Bull. Environ. Contam. Toxicol. (1991) 46:633–640

Bulletin of Environmental Contamination and Toxicology

Effects of Dieldrin Treatment on Physiological and Biochemical Aspects of the Toad Embryonic Development

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The widespread use of pesticides in veterinary, public health, household and industrial purposes has resulted in the ubiquitous presence of trace quantities of these compounds in the environment, However, the side effects of pesticides in wildlife have not always been predictable. The pollution of the aquatic environment may be one of the factors affecting frog and toad population survival (Harri et al. 1979; Cooke 1972; Dumpert and Zeitz 1984; Dial and Bauer 1984). These animals live both on land and in water, they are carnivorous and accumulate the pollutant that is in their prey organisms.

Dieldrin is a cyclodiene insecticide highly persistant in nature due to its chemical stability. The exposure of toad embryos to Dieldrin induces hyperactivity in the swimming larvae and inhibition of cholinesterases. However, the inhibition of these enzymes during early development is not life threatening (Llamas et al. 1985). The present report provides a physiological and biochemical study of the noxious effect of Dieldrin on the toad embryonic development.

MATERIALS AND METHODS

Bufo arenarum embryos were obtained as previously described (Pechen and Bazan 1974). Technical Dieldrin (100% pure) was added to the developing medium of experimental dishes dissolved in acetone (0.5 ml/l). The concentrations tested were 0.02; 0.2 and 2.0 mg/l. The normal stages of development and the timing of these events were controlled according to Del Conte and Sirling (1952). Due to the considerable variation in the percentages of mortality from one ovulation to another, we rendered it convenient to express the results as the ratio of the percentage of survival of the treated embryos as opposed to the control group.

Body weight and protein content: Twenty dejellied oocytes or embryos were randomly selected from control and Dieldrin (0.2 mg/l) containing dishes and weighed on aluminium planchettes. Protein determination was performed according to Lowry et al (1951) on 10 oocytes or embryos.

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Phospholipid determination: 100 dejellied larvae were homogenized with chloroform-methanol 2:1 (Folch et al. 1957). Individual phospholipids were separated by TLC and phosphorous concentrations were determined on each spot (Rouser et al. 1970).
Dieldrin determination: Bulked samples of 100 dejellied oocytes or embryos were homogenized with 17 ml of acetonitrile and filtered through filter paper with anhydrous sodium sulphate. The extract was partitioned with 10 ml of petroleum ether, washed twice with 2% sodium sulphate and concentrated to 1-2 ml. The extract was analyzed by gas chromatography with electron capture detector after florisil clean up. Column packing: 3% OV-17. Carrier gas: nitrogen at 60 ml/min. Column temperature: 200°C. Enzyme assay: Due to the great difference in activity between oocytes and larvae, homogenates that contained between 10 and 100 embryos were prepared. Appropriate volumes of homogenates were added to each enzyme assay. The reaction mixture of ATPase contained 5 mM ATP, 5 mM $MgCl_2$, 10 mM KCl, 100 mM NaCl, 20 mM buffer Tris-HCl pH: 8.5, and between 0.30 and 10 mg of protein in a final volume of 1.5 ml. Enzyme activity was measured at 30°C for 40 minutes. The reaction was stopped with the addition of 3 ml ice cold 10% TCA. The reaction mixture for 5´ nucleotidase contained 5 mM AMP. 10 mM $MgCl_2$, 10 mM buffer Tris-HCl pH: 8.5 and between 0.2 and 5 mg of protein in a final volume of 0.2 ml. Enzyme activity was measured at 30°C for 20 minutes. The reaction was stopped by the addition of 0.8 ml ice cold 0.2 M perchloric acid. The inorganic phosphate released was estimated by the method of Chen et al. (1956). Statistical analysis: Analysis for significance of differences between means were performed using student´s t test.

RESULTS AND DISCUSSION

When oocytes and early embryos were developed in a medium including Dieldrin insecticide, the cells took up the xenobiotic (Figure 1). At the external concentration of 0.2 mg/l maximum uptake was reached after 9 days of exposure. The greatest Dieldrin accumulation was 0.26 µg/embryo. It is noteworthy that the signs of severe poisoning and of mortality appeared far behind the peak of uptake (Figure 2); however, behavioral effects such as hyperactivity and disorientation began between 7 and 8 days of development.
Figure 2 illustrates the ratio between the percentage of survival of the treated embryos and the control group. It can be observed that in all the concentrations of Dieldrin assayed, this ratio is over the unit, which would indicate a protecting effect of the insecticide, at least during the first days of exposure. After 14 days, such protective effect began to dissapear for the 0.2 and 2.0 mg/l concentrations with the appearance of signs of severe poisoning (tail lashing, body twisting and a tendency to lie on the bottom) together with an increase in the mortality rate.

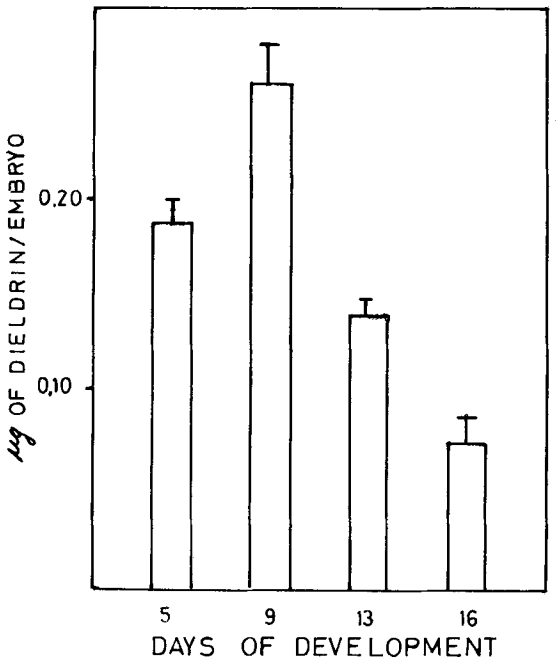

Figure 1 Uptake of Dieldrin into embryos and larvae. Embryos and larvae were developed in a medium containing 0.2 mg/l of Dieldrin. Details as in a Materials and Methods. Bars indicate the range of data from two experiments.

The growth rate was illustrated by the weight and protein content of the embryos.Figure 3 shows a drop in the dry weigth and protein content in the Dieldrin treated embryos and the control group. It is striking that the loss of dry weight reached a value of 50% in about 25 days of development. However, no difference in control and Dieldrin treated groups could be detected.
Phospholipid content and composition are presented in table 1. Long-time Dieldrin exposure produced only minor changes in the phopholipid composition of embryos. We observed a 17% decrease in the total phospholipid concentration after 16 days of treatment. No changes in the level of phosphatidylserine, sphingomyelin and phosphatidic acid were evidenced. However, there was a 56% decrease in cardiolipin after 16 days of development in both groups. By this time, we also observed a 60 % decrease in phosphatidylcholine and phosphatidylethanolamine in control larvae, while the decrease in the experimental group was lower (50%). A decrease was also observed in the level of phophatidylinositol in the control group, without the appearance of a similar change in the Dieldrin treated group.
The decrease in total phopholipids and the change in the pattern of individual phospholipids of Dieldrin treated embryos, may be related to the structural and functional abnormalities observed in the larvae. However, the decrease in total phospholipids is much smaller than the one in proteins. Therefore the expression of the results by proteins would indicate an increase rather than a decrease in phospholipid content.

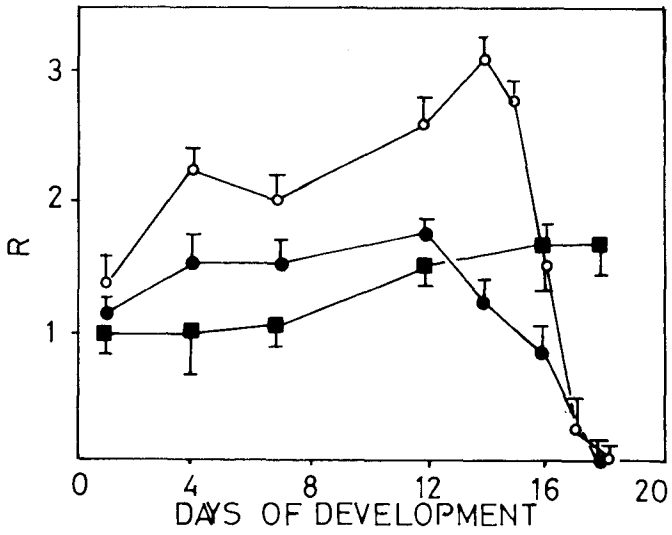

Figure 2. Effect of Dieldrin treatment on survival time. The plots of the ratio between the percentage of survival on treated embryos vs. the control group is presented on ordinate Abcisa: Days of Development. Results are expressed as mean ±SE (o) 2.0 mg/l of Dieldrin; (●) 0.2 mg/l of Dieldrin; (■) 0.02 mg/l of Dieldrin.

Figure 4 shows the activities of total Mg,Na,K - ATPase and 5'nucleotidase. Each enzyme is accumulated at its own rate during development.The activities of both enzymes were not significantly altered in crude homogenate of Dieldrin treated embryos.
The results obtained , especially with the concentration of 0.2 mg/ of Dieldrin seem to support the idea that initially the insecticide protects the development of the experimental groups.However, the mortality rate promptly increases after 14 days of exposure with 2.0 and 0.2 mg/l of Dieldrin (Figure 2).The protective effect previously mentioned could be due to the deleterious effect of Dieldrin on the growth of microorganisms (Lal & Saxena 1980) present in the developmental medium or associated to the jelly coat , as development was performed without the addition of antibiotics.Bufo arenarum embryos take up the insecticide from the developing medium and at the moment the maximum uptake was reached, Dieldrin related behavioral effects began tp be observable.By this time no indication of differences in weight and protein loss between the control and experimental group was evident, not even with a longer period of exposure (Figure 3). There is a range of 4 to 5 days between both maximum pesticide uptake and development of toxic symptoms followed by death. During this period an apparent decrease in Dieldrin uptake is evident (Figure 1).

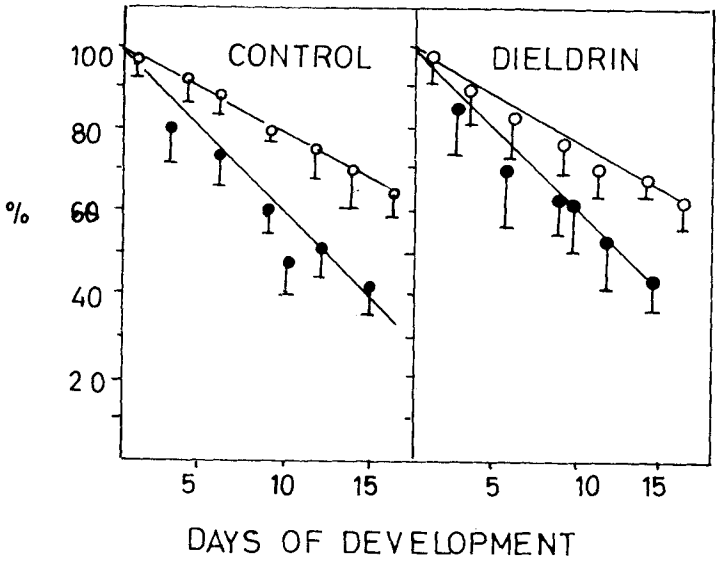

Figure 3. Effect of Dieldrin treatment on growth parameter. The percent of change with respect to oocyte in protein (●) and dry weight (o) are presented. Each point is the mean of duplicate experiments.

However, the amount of toxicant in the developing media remains constant.Under such circumstances we could assume that the pesticide stored in the embryo must be directly proportional to the lipid pool which is depleted during development (Balinsky 1965), thus greater detoxification of Dieldrin by storage is possible in oocytes or very young toad embryos. Fish eggs and larvae are also known to be resistant to cyclodiene pesticides than adult animals (Iyamoti et al 1968). Such results would indicate that resistance to Dieldrin gradually decreases with development.After 14 days of Dieldrin (0.2 mg/l) exposure, the embryos began to die. The timing for the appearance of toxic symptoms and death is in agreement with the one reported for other cyclodienes (Juarez and Guzman 1984; Hall and Swineford 1981).

The initial event underlying toxicity could be the accumulation of pesticides in the various lipidic pools of the oocyte or embryo.Interaction between pesticide and phospholipids has been reported (Buff and Berndt 1981;Lakowicz et al 1982; Omann and Lakowicz 1082).We observed a 17% decrease in total phospholipid content per embryo after prolonged exposure to Dieldrin, and minor changes in phospholipid distribution (Table 1). On the other hand a brief period of exposure produced an increase in whole embryo and microsomal phospholipid (Llamas et al 1981).

Table 1. Phospholipid Distribution in Control and Dieldrin Treated Embryos

Phospholipids	Control Days of Development 8 N=4		11 N=4		16 N=3		Dieldrin Treated Days of Development 8 N=4		11 N=3		16 N=3	
Total Phospholipids	4.83	±0.11	3.89	±0.08	3.16	±0.15	4.55	±0.23	3.62	±0.100	2.61	±0.15 a
Phosphatidyl Serine	0.240	±0.080	0.240	±0.030	0.250	±0.030	0.190	±0.050	0.24	±0.020	0.24	±0.03
Phosphatidyl Inositol	0.240	±0.050	0.200	±0.020	0.130	±0.020	0.100	±0.030 a	0.16	±0.001 a	0.10	±0.02
Phosphatidyl Choline	2.520	±0.220	1.710	±0.180	1.530	±0.300	2.410	±0.250	1.61	±0.200	1.19	±0.22
Phosphatidyl Ethanolamine	1.190	±0.210	0.930	±0.130	0.770	±0.100	1.150	±0.20	0.82	±0.170	0.62	±0.15
Sphingomyelin	0.310	±0.100	0.290	±0.080	0.320	±0.050	0.260	±0.050	0.27	±0.08	0.31	±0.05
Phosphatidic Acid	0.012	±0.002	0.019	±0.002	0.020	±0.0005	U		0.022	±0.0002	0.018	±0.004
Cardiolipin	0.130	±0.030	0.090	±0.010	0.070	±0.050	0.130	±0.050	0.12	±0.03	0.07	±0.03

Phospolipid content is expressed as μmol phospholipid - P per 100 embryos. Data represent mean ± standard deviation, N: number of samples, U: undetectable, a: $p \leq 0.05$

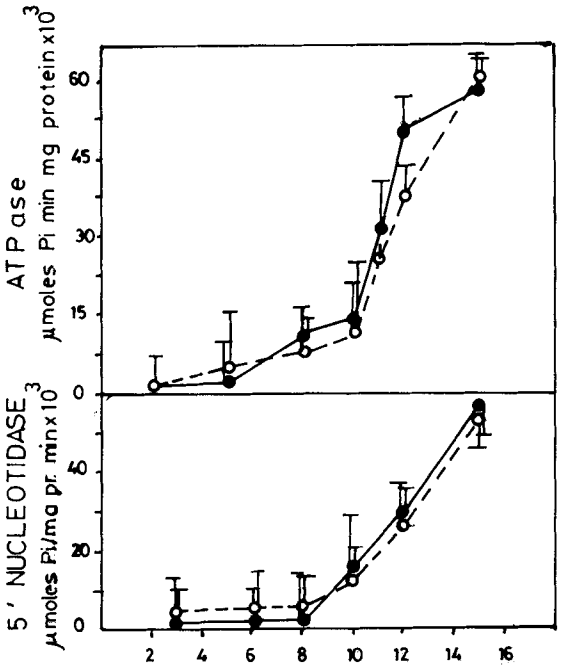

Figure 4. Activities of 5'nucleotidase and ATPase in Dieldrin treated embryos. The values shown are the average of at least three experiments from sequential samples of ovulated and fertilized eggs from the same toad. (o) Control; (●) Dieldrin 0.2 mg/l.

Altered lipid composition of cellular constituents may affect the affinity for Dieldrin by increasing its flux out, its distribution and the activities of membrane enzymes. However , the activity of 5'nucleotidase and ATPase were not sgnificantly altered in crude homogenate from Dieldrin treated embryos. The assay of total ATPase activity in oocyte and early embryos was difficult due to the high protein content of the homogenate and the very low activity of the sample, but despite the low specific activity found, the data presented in Figure 4 seems to indicate that Dieldrin had no effect in vivo on Mg,Na,K-ATPase.

We therefore conclude that while prolonged Dieldrin exposure produces an alteration in embryonic behavior, morphology (Llamas et al 1985) and phospholipid content and composition, the pesticide did not have a significant effect on body weight, protein content and membrane enzyme activities. Toad population has decreased considerably in many agricultural fields. The decline has been associated with anthropogenic activities, modification of their living habitat and pollution of the breeding sites with pesticides, oil, petrol or rubbish.The Dieldrin dosage used in our toxicological experiments is higher than the one reported in most aquatic environment. However, in countries where the use of these compounds have not been abolished, harmful Dieldrin levels appear in the toad breeding sites bordering agricultural lands recently treated.

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Received May 3,1990; accepted August 24,1990.