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

Jay Boyd Best, Michio Morita, James Ragin, and Jay Best, Jr.

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

Planarians are a ubiquitous component of the aquatic ecology of relatively unpolluted streams throughout the northern hemisphere (HYMAN 1951; KENK 1976). Since they are inexpensive to culture in the laboratory and model many of the toxicologically responsive systems of higher animals, they may provide an economical and useful organism for detection of environmental toxicants and toxicological screening (BEST 1981).

Mercury, especially in alkylated form, is a troublesome, toxic environmental pollutant that has been responsible for human epidemics of "Minamata disease" syndrom in several countries (BAKIR et al. 1973; IRUKAYAMA et al. 1962; IRUKAYAMA 1967; BERGLAND & BERLIN 1969). Inorganic mercury can, under natural environmental circumstances, undergo microbial conversion to methylmercury (JENSEN & JERNELOV 1969; BISOGNI & LAWRENCE 1973).

In view of the above, toxic responses of planarians to various aquatic habitat concentrations of methylmercury were investigated.

MATERIALS AND METHODS

Asexual planarians of the species, Dugesia dorotocephala, were used. These were obtained from laboratory cultures derived from wild stock collected from a small stream several miles NW of Fort Collins, CO. Our culture methods have been described previously (BEST et al. 1974). Culture and testing were conducted at 22 $\pm$ 1°C. Testing was done in 105 mm I.D., cylindrical, glass specimen bowls. Solutions of methyl mercuric chloride (MMC) were made up in the same water in which the planarians are normally maintained and which contain less than a fraction of a ppb of heavy metals. Experimental planarians were selected for homogeneous size (16 $\pm$ 1.5 mm length), normal morphology and tails tapering to a point; those with truncated tails, indicative of recent fissioning, were excluded. At zero time, 2,5 or 10 planarians were allocated to each of the specimen bowls along with 50 mL of water or solution. Daily observations (except for high concentrations where death occurs in hours) were made for the numbers of survivors, deaths, those with visible lesions and malformations, and fissionings. Tail fission fragments and debris from dead planarians were removed each day. If death occurred in a bowl, the water was changed; otherwise it was changed every third day. In the regeneration

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studies, surgically decapitated planarians were transferred into the various concentrations of MMC. All treatment and control groups to be compared were run concurrently. The planarians were not fed during the test period. For lower concentrations (20 to 0.03 ppb), where fissioning was the indicator response of interest, planarians were tested with groupings of 2, 5 and 10 per bowl for each concentration.

RESULTS AND DISCUSSION

One hundred percent lethality occurs within 5 h in 2 ppm MMC, a day in 1 ppm, and 5 days in 0.5 ppm (Fig. 1). No deaths occurred in 0.2 ppm over a 10-day period. The 5 (or 10) day LC_{50} is less than 0.5 and greater than 0.2 ppm.

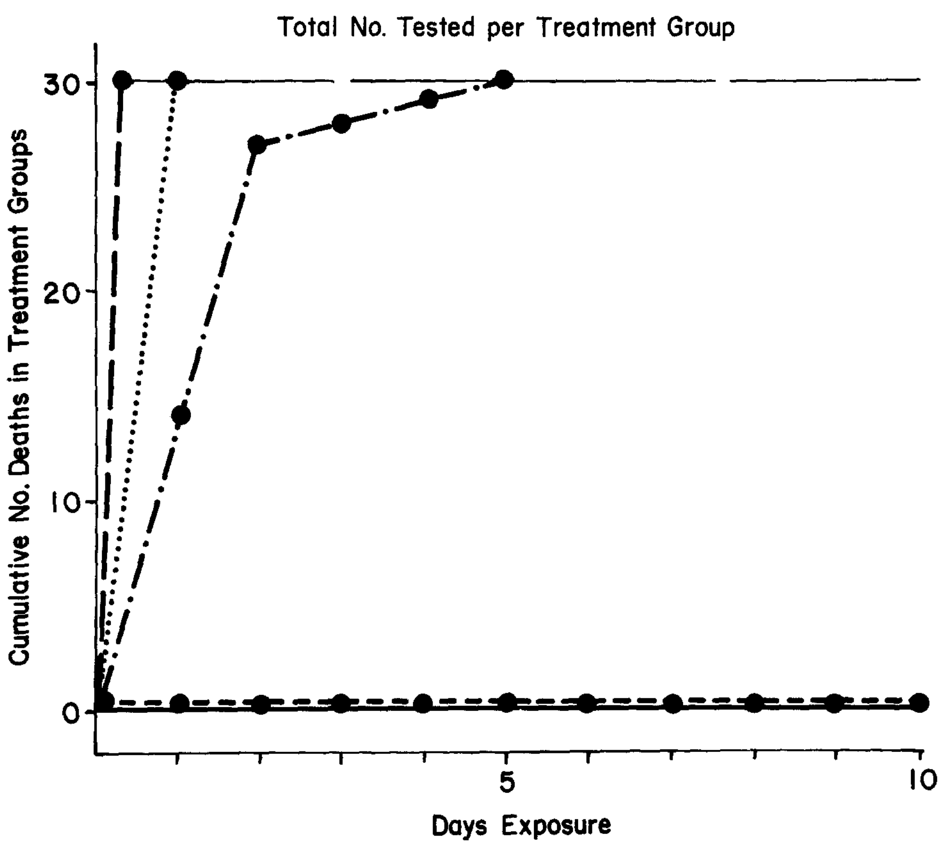

Figure 1: Lethality in planarians as a function of time in various aquatic concentrations of methylmercuric chloride. Cumulative number of deaths are shown as a function of time in 2.0 ppm (●——●), 1.0 ppm (●·········●), 0.5 ppm (●-·-·-●), 0.2 ppm (●------●), and 0 ppm (●———●) MMC. Thirty planarians, in groupings of five per bowl with 50 mL of solution per bowl, were used for each concentration.

With 0.2 ppm, other non-lethal toxic responses were evident. Varying degrees of head resorption, progressing caudally from the snout, were observed (Table 1). After one day in 0.2 ppm, 28 of the 30 planarians tested showed varying degrees of head resorption. With continuing exposure to the MMC, partial head regeneration and recovery toward more normal appearance occurs by 10 days, indicating compensatory adaptation. All 30 controls in water remained normal.

Table 1. Frequency and severity of head resorptions with exposure to 0.2 ppm aquatic concentrations of methylmercuric chloride (MMC).

Days in MMC	0	1	3	5	8	10
Normals	30	2	10	11	20	20
Sans snout	0	3	2	7	3	7
Sans anterior half-head	0	2	3	8	6	3
Sans head	0	23	15	4	1	0

"Teratogenic effects" were observed in the surgical decapitaties regenerating in 0.1 and 0.2 ppm MMC (Fig. 2). Comparison with controls 5 days after decapitation shows retardation of head regeneration in 0.1 and 0.2 ppm MMC, with it most severe in the 0.2 ppm group. Auricular development is still rudimentary at 10 days in 0.2 ppm. At 15 days, the partially regenerated heads of those in 0.2 ppm have resorbed so only their vestige remains. Regeneration of controls is complete by 15 days. No teratogenesis was evident in planarians regenerating new heads in 0.08 ppm MMC or less; however, these did exhibit behavioral indications of neurotoxicity, e.g. lethargy, slowing of movement, difficulty and incoordination in their righting (thigmotactic) response, and partial or complete paralysis of the caudal half.

Although no deaths, malformations, visible lesions or gross behavioral abnormalities were produced by 2 week exposures of intact planarians to concentrations of 20 ppb MMC or lower, these produced significant changes in fissioning incidence down to 0.03 ppb, the lowest concentration of MMC tested. Figure 3 shows the effect on 0.1 ppb MMC and increasing group size on fissioning. Under our experimental conditions each planarian can fission once or not at all. Increasing group size suppresses fissioning, an effect reported previously (BEST et al. 1969, 1974). Fissioning is almost completely suppressed after the third day in 0.1 ppb MMC.

This laboratory has previously shown that: increasing the number of planarians per bowl inhibits fissioning, isolation

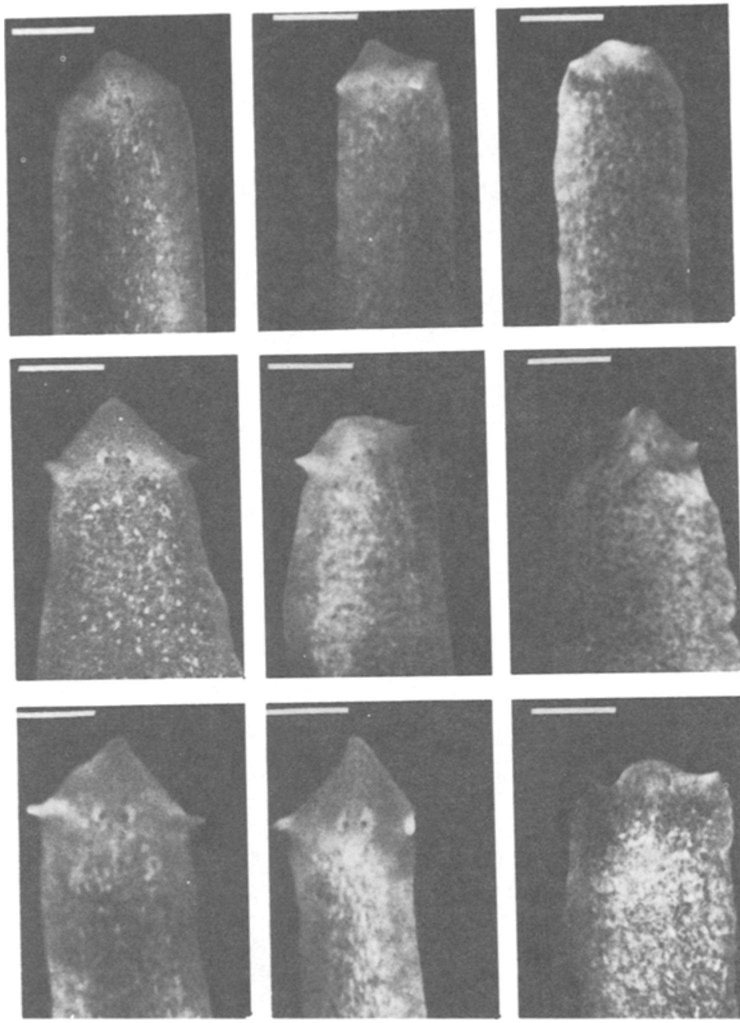

Figure 2: Teratogenic effects of MMC on head regeneration in planarians. All planarians were decapitated just behind the auricles at zero time. Photographs show head regeneration in controls (column one), 0.1 ppm MMC (column two) and 0.2 ppm (column three) groups at 5 days (row one), 10 days (row two) and 15 days (row three). Horizontal white bar in each photo (upper left) represents 1.0 mm.

releases it; the effect is not mediated by phermones discharged into the medium but involves direct cohort contact sensing by clumps of neural cilia located in the cephalic margins; the brain, receiving input from these social sensors, inhibits fissioning; the segmental nervous system facilitates fissioning and longer planarians are more apt to fission than shorter ones (BEST et al. 1969, 1974, 1975; PIGON et al. 1974). Dual antagonistic

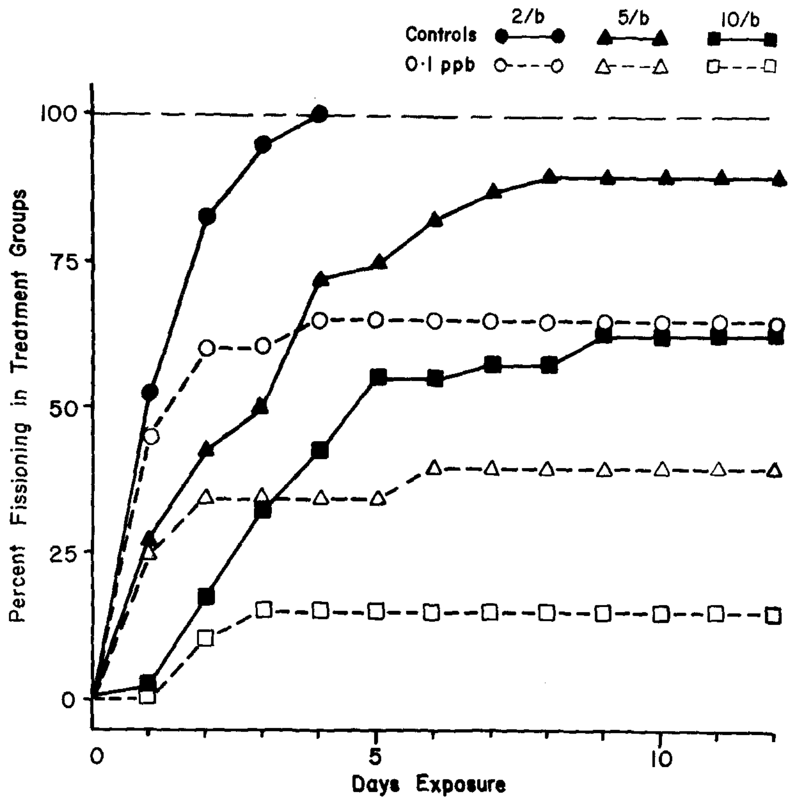

Figure 3: Joint effects of increasing group size and 0.1 ppb methylmercuric chloride on fissioning of asexual D. dorotocephala.

control by catecholaminergic neurons of the brain and serotonergic neurons of the segmental nervous system (WELSH & MOORHEAD 1960; WELSH & WILLIAMS 1969) is probably involved (BEST et al. 1975). The inhibitory effect of MMC on fissioning is thus most likely a reflection of its neurotoxicity. Neurotoxicity is also a primary symptom of chronic methylmercury poisoning in humans (BERGLAND & BERLIN 1969). It is also teratogenic in humans, primarily to neural and cephalic development. Selective blockade of nerve membrane sulfhydryl groups essential for normal nerve membrane depolarization appears likely to be involved in the toxicity of such mercury compounds (HUNEEUS-COX et al. 1966).

Toxic sensitivities found in the present studies compare favorably with those reported for other assay systems. Six months exposure to 3 ppb of MMC produces gross toxic symptoms in brook trout; no adverse effects, with two generations over a two-year period, occurred in less than 0.3 ppb (WOEBESER 1973). Full life cycle chronic tests with minnows yielded complete lethality with 0.5 ppb over a 3-mo period, reproductive defects at 0.13 ppb, no toxic effects below 0.08 ppb (MOUNT 1974). The 96-h LC_{50} for rainbow trout sac fry and fingerlings is 30 and 52 ppb respectively (WOEBESER 1973). EPA recommends that mercury levels in livestock tissues consumed by humans not exceed 0.5 ppm. The lower limit for ambient concentrations of mercury in unpolluted U.S. streams and ocean water is 0.03 ppb. The present studies thus indicate an adequate range of sensitivity and appropriate types of responses of planarians as a model assay system for detection of environmental mercury.

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