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Uptake, Distribution, and Metabolism of Endothall in Fish

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When bluegills were exposed in aquaria to water containing 2 ppm of [^{14}C]endothall, less than 1% of the herbicide was absorbed by the fish. The maximum concentration of endothall in the fish (0.1–0.2 ppm) was observed 12 hr after treatment; thereafter it did not change significantly up to 96 hr. Radioactivity was detected in viscera, flesh, scales, skin, and head. At all sampling times, the concentration of ^{14}C residues was highest in the viscera and lowest in the flesh. A small but detectable amount of ^{14}C was found in the blood 30 min after treatment. The herbicide was also absorbed by the fish when fed through the digestive tract. The fish did not metabolize endothall during the 48 hr after treatment.

The herbicide endothall (7-oxabicyclo[2.2.1]heptane 2,3-dicarboxylic acid) has been found to be effective in controlling certain aquatic weeds. The dipotassium salt of endothall is relatively nontoxic to fish; it has a medium tolerance limit ranging from 95 to 150 ppm for nine species of fish (Walker, 1963). A knowledge of the degree of accumulation of this herbicide by fish is important if they are to be used for human consumption. The herbicide, if accumulated by fish, may undergo metabolic transformation. The nature of these metabolites must be known in order to assess their possible toxicity to fish and man. Presently, no information is available in literature on the fate of the herbicide in fish. The present investigation was undertaken to study the uptake and metabolism of endothall in bluegills (*Lepomia macrochirus*).

MATERIALS AND METHODS

Uptake and Distribution of [^{14}C]Endothall. Bluegills were obtained from the National Fish Hatchery, North Attleboro, Mass. The fish were acclimated to the laboratory conditions for 2 weeks before exposing them to endothall. During acclimation, the fish were placed in holding aquaria containing aged tap water. The fish were maintained at about 20°. The fish used in these studies were 3–4 in. long and weighed 3–5 g. The fish were exposed to endothall in two ways: bathing and feeding. Uptake and distribution of endothall were determined by ^{14}C analysis of whole fish and selected fish tissues after various periods of exposure of the fish to [^{14}C]endothall. Fish were fasted for 48 hr before exposure to the herbicide.

Bath Exposure. Each fish was introduced into 500 ml of aged tap water containing 2 ppm of [^{14}C]endothall, labeled in positions 2 and 3 of the oxabicyclo ring. The water containing the fish was continuously bubbled with air alone or a mixture of ozone and air (to minimize the microbiological growth) during the exposure of the fish to the herbicide. The fish were removed from the treated water at 4, 12, 24, 48, 72, and 96 hr following treatment, rinsed with clean water three times, and weighed. To determine the amount of ^{14}C in the whole body, the fish were cut into small pieces and homogenized with 80% methanol in a Virtix homogenizer fitted with turboshear blades. The slurry was shaken for 30 min and centrifuged and the extract was decanted. The residue was refluxed with 80% methanol for 1 hr. The two extracts were combined; the amount of radioactivity in the pooled extract was measured by adding 1-ml aliquots to 15 ml of liquid scintillation solution and counting in a liquid scintillation counter. The amount of ^{14}C in the tissue residue was determined by solubilizing it in NCS tissue solubilizer (Amersham/Searle Co.) for 48 hr at 50°. Glacial acetic acid (0.02 ml/ml of solubilizer) was added to the solubilized tissue and the solution was counted for radioactivity using scintillation fluid containing Triton X-100. The samples were stored overnight at 0° in the dark before counting. The radioactivity in the methanol extract and in the tissue residue was combined to calculate the ^{14}C concentration in the fish.

To determine the distribution of radioactivity in various

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Table I. Whole Body Residues of Radioactivity in Bluegills Exposed to Water Containing 2 ppm of [^{14}C]Endothall

hr after exposure	^{14}C-Labeled residue[a] (expressed as μg of endothall/g fresh wt)
4	0.03
12	0.17
24	0.16
48	0.17
72	0.18
96	0.17

[a] The values represent the mean of two experiments each consisting of two replications.

Table II. Radioactive Residue in Tissues of Bluegills Exposed to Water Containing 2 ppm of [^{14}C]Endothall

Tissue	^{14}C-Labeled residue[a] (expressed as μg of endothall/g of fresh tissue) at hr after exposure		
	12	24	48
Viscera	0.24 (30.7)[b]	0.81 (54.9)	0.41 (37.3)
Flesh	0.046 (26.7)	0.034 (14.4)	0.044 (18.6)
Scales	0.19 (28.0)	0.18 (22.5)	0.35 (29.4)
Skin	0.10 (5.3)	0.057 (2.7)	0.068 (3.9)
Head + fins	0.043 (9.3)	0.053 (5.5)	0.08 (10.8)

[a] The values represent mean of two replications. [b] The values in parentheses represent percentage of the total radioactivity detected in various tissues.

Table III. Radioactive Residues in Tissues of Bluegills 48 hr after Exposure in the Feed of 10 mg of [^{14}C]Endothall/kg Body Weight

Tissue	^{14}C-Labeled residue[a] (expressed as μg of endothall/g of fresh tissue)
Viscera	13.29 (35.4)[b]
Flesh	1.04 (19.2)
Scales	8.37 (10.7)
Skin	2.49 (4.1)
Head + fins	2.30 (30.6)
Whole body	2.70[c]

[a] The values represent mean of eight replications. [b] The values in parentheses represent percentage of the total radioactivity detected in various fish tissues. [c] Micrograms/gram body weight.

organs, the fish were dissected into flesh, head and fins, skin, scales, and viscera after 12, 24, and 48 hr of exposure to [^{14}C]endothall. The amount of radioactivity in each tissue was determined by methanol extraction or solubilization with the NCS solubilizer as described for the whole body residues.

A separate experiment was conducted to determine the distribution of endothall in fish blood. The fish were introduced into water containing 2 ppm of [^{14}C]endothall. Groups of five fish were removed at 0.5, 1, 2, 3, and 4 hr after treatment and samples of blood were collected immediately. Blood samples were obtained by severing the caudal fin and collecting the blood from the dorsal aorta with a syringe containing 0.1 ml of heparin (50 mg/ml) as an anticoagulant. The blood was placed in 12 times its volume of NCS solubilizer. After 1 hr, 0.4 ml of a saturated solution of benzoyl peroxide in toluene was added and the mixture was shaken overnight at 50°. The bleached solution was then counted for ^{14}C in a liquid scintillation counter.

Feed Exposure. In these studies, the fish were force-fed [^{14}C]endothall at a rate of 10 mg of endothall/kg body weight (10 ppm). An appropriate amount of [^{14}C]endothall was placed in cellulose powder contained in a no. 5 gelatin capsule and the capsule was then force-fed. The experiment included eight replications. After 48 hr, the fish were removed from water and rinsed and the amount of ^{14}C in the whole body as well as in various tissues was determined as described above.

Metabolism of Endothall. The metabolic fate of endothall was followed in fish after they were exposed for 48 hr to water containing the ^{14}C-labeled herbicide. The 80% methanol extract from several fish was pooled and the extract was concentrated under vacuum to remove methanol. The remaining aqueous phase was extracted twice with petroleum ether to remove lipids. The amount of ^{14}C in each fraction was determined by liquid scintillation counting. The aqueous phase which contained essentially all the radioactivity was concentrated and chromatographed on thin-layer silica gel as well as cellulose plates. The solvent systems for developing silica gel and cellulose chromatograms consisted of ethyl acetate–chloroform–formic acid (40:50:50, v/v) and ether–formic acid–water (7:2:1, v/v) (Sikka and Rice, 1973). After drying, the chromatograms were scanned for detection of radioactivity in a Nuclear-Chicago Actigraph. Authentic [^{14}C]endothall was cochromatographed for comparison with ^{14}C-labeled compounds in the extract.

RESULTS AND DISCUSSION

Bluegills removed very small amounts of endothall from the treated water. Less than 1% of the total amount of the herbicide was absorbed by the fish during a 96-hr exposure. There was no measurable difference in the herbicide uptake by the fish when they were placed in water bubbled with air alone or a mixture of ozone and air. Table I shows the concentration of ^{14}C (expressed as endothall) in the fish at various periods after treatment. The concentrations of ^{14}C-labeled residues reported represent the sum of the radioactivity in the 80% methanol extract and in the extracted residue. The ^{14}C in the methanol extract comprised 85–90% of the total radioactivity detected in the tissue. The results show that the concentration of ^{14}C in the whole fish reached a maximum of 0.1–0.2 ppm 12 hr after treatment. Longer exposure up to 96 hr did not result in a significant change in the total ^{14}C concentration. These findings suggest that bioaccumulation of endothall by fish from water treated with the herbicide is unlikely. This is expected as endothall, due to its polar, hydrophilic nature, is less likely to partition from water into fish. Similar results have been reported with other polar, water-soluble pesticides and their metabolites (Sanborn, 1974; Schultz, 1973).

Table II shows the concentration of radioactivity in different tissues at various times after the fish were exposed to water containing [^{14}C]endothall. Radioactivity was detected in the viscera, flesh, skin, scales, and head and fins. At all sampling times, the concentration of ^{14}C was highest in the viscera and lowest in the flesh. The concentration of radioactivity in the viscera increased up to 24 hr after exposure and declined thereafter, which suggests that it was distributed to other tissues. The relative distribution of total radioactivity in the various tissues was in the following order: viscera > scales > flesh > head + fins > skin. The results of the Pennwalt Corporation (personal communication) have shown that, under field conditions, the residues of endothall in the flesh of fish sampled 24 hr or longer after treating the water with 0.5–5 ppm of the herbicide were below detectable limits (0.02 ppm) as determined by

gas–liquid chromatography. In the fish removed within 24 hr of treatment, endothall concentration in the flesh ranged from 0.02 to 0.1 ppm.

After 30-min exposure to water containing 2 ppm of [^{14}C]endothall, ^{14}C-labeled residues (expressed as endothall) ranging from 0.02 to 0.04 ppm were detected in the blood. After 60 min, the concentration of ^{14}C in the blood increased up to 0.08 ppm. Longer exposure up to 4 hr did not significantly increase the level of radioactivity in the blood.

The concentration of ^{14}C in the various tissues, 48 hr after the fish were fed [^{14}C]endothall through the digestive tract, is shown in Table III. The results show that the herbicide is absorbed by the intestinal tract though the fish were observed to eliminate 73% of the administered herbicide during this period. In the fish fed ^{14}C-labeled herbicide through the digestive tract, like the bath-exposed fish, the concentration of radioactivity was highest in the viscera and lowest in the flesh. The pattern of distribution of radioactivity in the various tissues in the feed-exposed fish was similar to that observed for the bath-exposed fish, with the exception that the scales had a relatively lower proportion of the total radioactivity.

These findings have demonstrated that bluegills absorb endothall directly from water as well as through the intestinal tract. Adsorption of endothall on the scales also contributes to the herbicide residues in the fish. This is supported by the observation that the proportion of ^{14}C in the scales decreased when the fish were fed endothall through the digestive tract.

Thin-layer chromatographic analysis of the methanol extract from the fish treated with the herbicide for 48 hr showed that all the ^{14}C in the alcohol extractable fraction was present in the form of unchanged endothall (R_f 0.48 and 0.75 on silica gel and cellulose plates, respectively). The ^{14}C in the extract co-chromatographed with authentic [^{14}C]endothall. In contrast to aquatic microorganisms which were found to readily degrade endothall (Sikka and Saxena, 1973), bluegills do not appear to be capable of metabolizing the herbicide.

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The Mechanism of Chlorobiphenyl Metabolism

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4-Chlorobiphenyl was administered to rabbits and the major urinary metabolites were identified as 4′-chloro-4-biphenylol and 4′-chloro-3,4-biphenyldiol. It was also shown that 4′-chloro-4-biphenylol was also converted into the diol as well as its monomethyl derivatives. The biohydroxylation pathway was further investigated using 4′-[^{2}H]-4-chlorobiphenyl as a substrate. The 4′-chloro-4-biphenylol metabolite retained 79% of the deuterium and the results are consistent with the intermediacy of an arene oxide in the first hydroxylation reaction. The diol metabolite retained ca. one-half the deuterium found in the phenol (39%) and it is therefore not formed directly from the arene oxide but by direct hydroxylation of the phenolic metabolite. The sequence of hydroxylation of chlorobiphenyl is, therefore, analogous to the stepwise hydroxylation of phenylalanine to give 3,4-dihydroxyphenylalanine and butamoxane to give 6,7-dihydroxybutamoxane.

Polychlorinated biphenyls (PCB) are industrial compounds which are now recognized as among the most widespread pollutants in the global ecosystem (Hutzinger et al., 1974b; Risebrough et al., 1968; Fishbein, 1973). Residues have been identified in both the terrestrial and aquatic environments (Koeman et al., 1969; Harvey et al., 1974; Jensen et al., 1969; Hom et al., 1974) as well as in animals (Bagley et al., 1970; Butler, 1973; Prestt et al., 1970) and humans (Biros et al., 1970; Jamieson et al., 1973). Recent work has shown that both commercial PCB mixtures and pure isomeric chlorobiphenyls are metabolized by animals (Hutzinger et al., 1972, 1974a; Safe et al., 1974, 1975a,b; Burse et al., 1974; Greb et al., 1973; Yoshimura and Yamomoto, 1973; Block and Cornish, 1959; Goto et al., 1974; Gardner et al., 1973), plants (Moza et al., 1973, 1974), and microorganisms (Wallnofer et al., 1973; Ahmed and Focht, 1973; Maas et al., 1975) to give a range of hydroxylated metabolites. The major rabbit urinary metabolites of 2,2′,5,5′-tetrachlorobiphenyl were 2,2′,5,5′-tetrachloro-3-biphenylol, 2,2′,5,5′-tetrachloro-4-biphenylol, and *trans*-3,4-dihydro-2,2′,5,5′-tetrachloro-3,4-biphenyldiol and these all can conceivably be formed from the 3,4-epoxy intermediate. NIH rearrangement (Jerina, 1974; Jerina and Daly, 1974; Daly et al., 1972) of this intermediate (i.e., *trans*-3,4-dihydro-2,2′,5,5′-tetrachloro-3,4-epoxybiphenyl) could yield the two phenolic metabolites and hydrolysis of the epoxide would yield the dihydrodiol product. The experiments described in this paper are concerned with the mechanism of PCB hydroxylation in the rabbit using 4-chlorobiphenyl, 4′-chloro-4-biphenylol, and 4′-[^{2}H]-4-chlorobiphenyl as model substrates.

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MATERIALS AND METHODS

Chlorobiphenyl Substrates. 4′-Chloro-4-biphenylol was synthesized as described (Savoy and Abernethy, 1942).