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Evaluation of *Drosophila melanogaster* as an alternative animal for studying the neurotoxicity of heavy metals

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Heavy metals cause irreversible neurobehavioral damage in many developing mammals, but the mechanisms of this damage are unknown. The influence of three heavy metal compounds, triethyllead chloride, lead acetate and cadmium chloride, on lethality, development, behavior and learning was studied using the fruit fly, *Drosophila melanogaster*. This animal was used because it allows hundreds of subjects to be assayed very easily in individual experiments and because it is a system in which toxicological questions might be answered by using the techniques of modern molecular genetics. When triethyllead chloride, lead acetate or cadmium chloride was placed in the medium, the larval LC_{50} (± standard error) was found to be 0.090 ± 0.004, 6.60 ± 0.64 and 0.42 ± 0.04 mM, respectively. Each of the tested compounds produced a dose-related delay in development. In particular, they caused a delay in the development of larvae to pupae. When larvae were reared on medium containing triethyllead chloride (0.06 mM), lead acetate (3.07 mM) or cadmium chloride (0.11 mM), phototaxis, locomotion and learning were not inhibited. Since significant neurobehavioral effects were not observed under the experimental conditions used, *Drosophila* does not appear to be an appropriate animal for the genetic dissection of such effects of heavy metals during development.

Keywords: *Drosophila melanogaster*, evaluation, neurotoxicity, heavy metals

Introduction

Triethyllead chloride, lead acetate and cadmium chloride are known to cause behavior alterations in developing mammals. Triethyllead chloride, a metabolite of tetraethyllead (Cremer 1959), causes a variety of neurobehavioral disorders including motor excitability, disorientation and memory impairments in rats (Walsh & Tilson 1984) and growth retardation in developing mice (Odenbro *et al.* 1988). Lead acetate causes behavioral effects in rats (Baraldi *et al.* 1985, Massaro *et al.* 1986), growth retardation in rats (Hammond *et al.* 1989), visual damage in monkeys (Reuhl *et al.* 1989) and perceptual deficiencies in humans (Bonithon-Kopp *et al.* 1986). Cadmium chloride has been shown to cause locomotor alterations, growth retardation in rats (Smith *et al.* 1985, Ali *et al.* 1986), and perceptual deficiencies in humans (Bonithon-Kopp *et al.* 1986). A generally accepted theory as to the mechanisms of the neurotoxicity of each of these heavy metal compounds is lacking, although many have been offered (Lampert & Schochet 1968, Manalis & Cooper 1973, Kober & Cooper 1976, Petite *et al.* 1983, Cremer 1984). This lack may be due to the complexity of behavior and learning processes in mammals. If so, the study of a simpler animal might help understand learning and behavior deficiencies resulting from heavy metal exposure.

We chose *Drosophila melanogaster* as a potential animal model for a number of reasons. First, the genetic heritage of *Drosophila* is unparalleled among complex metazoans, and we hoped that the sophisticated genetic and molecular genetic arsenal that has proven so successful in elucidating such complicated processes as the molecular basis of embryological pattern formation could be brought to bear on the mechanisms of heavy metal toxicity. Second, hundreds or even thousands of flies can be

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used easily for each experiment. Such numbers are usually difficult to obtain with mammals. Since behaviors are not easy to quantitate, the use of large numbers of animals might be expected to increase the significance of behavioral paradigms. Finally, such an animal offers a less expensive alternative to the use of mammals.

The life cycle of *Drosophila* at 25 °C is summarized as follows (Figure 1). The adult fly lays its eggs; the eggs hatch into larvae in approximately 1 day; larvae pass through the first, second and third instar developmental stages and pupate within 4–5 days. The pupae eclose (hatch) into adult flies in 4–5 days. Under these experimental conditions, a new generation of flies is produced every 9–10 days.

The central nervous system (CNS) of a holometabolous insect serves two very different animals, the larva and the fly. It changes continuously from the beginning of embryogenesis to the emergence of the fly from the pupal case (Konkel *et al.* 1980). The primary change of the CNS from the first to the third instar is a 30-fold increase in its size. At the end of the larval stage, most of the adult components are recognizable. However, the majority of the changes in the fly's nervous system occur during metamorphosis from pupa to fly. The brain and ventral ganglion elongate and the cervical connection forms from the separation of the sub-ganglia from the thoracic centers. The larval and adult nervous systems are derived from the same or very closely related embryonic precursors.

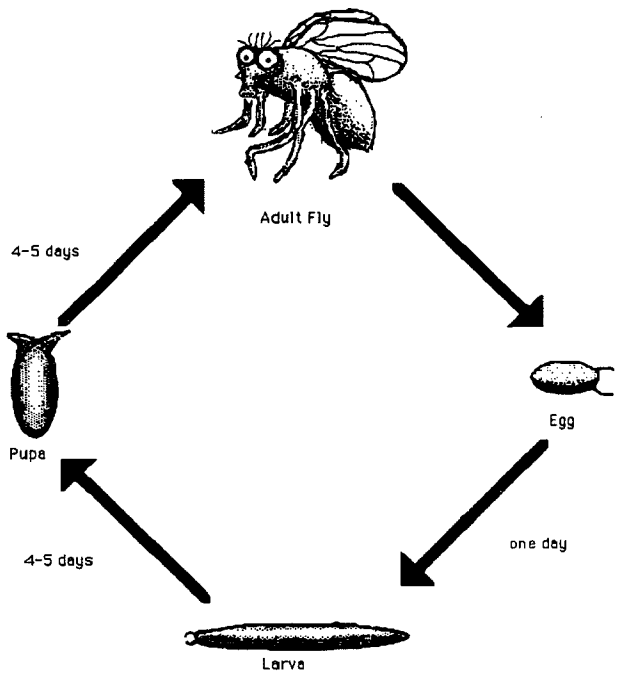

Figure 1. *D. melanogaster* development at 25 °C.

Although *Drosophila*, obviously, is extremely different from the human, the basic neuronal units and support cells of the CNS are similar. There is, however, a large body of information dealing with the neurotoxicity of heavy metal compounds in mammals but very little in *Drosophila*. The behaviors of *Drosophila* might be less complex and more easily quantitated than that of mammals. Other advantages for their use are that they can be quickly raised and trained and many different behavioral assays using them are available.

Most importantly, *Drosophila* is well characterized genetically. The entire genome of *Drosophila* has been thoroughly mapped, and many markers and behavioral mutants are available which could help dissect genetically the mechanisms of a compound's toxicity.

The present study shows that triethyllead chloride, lead acetate and cadmium chloride inhibit the development of *D. melanogaster*, but do not appear to effect phototaxis, locomotion or learning under the experimental conditions used.

Materials and methods

The Barton strain of *D. melanogaster* was used for all experiments.

Normal Drosophila *medium*

The medium in which the larvae mature to pupae was prepared as follows: 5 g of agar (Sigma, St Louis, MO, USA) was added to 1 l of cold distilled water and mixed thoroughly. The water/agar mixture was brought to boiling, removed from the heat and Quaker oatmeal (942 g) was added to the mixture. It was then cooled to 70–75 °C and 1 g of 4-hydroxybenzoic acid methyl ester (methyl paraben; Nipagin m) (Sigma), suspended in 5 ml of 100% ethanol, was added as a fungicide. Finally, 62.5 g of Carolina Instant Fly Food (Burlington, NC, USA) was added. The mixture was then decanted into 25 ml plastic vials, capped with foam stoppers, put in plastic bags and stored in the cold.

Grape-juice agar

Grape-juice firm agar was used for egg collection and LC_{50} experiments. It was prepared as follows: 200 g agar (Sigma), 2,178 g sucrose (EM Scientific, Gibbstown, NJ, USA), 435 g dextrose (Baker, Phillipsburg, NJ, USA), 2.56 l Welch's Grape Juice, and 5.57 l distilled water were mixed and boiled. The solution was cooled to 60 °C; 84 ml of a mixture of 41.8% propionic acid (Fischer Scientific, Fair Lawn, NJ, USA) and 4.15% H_3PO_4 (Baker), followed by 206.25 ml of 1 M NaOH (Fischer Scientific) were added. The hot solution was decanted into Petri dishes which were covered immediately and stored at 4 °C.

Chemicals

Lead acetate and cadmium chloride (Baker) were, according to the labels, 100.3 and 100.5% pure, respectively. Triethyllead chloride (Alfa Products, Danvers, MA, USA) was washed with cold ether and dried before use. Solutions of these compounds were added individually, with mixing, to the normal medium while the medium was warm and fluid.

The odorants for the olfactory learning study were 0.1% 3-octanol or 0.1% 4-methylcyclohexanol diluted with 100% ethanol. They were obtained from Aldrich (Milwaukee, WI, USA).

LC_{50} 11 day larval assay

The experimental procedure for determining the larval LC_{50} was modified from previously published assays (Sorsa & Pfeifers 1973, Christie *et al.* 1983). A plate of grape-juice agar with yeast sprinkled on top was placed in the *Drosophila* rearing cage. Eggs were collected for 0–3 h and removed gently from the agar with a gentle stream of water and a soft paint brush. This slurry of eggs, water and yeast was strained through a 200 μm sieve to remove dead flies and other large particles and then through a 125 μm sieve to collect the eggs. Forty-five eggs were placed on a 0.5 cm^2 piece of grape-juice agar, which was transferred to a 25 ml plastic vial containing normal medium and various concentrations of the heavy metals. The vials were placed in a 25 °C incubator at approximately 50% relative humidity. The endpoint of the LC_{50} assay was the pupariation stage of development. The number of larvae which survived and pupariated within 11 days was counted. The LC_{50} data were obtained from at least three separate experiments. For each concentration of heavy metal, five vials were used. Each vial contained 45 eggs.

Developmental analysis

The development of larvae reared on normal medium and on medium containing heavy metal ions was observed at two developmental time points, pupariation and eclosion. Pupariation is defined as the point at which the third instar larva stops moving, inverts its spiracles and condenses into the white puparium form. Eclosion is the stage at which the flies hatch from the pupae. Flies reared on normal medium will pupariate within 4–5 days and will eclose 4–5 days after pupariation. If the time of pupariation or the time of eclosion increased after treatment with heavy metal, then larval development or pupal development was considered to be inhibited.

Behavioral assays

These are described in the relevant table legends.

Results

The general approach was to determine first the LC_{50} of heavy metal compounds for *D. melanogaster*, use this information to choose the doses to be used and then use established behavioral assays to search for any induced neurobehavioral lesions.

Toxicity studies

The LC_{50} values ($\pm$ standard error) using Barton strain larvae were 0.090 ± 0.004, 0.42 ± 0.04 and 6.60 ± 0.64 mM for triethyllead chloride, cadmium chloride and lead acetate, respectively (Figure 2). No lethality was observed at 0.06, 0.11 and 0.31 mM, respectively. The concentrations at which the percent of surviving larvae began to differ significantly from the controls was 0.076 mM triethyllead chloride ($P < 0.01$), 0.273 mM cadmium chloride ($P < 0.001$) and 4.61 mM lead acetate ($P < 0.001$) according to the unpaired *t*-test (Figure 2).

Developmental studies

An inhibition of *Drosophila* development by the heavy metal compounds was observed (Figure 3). Triethyllead chloride, lead acetate and cadmium chloride delayed *Drosophila* development specifically during the larval stages of development (Figure 4). The lowest concentration significantly extending larval development was 0.061 mM triethyllead chloride ($P < 0.001$), 1.23 mM lead acetate ($P < 0.01$) and 0.06 mM cadmium chloride ($P = 0.027$). Significant delays of development occurred before a lethal dose was reached. No significant delays in pupal development were detected with triethyllead (Figure 5), lead acetate or cadmium chloride (data not shown).

Benzer phototaxis

Phototaxis is the attraction of a fly to light. Flies which exhibit a positive phototaxis move toward a light source and are given a low phototactic index. Flies which display a negative phototaxis move away from the light source and are given a high phototactic index. Flies reared on medium containing a NOEL dose of triethyllead chloride, lead acetate or cadmium chloride did not have phototactic indices significantly different from the control groups (Table 1). This behavioral assay is fairly precise since the percent relative standard error was $6.41 \pm 1.33\%$ and the phototactic index of the control groups did not differ significantly day to day. The phototactic index of the *eya* mutant, which has no eyes, was significantly different from that of the normal control flies ($P < 0.001$). More importantly, the phototactic index of the *eya* flies was not significantly different in the presence or absence of the

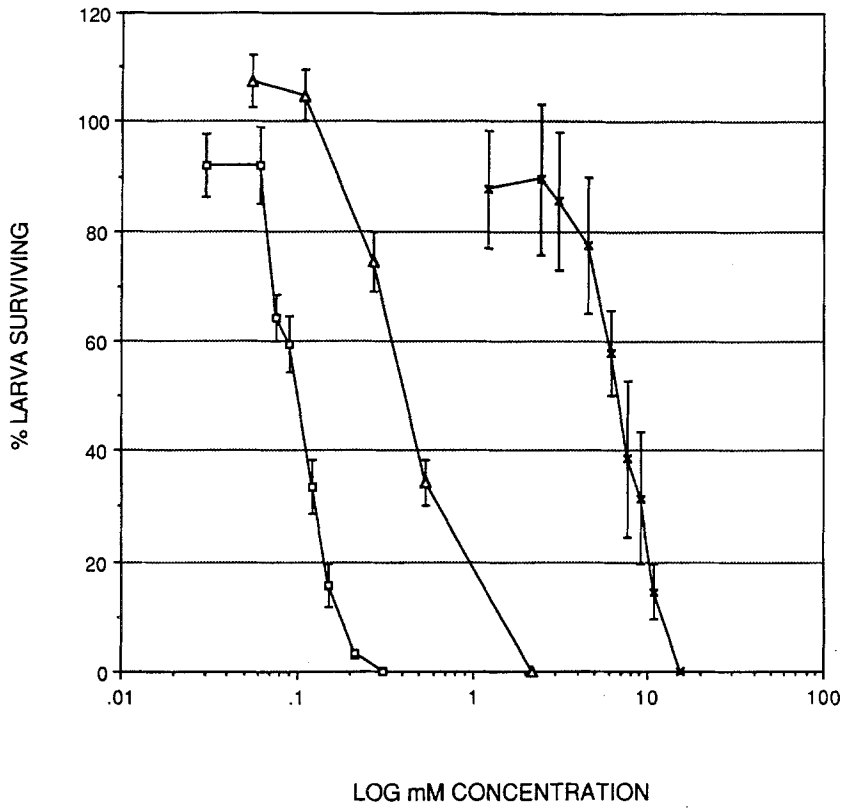

Figure 2. LC_{50}'s of triethyllead chloride (□), cadmium chloride (△), and lead acetate (×). The endpoint of the LC_{50} determination was the pupariation stage of development; the number of larvae surviving into pupariation within 11 days was counted. For each metal compound, three to five separate experiments were performed. For each experiment, each concentration of metal compound was tested with five vials. Each vial contained 45 eggs. For this figure, approximately 18 150 eggs were used.

light source ($P < 0.643$). This indicates that any heat produced from the lamp did not affect the phototaxis indices.

Reactivity

The Connolly paradigm measures the movement of a fly in a new environment. There appeared to be no significant influence of sub-lethal doses of triethyllead chloride, lead acetate or cadmium chloride on *Drosophila* reactivity when this assay was employed (Table 2). The description of the assay is in the legend of Table 2. Both locomotor mutants, *tyr-1* and *hk-2*, had altered reactivity scores, signifying the assay could detect differences in activity. The *tyr-1* mutant has a defective phenol oxidase enzyme in which dopamine levels are decreased as compared to control (Burnell & Daly, 1982). Dopamine levels are inversely related to activity (Tunnicliff *et al.*, 1969). The *hk-2* mutant has altered patterns of neurophysiological activity in its ganglionic center (Ikeda & Kaplan, 1970) and shows reduced activity because it jumps, falls over and thrashes hyperactively without crossing many centimeter lines (Burnet *et al.* 1974).

When control and triethyllead chloride treated flies were starved for 24 h before the Connolly assay, the activity indices of the control and the triethyllead chloride treated flies increased significantly as compared to the flies starved for 1 h. The reactivity of the triethyllead chloride group did not differ significantly from that of the controls in the 24 h starvation assay (Table 2). Female flies had higher reactivity indices than the male flies, but the triethyllead treatment did not alter either sex's reactivity. So, even with additional stress to the fly, the triethyllead chloride did not cause an activity alteration.

Spontaneous 12 h activity

No influence of triethyllead on *Drosophila* spontaneous activity was detected using the assay system of Lints *et al.* (1984) which measures spontaneous activity of individual flies and is described in the

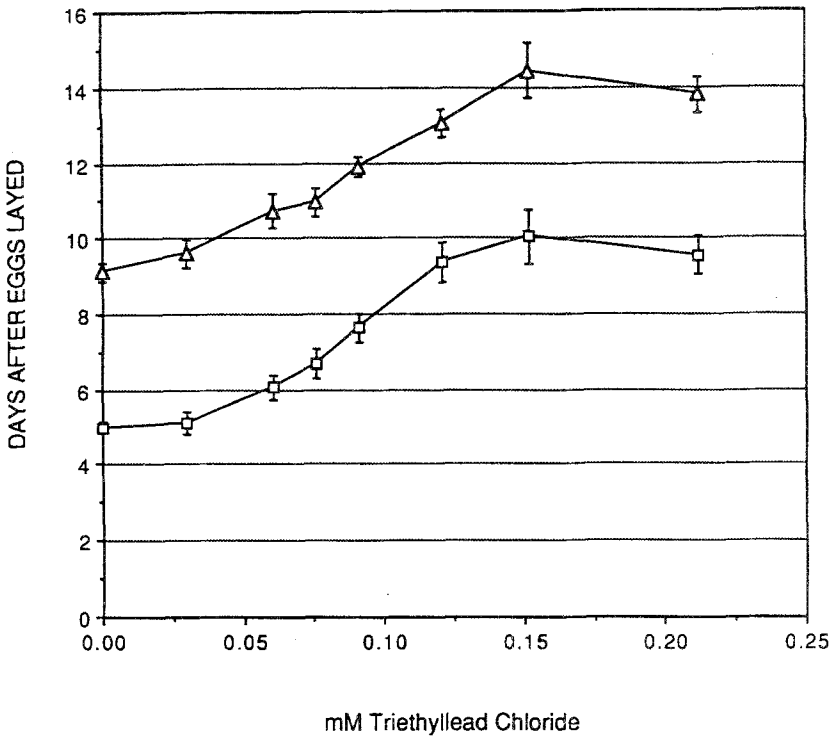

Figure 3. *Drosophila* development was delayed significantly by triethyllead chloride. Five experiments were performed. Each experiment had five vials and there were 45 eggs per vial for each concentration. The procedure was the same as in Figure 2. Days of eclosion (△); days of pupariation (□).

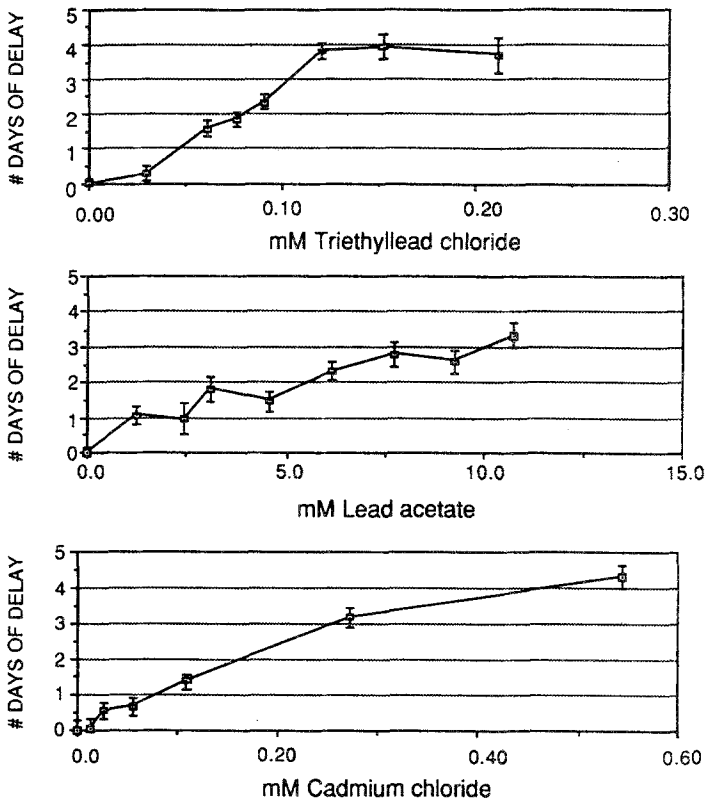

Figure 4. Larval development was delayed by triethyllead chloride, lead acetate or cadmium chloride.

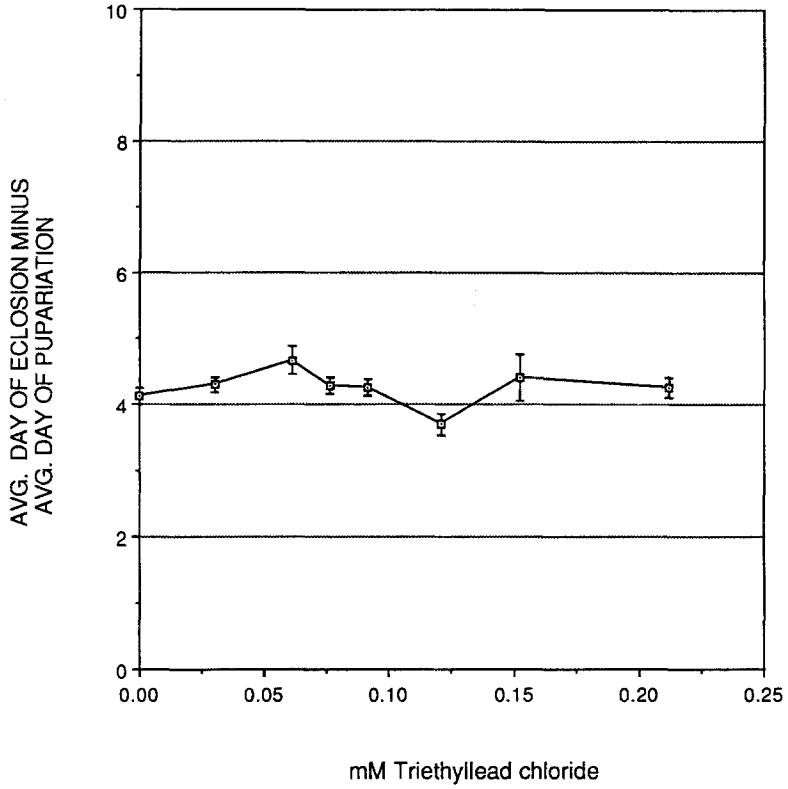

Figure 5. Pupal development was not delayed by triethyllead chloride. The average time of pupal development to fly was calculated by subtracting the average day of pupariation from the average day of eclosion. The number of observations was the same as in Figure 2.

Table 1. Triethyllead chloride, lead acetate or cadmium chloride did not alter *Drosophila* fast phototaxis

Agent	Phototactic index (mean ± SE)	*n*	*P* value
Control	2.15 ± 0.09	20	
Triethyllead chloride (0.06 mM)	2.04 ± 0.10	20	0.803
Control	1.95 ± 0.11	20	
Lead acetate (3.07 mM)	1.86 ± 0.11	20	0.564
Control	1.76 ± 0.08	20	
Cadmium chloride (0.10 mM)	1.73 ± 0.08	20	0.801
Control	2.11 ± 0.09	20	
eya Control in light	4.50 ± 0.08	10	<0.001
eya Control in dark	4.64 ± 0.10	10	<0.001

The fast phototaxis model of Benzer (1967) was used except that only six different tubes were used for the countercurrent distribution while the original assay used 15. The light source was a lamp with a 6.5 V bulb connected to a STACO ajusto-volt (Dayton, OH, USA) which was adjusted to emit 800 μEinsteins $m^{-2}\,s^{-2} \times 0.1$ watts m^{-2} 10 lux at the distance of 2 cm. This was the lowest intensity of light which produced maximum phototactic response from the control groups. The phototactic index or average tube number was used to quantitate the degree of phototaxis. The phototactic index is the sum of the number of flies in each tube (Fly #) multiplied by their tube number (Tube #) all of which was divided by the total number of flies (Total fly #) or phototactic index = Σ(Fly # × Tube #)/Total fly #. A phototactic index of 1 indicates a highly positive phototactic response. A phototactic index of 6 indicates a highly negative phototactic response. Larvae were reared on the NOEL doses of the heavy metals at 25 °C with 12 h light/dark cycle (8:00 a.m.–8:00 p.m. light). From them, 20–30 flies eclosed and were used for each observation (*n*). The *eya* mutant was used as a control. Eyes are completely absent in this mutant, but general activity levels are approximately normal.

Table 2. *Drosophila* reactivity was not influenced by triethyllead chloride, lead acetate or cadmium chloride.

	Reactivity index ± SE	*n*	Mann–Whitney *P* value
A: 1 h starvation			
control	13.6 ± 1.1	250	
triethyllead chloride (0.06 mM)	13.3 ± 1.6	100	0.857
lead acetate (3.07 mM)	11.0 ± 1.2	100	0.151
cadmium chloride (0.10 mM)	13.2 ± 1.3	100	0.942
tyr-1 mutant	22.7 ± 2.3	100	<0.001
hk-2 mutant	6.7 ± 1.7	100	0.001
B: 24 h starvation			
control male	22.6 ± 2.6	36	
triethyllead chloride (0.015 mM)	20.7 ± 2.9	35	0.628
triethyllead chloride (0.06 mM)	23.7 ± 3.7	26	0.785
C: 24 h starvation			
control female	26.0 ± 2.5	43	
triethyllead chloride (0.015 mM)	24.4 ± 2.9	33	0.678
triethyllead chloride (0.06 mM)	22.9 ± 2.6	36	0.392

The Connolly (1966) paradigm tests the reactivity of a fly to a new environment. After eclosing, flies were sexed and transferred to a fresh vial containing medium and yeast. In (A) the flies were removed from food at 1:00 p.m., and the experiments were carried out between 2:00 p.m. and 5:00 p.m. the same day. In (B) and (C), the flies were removed from food 24 h before the experiment began. Each individual fly was funnelled into the arena and the lid was closed. The fly was allowed to rest 90 s in the arena and then the test began. The reactivity of individual flies was measured by counting the number of squares (1 cm^2) the fly crossed during 1 min in a Plexiglass arena (12 × 15 × 0.5 cm) on the bottom of which was the cm grid. The reactivity index (Connolly 1966, 1967) is the number of squares the individual fly entered during the 1 min testing period. The experimental conditions were 22.6 ± 0.2 °C and 43.9% relative humidity; *n* represents the number of individual flies tested.

legend of Table 3. Also, no influence on circadian rhythm was detected. The male's activity was high at the beginning, low in the middle, and highest during the end of the 12 h light cycle. The female's activity varied throughout the day, but it generally followed the same activity pattern of the males at a slightly elevated level of activity.

Since the heavy metals were expected to cause hyperactivity, we chose to test the flies during their lowest activity period from 12:00 noon to 3:00 p.m. The observations were increased to every 5 min to increase the sensitivity of the assay. No influence of triethyllead chloride, lead acetate and cadmium chloride on spontaneous activity was detected using this shorter variation of the Lints paradigm (Table 3). However, *tyr-1* mutant flies and flies treated with ether had significantly lower activity scores than the controls indicating that the assay can detect changes in locomotion.

Learning

Using a sub-lethal dose of triethyllead chloride, lead acetate or cadmium chloride, no effect on *Drosophila* short-term learning was detected using the Dudai *et al.* (1976) assay (Table 4). The assay is described in the legend of Table 4. That the assay does measure learning is shown by comparing controls with and without shock or with and without training. We also examined the influence of lead acetate or cadmium chloride on long-term memory. None was found (Table 4).

Patterns of gene expression

Molecular genetic studies of *Drosophila* nervous system development have led to the use of many molecular markers of CNS organization. For example, the homeotic genes code for DNA-binding regulatory proteins that normally display distinct segmental domains of expression in the developing CNS. During our studies of triethyllead chloride, we utilized monoclonal antibodies against the protein products of two homeotic genes, *Ubx* and *Scr*, to probe for pattern defects in the CNS of treated third instar larvae. We found no significant changes in the patterns of antibody staining at either of the triethyllead concentrations tested, 0.03 and 0.06 mM (data not shown).

Table 3. *Drosophila* spontaneous activity was not influenced by triethyllead chloride, lead acetate or cadmium chloride.

	Average ± SE (%)	*n*	*P* value
A[a]			
control male	45.4 ± 2.1	62	
triethyllead chloride (0.015 mM)	44.4 ± 2.5	55	0.760
triethyllead chloride (0.061 mM)	43.5 ± 2.7	60	0.593
triethyllead chloride (0.122 mM)	40.0 ± 5.6	13	0.220
control female	58.5 ± 2.3	63	
triethyllead chloride (0.015 mM)	52.7 ± 2.9	62	0.117
triethyllead chloride (0.061 mM)	60.0 ± 2.5	64	0.925
triethyllead chloride (0.122 mM)	57.9 ± 4.8	25	0.893
B[b]			
control male	36.9 ± 1.0	79	
0.061 mM triethyllead chloride	27.8 ± 1.4	40	0.062
3.07 mM lead acetate	31.3 ± 1.0	75	0.164
0.10 mM cadmium chloride	30.9 ± 1.0	76	0.130
tyr-1 mutant	20.5 ± 0.7	80	<0.001
ether	27.2 ± 1.3	39	0.045

[a]This assay was developed by Lints *et al.* (1984). Larvae were reared on NOEL doses of the heavy metal at 25 °C with 12 h light/dark cycle (8:00 a.m.–8:00 p.m. light). On eclosion, 0–1 day old male flies were placed in vials containing the standard medium and yeast. Eighteen hours before the experiment was to begin, 5–7 day old flies were individually placed in separate 60 × 15 mm Petri dishes (Falcon), containing 10 ml of normal medium. Every 15 min each fly was checked for activity. If the fly was walking, running, attempting to fly or actively eating, it was considered active and given a score of one point. If the fly was standing still or eating without moving its legs, then the fly was considered inactive and given a score of zero. The activity in each 15 min observation period was scored as all-or-nothing. A total of 48 observations were made for each fly. Total inactivity for the day was scored as 0 (0% activity) and total activity was scored at 48 (100% activity). The duration of the experiment was 12 h (8:00 a.m.–8:00 p.m.) at 22 °C. Thus, the activity index is a percentage of total activity in the entire 12 h period. *n* = number of flies tested.

[b]The second series of experiments differed from the first in that they were carried out from 12:00 noon to 3:00 p.m. and in that the observations of activity were made every 5 min, totaling 36 observations per fly during the 3 h period. *n* = number of flies tested.

Discussion

The LC_{50} values obtained in the present experiments (Figure 2) are different from those obtained from the work of others (Sorsa & Pfeifers 1973, Magnusson & Ramel 1986). This may be because LC_{50} and NOEL are different for each strain of *D. melanogaster* as suggested by Magnusson & Ramel (1986). Christie *et al.* (1983) found that the larval LC_{50} of cadmium chloride was 0.047 mM using the Canton S strain; in our experiments using the Barton strain, the larval LC_{50} was 0.42 mM cadmium chloride (Figure 2). Other explanations for the difference in LC_{50} values may be composition of medium and method and timing of feeding.

Larvae reared on medium containing triethyllead chloride, lead acetate or cadmium chloride took a longer time to develop than did the control larvae. Sorsa & Pfeifers (1973) also observed a delay when the medium contained 0.006 mM cadmium chloride. In our experiments, however, the stage where the developmental delay manifested itself was delineated. Each of the three heavy metal ions induced a delay specifically during the larval stages.

The first significant delay of development was noted at the concentrations of 0.06, 1.23 and 0.06 mM triethyllead chloride, lead acetate and cadmium chloride, respectively (Figure 4). Sorsa & Pfeifers (1973) using the Porvoo-wild strain found that 0.27 mM cadmium chloride caused a significant delay of egg to fly development with no lethality.

There are many established behavioral assays for determining abnormal behaviors. We concentrated on phototaxis, locomotion and learning because these paradigms have been used extensively with *Drosophila* and the latter two are influenced by heavy metal ions in mammals. While others have studied the influence of caffeine, theophylline, neostigmine (Folkers & Spatz, 1984) and formamadines (Dudai *et al.* 1987) on *Drosophila* behavior, they exposed and tested adult flies. The interesting work reported by Cohn *et al.* (1990) showed that lead acetate caused a delay in larval development, but we wished to determine whether *Drosophila* would be an appropriate model for detecting behavioral and learning deficiencies during development. For example, lead causes such CNS effects in 0.5–5 year old children (Davis & Svendsgaard 1987). The

Table 4. Triethyllead chloride, lead acetate or cadmium chloride did not alter *Drosophila* learning or memory

Agents	Learning index ± SE	*n*	*P* value
A: learning testing[a]			
control	0.41 ± 0.06	12	
triethyllead chloride (0.06 mM)	0.36 ± 0.04	12	0.471
control	0.38 ± 0.03	8	
lead acetate (3.07 mM)	0.46 ± 0.06	8	0.220
control	0.36 ± 0.05	19	
cadmium chloride (0.10 mM)	0.27 ± 0.04	11	0.153
control without shock	0.02 ± 0.03	12	<0.001
control without training	−0.01 ± 0.04	8	<0.001
B: memory testing[b]			
control	0.29 ± 0.06	14	
3.08 mM lead acetate	0.28 ± 0.05	14	0.895
0.10 mM cadmium chloride	0.31 ± 0.05	14	0.830

[a]This assay was a variation of that described by Dudai *et al.* (1976). The learning assay coupled an odorant (3-octanol) with an electric shock during two training sessions. One minute following the training sessions, the flies were tested with two compounds, one (3-octanol) which was and one (4-methylcyclohexanol) which was not associated with the electrical shock. The learning index equals the number of flies which avoided the shock-associated odor minus the number of flies which avoided the control odor all divided by the total number of flies tested. For each trial (*n*), 20–30 flies were used. The flies, 2–7 days old, were reared on the medium containing the NOEL dose of metal compound at 25 °C, 40–50% relative humidity with a 12 h light/dark cycle. One day before the experiment began, the flies were placed in a clean vial containing normal medium and yeast. One hour before the experiment, 20–30 flies were placed into the tubes in which they were to be trained, in order to allow the flies to acclimate to the new environment. The experiment was controlled by 'training' the flies without the adverse stimuli and by testing the flies without training. The shocking grids were obtained from Circuit Engineering & Development (Tucson, AZ, USA). They were made from a copper-engraved sheet of flexible fiberglass circuit board. The design was taken from Quinn *et al.* (1974). The shocking tube contained an electric grid on which 20 μl of a 1% solution of 3-octanol had been pipetted and dried. The odor remained fresh since a training tube was never used for more than 2 h, and the grids were washed thoroughly with water and soap, and rinsed with ethanol. Attached to the electric grid were leads, connected in series to 10 batteries of 9 V, providing an electric current of 85–88 V. The shock rarely killed the flies. Since it did seem to stun them, they were allowed to rest for 30 s after each shocking session.

[b]The same learning paradigm was used for the memory study, except that the time between training and testing was increased from 1 min to 10 h.

larvae, therefore, were exposed to the metal compounds and the resulting adults were tested for behavioral alterations.

The present experiments did not detect any effect of triethyllead chloride, lead acetate or cadmium chloride on *D. melanogaster* behavior. This might be due to a number of reasons. First of all, the damage caused by these heavy metals may not be manifested in the type of *Drosophila* behavior studied in the present experiments. Secondly, the dose of heavy metal compound given in the present experiments might have been inadequate to cause neurobehavioral damage. Thirdly, the physiology and structure of the CNS of flies may be so different from that of mammals that they are not really a valid model system for studying CNS effects of heavy metals.

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