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Acute Toxic Responses of the Freshwater Planarian, *Dugesia dorotocephala,* to Chlordane

Jay Boyd Best, Michio Morita, and Bruce Abbotts

Department of Physiology and Biophysics, Colorado State University, Fort Collins, CO 80523

Freshwater planarians are an important component of the aquatic ecology of relatively unpolluted streams of the northern hemisphere (HYMAN 1951; KENK 1976). Since they are inexpensive to culture in the laboratory and model many of the toxicologically vulnerable systems of higher animals, they may provide an economical organism for toxicological screening and bioassays (BEST 1972, 1981). The following studies were undertaken to ascertain their sensitivity and toxic responses to aquatic concentrations of chlordane.

MATERIALS AND METHODS

Planarians used were laboratory-cultured, asexual, Dugesia dorotocephala, derived from wild specimens collected from a small stream northwest of Fort Collins, CO, one to five years previously. They were maintained in aged tap water in the laboratory. Conditions of culture were similar to those described previously (BEST et al. 1974). Planarians of normal conformation (Fig. 1A), with tails tapering to a point, indicating fissioning competence, and 16.5 $\pm$ 1.5 mm in length, when extended, were selected from the culture pans 3 days prior to zero time. These planarians were last fed 1 to 2 days before such selection. Immediately following selection, these were placed in glass specimen bowls with 100 mL of aged tap water and 60 planarians per bowl. This population density effectively suppresses fissioning during this 3-day pre-experimental acclimation period (BEST et al. 1974).

The various aqueous concentrations of chlordane were prepared from a commercial emulsifiable chlordane concentrate (EPA Reg. No. 1348-58) compounded by the Selco Supply Co., Severance, CO. This contained 73% chlordane, 23% aromatic petroleum derivatives solvent and 4% "inert" components. Concentrations of the various aqueous solutions tested are expressed in terms of uL of this chlordane concentrate per liter of aged tap water, i.e. as λ/L. Although the concentrate had the consistency of a reddish-brown oil, it readily formed a finely dispersed stable emulsion upon addition to water.

At zero time, ten planarians and 50 mL of water, or chlordane solution, were allocated into each of 42 cylindrical glass specimen bowls, 105 mm inside diameter. Twelve bowls of ten planarians each (120 planarians) were used as water controls, 5 bowls of ten planarians each (50 planarians) were used for each aqueous con-

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centration of chlordane tested. Observations were made at the same time each day for 13 days following zero time. Tail fragments of fissionings and debris from dead planarians were removed, and the solutions changed, immediately following each day's observations. Control and treatment groups were run concurrently on the same laboratory table top at 22 ± 1°C. Planarians were not fed throughout the period of acclimation or the experiment.

RESULTS AND DISCUSSION

Latency of lethality decreases and rate of death increases as the concentration of chlordane is increased (Fig. 2). No lethality was observed over the 13-day period in either controls or those exposed to chlordane concentrations of 0.2 λ/L or less. The response curves of Fig. 2 indicate a 5-day LC50 between 5 and 10 ppm and a 10-day LC50 between 1 and 5 ppm.

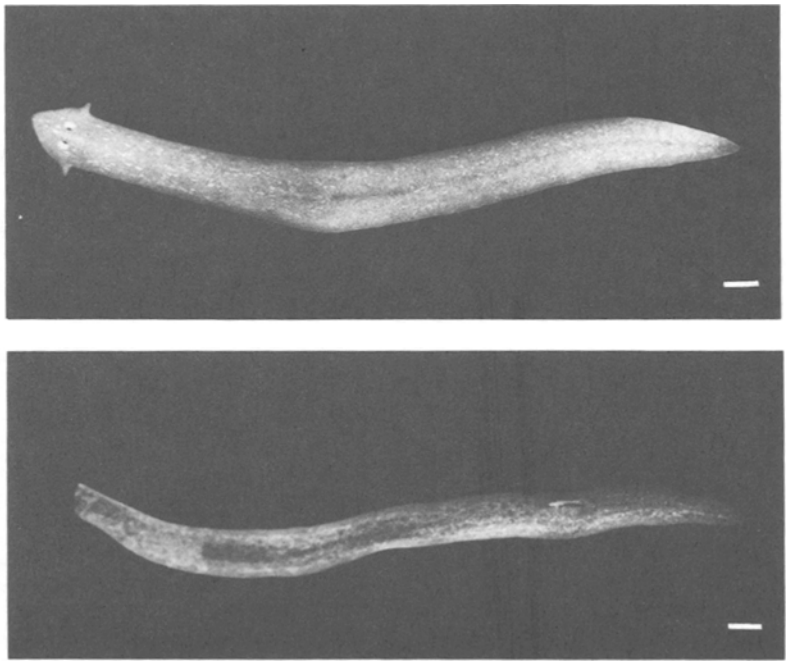

Figure 1. Photographs of normal D. dorotocephala (A) and one showing lesions typical of sublethal exposure to chlordane (B). Horizontal bars represent one mm.

Head lesions or resorptions, an abnormally extended "serpentine" appearance and protruding pharynx (Fig. 1B) are characteristic sublethal responses of these planarians to chlordane. The percentages of surviving planarians with such head lesions or resorptions as a function of chlordane concentration and duration of exposure (Fig. 3) provide sublethal toxic responses that are somewhat more sensitive than lethality: the 5-day TXC50 lies between 1 and 5 ppm

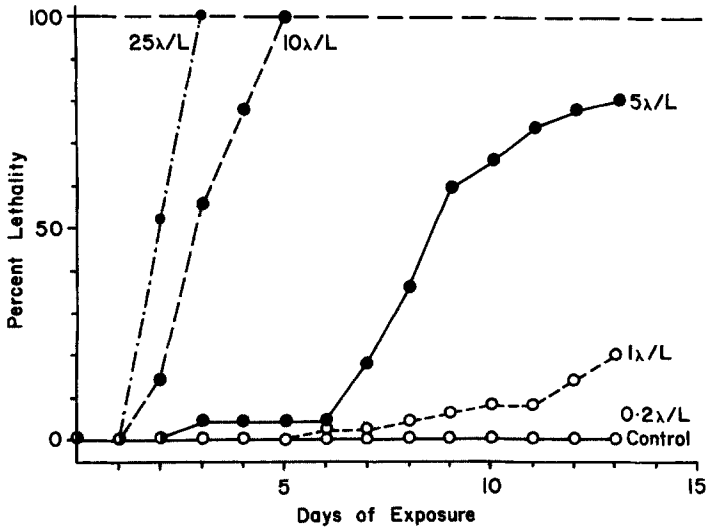

Figure 2. Percent lethality of D. dorotocephala as function of duration of exposure to aquatic concentrations of chlordane.

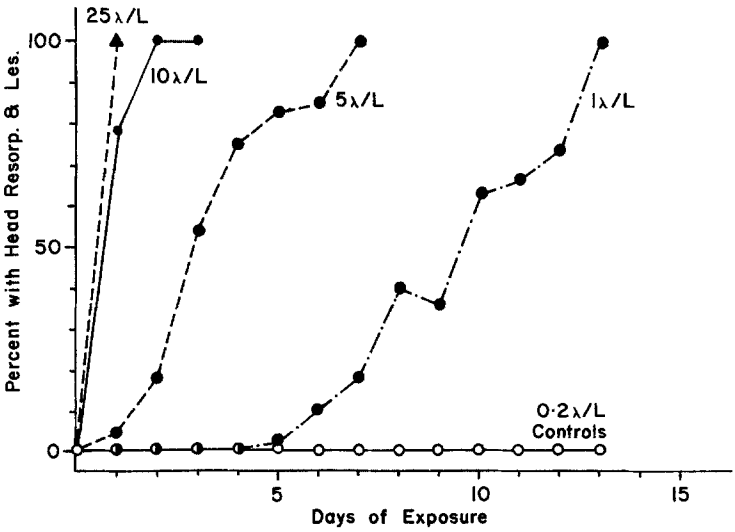

Figure 3. Percent D. dorotocephala with head lesions as function of duration of exposure to aquatic concentrations of chlordane.

and the 10-day TXC50 between 0.2 and 1 ppm. As in the case of lethality, no such head lesions developed in the planarians exposed to 0.2 λ/L or less over the 13-day test period. Latency increases and rate of increase in incidence with time decreases as the chlordane concentration is decreased from 25 to 1 λ/L. The slight increase in incidence observed between the 8th and 9th day for the 1 λ/L curve of Fig. 3 is a computational artifact resulting from the fact that the percentages with lesions are based upon survivors and several with head lesions died in that time interval.

The incidence of fissioning within the initial 10 days is a biphasic function of aqueous chlordane concentration (Fig. 4), i.e. low concentrations increase fissioning incidence above control values, higher concentrations suppress it.

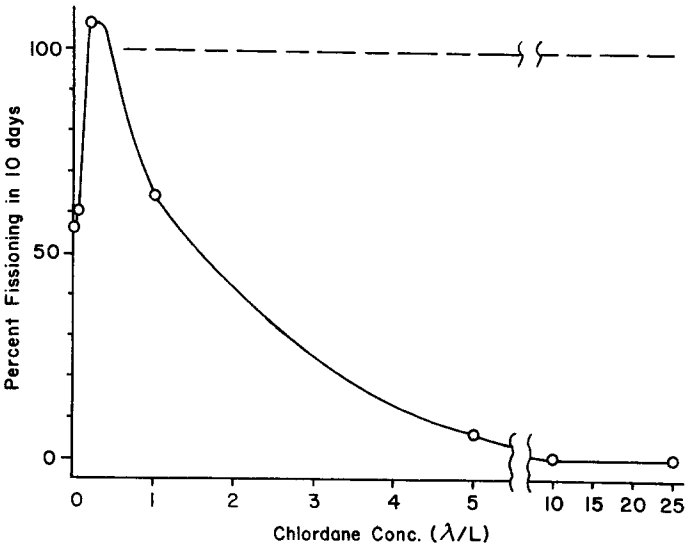

Figure 4. Percent D. dorotocephala fissioning in 10 days in various aqueous concentrations of chlordane.

Previous studies (BEST et al. 1969, 1974, 1975; PIGON et al. 1974) have elucidated major features of fissioning control in this species. Elevated population densities inhibit fissioning, isolation or low population densities increase it. Pheromones released into the environment are not involved. Cohort sensing involves contact chemoreception by clumps of neural cilia distributed in the cephalic margins. With such sensory input, the brain exerts an inhibitory effect on the segmental plexus centers that actually organize and orchestrate the events of fissioning. Monoaminergic neurons appear involved in this control system. The segmental nervous system is facilitatory to fissioning and longer

planarians are more apt to fission than shorter ones. The effects of chlordane on fissioning incidence are thus probably indicative of neurotoxicity. The atypical serpentine extended posture and pharyngeal protrusion are also suggestive of neurotoxicity.

These effects of chlordane on fissioning differ from those observed with other toxicants, e.g. methylmercury, cadmium and copper, in that these monotonely suppress fissioning with increasing concentration of the toxicant and use of the same population densities of 10 planarians per bowl (BEST & MORITA 1980). It is also rare for fissioning incidence to exceed 100 % with the general experimental conditions used in this study, i.e. restriction of the observational period to two weeks or less and daily removal and discarding of tail fission fragments. Secondary fissionings of anterior fragments within two weeks of an initial fissioning have never been observed in the tens of thousands of such planarians similarly used in comparable experiments with a variety of other compounds.

Acute LDLo values* of 40, 240 and 20 mg/kg, respectively for oral administration to humans, intraperitoneal administration to mice and intravenous administration to rabbit, and acute LD50 values of 283, 430 and 100 mg/kg, respectively for oral administrations to rat, mouse and rabbit, have been reported (CHRISTENSEN et al. 1976). Thus, in such acute bioassays, planarians are approximately two orders of magnitude more sensitive than mammals to chlordane.

Usage of a commercial emulsifiable chlordane preparation, instead of pure chlordane, deserves comment. Since it is in the form of similar preparations that most chlordane was introduced into the environment as a toxic contaminant in agricultural spraying, it seemed more pertinent to assess its toxicity in this form. Our data do not rule out the possibility that the aromatic petroleum distillates, and surfactants possibly contained in the "inert components", may potentiate chlordane toxicity, but we do not think this is a major effect. Similar reactions of convulsions and death, LC50 between 1.2 and 2 ppm, were observed in goldfish with pure chlordane in 0.4 mL acetone/L water (DAVIDOFF & SABATINO 1954).

Acknowledgments.

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* Lowest lethal dose reported in the literature.

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