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Transfer of ^{14}C-Toluene from Mosquito Larvae to Bluegill Sunfish

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Toluene is one of the main water-soluble fractions of refined petroleum products such as gasoline (BERRY and BRAMMER 1977, BERRY and STEIN 1977) and jet fuels (R. L. PUYEAR, personal communication). Previous studies have demonstrated the uptake of toluene from aqueous solutions by mosquito larvae (BERRY et al. 1978) and bluegill sunfish (BERRY 1979). The transfer of toluene from fourth-instar mosquito larvae to bluegills feeding on such larvae is reported in this paper.

EXPERIMENTAL

Toluene-water solutions were prepared by adding 0.5 mℓ of ^{14}C-toluene (S.A. = 4mCi/mmol) to one liter of water in a one liter flask. This mixture was then stirred for 3 hrs in a stoppered flask, placed in a separatory funnel and permitted to separate for 20 hrs. The lower 950 mℓ of this solution was removed to another flask and stirred for 10 minutes. This mixture was transferred to a 1.5 liter glass bowl and 500 fourth-instar larvae of the mosquito Aedes aegypti were added to the solution and permitted to incorporate radioactive toluene for 3 hrs. At the end of the 3 hr exposure period, a group of 5 mosquito larvae were added to each of 5 scintillation vials. Tissue solubilizer (1 mℓ Soluene-350) was added to each vial containing 5 larvae and tissue digestion was permitted to proceed for the next 24 hrs at room temperature (22°C). Following digestion, 10 mℓ of scintillation cocktail (Dimilume-30) was added to each vial. Prepared vials were counted on a scintillation spectrometer to determine the amount of ^{14}C-toluene accumulated by the mosquitoes.

Twenty-five bluegill sunfish (mean wt = 6.77 gm) were placed individually in separate 500 mℓ beakers of

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clean water. Fish were not fed for 48 hrs prior to experimentation. Each fish was given 10 exposed mosquito larvae on which to feed. Although the period of time to consume 10 larvae varied between fish, all fish had eaten 10 larvae within 45 minutes. All 25 fish were then placed in a 15 liter aquarium containing untreated water. At selected time periods (1, 4, 8, 24 and 48 hrs) following their ingestion of ^{14}C-toluene exposed larvae, 5 bluegills were selected at random and sacrificed. The spleen, gall bladder, liver, stomach, gut and kidney were excised from these fish, placed in scintillation vials, weighed, and 1 mℓ of tissue solubilizer added. Following 24 hrs of tissue digestion, 10 mℓ of scintillation cocktail was added to each vial and the amount of ^{14}C-toluene present in each organ was analyzed by scintillation counting. For each time period, a single control fish which had been fed unexposed mosquito larvae was also sacrificed and analyzed for organ concentration of radioactive toluene.

RESULTS and DISCUSSION

The mean uptake of ^{14}C-toluene by fourth-instar mosquito larvae which were fed to bluegill sunfish was 368 ± 42 and 1598 ± 110 counts per minute (cpm) for experiments I and II, respectively. The large difference in the uptake of toluene by mosquito larvae during the two experiments was not found in previous studies (BERRY et al 1978), but can be explained by differences in the amount of ^{14}C-toluene in solution during larval exposures. Radiometric analysis of water samples from ^{14}C-toluene solutions in which the mosquitoes were exposed revealed a 10-fold difference in the amount of labeled toluene in solution between experiments (i.e., 685 cpm and 6946 cpm in experiments I and II, respectively). Values for control larvae were 27-39 cpm for both experiments.

The concentrations of ^{14}C-toluene found in specific bluegill organs for the various times post-feeding on exposed mosquito larvae are shown in Table 1 for experiments I and II. Control values for bluegill organs ranged from 29-40 cpm. Examination of Table 1 demonstrates that, with the exception of the stomach and intestine, there was no difference in the concentration of ^{14}C-toluene in the organs of experimental and control fish. The large accumulations of toluene in the stomach at hours 1, 4 and 8 as well as the modest concentration in the intestine during the intermediate time periods is not surprising. This would be expected as the ingested toluene-treated larvae pass along the alimentary canal. It is obvious that a very insignificant amount

TABLE I

Organ concentration of ^{14}C-toluene from bluegills fed toluene-treated mosquito larvae.

Organ*		Time Post-Feeding (hr) 1	4	8	24	48
Sp	1	30.0 (1.9)#	30.2 (0.3)	30.9 (1.4)	38.5 (4.5)	37.6 (1.9)
	2	36.6 (5.0)	40.0 (4.7)	41.0 (4.1)	39.4 (3.6)	37.4 (4.3)
GB	1	29.6 (2.1)	27.7 (1.9)	30.5 (1.1)	32.5 (4.0)	29.7 (0.8)
	2	31.5 (3.3)	35.5 (2.6)	34.3 (2.9)	38.3 (4.3)	38.0 (2.6)
L	1	34.3 (0.9)	34.9 (3.2)	36.2 (5.9)	42.7 (5.0)	42.7 (7.8)
	2	39.0 (6.2)	42.2 (8.1)	39.4 (9.3)	37.4 (4.3)	34.8 (3.1)
ST	1	102.0 (77.5)	109.1 (76.8)	121.1 (23.4)	66.7 (22.4)	39.5 (4.0)
	2	491.8 (264.3)	338.8 (156.4)	48.2 (16.2)	35.6 (1.8)	32.4 (3.4)
I	1	34.5 (2.4)	36.8 (2.2)	43.6 (7.3)	52.0 (9.3)	35.2 (1.2)
	2	46.6 (12.2)	72.6 (44.5)	55.2 (14.8)	49.2 (7.0)	34.4 (2.5)
K	1	31.6 (1.6)	32.4 (3.3)	32.3 (1.0)	33.8 (3.0)	32.0 (2.4)
	2	34.6 (2.6)	32.8 (3.4)	30.8 (1.9)	33.0 (2.0)	31.0 (3.2)

*Sp=Spleen, GB=Gall Bladder, L=Liver, St=Stomach, I=Intestine, K=Kidney, 1=Experiment I, 2=Experiment II.

#Values are the Mean counts per minute ($\pm$ S.E.) of organs from 5 fish.

of toluene, if any, leaves the digestive tract to be accumulated in other vital organs of the bluegill. This finding is in direct contrast to a previous study which demonstrated that large concentrations of ^{14}C-toluene are found in the spleen, gall bladder, gut and kidney of bluegills exposed to solutions of ^{14}C-toluene containing no food organisms (BERRY 1979). The results reported here support the suggestion of MACEK et al (1977) that bio-accumulation of chemicals within aquatic food chains is insignificant when compared to accumulation directly from water.

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