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Uptake, Metabolism, and Elimination of Diphenyl Ether by Trout and Stickleback

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Diphenyl ether is extensively used in perfumes, flavorings, and industrial solvents. It is also the major component of a widely-used heat transfer agent (Dowtherm A). In 1962, world usage of diphenyl ether as a heat transfer component was 3000000 kg (KIRK & OTHMER 1965). In spite of its industrial importance, little is known of its behaviour in the environment. It has been detected in European lake waters (GROB & GROB 1974), in Nova Scotian marine organisms (ADDISON 1977), and it has been accumulated by fish during experimental exposures (NEELY *et al.* 1974).

Diphenyl ether is metabolized by rabbit (BRAY *et al.* 1953) and by trout liver microsomes (HALL *et al.* 1978) to the 4-hydroxy derivative. Among its biochemical effects are inhibition of cytochrome oxidase (ABOOD & GERARD 1953) and of the uptake of 5-hydroxytryptamine by rat brain mitochondria (WALASZEK & ABOOD 1959). In this study, we examined the accumulation and excretion of diphenyl ether by trout and stickleback. We also described in detail the characteristics of trout liver microsomal diphenyl ether 4-hydroxylase (HALL *et al.* 1978).

MATERIALS AND METHODS

Rainbow trout (*Salmo gairdneri*) and speckled trout (*Salvelinus fontinalis*), obtained from Coldbrook Fish Hatchery, Coldbrook, Nova Scotia, were held in continuously flowing fresh water and fed *ad lib* with Ewoz Fish Chow until used. Stickleback (*Gasterosteous aculeatus*), collected at Cole Harbor Dyke, Cow Bay, Nova Scotia, were held in continuously flowing seawater at 2-4°C and fed Tetramin and brine shrimp until used.

^{14}C-Diphenyl ether was synthesized according to the method described by CROWDER *et al.* (1963) using ring labelled ^{14}C-phenol (Amersham Co., Oakville, Ontario). All other chemicals were of the highest purity commercially available.

Microsomes were prepared and the diphenyl ether hydroxylase was assayed by the method of HALL *et al.* (1978). Protein was determined by the method of LOWRY *et al.* (1951).

Uptake studies were carried out in an uptake tank containing 12 L of stagnant and aerated fresh water at 9°C. After the addition of 0.17 µmol of ^{14}C-diphenyl ether (specific activity, 9.60 MCi/Mmol), about 100 rainbow trout (3-4 g each) were put into the tank. The control fish were placed in another tank of fresh water without diphenyl ether at 9°C.

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At fixed intervals, 3 fish from each tank were removed and rinsed with fresh water. They were killed, weighed and quickly homogenized in glass distilled water (4 : 1 v/w) using a Polytron homogenizer (Brinkmann Co., Rexdale, Ontario). A small sample (0.5 mL) of the homogenate was oxidized in a Biological Material Oxidizer (R.J. Harvey Instrument Co., Hillsdale, New Jersey), and the evolved $^{14}CO_2$ was trapped in Oxifluor (New England Nuclear, Lachine, Quebec). The amount of radioactivity trapped was determined by a Beckman Liquid Scintillation Counter. All samples were corrected for 100% efficiency. Total counts in experimental fish were corrected for apparent "radioactivity" in samples of control fish.

After 8 h of exposure, fish from the experimental group were removed from water containing diphenyl ether, rinsed with fresh water, and then placed in 5 L of clean fresh water flowing at a rate of 1.3 L/min. At fixed intervals, 3 fish were removed, killed and homogenized; the ^{14}C was measured as described previously.

The uptake and elimination experiments with stickleback (about 0.3 g each) were done in the same way as the experiment with the trout, except that uptake studies were carried out in 14 L of stagnant and aerated seawater. At fixed time intervals, 3 fish were taken out, killed, weighed and the ^{14}C measured after oxidation.

RESULTS AND DISCUSSION

^{14}C-Diphenyl ether was rapidly taken up by rainbow trout and stickleback. The amount of radioactivity accumulated by the fish increased with exposure time to the chemical. At the conclusion of an 8 h exposure, radioactivities accumulated by rainbow trout and stickle back were equivalent to 0.46 nmol and 1.7 nmol diphenyl ether per g wet weight, respectively.

The radioactivities in trout and stickleback decreased biphasically with time when they were put in fresh flowing water (Fig. 1). About 35% and 55% of the radioactivities accumulated by rainbow trout and stickleback, respectively, were eliminated in the first phase of elimination. Thereafter, elimination of ^{14}C-diphenyl ether by both trout and stickleback almost stopped. The presence of a slow phase of ^{14}C-diphenyl ether elimination in the fish indicates that the chemical remains in trout and stickleback for long periods of time. Further studies are required to determine whether lengthy sequestration of diphenyl ether produces toxicity in fish.

To determine whether diphenyl ether metabolism played a role in the elimination of the chemical by the fish, the rate of diphenyl ether hydroxylation by speckled trout liver microsomes *in vitro* was determined under optimal incubation conditions. Hydroxylation of diphenyl ether by trout liver microsomes was linear up to 90 min, after which reaction rate slowed (Fig. 2). We also found that enzyme activity increased rapidly from pH 6.2 to 7.5, but decreased rapidly at higher pH levels (Fig. 3). Optimal temperature of incubation was 25°C (data not shown). The production of 4-hydroxydiphenyl ether by trout

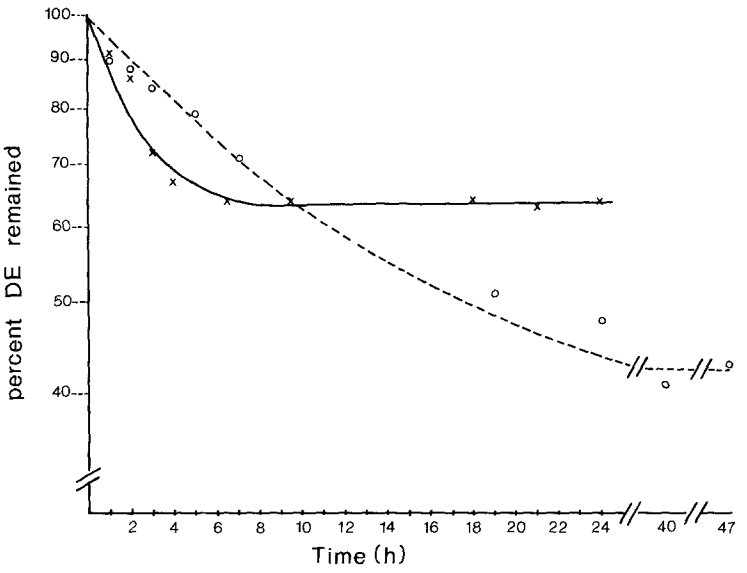

Fig. 1. Time course of diphenyl ether elimination from trout and stickleback. Trout x——x , stickleback o----o.

liver microsomes was linear up to 1.5 mg of microsomal protein/mL of reaction mixture (Fig. 4). The effects of substrate concentrations on enzyme activity at conditions optimized as described above are shown in Fig. 5. The apparent Km was 0.76 x 10^{-4} M with a Vmax of 0.30 nmol of 4-hydroxydiphenyl ether formed per mg microsomal protein per min, or 288 nmol of the product formed per g liver per h. No substrate inhibition was observed.

In the present study, the rate of diphenyl ether hydroxylation by trout liver microsomes appears to be low : 288 nmol/g liver/ h. However, this compares favorably with the values given for biphenyl hydroxylation by CREAVEN *et al.* (1965) and WILLIS AND ADDISON (1974) in trout liver homogenates; they found values of 220 nmol/g liver/h and 200 nmol/g liver/h, respectively.

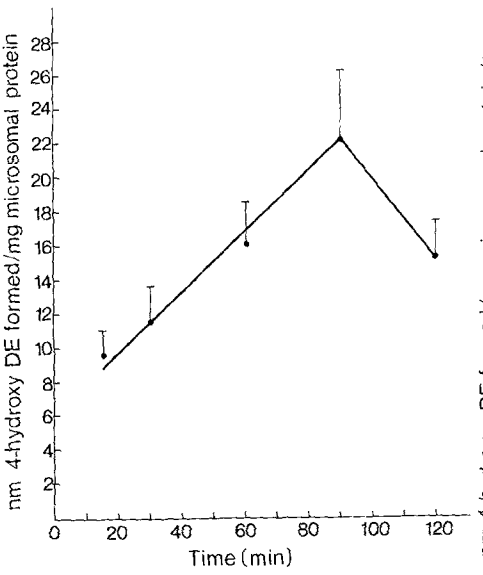

Fig. 2. Time course of the formation of 4-hydroxydiphenyl ether

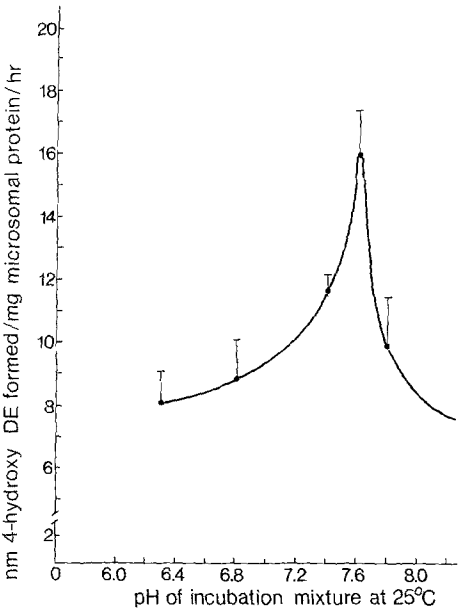

Fig. 3. pH *vs.* diphenyl ether hydroxylation

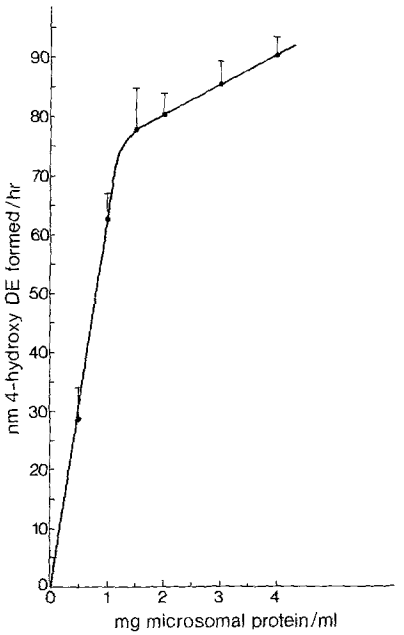

Fig. 4. Microsomal protein concentration *vs.* diphenyl ether hydroxylation

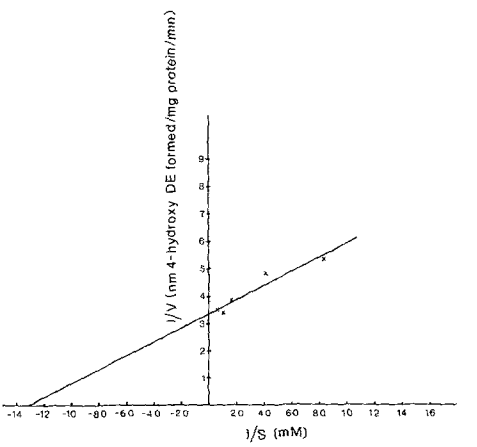

Fig. 5. Lineweaver-Burk plot of diphenyl ether hydroxylation

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