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Original Article

Uptake, Distribution, Metabolism and Excretion of Tebufenpyrad by Carp, *Cyprinus carpio*

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Uptake, distribution, metabolism and excretion of tebufenpyrad [*N*-(4-*tert*-butylbenzyl)-4-chloro-3-ethyl-1-methylpyrazole-5-carboxamide, Pyranica®], a synthetic acaricide, were studied with carp. Fish were exposed to ^{14}C-tebufenpyrad treated water (1.2 $\mu g/l$) for 28 days, followed by 14 days period for depuration. Bioconcentration factors (BCFs) of ^{14}C reached a steady-state plateau within 7 days of exposure and its maximum BCF was 864. HPLC analysis showed that less than 4% of the ^{14}C in whole fish was tebufenpyrad to give the maximum BCF of 29. Gall bladder bile contained the highest concentration of ^{14}C, followed by gastrointestinal tract with the contents and hepatopancreas. Tebufenpyrad was not detected in the bile. An enzymatic treatment suggested that 84% of the ^{14}C in bile were β-glucuronic acid conjugates. Major metabolic reactions of tebufenpyrad by carp were hydroxylation on the *tert*-butyl moiety, followed by oxidation to the carboxyl group and conjugation of the resultant metabolites with glucuronic acid. During the depuration phase, more than 98% of ^{14}C were excreted from fish within 7 days with a half-life of 0.5 days. The results of this study strongly suggest that active biotransformation and excretion play important roles to lower the bioaccumulative potential of tebufenpyrad by carp.

INTRODUCTION

Tebufenpyrad [*N*-(4-*tert*-butylbenzyl)-4-chloro-3-ethyl-1-methylpyrazole-5-carboxamide, Pyranica®] is a potent synthetic acaricide.[1] Before practical use of this acaricide in the field, it is important to evaluate the fate of this compound in the environment. The high lipophilicity (1-octanol/water partition coefficient [log *P*] of 5.04) of this compound may affect its uptake by aquatic biota. In many cases, bioaccumulation potentials of chemicals into fish are predicted from the relative lipophilicity measured by their log *P* values.[2] For example, the BCF value of a compound having the log *P* of 5.04 is predicted to be 2200 from an equation of "log BCF = 0.82·log *P* − 0.75" proposed by Hoshikawa *et al.*[3] On the other hand, many organic chemicals undergo biotransformation in fish *via* phase I (oxidation, reduction, *etc.*) and phase II (conjugation) reactions to more polar derivatives, which are more readily excreted than the parent compound.[4,5] The uptake, distribution, metabolism and excretion of tebufenpyrad by aquatic organisms have not been studied yet. This study is designed to evaluate the bioaccumulative potential of tebufenpyrad in carp.

MATERIALS AND METHODS

1. Chemicals

Tebufenpyrad labeled with ^{14}C at the C-3 position in the pyrazole ring was synthesized by Daiichi Pure Chemicals Co., Ltd., Ibaraki, Japan. The specific activity was 863 MBq/mmol (MW = 333.9) with radiochemical purity of greater than 99% as determined by HPLC and TLC under the conditions described later. To identify the metabolites of tebufenpyrad, a

Table 1 Authentic standards used in this study.

Chemical name	[Abbreviation]
N-(4-*tert*-Butylbenzyl)-4-chloro-3-ethyl-1-methylpyrazole-5-carboxamide	[Tebufenpyrad]
N-(4-*tert*-Butylbenzyl)-3-acetyl-4-chloro-1-methylpyrazole-5-carboxamide	[CO-M]
N-(4-*tert*-Butylbenzyl)-4-chloro-3-(1-hydroxyethyl)-1-methylpyrazole-5-carboxamide	[OH-M]
N-[4-(1-Hydroxymethyl-1-methylethyl)benzyl]-4-chloro-3-ethyl-1-methylpyrazole-5-carboxamide	[M-OH]
N-(4-*tert*-Butylbenzyl)-4-chloro-3-(2-hydroxyethyl)-1-methylpyrazole-5-carboxamide	[2OH-M]
N-[4-(1-Hydroxymethyl-1-methylethyl)benzyl]-4-chloro-3-(1-hydroxyethyl)-1-methylpyrazole-5-carboxamide	[OH-M-OH]
N-[4-(Hydroxymethyl-1-methylethyl)benzyl]-3-acetyl-4-chloro-1-methylpyrazole-5-carboxamide	[CO-M-OH]
N-(4-*tert*-Butylbenzyl)-4-chloro-3-ethylpyrazole-5-carboxamide	[DM-M]
N-[4-(1-Carboxy-1-methylethyl)benzyl]-4-chloro-3-ethyl-1-methylpyrazole-5-carboxamide	[M-CA]
N-(4-*tert*-Butylbenzyl)-3-carboxymethyl-4-chloro-1-methylpyrazole-5-carboxamide	[2CA-M]
N-[4-(1-Carboxy-1-methylethyl)benzyl]-4-chloro-3-(1-hydroxyethyl)-1-methylpyrazole-5-carboxamide	[OH-M-CA]
N-[4-(1-Carboxy-1-methylethyl)benzyl]-3-acetyl-4-chloro-1-methylpyrazole-5-carboxamide	[CO-M-CA]
4-Chloro-3-ethyl-1-methylpyrazole-5-carboxylic acid	[PCA]
4-Chloro-3-ethyl-1-methylpyrazole-5-carboxamide	[PAM]
4-Chloro-3-(1-hydroxyethyl)-1-methylpyrazole-5-carboxamide	[OH-PAM]
3-Acetyl-4-chloro-1-methylpyrazole-5-carboxamide	[CO-PAM]
4-Chloro-3-ethylpyrazole-5-carboxylic acid	[DM-PCA]
N-[4-(1-Methyl-1-sulfooxymethylethyl)benzyl]-4-chloro-3-ethyl-1-methylpyrazole-5-carboxamide	[M-OSO_3H]
N-[4-(1-Methyl-1-sulfooxymethylethyl)benzyl]-3-acetyl-4-chloro-1-methylpyrazole-5-carboxamide	[CO-M-OSO_3H]

series of authentic standards were supplied by Mitsubishi Kasei Corporation (Tokyo, Japan). The chemical names and abbreviations used in this study are shown in Table 1.

2. Test Fish

Carp (*Cyprinus carpio*) were obtained from a fish farm (Sankyo Suisan, Tokyo, Japan) and acclimated in dechlorinated-tap water maintained at 24–26°C for several months. The fish weighed between 13 and 24 g, and were fed a dry commercial pelleted food daily of an amount equivalent to 1% of body weight during the experiment. No mortalities or abnormalities in appearance or behavior in the fish were observed during the acclimation and test period.

3. Test System

The study was carried out under the continuous-flow through system. The system consisted of a 50-*l*-glass tank, two types of pumps for supplying the dilution water (dechlorinated-tap water) and stock solution (500 μg ^{14}C-tebufenpyrad/*l* water with 0.02% acetone). The stock solution was delivered at a rate of 2.4 *l*/day and continuously mixed with dilution water supplied at a rate of 1000 *l*/day, and then delivered to the tank at a renewal rate of 22 times per day. The nominal concentration of ^{14}C-tebufenpyrad in water during the exposure period was 1.2 μg/*l* (1/60 of the 96-hr LC_{50} value of 0.073 mg/*l* to carp). ^{14}C-Tebufenpyrad was not degraded in the stock solution and test water prior to the introduction of carp. The hydrolysis study showed that tebufenpyrad was stable in water under the conditions of pH 5–9 at 25–37°C for 28 days. The temperature, dissolved oxygen and pH of the test water throughout the study were 25.0–25.9°C, 5.7–7.8 mg/*l* and 7.3–7.8, respectively.

The uptake study was started by transferring 47 carp into the tank. After 28 days of the exposure period, 15 fish were transferred into the tank supplied with only ^{14}C-free water to start the 14 days excretion study.

4. Sampling and Analysis

To determine the concentrations of ^{14}C-tebufenpyrad equivalents (^{14}C radioactivity) in water, 5 ml of water sample was collected in a scintillation vial twice a week and then 10 ml of emulsifier scintillator ACS-2 (Amersham, U.K.) was added for radioanalysis with a Tri-carb 2200CA liquid scintillation spectrometer (Packard, U.S.A.). On days 0.17, 1, 3, 7, 14 and 28 of exposure and on days 1, 3, 7 and 14 of depuration, each two fish was sampled and homogenized. A portion (0.5 g) of the homogenized fish sample was air-dried and combusted by a Tri-carb 306 oxidizer (Packard) to measure radioactivity by liquid scintillation counting (LSC). The remaining homogenized samples were extracted three times with an equal volume of methanol. The solvent layers were combined and evaporated to dryness at 40°C to analyze the metabolites by HPLC under the condition A described later.

On day 28 of exposure and on day 7 of depuration, each three fish was collected to determine the ^{14}C-residue levels in tissues. Blood was collected from caudal sinus of each fish. Bile was collected by gall bladder puncture. Then, the fish were dissected into hepatopancreas, kidney, spleen, gastrointestinal tract including contents (GI tract), heart, gill, muscle, skin with scale, eye, air bladder, brain and residual carcass. Each sample was combusted by the oxidizer and radioactivity was measured by LSC. The mean value of combustion efficiency was 98.6±3.5%.

In addition, 15 fish were collected on day 28 of exposure to characterize the metabolites in the bile, hepatopancreas or GI tract. The hepatopancreas and GI tract were collected from five fish and then extracted three times with 20 ml of methanol. The solvent layers were combined and evaporated to dryness at 40°C for HPLC analysis under the condition A. Each extracted tissue was air-dried and combusted by the oxidizer to measure radioactivity by LSC. The bile was sampled by gall bladder puncture from 15 fish and diluted to 10 ml with methanol. A two-ml aliquot of bile-methanol solution was dried under nitrogen for HPLC analysis under the conditions A and B. The remaining 8 ml solution was dried under nitrogen, reconstituted in 5 ml of water and extracted with 5 ml of ethyl ether. A one-ml aliquot of water layer was dried under nitrogen, redissolved in water and then incubated with β-glucuronidase (5000 units) from bovine liver (Sigma Type B-1, U.S.A.) in 0.2 M acetate buffer (pH 5.0). After incubation at 37°C for 4 hr, the incubation mixture solutions were extracted once with an equal volume of ethyl ether. Both the water and ethyl ether layers were dried under nitrogen for HPLC analysis under the conditions A and B.

Each concentrated extract of the whole fish, hepatopancreas, GI tract and bile was dissolved in 200 μl of methanol containing 19 authentic standards for HPLC co-chromatography. The radioactivity in the extracts was measured before HPLC analysis to calculate the recovery of radioactivity.

5. HPLC

HPLC was carried out with a Hitachi 6000 Series liquid chromatograph (Hitachi, Tokyo, Japan) under the following two conditions: condition A; column, Senshu Pak C_6H_5-1250N (Senshu Science, Tokyo, Japan); flow rate, 1.2 ml/min; mobile phase (gradient system), 0.1% trifluoroacetic acid (TFA)/15% acetonitrile (CH_3CN)/water(H_2O)→50 min→0.1% TFA/40% CH_3CN/H_2O→20 min→0.1% TFA/60% CH_3CN/H_2O→5 min→0.1% TFA/100% CH_3CN; condition B; column, Inertsil ODS (Gasukuro Kogyo, Tokyo, Japan); flow rate, 1.2 m/min; mobile phase (gradient system), 0.1% TFA/25% CH_3CN/H_2O→25 min→0.1% TFA/30% CH_3CN/H_2O→25 min→0.1% TFA/100% CH_3CN.

Radioactive peaks were detected by a RAMONA 5LS radiodetector with a solid scintillator (Raytest, U.S.A.). The authentic standards used for co-chromatography were detected with an UV detector at 254 nm (Table 2). Integration to quantify the radioactivity of selected peaks was done with a PRO-3000 data processor (Daewoo, Korea).

RESULTS

1. ^{14}C-Levels in Water and Fish

The mean concentration of ^{14}C-tebufenpyrad equivalents in water during the exposure period was 1.18 μg/l with the standard deviation of 0.15. The measured concentration was very close to an expected nominal concentra-

Table 2 Representative HPLC retention times for authentic standards.

Compound	Retention time (min)	
	HPLC conditions A	B
OH-PAM	4.9	3.2
CO-PAM	8.9	5.4
DM-PCA	10.6	7.9
PAM	14.0	10.2
PCA	20.5	19.3
CO-M-OSO_3H	25.0	21.0
OH-M-OH	25.8	17.9
OH-M-CA	27.4	20.1
M-OH-CA[a)]	31.6	25.0
M-OSO_3H	31.6	31.7
CO-M-OH	37.6	33.4
CO-M-CA	39.2	34.2
M-OH	45.8	37.6[c)]
M-CA	46.8	37.6[c)]
OH-M	49.0[b)]	39.7[b)]
2CA-M	49.0[b)]	39.7[b)]
2OH-M	49.8	40.0
DM-M	58.4	43.4
CO-M	61.0	44.3
Tebufenpyrad	66.0	47.6

[a)] Not an authentic standard but a metabolite in rat identified by GC-MS and NMR analyses.[6)]
[b)] Two peaks completely overlapped under either HPLC condition.
[c)] Two peaks completely overlapped under HPLC condition B.

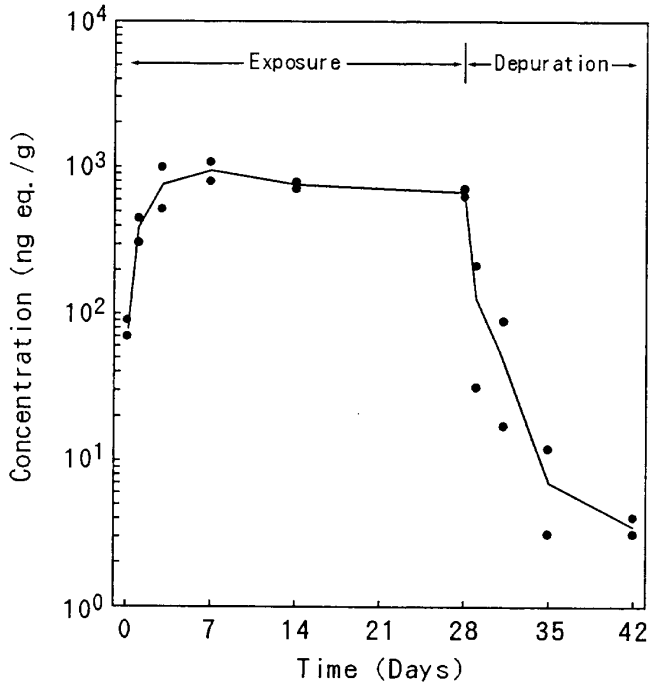

Fig. 1 Concentrations of ^{14}C-tebufenpyrad equivalents in carp during 28 days of exposure and 14 days of depuration.

tion of 1.2 $\mu g/l$.

The concentrations of ^{14}C in whole fish are shown in Fig. 1. The ^{14}C-levels in carp increased rapidly during the first 3 days and then slowly from 3 to 7 days. The bioconcentration factors (BCFs) of ^{14}C in whole fish reached a steady-state plateau within 7 days and its maximum BCF value was 864. During the depuration period, ^{14}C was rapidly excreted from carp. More than 66, 86, 98 and 99% of ^{14}C in whole fish were excreted within 1, 3, 7 and 14 days, respectively. The biological half-life in carp was calculated to be 0.5 day by the least square method when the logarithm of the ^{14}C in whole fish was plotted against time.

2. ^{14}C-Tissue Distribution

The ^{14}C-levels in tissues sampled on day 28 of exposure and on day 7 of depuration are shown in Table 3. Radioactivity was widely distributed in tissues, with the highest concentration found in the gall bladder bile (95,600±25,500 ng/g), followed by GI tract including contents (2680±552 ng/g) and hepatopancreas (1110±455 ng/g). Most of the ^{14}C were mainly distributed into the tissues related to the metabolism and excretion. Within 7 days of the depuration, more than 93% of the ^{14}C were eliminated from all tissues.

3. Biliary Metabolites

More than 97% of the ^{14}C in bile were partitioned in water and 1.8% in ethyl ether, indicating that most of the biliary metabolites were polar compounds. After β-glucuronidase treatment of the water extract, 21.0 and 76.3% of the ^{14}C in bile were partitioned into the water and ether layers, respectively. It appears that at least 76% of the ^{14}C in bile are the β-glucuronic acid conjugates. Representative HPLC radiochromatograms (condition A) for bile are shown in Fig. 2, together with those of the water and ether layers after enzymatic treatment. At least 14 peaks were found in bile, five of which had retention times identical to OH-M-OH, OH-M-CA, M-OH-CA

Table 3 Concentrations of radioactivity in carp tissues after 28 days of exposure to ^{14}C-tebufenpyrad (1.18 μg/l) and 7 days of depuration.

Tissue	28 days of exposure	7 days of depuration	
	Concentration[a] (ng eq./g)	Concentration[a] (ng eq./g)	Residual rate[b] (%)
Gill	291±357	2±1	0.7
Blood	15±3	0.3±0.6	2.0
Heart	134±36	ND[c]	0
Hepatopancreas	1110±455	16±8	1.4
Kidney	250±106	6±1	2.4
Spleen	109±18	7±7	6.4
Gall bladder bile	95,600±25,500	448±373	0.5
GI tract[d]	2680±552	30±22	1.1
Brain	58±2	1±2	1.7
Muscle	65±50	ND[c]	0
Skin with scale	112±64	0.3±0.6	0.3
Eye	36±7	1.0	2.8
Air bladder	271±188	3±6	1.1
Residual carcass	124±52	2±2	1.6

[a] The mean values ±standard deviations of three fish, expressed as ^{14}C-tebufenpyrad equivalents.
[b] % of ^{14}C residues relative to the tissue concentrations at 28 days of exposure.
[c] Not detectable (<0.5 ng eq./g).
[d] Gastrointestinal tract with its contents.

and M-OSO$_3$H, M-OH and M-CA, while tebufenpyrad was not detected in bile. Following treatment with β-glucuronidase, two major peaks [peaks "c" and "d" (Fig. 2-i and -i')] detected in bile were shifted to M-CA (peak "e") and M-OH (peak "h") peaks (Fig. 2-ii and -iii), respectively. Thus, the former "c" seemed to be an ester glucuronide of M-CA (M-CA-glu) and the later "d" an ether glucuronide of M-OH (M-O-glu). These shifts were also observed by a two-dimensional TLC co-chromatography (1st solvent, chloroform/acetonitrile=3/1; 2nd solvent, ethyl ether/*n*-hexane=4/1). The radioactivity on the TLC plate was measured by LSC after scraping the radioactive or UV detectable area, and the percentages of M-OH and M-CA were same as those of HPLC analysis.

The percentages of biliary metabolites determined by two HPLC conditions are shown in Table 4. Each conjugated metabolite was quantified by subtracting the amount of individual free metabolite in bile from the sum amount of the corresponding free metabolite in the water and ethyl ether extracts after the enzyme treatment. The result of HPLC condition A closely agreed with that of condition B. HPLC analysis showed that 10 metabolites were present in bile, accounting for more than 90% of the ^{14}C in bile. And 84% of the ^{14}C were β-glucuronic acid conjugates. Of these metabolites, M-CA-glu and M-O-glu amounted to 47 and 24%, respectively. In addition, the sulphate conjugate of M-OH [M-OSO$_3$H] amounted to 6.5 (condition A)–8.0% (condition B). Under HPLC condition A, M-OSO$_3$H peak overlapped with that of M-OH-CA, *N*-[4-(1-carboxy-1-hydroxymethylethyl)benzyl]-4-chloro-3-ethyl-1-methylpyrazole-5-carboxamide, found in rat.[6] Under HPLC condition B, M-OSO$_3$H peak overlapped with those of both M-O-glu and M-CA-glu. However, it was possible to distinguish M-OSO$_3$H from these three metabolites because it was not extracted by ether and not easily hydrolyzed by enzymatic treatment with β-glucuronidase. M-OH-CA was identified by HPLC co-chromatography with a M-OH-CA which has been identified in rat by GC-MS and NMR analyses.[6] Thus, the major unknown metabolite in bile could be the ether or ester glucuronide of M-OH-CA (8.7% in condition

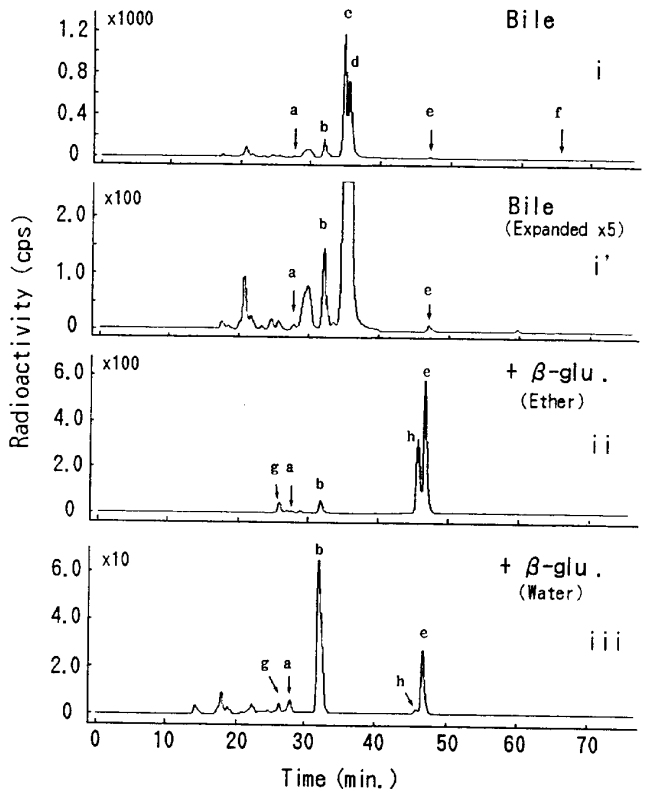

Fig. 2 HPLC chromatograms for biliary metabolites analyzed under condition A.

Bile (i and i') without β-glucuronidase treatment, ether (ii) and water (iii) extracts with β-glucuronidase treatment. a: OH-M-CA, b: M-OH-CA and M-OSO_3H, c: M-CA-glu, d: M-O-glu, e: M-CA, f: tebufenpyrad, g: OH-M-OH, h: M-OH.

A, 9.0% in condition B). As minor metabolites, the glucuronides of the OH-M-OH and OH-M-CA seemed to be present in bile.

4. Metabolites in Hepatopancreas and GI Tract

More than 99% of the ^{14}C in both tissue samples were extracted. The percentages of metabolites in hepatopancreas and GI tract characterized by HPLC under condition A are shown in Table 5. In both chromatograms of hepatopancreas and GI tract, at least 20 peaks were found, five of which had retention times identical to OH-M-OH, OH-M-CA, M-OSO_3H, M-OH and M-CA. Tebufenpyrad was detected in a trace amount (0.5%) in hepatopancreas, while not in GI tract. M-OH and M-CA amounted to 1.4 and 2.9% of the ^{14}C in hepatopancreas, and 2.5 and 8.9% in GI tract, respectively. Two major metabolites, M-CA-glu and M-O-glu found in bile, were also present

Table 4 Relative amounts of metabolites identified in carp bile[a] (expressed as percentages of the ^{14}C in bile).

	Metabolite	HPLC conditions A	HPLC conditions B
Free	OH-M-OH	<0.1	0.12
	OH-M-CA	0.33	0.88
	M-OH	<0.1	0.72[b]
	M-CA	0.50	
Sulphate	M-OSO_3H	6.5	8.0
Glucuronides	OH-M-OH-glu	3.5	3.5
	OH-M-CA-glu	1.1	0.42
	M-CA-glu	46.7	70.9[c]
	M-O-glu	24.1	
	M-OH-CA-glu[d]	8.7	9.0
	Total	84.1	83.8
Other unknowns		8.6	6.5
Total ^{14}C in bile (%)		100	100

[a] The bile was collected from 15 carp and combined before analysis.

[b] Two peaks completely overlapped under HPLC condition A.

[c] Estimated as sum of M-OH and M-CA after β-glucuronidase treatment, subtracting the free metabolites in bile.

[d] Identified by HPLC co-chromatography with a M-OH-CA in rat,[6] after enzymatic treatment.

Table 5 Relative amounts of metabolites in hepatopancreas and GI tract[a] analyzed by HPLC under condition A (expressed as percentages of the ^{14}C in each tissue).

Metabolite	Hepatopancreas	GI tract
OH-M-OH	1.2	1.4
OH-M-CA	0.4	1.4
M-OH	1.4	2.5
M-CA	2.9	8.9
M-OSO_3H and/or M-OH-CA	27.1	24.6
M-CA-glu	42.1	28.4
M-O-glu	11.4	12.4
Tebufenpyrad	0.5	ND
Others	12.2	19.5
Unextracted	<1	<1
Total	100	100

[a] The tissues were collected from five carp and combined before analysis.

ND: not detectable.

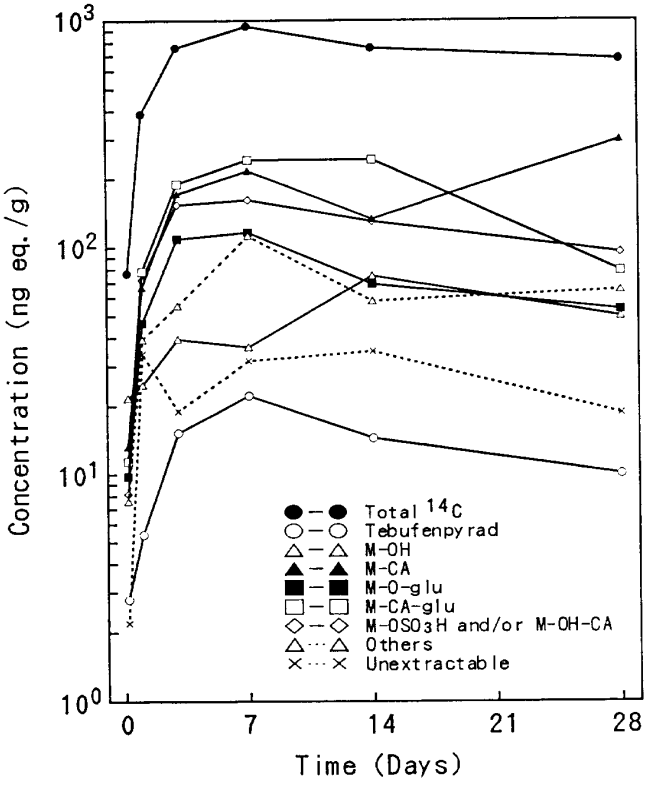

Fig. 3 Concentrations of ^{14}C-tebufenpyrad and its metabolites in carp during 28 days of exposure (analyzed by HPLC under condition A).

in both tissues, amounting to 42 and 11% in hepatopancreas, and 28 and 12% in GI tract, respectively. In addition, an overlapped peak with M-OH-CA and M-OSO_3H was found, amounting to 27% in hepatopancreas and 25% in GI tract.

5. Metabolites in Whole Fish

More than 91% of the ^{14}C in carp were extracted except for one fish (78%) sampled on day 1 of exposure. The time course changes of concentrations of tebufenpyrad and its metabolites in carp during 28 days exposure period are shown in Fig. 3. The concentrations of tebufenpyrad and its metabolites rapidly increased in whole fish during the first 3 days and reached a steady-state plateau within 7 days. Based on these data, BCF of tebufenpyrad was calculated (Table 6) with the maximum value 29.

DISCUSSION

Most of the ^{14}C in fish were mainly distributed into the tissues related to the metabolism and excretion, such as bile, GI tract with contents and hepatopancreas. Similar distribution patterns were observed in the whole-body autoradiograms of the exposed fish (results not shown). The high concentration of ^{14}C in bile was consistent with its role in excretion. When carp were subsequently transferred to fresh water, more than 66, 86, 98 and 99% of total ^{14}C in carp were eliminated within 1, 3, 7 and 14 days, respectively. And more than 93% of ^{14}C were excreted from all tissues within 7 days of the depuration. These results indicate that tebufenpyrad is actively metabolized to polar metabolites in carp and the latter are rapidly eliminated from them, mainly *via* bile.

Table 6 Bioconcentration factors (BCFs) of total ^{14}C and tebufenpyrad.

Uptake time (day)	Conc. in water[a] (ng eq./ml)	BCFs[b] based on	
		Total ^{14}C	Tebufenpyrad
1	1.06	299, 433	5.7, 3.9
3	1.16	452, 855	14 , 8.6
7	1.19	864, 722	29 , 10
14	1.18	666, 614	7.3, 17
28	1.18	586, 551	11 , 6.6

[a] Mean concentration of ^{14}C-tebufenpyrad equivalents in water (ng eq./ml) during the exposure period of day 0 and days N (N=uptake time), Cw.

[b] Data from two carp. BCFs calculated by the following equation.

$$\text{BCFs} = \frac{\text{Concentrations of } ^{14}\text{C-tebufenpyrad equivalents in whole fish (ng eq./g)}}{\text{Cw (ng eq./ml)}}$$

Proposed metabolic pathways of tebufenpyrad in carp are shown in Fig. 4. The major metabolic reactions were hydroxylation (M-OH) on the *tert*-butyl moiety, followed by oxidation to the carboxyl group (M-CA), and their β-glucuronic acid conjugation (M-O-glu and M-CA-glu). Sulphate conjugation of M-OH (M-OSO_3H), hydroxylation on the *tert*-butyl moiety of M-CA (M-OH-CA) and its β-glucuronic acid conjugation (M-OH-CA-glu) were also proposed. The free precursors of all these conjugates were also found in rat.[6] The metabolites cleaved at the amide or benzylmethylene carbon-nitrogen bond of tebufenpyrad, such as PCA, PAM, OH-PAM, CO-

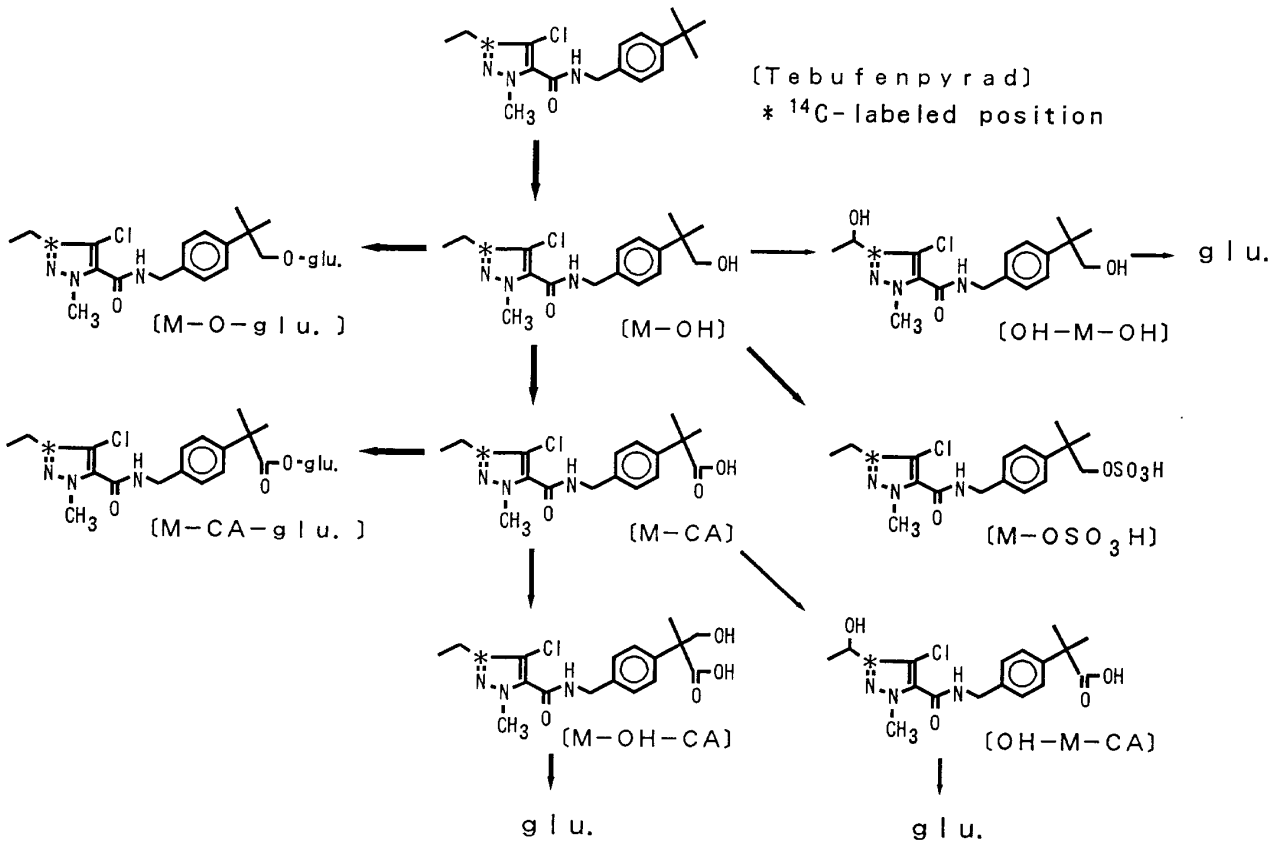

Fig. 4 Proposed metabolic pathways for tebufenpyrad in carp.
glu: β-glucuronide.

PAM and DM-PCA, were not found in carp when analyzed by HPLC co-chromatography with authentic standards of the pyrazole moiety. Even if all of the unidentified metabolites in bile were the cleaved metabolites, the amounts were less than 10%.

The amounts of M-OH and M-CA, as free precursors to the glucuronic acid conjugates, in the GI tract and its content were higher than those in bile. Such observations suggest the possibility that enterohepatic circulation takes place in carp, although the occurrence level is unknown at present. However, even if these metabolites were reabsorbed, they were rapidly reconjugated and readily excreted from carp.

From these results, it seems likely that the considerably lower bioaccumulation potential of tebufenpyrad by carp, than predicted by its high lipophilicity, is due to the active metabolism and rapid excretion *via* bile.

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要　　約

テブフェンピラドのコイにおける吸収・分布・代謝・排泄

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テブフェンピラド〔*N*-(4-*tert*-butylbenzyl)-4-chloro-3-ethyl-1-methylpyrazole-5-carboxamide〕のコイにおける吸収・分布・代謝・排泄を検討した．^{14}C 標識体濃度 1.2 $\mu g/l$ に設定した連続流水式の飼育水中でコイを28日間暴露後，引き続き清水中で14日間の排泄試験を行なった．全 ^{14}C の生物濃縮係数は暴露7日間で平衡に達し，最大で864倍であったが，テブフェンピラドのそれは最大でも29倍であった．全 ^{14}C は主に胆嚢，胃腸管，および肝膵臓に分布した．胆汁中にはテブフェンピラドは検出されず，84% が β-グルクロニダーゼ処理後，加水分解を受けた．主要代謝反応は *tert*-ブチル基の水酸化，それを経由したカルボキシル基への酸化およびそれらの β-グルクロン酸抱合化であった．全 ^{14}C の排泄は速く7日目には98% 以上が魚体から排泄された．以上の結果から，テブフェンピラドはコイ体内で活発に代謝を受け，主に胆汁を経て速やかに体外へ排泄されると推察した．