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Toxicity of DDT to the Different Life Stages of the Mummichog *Fundulus heteroclitus* (Wabum)

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The use of DDT (an organochlorine insecticide) has been banned in Europe and North America and other industrialized nations. However, in other parts of the world, it is intensively used as an insecticide to control vectors of diseases such as malaria, plague, typhus fever, yellow fever, sleeping sickness, and river blindness, and to control agricultural pests. It is also used in forestry management to control pests such as the spruce bud-worm and the Dutch Elm disease (Laws 1981). DDT is a very effective insecticide with a relatively low toxicity towards humans (Pine et al. 1980). Its use as an insecticide is still recommended by the Food and Agriculture Organization of the United Nations.

Today, DDT is still ubiquitous in the environment due to its past wide use, and characteristic persistence in the environment (Kaloyanova and Batawa 1991). In emergency cases, DDT may sometimes still be used in developed nations. For example, it was used to spray 1700km^2 of Douglas fir in Oregon to control an outbreak of tussock moth in 1994 two years after it had been banned. Organochlorine residues, DDT inclusive, have been reported in tissues of feral aquatic species (marine mammals and fish eating birds). Reproductive failures in some of marine organisms have been attributed to possible estrogen inhibition by DDT. DDT is insoluble in water but soluble in organic solvents, stable to air, light, heat and carbon dioxide. It may persist for more than 10 years in the soil and accumulates in living organisms through trophic transfer.

The objective of this study was to obtain acute bioassay data on various life stages of the mummichog; *Fundulus heteroclitus*. The data were; the 96-hLC_{50}, which is the concentration of the DDT that is lethal to 50% of the population in 4 days. The MATC (maximum acceptable toxicant concentration); the geometric mean of the NOEC and LOEC. The NOEC (no observable effect concentration) - the highest concentration of the toxicant with no adverse effect on the survival of the fish; the LOEC (lowest observed effect concentration)- the lowest concentration tested having a significant toxic effect (Rand 1995) and the 'safe' concentrations. These types of toxicity generated data are useful in establishing water quality criteria and standards as well as in ecological risk assessment. The application of aquatic toxicity results to risk assessment is becoming increasingly important since it supplies useful quantitative data used by environmental policy makers.

MATERIALS AND METHODS

The common mummichog order- cypriniformes is known to occupy an important

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niche in the trophic structure of the east coast (United States) tidal marshes: they function as both prey and predator in these marshes (Kneib 1986). Adult and subadult mummichogs (3-7g; 2-35cm) were collected using minnow traps from tidal creeks located on Wadmalaw Island about 30 miles S. East of the Center for Coastal Environmental Health and Biomolecular Research at Charleston, South Carolina.

Fish were acclimated for 7- 10 days in a 260L fiberglass tank. Fish were stocked for acclimation at a density of approximately 1 fish/2.5L of seawater. Seawater was collected from surface water adjacent to the fish collection site. The seawater was filtered and recirculated by use of Magnum 350 canister filter and aerated using Maxima® 805 air pumps. An ultraviolet sterilizing unit (1.5L capacity, Angstrom 2537® Model AN - 5) was connected to the pumps. Mass mortalities of mummichogs were observed in the holding tanks not fitted with the sterilizing units for periods lasting more than two weeks. Fish were fed twice daily (at 1000 and 1500 hr.) on weekdays and once on weekends during holding times with flake food (TETRA SM 80®) to satiation. The adults were held at 14 hr light: 10 hr dark regime provided by two 20W 'Cool White' florescent tubes.

Static renewal bioassays were used for acute toxicity tests according to the protocol by Sprague (1969). Each replicate consisted of seven 8-L glass aquaria These were fitted into environmental incubation chambers (REVCO)® capable of maintaining constant temperature and daylength regimes (12 hr light and 12 hr dark). Six different concentrations (in geometric increments) of DDT were used. The seventh aquarium served as a carrier (acetone) control without DDT. All test concentrations and the control contained the same acetone concentration (0.1%). Research grade DDT was procured from SIGMA Chemical Co., St. Louis, Missouri.

Adult fish were not fed during testing. All of the containers were aerated by use of (Maxima A805) aquaria pumps inside the environmental chambers. DDT was initially dissolved in acetone to make the stock solution used in the exposure experiments. Adult (ca. 1 year old) and subadults (ca 2 months) mummichogs were exposed to 0, 4, 8, 16, 32, and 64 µg/L of DDT for a 96-h period. Mortalities in the tanks were observed at 3, 6, 12, 24, 48, 72, and 96-h (Sprague 1969; EIFAC 1975). Dead fish were removed as soon as they were observed. Two- day old mummichog larvae were similarly exposed to 0, 2, 4, 8, 16, and 32 µg/L of DDT. Exposures were carried out in 300-mL wide mouth glass jars in the environmental chamber. All other conditions were similar to the adult exposure regime.

The 96-h LC_{50} and the 95% confidence intervals were estimated by the USEPA probit analysis program (Lazorchak 1994). Significant differences between the various 96-h LC_{50}s were based on the method of 'Standard Error of the Difference' (Finney 1964).

From laboratory observations, the eggs of the *F. heteroclitus* produced in the laboratory hatched from 10 - 12 days after fertilization. Fertilized eggs were held in prefabricated floating plastic exclusion containers (100-mL). These were kept in Midland hatching jars installed within a 260-L acclimation tank for 7-8 days. The eggs were then transferred to 300-mL jars in 200-mL of acclimation water, On the ninth day following fertilization near completion of embryonic development, 10 eggs per treatment were exposed to 0, 2.0, 4.0, 8.0, 16.0 and 32.0 µg/L of DDT. The choice of these concentrations was based on the calculated MATC from the acute toxicity results with adult minnows. Hatching of the

embryos was observed daily as the DDT solution was renewed. Dissolved oxygen, pH, temperature and salinity were measured daily from the control. Dissolved oxygen and temperature were measured with YSI Model 55 meter (Yellow Springs Instrument Co., Ohio, USA), salinity with an optical refractometer (Spartan Model A 366ATC) and the pH with a pH meter (pH Tester 2™).

RESULTS AND DISCUSSION

The results of the acute toxicity of DDT on the adults, juveniles and two-day old larvae are presented in Table 1.

Table 1. The 96-hLC_{50}s and the associated toxicity endpoints (LOEC, NOEC MATC, AF etc) of the adults, juveniles and newly hatched larvae of the mummichog, *F. heteroclitus*.

Life Stage	96-h LC_{50} (µg/L)	95% CI (µg/L)	NOEC (µg/L)	LOEC (µg/L)	AF	MATC (µg/L)	Safe Con' (µg/L)
Two- day old larvae	11.3*	8.8,14.3	4.0	4.0	0.1-0.4	5.6	0.6 -2.6
Two-month old Juveniles	16.6	12.4,22.3	4.0	8.0	0.1-0.4	5.6	1.6-6.6
Adult	20.3	14.4, 28.6	8.0	16.0	0.1-0.4	11.3	2.0-8.1

* Significantly different from the adult 96-h LC50 (p=0.05) (Finney 1964)

The 96-h LC50s and the associated 95 % confidence intervals are 20.3 (14.4-28.6) µg/L for the adults, 16.6 (12.4-22.3) µg/L for the juveniles and 11.3 (8.8-14.3) µg/L for the larvae. The NOECs were 8.0, 4.0 and 4.0 µg/L for the adults, juveniles and larvae respectively. The LOECs were 16, 8 and 8 µg/L in the same order. The MATCs were estimated at 11.3, 5.6, and 5.6 µg//L respectively. A hypothetical application factor (AF) of 0.1-0.4 (Warren 1971; Sprague 1976; Abel 1986) was used to estimate the 'safe' concentration. The values ranged from 1.1 - 8.1 µg/L for the different life stages.

When the nine day old egg/embryo were exposed to DDT till hatch (approximately 4 days) at concentrations below and above the adult 96-h LC_{50} (2-32 µg/L, successful hatching of the eggs in all concentrations was observed, The average percent hatch was 94% (see Table 2).

The transparent nature of the fertilized fish eggs made it possible to observe the developing embryo. By the 9th day post-fertilization, the lens of the eye had filled the optic cup, the retina was heavily pigmented, and the eyes occupied over 50% of the head. Cardiac concentration and sporadic uncoordinated movements of the fins were observed under the microscope. The newly hatched larvae were observed to be actively swimming

Table 2. Effect of sublethal concentrations of DDT on the hatching of mummichog eggs.

		No. of eggs hatched on each day					
		Days					
Exposure concentration (μg/L)	No. of eggs exposed	1	2	3	4	5	Final hatch (%)
0	10	1	2	3	10	10	100
2	10	0	4	6	8	8	80*
4	10	0	2	6	10	10	100
8	10	0	5	6	8	8	80
16	10	0	1	5	8	8	100
32	10	0	1	10	10	10	100

- All eggs not viable to last day of hatching
-

as soon as they hatched. The caudal and pectoral tins were developed the operculum and pectoral fins were almost continuously rhythmically active. There is coordination of the lower jaw and operculum. They soon accepted food organisms such, brine shrimp, (Artemia) larvae.

The average water quality parameters measured during the exposure experiments were dissolved oxygen 6.7 ∀ 0.94 mg/L, pH of 7.8 ∀ 0.4, temperature, of 21.7 ∀ 1.06°C, and salinity, of 20.7 ∀ 1.6λ. The water quality ranges were within the acceptable range for the normal functioning of the mummichog (Kneib, 1986).

In aquatic toxicology testing, a variety of data are generated which is useful for setting water quality standards and criteria as well as conducting of ecological risk assessments. Ecological risk assessment has been defined by Suter (1995) as the process of estimating and characterizing the likelihood that an adverse effect of human actions on the non-human environment will occur, or are occurring, or have occurred. It is a general methodology for basing regulatory decisions on the expected magnitude of effects and the uncertainty concerning those effects. These data are usually the numeric summary of the results of a toxicity test.

Sometimes the end point data may be predicted or estimated from the toxicity tests. Examples of such data or end point results may include such measurements and observations as the time dependent LC_{50}, NOEC, LOEC, MATC, AF, and 'safe' levels already defined. In the scheme of ecological risk assessment, acute or chronic toxicity test

is a very important component of the steps required to generate the needed information for making environmental policy decisions about the regulation and/or the use of the pesticide/pollutant. The formulation of water quality standards from toxicological data remains that of using mean lethal concentrations (LC_{50}), as the basis for estimating concentrations which will not only fail to kill the organisms, but which will also allow them to survive, grow and reproduce normally (Mount and Stephan 1967; Warren 1971; McKim 1977)

It seems likely that DDT will continue to be used on a large scale in many parts of the world for public health reasons and to support agricultural production. In the present study, it was shown that DDT is toxic to the larvae, juvenile and the adults of the mummichog at the indicated exposure levels. The estimated 96-h LC_{50} reported here is close to the ranges of DDT on other fish species of similar sizes and group such as the gold fish - *Carassius auratus* (21 µg/L); fathead minnow-*Pimephales promelas* (19 µg/L); and the mosquito fish - *Gambusia affinis* (19 µg/L) (Verschueren 1983).

Previous studies with fish and the early life stages (McKim 1977; Woltering 1984) have demonstrated that in a majority of cases, the embryo and larval stages were the most sensitive life cycle stages as was the case in this study here with the larval stage. One of the advantages of using the larval stages in bioassay tests apart from the sensitivity is that it provides a good estimate of chronic test that cuts short the task of performing whole life cycle tests to get similar results. Recently, tests running for months have been replaced with tests that can be completed in days. Chapman (1995) and Woltering (1984) suggest that an abbreviated embryo-larval lethality test (approximately 4-8 days post hatch) would significantly reduce the duration and cost of screening chemicals with no appreciable impact on estimating the MATC for chemical hazard assessment.

We report that eggs exposed to DDT concentrations even above the adult 96-h LC_{50} had no adverse effect on the hatching success of the eggs. All eggs in the different concentration hatched successfully at the average rate of 94%. Woltering (1984) reports that egg hatching is not a good endpoint indicator for sublethal toxicity. One of the possible reasons for the eggs not responding to exposure to DDT may be by reason of the protective covering offered to the eggs by the membranes external to the embryo. The mummichog embryo is covered by the chorion, the vitelline membrane and the previtelline membrane. These membranes may not permit the easy diffusion of DDT or other contaminants to the embryo to affect it adversely. On hatching of the embryo, mass mortality of the embryo resulted after two days even in rather low concentrations.

Application factors (AF) have been used for sometime in aquatic toxicology. This is the factor with which one may multiply the 96-h LC_{50} to obtain an estimate of 'safe' concentration of a toxicant or waste. Factors ranging from 0.1 - 0.4 of the LC_{50} has been used. A number of toxicologists including Sprague (1976) advocated or used the factor of 0.1. Abel (1986) also suggests that multiplying the lethal threshold concentration by a factor of 0.1 should give a rough indication of the acceptable level of a toxicant

When the factors of 0.1-0.4 were applied to the 96-h LC_{50}s obtained in this study, 'safe' levels of 2.0 - 8.1 mg/L for the adults, 1.6 - 6.6 mg/L for the juveniles and 1.1 - 4.5 for the larvae were obtained. Application factors in toxicity can be empirically estimated by dividing the MATC by the 96-h LC_{50} or the time independent 96-h LC_{50} in longer tests

(Abel 1989). The MATC refers to a hypothetical toxic threshold concentration in a range bounded at the lower end by the NOEC and at the higher end by the LOEC in a full or partial chronic test. This parameter can be estimated from the shortened embryo larval test as the geometric means of the LOEC and the NOEC, which are in this case 11.3 µg/L for the adults and 5.6 µg/L of DDT for the juveniles and the larvae. The NOEC, LOEC, and the MATC of the juveniles were similar to those of the larvae. The adult 96- h LC_{50} was significantly higher than that of the one - day old larvae, but not of the juveniles. The MATC estimated from juvenile/larvae acute bioassays was found to be protective of the adults, but the reverse may not be the case.

The recommended MATC for all the life stages is 5.6 µg/L or approximately 30% of the adult 96-h LC_{50}. Since the mode of action of DDT is known to affect the nervous system of fish, the above exposure results forms the basis for further investigative study of the chronic effects of DDT as possible endocrine disruptors in fish and wildlife populations.

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