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In Vitro Metabolism of Carbaryl by Liver Explants of Bluegill, Catfish, Perch, Goldfish, and Kissing Gourami

The purpose of this study was to demonstrate the metabolic role of livers of five species of fish toward insecticide carbaryl (Sevin), 1-naphthyl *N*-methylcarbamate. Carbaryl (naphthyl-^{14}C or *N*-methyl-^{14}C) was applied to a growth medium containing liver explants of the five species. Each mixture was incubated for 18 h after which the medium was analyzed by diethylaminoethyl (DEAE)–cellulose column chromatography. Based on the metabolic profile analysis, hepatic tissues from each species all performed the metabolic processes of demethylation and/or hydrolysis, hydroxylation, and oxidation, followed by conjugation. The most significant common metabolite in all species chromatographed as a dihydrodihydroxycarbaryl glucuronide.

Sullivan et al. (1972a) reported a potential in vitro method for the selection of species metabolically similar to man for use in chronic toxicity studies. The technique is simple, rapid, reproducible, and organ specific (Chin et al., 1974, 1979) and therefore is very practical in estimating major metabolic differences among test species without resorting to the actual dosing of animals with test chemicals. The purpose of this study is to elucidate the metabolic role of livers of several species of fish using the in vitro method because increased importance of metabolic fate studies have been emphasized in fish in recent years.

As a prototype the carbamate insecticide carbaryl (Sevin) 1-naphthyl *N*-methylcarbamate was used because extensive metabolic information is available in man and in many other species as reviewed by Knaak (1971). The designation of major metabolites of carbaryl is based on chromatographic behavior and fluorescence characteristics rather than actual isolation and structural verification of the metabolites.

MATERIALS

1-Naphthyl-^{14}*C* *N*-methylcarbamate (carbaryl) of specific activity 30 μCi/mg and 1-naphthyl *N*-methyl-^{14}*C*-carbamate of specific activity 11.3 μCi/mg were provided by the Union Carbide Corporation Technical Center, South Charleston, WV. The carbogen used was the commercially available oxygen/carbon dioxide mixture (95:5). Trowell (1959) T8 medium and penicillin–streptomycin mixture (Catalog No. 12603F) were purchased from Microbiological Associates, Bethesda, MD. Kissing gourami (*Helostoma temmincki*), weighing 6–8 g, and goldfish (*Carassius auratus*), weighing 28–33 g, were purchased from a local pet shop. Because these fish had very small amounts of liver, respective liver specimens from these fish

0021-8561/79/1427-1395$01.00/0

were pooled and used for in vitro studies. Bluegills (*Lepomis macrochirus*), weighing 220–270 g, channel catfish (*Ictalurus punctatus*), weighing 77–110 g, and yellow perch (*Perca flavescens*), weighing 80–250 g, were acquired from the Pennsylvania Fish Hatchery at Linesville, PA. These specimens were furnished through the offices of Mr. James Meade, III, of the Benner Springs Research Station, and of Mr. Thomas Clark at Linesville, PA.

METHODS

The incubation procedure of Sullivan et al. (1972a), DEAE–cellulose chromatography of Knaak et al. (1965) modified by Sullivan et al. (1972b), and thin-layer analysis of Dorough and Casida (1964) were employed. Silica gel chromatography of the ether-extractable neutrals was performed according to methods described by Sullivan et al. (1972b).

RESULTS

The quantitative data of in vitro metabolites of carbaryl by five fish livers using naphthyl-^{14}C and *N*-methyl-^{14}C are shown in Tables I and II, respectively. These data are given as percentage of radioactivity found in the medium. Comparison of percentages in Tables I and II for the two labels indicate that the majority of the metabolites were intact carbamates. Peak identifications were based primarily on chromatographic positions of known compounds as reported for the rat by Knaak et al. (1965) and Sullivan et al. (1972a).

All species produced a high percentage of neutrals (peaks A and B) and dihydrodihydroxycarbaryl glucuronide (peak D). Peak E (an unknown material) was found in the goldfish, bluegill, and catfish with naphthyl label only. Peaks G (naphthyl glucuronide) and J (naphthyl sulfate) were minor metabolites for all aquatic species tested. Significant amount of peak K (an unknown metabolite) was found in the kissing gourami, goldfish, and bluegill but minor in the other fish species studied.

The quantitative data of silica gel chromatographic analysis of the ether-extractable neutrals are shown in Table III. The peaks are given as percentage of applied radioactivity found in the growth medium and are numbered 1 through 8 to distinguish them from anionic metabolites isolated from DEAE–cellulose chromatography. 1-Naphthol (peak 1) was a minor metabolite in all species. Unchanged carbaryl (peak 2) remaining in the medium after incubation with the respective liver tissue increased in ascending order for kissing gourami, bluegill, goldfish, perch, and catfish. Kissing gourami liver was approximately four times more active than that of bluegill and it was eight times more active than those of catfish, goldfish, and perch. No histopathologic changes were found on either treated or untreated livers which could explain the lesser activity of bluegill, catfish, goldfish, and perch.

4-Hydroxycarbaryl (peak 4) was an important neutral component in the catfish. Peak 7 may be a mixture of 3,4 or 5,6 isomers of dihydrodihydroxycarbaryl. In the gourami, the majority of radioactivity in peak 7 was associated with the 5,6 isomer of dihydrodihydroxycarbaryl. In the goldfish, the majority of the radioactivity in peak 7 was the 3,4 isomer of dihydrodihydroxycarbaryl.

DISCUSSION

Recent studies demonstrated that microsomal enzymes which metabolize foreign compounds are present in fish and other marine animals (Creaven et al., 1965, 1967). Their work showed that trout liver preparations can hydroxylate and *O*-dealkylate biphenyl. The present work verifies that fresh water fish livers are capable of performing the metabolic processes of demethylation and/or hy-

Table I. Tentative in Vitro Derived Metabolites of Carbaryl-*naphthyl*-^{14}C in Fish Livers[a]

species	chromatographic fraction in order of elution															
	A	B	C	D	E	F	G	H	I	J	K	L	M			
	neutrals to DEAE		UK[b]	dihydrodihydroxycarbaryl glucuronide, major aglycon	UK[b]	hydroxycarbaryl glucuronide	naphthyl glucuronide	UK[b]	hydroxycarbaryl sulfate	naphthyl sulfate		UK[b]			pooled fraction[c]	
kissing gourami[d]	56.1		0	24.4	0	5.9	1.3	1.2		1.7	4.0	1.6	1.2		2.6	
goldfish[d]	46.6		0	20.0	1.9	2.5	0.8	0.9		1.2	7.5	8.2	1.8		3.6	
bluegill[e]	51.7		0	16.2	1.2	2.6	0.8	Tr	3.1	2.0		13.8	1.0		2.8	
catfish[e]	78.3		0	8.8	0.7	3.7	1.3	Tr	0.8	1.3		2.7	0.7		1.6	
perch[e]	60.6		0	13.2	Tr	11.9	2.9	Tr	4.1	0.8		1.5	1.6		3.4	

[a] Calculated as percent of ^{14}C found in the medium. All tissues were incubated at 22 °C. [b] UK = unknown. [c] Combined fractions from areas of chromatogram not containing a distinguishable peak. [d] Holding tank temperature was 22 °C. [e] Holding tank temperature was 10 °C.

Table II. Tentative in Vitro Derived Metabolites of Carbaryl-*N*-methyl-^{14}C-in Fish Livers[a]

	chromatographic fraction in order of elution												
	A	B	C	D	E	F	G	H	I	J	K	L	M
	neutrals to DEAE	UK[b]	dihydro-dihydroxy-carbaryl glucuronide, major aglycon	UK[b]	hydroxy-carbaryl glucuronide	naphthyl glucuronide	UK[b]	hydroxy-carbaryl sulfate	naphthyl sulfate		UK[b]		pooled fraction[c]
goldfish[d]	47.0		29.0	0	4.1	1.2	1.0	2.1	0.6	4.9	0	0	10.0
bluegill[e]	55.3		18.3	0	1.8	0.5	0	1.7	0		0		5.4
catfish[e]	61.3		12.1	0	1.1	0	0	0.6	0		0		3.9
perch[e]	52.9		15.2	0	13.3	0	0	Tr	0		0		3.6

[a] Calculated as percent of ^{14}C found in the medium. All tissues were incubated at 22 °C. [b] UK = unknown. [c] Combined fractions from areas of chromatogram not containing a distinguishable peak. [d] Holding tank temperature was 22 °C. [e] Holding tank temperature was 10 °C.

Table III. Distribution of Ether Extractable ^{14}C-Labeled Compounds from in Vitro Fish Liver Expressed as Percent of ^{14}C Applied on the Column

	chromatographic fraction in order of elution										
	1	2	3	4	5	6	7	8			
	1-naphthol	carbaryl	UK[b]	UK[b]	UK[b]	UK[b]	dihydro-dihydroxy-carbaryl	UK[b]			
	fractions										
species	110-130	220-260	270-290	330-350	351-380	381-410	420-510	530-570	neutrals from DEAE	neutrals extracted by ether	unchanged carbaryl in medium
kissing gourami[c]	2.2	5.8	0		10.7		67.1	9.4	56.1	70	2.3
goldfish[c]	Tr	68.9	1.3	5.9	0	0	14.1	2.4	46.6	84	26.9
bluegill[d]	Tr	24.4	0	2.4	0	5.0	28.8	23.0	51.7	81	10.2
catfish[d]	2.8	44.0	0	17.9	0	Tr	11.5	2.1	78.3	86	29.6
perch[d]	2.2	54.9	0	2.2	0	Tr	24.2	3.8	60.6	87	28.9

[a] All tissues were incubated at 22 °C. [b] UK = unknown. [c] Holding tank temperature was 22 °C. [d] Holding tank temperature was 10 °C.

drolysis, hydroxylation, and oxidation, followed by conjugation.

Based on the metabolic profiles obtained with five species, the metabolic pathways found with mammalian systems (Sullivan et al., 1972a,b) are operative also in fish species. The most significant conjugated metabolite found in all fish species studied had chromatographic characteristics of 5,6-dihydro-5,6-dihydroxycarbaryl glucuronide. Hydrolysis studies by β-glucuronidase of this fraction obtained from the kissing gourami had shown that this peak may have contained the glucuronide of the 3,4 isomer also.

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Uptake of Glyphosate and *N*-Nitrosoglyphosate from Soil by Oat Plants

Absorption of glyphosate and *N*-nitrosoglyphosate from soil treated with high rates of these compounds and their translocation in oat plants was observed in a greenhouse experiment. *N*-Nitrosoglyphosate moved more readily into the root and subsequently into the shoot of oat plants than glyphosate. Formation of *N*-nitrosoglyphosate in soil and its uptake by plants under normal field conditions is not expected.

The herbicide glyphosate [*N*-(phosphonomethyl)glycine] provides effective control of most herbaceous perennial weeds through postemergence application (Spurrier, 1973). For this purpose it is applied to weed foliage at rates up to 4.2 kg/ha, often as a spot treatment. Formation of the *N*-nitroso derivative of glyphosate in various nitrite-treated soils under in vitro conditions has been recently demonstrated in the laboratory (Khan and Young, 1977). The *N*-nitrosoglyphosate formed was persistent in soil up to about 4 months. The formation of the *N*-nitroso derivative of glyphosate in soil at average recommended rates of application is not expected under normal field conditions due to the high rates of glyphosate and nitrite required for its production. However, in instances when nitrite accumulates temporarily in high concentrations (Chapman and Liebig, 1952), the use of exceptionally high levels of glyphosate raises the possibility of the herbicide undergoing N-nitrosation in soil (Khan and Young, 1977) and being subsequently taken up by plants. The herbicide glyphosate can be taken up by plant roots and translocated to the shoots (Sprankle et al., 1975). It has been shown that *N*-nitrosodimethylamine is stable in soil (Tate and Alexander, 1975, 1976) and can be translocated into vegetable crops (Dean-Raymond and Alexander, 1976).

The investigation reported here is an extension of the study on possible formation of *N*-nitroso derivatives of pesticides in soil. Its purpose was to investigate whether uptake of *N*-nitrosoglyphosate by plants would occur from soil and to compare this uptake with that of glyphosate.

EXPERIMENTAL SECTION

Chemicals. All solvents were of pesticide grade and used as received. Glyphosate, as its isopropylamino salt (96.7% purity), was obtained from Monsanto Commercial Products Co. (St. Louis, MO).

Synthesis of *N*-Nitrosoglyphosate. To a stirred solution of 1.157 g of glyphosate isopropylamine salt in 50 mL of 0.12 N hydrochloric acid was added 3 g of sodium nitrite. The stirring was carried out in the dark overnight. The *N*-nitrosoglyphosate thus prepared (yield = 1.0 g) was kept in the dark and refrigerated when not in use. The purity of the compound was checked by its TLC properties and UV spectra (Young et al., 1977). Many *N*-nitrosamines are potent carcinogens. Although the carcinogenic properties of *N*-nitrosoglyphosate are unknown at this time, safety precautions such as those outlined in the National Cancer Institute Safety Standards for Research Involving Chemical Carcinogens to prevent skin contact and inhalation must be exercised at all times.

Soil Treatment and Greenhouse Experiment. (*a*) *Preliminary Experiment.* Fox sandy loam soil (300 g), previously steam sterilized to prevent microbial herbicide degradation, was spread evenly in individual 21 × 29 cm plastic trays. The soil was sprayed with successive applications of glyphosate solution (1% AI) to achieve four replicates of soil concentrations of 25–625 ppmw. The

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