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Dose-Response Studies Using Ethylene Dibromide (EDB) in *Hydra Oligactis*

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Ethylene dibromide (EDB;DBE) is widely used as an antiknock additive in leaded gasoline (Stecher 1968). Increasing amounts of this chemical is also finding utility as a fumigant for fruits and vegetables (Brown 1984). There is widespread evidence that EDB is a carcinogen (Olson 1973), a mutagen (Kale Baum 1981), and causes reproductive dysfunction (Geddes 1982).

Even though the use of EDB as a soil fumigant has been banned by the EPA (Brown 1984), it is still used as a grain and fruit fumigant. The appearance of residues of this chemical in agricultural products, and the detection of it in groundwater supplies as a result of runoff from agricultural areas, has caused a great deal of concern in the U.S. (Sun 1984). Still, there is limited information available on the effects of EDB on aquatic animal systems. In one of the few studies on aquatic animals, EDB was found to have a 48-h LC50 of 4.8 mg/L for the common snook, and 6.2 mg/L for sheepshead minnow juveniles (Landau & Tucker 1984).

The current report examined the effects of EDB on mortality in Hydra oligactis. Toxicity tests were performed in a series of studies. The first study examined acute toxic responses in adult Hydra. This study employed two control groups and seven experimental groups. Each group consisted of two separate trials with 25 animals per trial. The first control group was exposed to 1500 mg/L acetone dissolved in APW. The acetone control was necessary since we employed acetone as an emulsifier to insure mixing of the EDB with water (19:1; acetone:EDB). Our acetone control contains an acetone level equal to the total solute concentration of our highest EDB level tested. Different experimental groups were treated with 7.50, 18.50, 30.00, 41.25, 52.50, 63.75, or 75.00 mg/L EDB. Fresh media was prepared daily following feeding. The hydras were monitored and observation of feeding behavior, motility, and mortality were performed at 24-h intervals through 72-h.

The second study examined the acute toxicity of EDB in adult hydras and their F1 progeny following pretreatment of the adults with a sublethal concentration (5 mg/L) of EDB for 14 days. This study employed two control groups and seven experimental

groups consisting of 17 animals per group. Three consecutive trials were performed. The two control groups used in this study were an APW and an acetone-APW control group, with a final concentration of 6000 mg/L acetone. Different experimental groups were exposed to the following concentrations of EDB for a 72-h interval: 25, 50, 100, 150, 200, 250, and 300 mg/L, respectively.

RESULTS AND DISCUSSION

When comparing the untreated control group to the acetone-conditioned controls no significant differences were found between the two control groups at any observation period. Therefore, for statistical comparisons all control values were represented by acetone-APW controls. Animals treated with less than 52.5 mg/L after 48-h displayed 100% survival. Treatment with 52.5 mg/L caused 12% mortality. These values increased to 32% in 63.75 mg/L, and 66% in 75 mg/L. After 72-h of exposure animals treated with less than 41.25 mg/L exhibited no mortality. Treatment with 41.25 mg/L caused 44% mortality. The mortality rate increased to 56% in 52.5 mg/L, 74% in 63.75 mg/L and 100% in 75 mg/L (Table 1).

Table 1. Effects of ethylene dibromide on mortality in *Hydra oligactis* after 48 and 72-h exposure

Treatment	$\bar{X}$% Mortality ± S.D. at 48-h	$\bar{X}$% Mortality ± S.D. at 72-h
1500 mg/L Acetone , n=50	0 ± 0	0 ± 0
30.00 mg/L EDB , n=50	0 ± 0	0 ± 0
41.25 mg/L EDB , n=50	0 ± 0	46 ± 5.7**
52.50 mg/L EDB , n=50	12 ± 5.7*	56 ± 5.7**
63.75 mg/L EDB , n=50	32 ± 5.7*	74 ± 8.5**
75.00 mg/L EDB , n=50	66 ± 2.8*	100 ± 0.0**

*Significantly different from control ($p < 0.05$, t-test).
**Significantly different from control ($p < 0.001$, t-test).

Figure 1 is a graphic representation of a probit analysis of the 48 and 72-h data from the treatments with EDB. The theoretical LC50 was determined from the curve to be approximately 70 mg/L after 48-h and 50 mg/L after 72-h. The Pearson's Product-Moment-Correlation Coefficient (r) value for the 48-h curve is 0.921 and at 72-h it was 0.987. Both r values represented significant correlations between increased EDB concentration and increased mortality.

In pre-exposed hydras, no significant differences in mortality occurred between the APW and Acetone-APW control groups, thus for

statistical comparisons all control values were represented by acetone-APW controls. After 24-h exposure, 150 mg/L EDB caused 12% mortality. This rate increased to 29% in 200 mg/L, and 52% in 300 mg/L. After 48-h in 200 mg/L 47% of the animals died; and in 300 mg/L 58% mortality occurred. The mortality rates increased after 72-h in 200 mg/L to 65% and 76% in 300 mg/L (Table 2). Figure 2 presents a graphic representation of these data, and clearly illustrates the "upward shift" in the LC50 following pre-exposure to the toxin.

Table 2. Effects of ethylene dibromide on pre-exposed Hydra oligactis (parents) at 24, 48, and 72-h

Treatment		% Accumulated Death 24 h	48 h	72 h
APW	; n=17	0.00	0.00	0.00
6000 mg/L APW/ACE	; n=17	0.00	0.00	0.00
25 mg/L	EDB; n=17	0.00	0.00	0.00
50 mg/L	EDB; n=17	0.00	0.00	0.00
100 mg/L	EDB; n=17	0.00	0.00	0.00
150 mg/L	EDB: n=17	11.76*	11.76*	11.76*
200 mg/L	EDB; n=17	29.41*	47.05*	64.69*
250 mg/L	EDB; n=17	23.53*	23.53*	41.17*
300 mg/L	EDB; n=17	52.00*	57.88*	75.52*

*Significantly different from control ($p < 0.05$, t-test)

Table 3. Effects of ethylene dibromide on pre-exposed Hydra oligactis offsprings (F1) at 24, 48, and 72

Treatment		% Accumulated Death 24 h	48 h	72 h
APW	; n=17	0.00	0.00	0.00
6000 mg/L APW/ACE	; n=17	0.00	0.00	0.00
25 mg/L	EDB; n=17	0.00	0.00	0.00
50 mg/L	EDB; n=17	0.00	0.00	0.00
100 mg/L	EDB; n=17	0.00	0.00	0.00
150 mg/L	EDB: n=17	10.00	20.00	20.00
200 mg/L	EDB; n=17	0.00	0.00	20.00
250 mg/L	EDB; n=17	0.00	0.00	10.00
300 mg/L	EDB; n=17	0.00	20.00	40.00

*Significantly different from control ($p < 0.05$, t-test)

In pre-exposed F1 offsprings, the mortality rates were significantly lower than the pretreated parents. After 24-h exposure 10% mortality occurred in the group exposed to 200 mg/L.

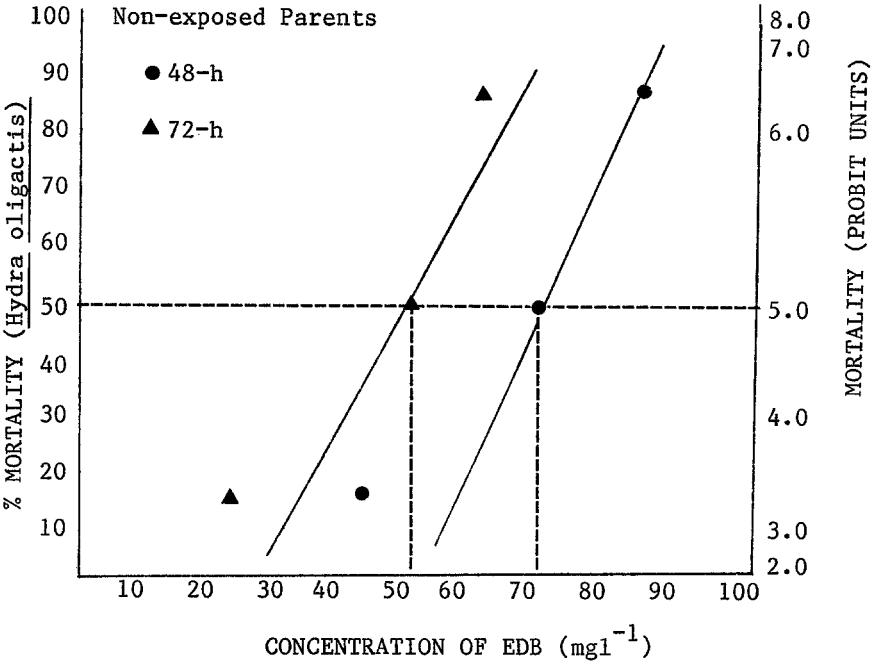

Figure 1. Dose-response relationship between lethality and concentration of EDB following 48 & 72-h continuous exposure

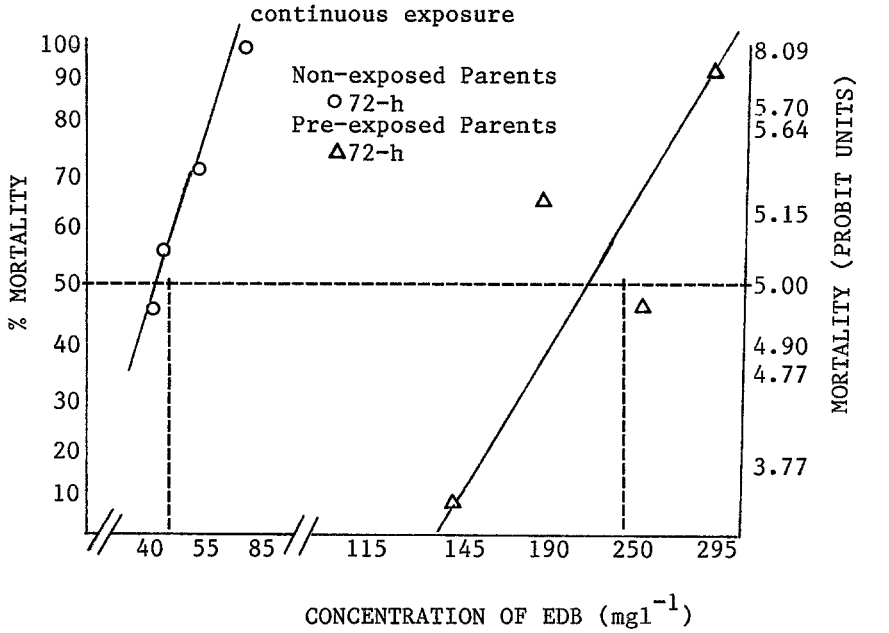

Figure 2. Comparative effects of EDB treatment on pre-exposed vs. non-pre-exposed Hydra oligactis: Dose-response mortality rates at 72-h

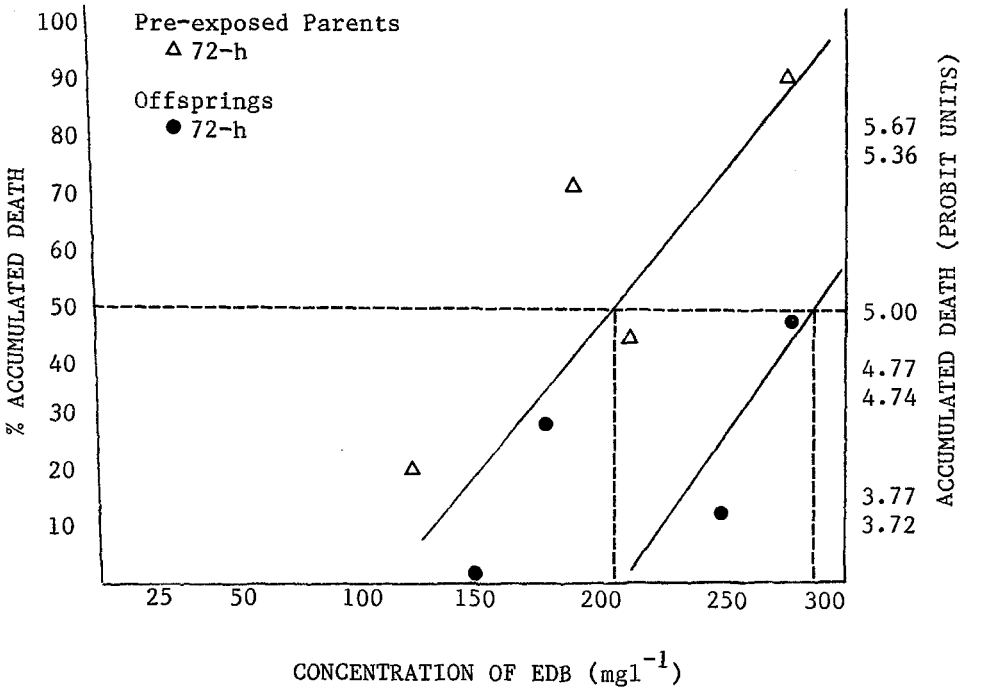

Figure 3. Comparative effects of EDB treatment on pre-exposed Hydra oligactis (Parents) vs. their offsprings (F1 generation): Dose-response mortality rates at 72-h

The mortality rate increased to 20% in the same group after 48-h, with 20% mortality occurring in 300 mg/L. After 72-h 20% mortality was observed in the 200 mg/L group, 10% at 250 mg/L and 40% at 300 mg/L (Table 3).

Figure 3 shows a probit plot of the LC50 for pre-exposed parents and their progeny. The offsprings show a significantly greater tolerance for the toxin than even their pre-exposed parents. It is clear from this result that the resistance to EDB toxicity is inheritable, however, the mechanism of this inherited tolerance is unclear. Reproduction was by budding, thus the offspring contain many cells which were part of the parent body (blastogenesis). Therefore, it is impossible to determine without further investigation whether we are dealing with somatic or genetic inheritance.

This study demonstrated the toxic nature of EDB in Hydra oligactis. The LC50 in untreated Hydra was 50 mg/L after 72-h exposure. In Hydra pretreated with sublethal concentrations, tolerance for the chemical developed as indicated by the increased levels required to kill the animals. This tolerance was also acquired by offspring of parents pre-exposed to the toxin. The offspring were able to tolerate even higher levels

than parents, which is an interesting result and may have implications for uptake and accumulation of the chemical in the tissues of the exposed animals. EDB is not believed to be accumulated to the extent that other brominated hydrocarbons are (Kowalski 1978). However, because EDB may be metabolized to other derivatives, bromoacetaldehyde or bromoethyl glutathione (Vanbladeren et al. 1983), the accumulation of these intermediates may play a role in the toxicity and/or the development of tolerance to subsequent exposure. Another possible consequence of sublethal exposure is the occurrence of a mutation which renders the offspring tolerant to increased levels. EDB has been demonstrated to increase acetyltransferase activity in F1 progeny in rats when male parents were exposed to low levels of EDB (Hsu et al. 1985).

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