Studies on the Metabolism of Chlorophenols in Fish—II Turnover of Absorbed PCP in Goldfish

Kunio KOBAYASHI*¹ and Hiroshi AKITAKE*²

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A study has been made regarding the turnover of absorbed pentachlorophenol (PCP) in various organs of goldfish, *Carassius auratus*, during absorption in a PCP-medium (0.2 ppm), its subsequent excretion in running water, and also its decomposition using ¹⁴C labeled PCP.

PCP absorbed by fish from the medium was accumulated in various organs, especially the gall bladder. The biliary concentration of PCP reached a value of 539 µg/g after a 24-hr exposure, and displayed a further increase even after fish had been transferred to clean, running water for 24 hr, whereas a decrease was observed in all other organs examined. PCP in the gall bladder eventually reached a level of 1,077 µg/g, corresponding to a concentration factor of 5,400, which accounted for 41 % of the total PCP detected in fish after transfer to running water for 24 hr.

Some decomposition of PCP in fish was confirmed by a slight increase in the specific activity found in the gall bladder. It is probable that a large proportion of the PCP and ¹⁴C found in the gall bladder had been transferred from the hepatopancreas after detoxification by conjugation or decomposition.

The characteristic accumulation of PCP in the gall bladder indicates that fish can dispose of PCP by active elimination such as conjugation and decomposition, although BRODIE *et al.* (1962) have concluded that fish dispose of drugs by passive diffusion through the gills.

Though many studies have been made of the toxic effects of pentachlorophenol (PCP) on fish, few workers have dealt with the turnover of PCP in fish. KUMADA *et al.*¹⁾ determined PCP in several tissues of carp killed by PCP, but they could not ascertain the rate of turnover because of the small numbers of fish used in their experiments. TOKUNAGA²⁾ reported the distribution of PCP in several organs of carp which were placed in ¹⁴C labeled PCP (¹⁴C-PCP) media, but did not study the change in the distribution of PCP with exposure time.

In the preceding paper,³⁾ the absorption and excretion of PCP in goldfish, *Carassius auratus*, was reported. The present paper deals with the turnover of absorbed PCP in various tissues of goldfish, during both absorption in a PCP-medium and subsequent excretion in running water, and also with the confirmation of the decomposition of PCP in the various tissues.

Materials and Methods

¹⁴C labeled sodium pentachlorophenolate used in this experiment was the same as

*¹ 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)

*² Fisheries Section of Hyogo Prefecture, Kobe, Japan (秋武 宏: 兵庫県農林部水産課)

Table 1. Changes in radioactivity and amount of PCP accumulated in various tissues of goldfish, during exposure to ^{14}C -PCP medium

Tissue	Exposure time											
	1 hour			5 hours			12 hours			24 hours		
	Total weight (g)	Total PCP (μg)	Total ^{14}C (cpm)	Total Weight (g)	Total PCP (μg)	Total ^{14}C (cpm)	Total weight (g)	Total PCP (μg)	Total ^{14}C (cpm)	Total weight (g)	Total PCP (μg)	Total ^{14}C (cpm)
Gills	6.23	25.9	3311	6.69	67.2	7885	5.33	102.5	11624	5.78	124.1	13480
Blood*	6.35	61.7	7102	7.31	236.2	27276	5.51	305.1	35663	5.55	332.3	36486
Hepatopancreas	10.77	25.4	3722	10.55	117.6	13567	9.63	173.7	19663	8.60	227.4	26850
Gall bladder	0.64	tr.	174	0.87	37.8	3834	0.50	108.1	14458	0.64	342.1	49913
Kidney	1.62	tr.	911	1.57	30.6	3501	1.38	53.3	6073	1.33	47.1	5194
Spleen	0.49	tr.	150	0.50	tr.	411	0.40	6.7	730	0.48	13.5	1315
Heart	0.39	tr.	214	0.52	8.7	1107	0.33	9.5	1128	0.33	9.6	1115
Gonad	3.11	tr.	683	2.41	6.6	729	5.92	43.8	4848	1.59	17.2	1686
	12.97	7.4	1403	20.26	54.5	6126	1.68	17.3	2185	3.15	121.3	13342
Air bladder	1.16	tr.	248	1.43	10.9	1264	1.01	17.3	2005	1.05	20.4	2415
Digestive tract	7.69	11.8	1203	7.59	67.5	7752	5.41	122.6	13907	6.41	183.9	20874
Brain	0.87	tr.	244	0.86	8.4	740	0.76	11.0	1317	0.82	15.0	1741
Muscle	49.95	39.0	4356	56.47	116.9	13345	44.50	216.2	24671	42.96	211.0	23546
Vertebra	2.47	tr.	392	3.60	17.4	1990	2.08	18.6	2179	1.85	18.8	2306
Skin	4.33	12.5	1607	4.69	42.1	5035	3.46	71.0	8134	3.52	79.1	8926
Scale	9.46	33.4	4122	11.11	91.4	9958	8.55	103.5	11991	8.95	134.7	14687
Remainder	93.32	108.2	12542	107.24	241.3	34123	87.15	532.6	63056	91.94	577.6	68152
Whole body	211.82	325.3	42384	243.67	1155.1	138643	183.60	1912.8	223632	184.95	2475.1	292028
Number of fish	♂ 2, ♀ 3			♂ 1, ♀ 4			♂ 4, ♀ 1			♂ 1, ♀ 4		

^{14}C -PCP medium: PCP, 0.2 ppm; ^{14}C -activity, 24 cpm/ml; temperature, 18°C.

* The weight of blood was calculated as 3% of that of whole body.

Table 2. Change in retention of radioactivity and PCP in various tissues of goldfish, during culture in running water after a 24-hour exposure to ^{14}C -PCP medium

Tissue	Excretion time												
	1 hour			5 hours			12 hours			24 hours			
	Total weight (g)	Total PCP (μg)	Total ¹⁴ C (cpm)	Total weight (g)	Total PCP (μg)	Total ¹⁴ C (cpm)	Total weight (g)	Total PCP (μg)	Total ¹⁴ C (cpm)	Total weight (g)	Total PCP (μg)	Total ¹⁴ C (cpm)	
Gills	6.13	119.3	13448	5.53	98.2	10804	5.35	78.8	8641	5.02	65.3	6871	
Blood*	6.25	261.5	31559	6.19	258.7	31351	5.57	208.8	25013	5.77	196.7	19785	
Hepatopancreas	10.79	246.4	29442	9.57	191.5	24149	8.33	135.8	15902	9.60	128.8	13717	
Gall bladder	0.54	248.8	36399	0.56	363.3	54458	0.55	366.1	61721	0.54	580.5	83647	
Kidney	1.31	57.5	6644	1.64	64.8	7569	1.37	44.2	4823	1.31	36.5	4242	
Spleen	0.53	9.4	944	0.72	10.7	1201	0.55	11.4	1044	0.66	tr.	638	
Heart	0.38	10.8	1355	0.41	10.1	1251	0.39	8.9	886	0.38	5.6	710	
Gonad {	Testis	3.73	37.0	4432	7.64	49.8	5781	6.01	31.7	3306	4.90	21.6	2272
	Ovary	6.01	54.0	6121	—	—	—	—	—	2.47	8.9	1018	
Air bladder	1.45	23.6	2825	1.33	20.7	2576	1.10	14.8	1730	1.33	10.6	1342	
Digestive tract	6.92	362.1	45478	7.31	247.1	31625	5.56	139.0	18905	5.74	118.2	11914	
Brain	0.85	15.2	1835	1.00	14.6	1762	0.92	9.5	1098	0.87	7.0	723	
Muscle	47.39	226.5	27220	46.18	229.6	25604	44.61	147.6	17331	45.00	109.3	11435	
Vertebra	1.99	21.5	2522	2.08	17.3	2329	1.91	14.1	1668	1.87	12.6	1257	
Skin	3.90	88.0	10327	4.32	85.6	9762	3.73	51.9	6036	3.45	52.3	5536	
Scale	9.89	144.1	16268	10.34	127.8	15285	9.46	89.9	10960	9.41	87.8	9144	
Remainder	100.46	693.0	80801	101.61	684.6	78525	90.25	385.5	46722	94.01	278.2	29518	
Whole body	208.52	2618.7	317620	206.43	2474.4	304032	185.66	1738.0	225786	192.33	1719.9	203769	
Number of fish	♂ 2, ♀ 3			♂ 5, ♀ 0			♂ 5, ♀ 0			♂ 4, ♀ 1			

Running water: temperature, 18°C; water supply, 5 l/min; PCP, free.

* The weight of blood was calculated as 3% of that of whole body.

described in a previous paper.⁴⁾ The specific activity was ca. 0.1 mCi/g-PCP.

Forty goldfish having an average body weight of 40 g each were placed in 200l of a ^{14}C -PCP medium (PCP, 0.2 ppm; ^{14}C activity, 24 cpm/ml) at 18°C. After 1, 5, 12 and 24 hr, five fish were taken out and rinsed with PCP-free water and dissected after collecting blood by cutting the tail off. After the treatment for 24 hr the remaining twenty fish were transferred from the ^{14}C -PCP medium to running water. At 1-, 5-, 12- and 24-hr time intervals, five fish were removed from the running water and dissected as described above.

Aliquots of the various organs were assayed for PCP content by the 4-aminoantipyrine method and ^{14}C was measured by a liquid scintillation counter, Aloka LSC-201, as described previously.⁴⁾

Results

Table 1 shows the weights, total amounts of PCP and total ^{14}C content of various organs collected from five fish in each group exposed to the ^{14}C -PCP medium. Table 2 shows the same data for those fish which were kept in running water for various periods of time after exposure to the ^{14}C -PCP medium.

Six of the tissues shown in Tables 1 and 2, namely gall bladder, blood, kidney, digestive tract, hepatopancreas, and gills were selected and subjected to further study, because they were thought to play an important role in the turnover of PCP in fish. Fig. 1 shows the changes in concentration of PCP in these tissues as well as the muscle and the whole body of fish during exposure to PCP medium and running water.

Fig. 1 shows a characteristic accumulation curve for PCP in the gall bladder. Though the PCP concentration in the gall bladder was the lowest among the tissues assayed

Fig. 1. Changes in concentration of PCP in various tissues of goldfish during exposure to PCP medium and culture in running water after a 24-hr exposure.

after a 1-hr exposure, it rapidly increased with time, until it reached values of 43.6 $\mu\text{g PCP/g}$ at 5-hr and 539 $\mu\text{g PCP/g}$ at 24-hr. Even after transfer of the fish from the PCP-medium to running water the PCP concentration in the gall bladder increased still more and reached a value of 1,077 $\mu\text{g PCP/g}$ after 24 hr culture in running water. This corresponds to a concentration factor of 5,385 times that of the PCP-medium.

The PCP concentration in each organ, except the gall bladder, gradually increased with the exposure time in the PCP-medium, and after the transfer of the fish to running water, it gradually decreased. With the exception of the gall bladder the highest concentration of PCP occurred in blood, followed by kidney, digestive tract, hepatopancreas and gills. Muscle always showed the lowest concentration among all the tissues examined (Fig. 1).

Fig. 2 shows the variation with time in the distribution of PCP in various organs of the fish during the experiment. The blood and the gills showed relatively high amounts accounting for 17% and 8%, respectively, of the total body content in early periods of exposure, but the amounts subsequently declined and eventually reached levels of about 10% and 4% at the end of excretion period. The amounts in the hepatopancreas and the kidney were almost constant in both absorption and excretion.

Though the amount of PCP in the gall bladder was only 0.4% of the total body content after a 1-hr exposure, it rapidly increased with longer exposure. Even after transfer of the fish to running water, the amount of PCP in the gall bladder continuously increased whereas all other tissues decreased. The amount of PCP in the gall bladder eventually accounted for 41% of that contained in whole body. The amount in the digestive tract increased with exposure time, and immediately after the transfer of the fish to running water it increased sharply and then gradually decreased. The amount in the muscle was about 10% of the total body burden during exposure, but it gradually decreased when the fish were transferred to running water.

Table 3 shows the variation of the specific radioactivity in each tissue based on data

Fig. 2. Variation in distribution of PCP into various tissues of goldfish during exposure to PCP medium and culture in running water after a 24-hr exposure.

Table 3. Variation of specific radioactivity in various tissues of goldfish, during exposure to ^{14}C -PCP medium and culture in running water after a 24-hour exposure
Values given are expressed as ^{14}C -cpm/ μg -PCP.

Tissue	Exposure time (hr)				Excretion time (hr)			
	1	5	12	24	1	5	12	24
Gills	128	117	113	109	113	110	110	105
Blood	115	116	117	110	121	121	120	101
Hepatopancreas	146	115	113	118	120	126	117	107
Gall bladder	—	102	134	146	146	150	169	144
Kidney	—	115	114	110	116	117	109	116
Spleen	—	—	109	98	101	113	91	—
Heart	—	128	119	116	126	124	100	126
Gonad { Testis	—	111	111	98	120	116	104	105
Ovary	190	112	126	110	113	—	—	115
Air bladder	—	117	116	118	120	125	117	127
Digestive tract	102	115	113	114	126	128	136	101
Brain	—	88	119	116	121	121	115	103
Muscle	112	114	114	112	120	112	117	105
Vertebra	—	115	117	122	118	134	118	100
Skin	128	120	115	113	117	114	116	106
Scale	124	109	116	109	113	120	122	104
Remainder	116	141	118	118	117	115	121	106
Whole body	130	120	117	118	121	123	130	118

shown in Tables 1 and 2. Throughout both absorption and excretion, the specific activity in each tissue, except the gall bladder, was almost equal to that of the ^{14}C -PCP medium (120 cpm- $^{14}\text{C}/\mu\text{g}$ -PCP). The specific activity in the gall bladder, however, gradually increased with exposure time and ranged from 144 to 169 cpm- $^{14}\text{C}/\mu\text{g}$ -PCP during culture in running water.

Discussion

The abrupt decrease of the PCP concentration in the blood immediately following the transfer of the fish to running water (Fig. 1) might be due to passive diffusion through the gills, as reported by BRODIE *et al.*⁵⁾

In the gall bladder, however, the PCP concentration reached a level of 1,077 $\mu\text{g}/\text{g}$ corresponding to 5,385 times that of the original medium and the amount of PCP in the gall bladder accounted for 41% of that in whole body after 24 hr culture in running water (Figs. 1 and 2). The increase in specific activity, observed in the gall bladder, indicates decomposition of a part of the PCP in the fish (Table 3). It will be shown in a subsequent paper that most of the PCP excreted from goldfish has been conjugated with sulfate, as has also been found in a shell-fish.^{6,7)}

It would seem that most of PCP transferred to the hepatopancreas was detoxified by sulfate conjugation or by decomposition and then partly transferred to the gall bladder and

bile, though BRODIE *et al.*⁵⁾ have concluded that fish can only dispose of lipid-soluble substances by passive diffusion through the gills and that detoxification by either conjugation or oxidation does not occur.

It seems that the slower decrease of the PCP concentration found in fish after the concentration has reached approximately 20% of the initial value which they had before transfer to running water, as reported in a preceding paper,³⁾ is due to storage of PCP in the gall bladder.

The abrupt increase of PCP concentration in the digestive tract which was observed immediately after transfer of the fish from the PCP-medium to running water (Figs. 1 and 2) might be due to an initial secretion of bile stored in the gall bladder which contained PCP in a high concentration.

With the exception of the gall bladder the specific radioactivity of all organs was almost the same as the ¹⁴C-PCP medium, though there were some exceptions (Table 3) which were probably due to the analytical error. This suggests that the decomposition of PCP in fish is of little consequence in detoxification of PCP, when compared to sulfate conjugation as will be shown in a subsequent paper.

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