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Acute Toxicity of Toluene to Three Age Groups of Fathead Minnows (*Pimephales promelas*)

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Toluene is a low molecular weight, monocyclic, aromatic hydrocarbon (specific gravity 0.866) that is widely used as an organic solvent. It is also a major component of the water soluble fraction of refined oils (PUYEAR et al. 1981). Transportation related spills of petroleum fuels and discharge of toluene from research institutions and industry are two major routes of entry of toluene into the aquatic environment (PARKER et al. 1976)

Like other aromatic hydrocarbons, toluene is toxic to aquatic organisms (BERRY & BRAMMER 1977; POTERA 1975; PICKERING & HENDERSON 1966). Early life stages of fishes are sensitive to toxic stress and are helpful in evaluating toxic effects of a compound (ERNST & NEFF 1977). However, most toxicity tests with toluene on teleosts have been performed under static conditions on post-embryonic organisms.

This study was designed to determine the 96-h LC50 (lethal concentration for 50% of the population) and LOEC (lowest observed effect concentration) of toluene on the fathead minnow (Pimephales promelas Raf.). Ninty-six-hour LC50s were determined for embryos, 1-day posthatch protolarvae, and 30-day-old fish. The LOEC was determined only for 32-day old fish.

METHODS

Several bioassay systems have been designed to provide continuous or intermittent supply of control water and water-toxicant mixtures to a series of exposure tanks (LEMKE et al. 1978; PELTIER 1978; BRUNGS & MOUNT 1970). These systems typically are larger than necessary for toxicity tests with fish embryos and larvae. A small enclosed mini-diluter system described by BENOIT et al. (1981) and a larger multichannel toxicant injector diluter system described by DEFOE (1975) were used as exposure systems during this study.

The Defoe diluter was used to perform one 96-h LC50 test with 30-day-old fathead minnows, and one 32-day embryo-larval test. The diluter board of the Defoe diluter was set to deliver a flow rate of 1 L every 5 min to each of twelve 5-L glass exposure tanks. This minimized volatilization of toluene, and maintained constant dissolved oxygen levels in the tanks.

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Glass jars (100-mL) fitted with stainless-steel screen bottoms served as egg cups for the embryo-larval test. Egg cups were suspended from a motorized rocker arm assembly, which provided a gentle up-and-down motion and allowed constant exchange of water. The entire system was enclosed in clear plastic and maintained under negative air pressure to protect workers from toluene vapors. Filtered water from Lake Superior (pH 7.6, hardness 45 mg/L $CaCO_3$, 25 C) was used as dilution water. LC50 tests were performed according to standard procedures described by the U.S. EPA (1975).

A 32-day embryo-larval test was conducted to determine the LOEC of toluene on fathead minnow weight. Embryo-larval test procedures followed guidelines outlined by U.S. EPA (1971). After 32 days, fish were weighed individually, and mean weights for each toluene concentration were calculated. Significant differences (alpha=0.05) in mean fish weight for each concentration were determined by Duncans multiple range test (STEELE & TORRIE 1960). Fathead minnows used for tests with the Defoe diluter were from stock cultures at the U.S. EPA Laboratory, Duluth, MN. Toluene concentrations were determined with a Baird-Automic model SF100 ratio recording spectrofluorimeter.

A mini-diluter system described by BENOIT et al. (1981) was used to determine 96-h LC50s for embryos, 1-day old protolarvae, and 30-day-old fish. Carbon filtered Fargo city tap water (pH 8.3, hardness 80 mg/L $CaCO_3$) maintained at 25 C was used as dilution water. Fathead minnows used in the mini-diluter were from stock cultures in our laboratory established from cultures at the U.S. EPA Environmental Research Laboratory, Duluth, MN. Test procedures followed those recommended by U.S. EPA (1975). Toluene concentrations in the exposure tanks were determined as described by PUYEAR et al. (1981).

The 96-h LC50s and their confidence limits were calculated with an interactive computer program developed by HANES et al. (1980). This program essentially follows the probit analysis except it does not require partial kills. Values were considered significantly different (alpha=0.10) when the 90% confidence limits about the LC50s did not overlap (AMERICAN PUBLIC HEALTH ASSOCIATION 1976).

RESULTS

Toluene concentrations and the number of fathead minnows in each of the twelve LC50 tests are listed in Table 1. The 96-h LC50s for the three age groups of fathead minnows used in this study are given in Table 2. The 96-h LC50s calculated for embryos ranged from 55-72 mg/L and were not significantly different from each other. The 96-h LC50s for one-day post-hatch protolarvae had a smaller range (25-36 mg/L), and were also not significantly different from each other. The 96-h LC50s for 30-day-old fathead minnows obtained with the mini-diluter were 26, 30, and 31 mg/L, and none of these values were significantly different. The test

with 30-day-old fish and the Defoe diluter gave a 96-h LC50 value of 18 mg/L, which is significantly different from the other three values for 30-day-old fish.

Table 1. Toluene Dilution Series Used in the LC50 Tests

Embryos		Protolarvae		30-Day-Old Fish	
Conc.	No.	Conc.	No.	Conc.	No.
105	10	65	12	123	6
56	10	37	12	67	6
25	10	17	12	26	6
11	10	10	12	12	6
4	10	3	12	4	6
0	10	0	12	0	6
110	16	61	20	115	10
46	16	45	20	45	10
19	16	17	20	18	10
9	16	8	20	9	10
4	16	4	20	5	10
0	16	0	20	0	10
120	16	74	20	123	10
44	16	40	20	64	10
12	16	15	20	26	10
8	16	10	20	11	10
4	16	4	20	5	10
0	16	0	20	0	10
112	30	115	10	24	20
40	30	70	10	14	20
18	30	33	10	6	20
8	30	12	10	4	20
5	30	4	10	2	20
0	30	0	10	0	20

This table lists the toluene concentration (mg/L) in each exposure tank and the number of fish per tank for each of the twelve 96-h LC50 tests reported.

There was no significant difference between the 96-h LC50s obtained with the mini-diluter for protolarvae and 30-day-old

Table 2. 96-h LC50 Values of Toluene for Fathead Minnows

30-Day-Old Fish	Protolarvae	Embryos
LC50 in mg/L	LC50 in mg/L	LC50in mg/L
30 (23-42)	36 (29-44)	72 (55-107)
31 (24-44)	25 (21-29)	66 (56-78)
26 (21-33)	27 (23-32)	59 (51-68)
18 (16-20)	28 (21-34)	55 (46-66)

Ninety-six-hour LC50s of toluene for each of the three age groups of fathead minnows. Ninty percent confidence limits are shown in parentheses.

fish. However, values for both the protolarvae and the 30-day-old fish differ significantly from the LC50s for embryos.

Because growth of fish larvae is a sensitive indicator of toxic stress, mean fish weight for each toluene concentration was used as an index of larval growth in the embryo-larval test. Based on mean weight, the LOEC of toluene on 32-day-old fathead minnows was 6 mg/L (see Table 3).

Table 3. 32-Day Embryo-Larval Test Data

	Mean Weight, g	Number of Fish	Concentration of Toluene, mg/L
a	0.202	31	0
a	0.196	20	4
b	0.164	35	6
c	0.075	33	9
d	0.034	16	15

This table lists the number of fathead minnows surviving and their mean weight for each concentration at the end of the 32-day embryo-larval test. Means with the same letter are not significantly different (alpha=0.05).

DISCUSSION

The LC50s reported for toluene and fathead minnows in our study are similar to those reported for other teleosts: 54 mg/L for Japanese medaka embryos, Oryzias latipes (STOSS & HAINES 1979); 59 mg/L for adult guppies, Poecilia reticulatus (PICKERING & HENDERSON 1966); 42 mg/L for adult fathead minnows (PICKERING & HENDERSON 1966); 23 mg/L for adult goldfish, Carassius auratus (BRENNIMAN et al. 1976); and 13 mg/L for the adult bluegill, Lepomis macrochirus (U.S. EPA 1978).

LC50s of 30-day-old fathead minnows obtained in our study with the Defoe and mini-diluter systems were significantly different. The variation in results may be due to the differences in diluter systems, water quality or stock cultures of fathead minnows used. The toxicity test that gave a miminum value (18 mg/L toluene) was performed with a Defoe diluter in the U.S. EPA laboratory, Duluth, MN. The remaining tests (30, 31, 26 mg/L toluene) were done with a mini-diluter at North Dakota State University.

There is a significantly greater resistance of embryos to the toxic effects of toluene when compared to protolarvae or 30-day-old fish. It is not unusual for embryos to be more resistant to toxicant stress than larval or adult forms (JOHNSON & JULIN 1980). The greater resistance of embryos to toluene in our study may be due eithor to their lower metabolic rate or the result of toluene being sequestered in the lipid-rich yolk, thus reducing the rate at which it is metabolized.

The LC50 and LOEC values reported in this study may be useful for preliminary screening of the effects of toluene on the fathead minnow. However, care must be taken in utilizing results of single species toxicity tests for risk assessment of a chemical in the environment. Toluene concentrations and water quality characteristics were rigidly controlled during our tests. The fate of low molecular weight hydrocarbons like toluene introduced into the aquatic environment may be quite different from that in the laboratory; dissolution, elmulsification, sorption to suspended particulates and sediments, photochemical modification, biodegradation and bioaccumulation are all factors affecting the fate and effect of toluene in the aquatic ecosystem (MALINS 1977). Studies are needed to elucidate the fate of toluene in the aquatic environment to determine if the toxic limits reported in the present study represent realistic environmental concentrations. Further work is also necessary to characterize the sublethal effects of aromatic hydrocarbons such as toluene on teleostean development.

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