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Diurnal Fluctuations in Toxicity in Two Fish Species: *Gambusia affinis* and *Notropis ludibundis*

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The most convenient and direct method for measuring toxic effects of chemicals on organisms is the acute LC_{50} test. Data obtained from these tests can provide both a rank order of chemical toxicity and relative susceptibilities of organisms to toxicants. Most acute toxicity tests are limited multi-day experiments designed so that termination occurs during working hours (e.g. daylight hours). Also, these tests do not routinely include a record of time of death of individuals during the acute exposure period; rather, total mortality at the end of the exposure period is the variable of interest. Consequently, there has been little opportunity or reason to note potential effects of photoperiod on sensitivity to chemicals. The results presented in this paper, which indicate diurnal variation in mortality, were obtained during a study designed to investigate the relationship between individual genetic variability and fluctuating asymmetry on an individual's tolerance to pesticide exposure. Because animals were screened for death at three hour intervals during a 96 hour exposure, data were available to test the null hypothesis of no photoperiod effect on mortality.

MATERIALS AND METHODS

Experimental organisms were two fish species native to Kansas. Sand shiner minnows *(Notropis ludibundis)* were seined from the Arkansas River near Maize, KS, while mosquitofish *(Gambusia affinis)* were collected from the Ninnescah River below Cheney Reservoir, Sedgwick Co., KS. Fish were acclimated to the laboratory for at least three weeks prior to use in toxicity testing. Fish were fed twice daily with Tetrafin® and were held in 400 L tanks filled with aerated dechlorinated tap water. Adult, non-pregnant fish were randomly selected for use in all toxicity tests. Only female mosquitofish were used in toxicity tests because of the large size discrepancy between male and female mosquitofish and overt sensitivity of the males to the pesticides used in our study. It was not possible to determine sex of sand shiners by external examination. Therefore, both sexes of sand shiners were included in the tests.

Fish were exposed to two different pesticides during this study, parathion and

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lindane. Lindane (γ-hexachlorocyclohexane)is a persistent organochlorine insecticide that is "insoluble" in water, acutely toxic to fish, and is considered to be a possible carcinogen. Lindane was banned for most uses in the United States in 1983, but is presently used in the United States for household uses. Parathion (o-diethyl o,p-nitrophenyl phosphorothioate) is a "less-persistent" organophosphate insecticide which is moderately soluble in water and also acutely toxic to fish. Lindane and parathion were ordered from Chemical Services (West Chester, PA) and their respective purities were > 97%.

Preliminary 96 hour LC_{50} tests were conducted in order to define the concentration range for the subsequent time-to-death (TTD) tests. Acute toxicity tests followed methods of Lydy et al. (1990). The fish were dosed in 22 L aquaria filled with 10 L of dechlorinated tap water. A total of five concentrations and two controls (negative and solvent control) were used with 20 individuals in each concentration and control. Pesticides were dissolved in an acetone carrier and each treatment tank received 0.5 mL of the acetone-pesticide mixture, while the solvent control tank received 0.5 mL of acetone. Tanks were maintained at a constant temperature of 22°C ± 1°C on a 14.5:9.5 L:D photoperiod in a Sherer Dual Jet® (New Hartford, NY) walk-in environmental chamber. The chamber was illuminated by four 50 watt cool white fluorescent light bulbs which produced from 1.011 to 3.590 $\mu mol/s^{-1}/m^{-2}$ of visible light within the testing area. Dissolved oxygen, pH, conductivity and ammonia levels were checked at the beginning and end of the 96 hour testing period and were found to be within U.S. Environmental Protection Agency guidelines for both initial and final water parameters. Temperature was maintained at 22°C ± 1°C, while dissolved oxygen levels were > 60% in all of the toxicity tests. Conductivity remained between 330-360 µS/cm and pH ranged from 7.8-8.2. Finally, ammonia levels did not exceed 1 mg/L among all of the tests. At the end of each experiment, LC_{50} values and 95% confidence limits were calculated using a Trimmed Spearman-Karber computer program (Hamilton et al. 1977). LC_{50} values were determined by plotting the preliminary acute toxicity data on log-probit graph paper. TTD tests were then initiated using 22 L aquaria filled with 10 L of dosed water. A maximum density of 14 fish/aquaria was established to achieve a total of 200 individuals per test. TTD tests were conducted in the same manner as the acute 96 hour toxicity tests with the exceptions that a single concentration was used (LC_{70}) instead of serial dilutions and that dead fish were removed approximately every three hours throughout the experiment. The LC_{70} value was used for the TTD tests rather than the LC_{50} to maximize numbers of deaths for statistical comparisons.

RESULTS AND DISCUSSION

The toxicity of the two insecticides varied significantly between the two fish species (Table 1). *Notropis ludibundis* (Family Cyprinidae) was more

susceptible to lindane (e.g. lower LC_{50} values) than *G. affinis* (Family Poecillidae), while an opposite trend was noted for parathion. Differential insecticide susceptibility of fish from distinct systemic groups has been previously shown. For example, the relative toxicity of parathion to fish belonging to the sunfish family (Centrarchidae) and minnow family (Cyprinidae) differed by a factor of 12 (Macek and McAllister 1970). Pickering et al. (1962) found 13 insecticides produced differences in acute toxicity ranging from 4- to 900-fold between bluegills *(Lepomis machrochirus)* and guppies *(Poecilia reticulata)*, which are sensitive, and goldfish *(Carassius auratus)* and fathead minnows *(Pimephales promelas)*, which are generally more tolerant of chemical exposure.

Table 1. Acute toxicity of parathion and lindane to *Notropis ludibundis* and *Gambusia affinis*.

Pesticide	Species	LC_{50} (ppb)[1,2]	LC_{70} (ppb)
Lindane	*N. ludibundis*	132 (116-151)[a]	140
	G. affinis	263 (241-287)[b]	290
Parathion	*N. ludibundis*	3517 (3203-3861)[d]	3800
	G. affinis	2041 (1694-2458)[c]	2300

[1] 95% confidence limits are given in parentheses.
[2] For a given compound, LC_{50} values with the same letter are not significantly different.

Toxicity also varied significantly between chemicals, with lindane being more toxic to both fish in comparison to parathion (Table 1). Pickering et al. (1962) suggested that organophosphate insecticides are generally less toxic to fish than organochlorine insecticides. LC_{50} values for parathion were 1,300 µg/L for fathead minnows and 2,700 µg/L for goldfish, while LC_{50} values for lindane were much lower for the same species (fathead minnows = 62 µg/L and goldfish = 152 µg/L). These results agree with those found in this study and are not surprising since organophosphate insecticides were designed to replace organochlorine insecticides, the latter having less specific modes-of-action and longer environmental half-lives (Matsumura 1985).

Over each 96 hour test period, mortality was checked at 20 three hour intervals in the light and 12 three hour intervals in the dark. The proportion of fish that died during each successive 3 hour interval, based on the total

number that died, was determined. As depicted in Figure 1, cumulative mortality rates of both species decreased during periods of darkness in 96 hour tests conducted for lindane exposure. Non-parametric Mann-Whitney tests were used to analyze differences in mean mortality rates between the light and dark phases because statistical variances were heteroscedastic. During the lindane exposure, a total of 162 (81%) mosquitofish and 97 (49%) sand shiners died. Average mortalities were significantly greater during light intervals than dark intervals (Table 2) for both the sand shiner (X^2 = 7.53, d.f. = 1, p = 0.006) and the mosquitofish (X^2= 4.15, d.f. = 1, p = 0.042).

Table 2. Average values (x ± SE) for proportions of fish that died during each 3 hour interval within photophase and scotophase after lindane and parathion exposure. Sample sizes are given in parentheses.

Pesticide	Species	Photophase	Scotophase
Lindane	*N. ludibundis*	0.038 ± 0.009 (20)	0.011 ± 0.005 (12)
	G. affinis	0.041 ± 0.008 (20)	0.015 ± 0.004 (12)
Parathion	*N. ludibundis*	0.023 ± 0.005 (22)	0.017 ± 0.006 (10)
	G. affinis	0.031 ± 0.008 (18)	0.035 ± 0.008 (12)

TTD tests were also conducted to assess parathion effects on the two fish species (Table 2). As noted in Figure 2, no diurnal trend in mortality was evident for either species. A total of 137 *G. affinis* (68.5%) and 105 (52.5%) *N. ludibundis* died during testing. Average mortalities during three hour intervals within the photophase (daylight) were not significantly higher than average mortalities within the scotophase (darkness) (Table 2).

The occurrence of diurnal variation in mortality rates noted in TTD tests for lindane, but not parathion, may be related to differences in the modes of action of the pesticides coupled with the decreased metabolic activity of the fish during the scotophase. At night, melatonin concentrations increase in fish (Falcon and Collin 1991) and these higher melatonin levels have been associated with decreased locomotor activity, decreased respiration rates, and promotion of sleep (Kavaliers 1979; Hadley 1996; Iigo et al. 1994). Landrum and Stubblefield (1991) showed that accumulation of hydrophobic organic chemicals occurs by passive diffusion across the respiratory membrane of aquatic organisms and this accumulation is related to the metabolic activity

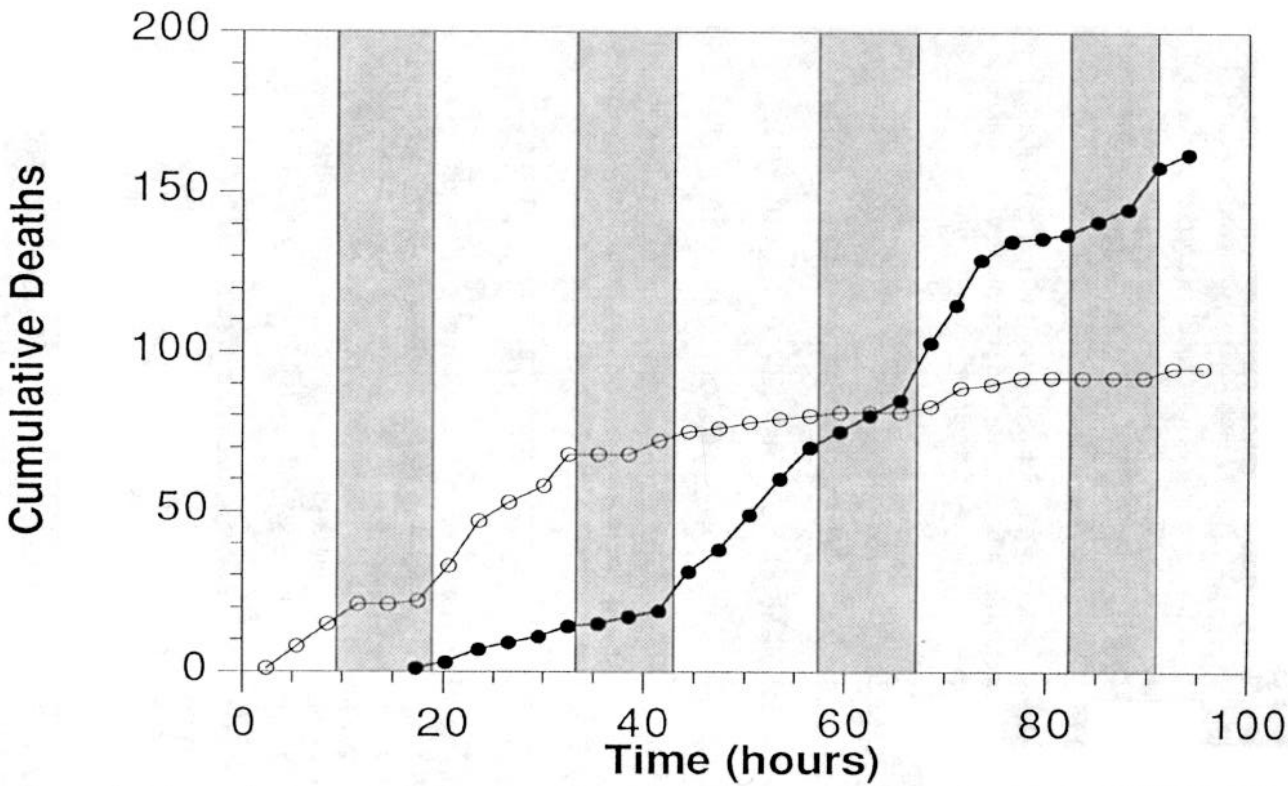

Figure 1. Time to death test with lindane. Filled in circles represent *Gambusia affinis* and open circles represent *Notropis ludibundis*. Grey areas indicate the scotophase.

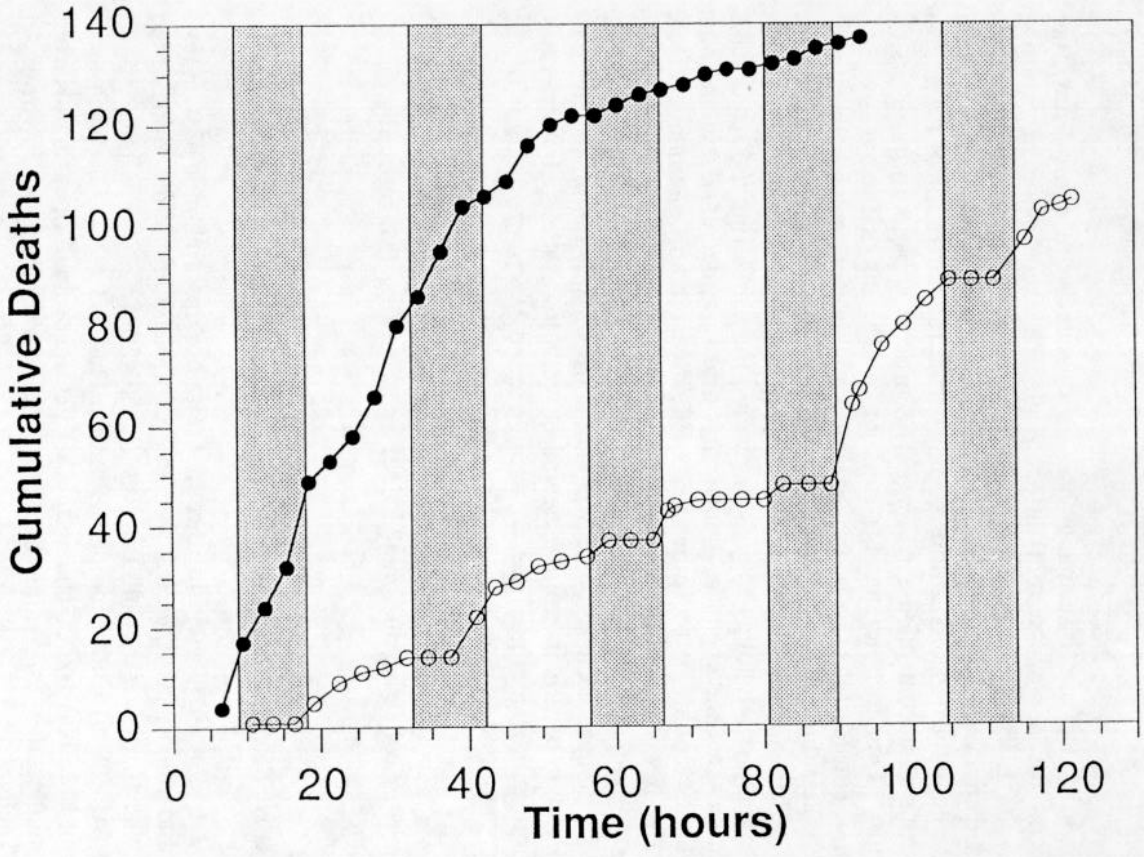

Figure 2. Time to death test with parathion. Filled in circles represent *Gambusia affinis* and open circles represent *Notropis ludibundis*. Grey areas indicate the scotophase.

of the organism. Therefore, uptake of hydrophobic organic chemicals across the gills of fish should decrease with decreased respiration rates. The decrease in toxicity noted for lindane during scotophase may be due to a decreased uptake caused by lower respiration and metabolic activity at this time. The mode of action of parathion is more complicated than lindane; these differences in mode of action may explain the noted differences in toxicity.

Although both of the insecticides used in this study are neurotoxins, their effects on the nervous system differ. While the exact mode of action of lindane is still being debated, Matsumura and Ghiasuddia (1983) have proposed that lindane suppresses the aminobutyric acid (GABA) - induced Cl^- uptake in the neuron resulting in only partial repolarization of the neuron and a state of uncontrolled excitation. This process appears to be reversible in nature. On the other hand, parathion, which acts at the synapse of a neuron, is considered to be an "irreversible" inhibitor of acetylcholinesterase (AChE). Parathion is also biotransformed into paraoxon which is a more active AChE inhibitor than the parent compound (Matsumura, 1985). We propose that as metabolic activity slows within the fish during the scotophase, respiration rates and chemical uptake rates also slow, decreasing the amount of pesticide binding at receptor sites. Effective binding of lindane at the active site is expected to decrease because it is a reversible inhibitor. In contrast, effective binding of parathion/paraoxon should remain unchanged during scotophase because these compounds irreversibly inhibit the receptor sites. Even if parathion uptake decreases with lowered respiration rates, inhibition should continue due to uninterrupted biotransformation of the parent compound to paraoxon in the dark.

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