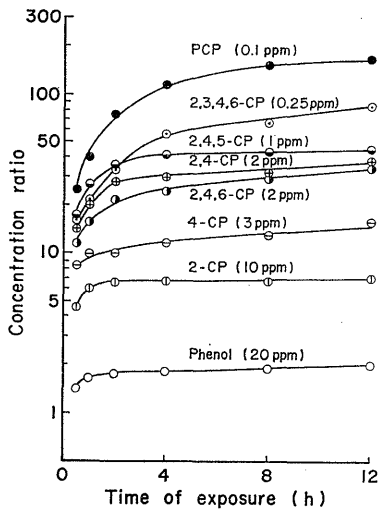

Fig. 3. Accumulation of various chlorophenols by goldfish.

of Cl-atom number from 1.9 in 60 ppm phenol to 475 in 0.2 ppm PCP within 24 h exposure.

As shown in Fig. 3, the concentration ratios of chlorophenols in their sublethal media also increased with increasing the Cl-atom number from 2 in 20 ppm phenol to 165 in 0.1 ppm PCP at 12-h exposure, taking more time to reach maximal levels.

From these results, it seems that an increase of the Cl-atom number in chlorophenols promotes an accumulation of the chlorophenols by the fish and leads their concentrations in the fish to a certain lethal level (roughly 100–200 μg/g body weight) even when the fish were exposed to rather low concentration media, and consequently increases the toxicity to fish, although the chemical form, locality and physiological activity of the chlorophenols in tissues of the fish must be concerned.

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