A PROMISING INDICATOR OF NEUROBEHAVIORAL TOXICITY USING THE NEMATODE *CAENORHABDITIS ELEGANS* AND COMPUTER TRACKING

PHILLIP L. WILLIAMS AND DAVID B. DUSENBERY
School of Biology
Georgia Institute of Technology
Atlanta, GA 30332

A promising screening test for neurotoxicity has been developed using a computer tracking system and a species of nematode, Caenorhabditis elegans. *The animals are viewed in dark-field illumination by a video camera interfaced directly to a microcomputer. Several hundred nematodes are tracked simultaneously and rates of locomotion and frequency of change of direction are reported in real time. This system can rapidly obtain reliable data on a variety of behavioral parameters relating to locomotion and response to sensory stimulation. Initial testing has examined the effects of six chemicals on locomotion. Four metals (copper, beryllium, mercury, and lead) and two organophosphate pesticides (malathion and vapona) have been studied. Copper and beryllium were chosen as chemicals that have not been shown to be neurotoxins and the other four chemicals were chosen as substances known to be neurotoxins. Our findings indicate that the rate of movement of exposed nematodes compared to the rate of movement of vehicle controls may prove to be useful as an indicator of neurotoxicity.*

INTRODUCTION

It has been recognized that many toxic chemicals affect behavior at concentrations below those causing physiological changes. Over 25% of the industrial

1. Corresponding Author: Dr. Phillip L. Williams, A.T. Kearney, 1100 Abernathy Road, Suite 900, Atlanta, Georgia 30328, (404) 393-9900.

2. Abbreviations: LC50, lethal concentration for 50% of population; LD50, lethal dose for 50% of populations; LC16, lethal concentration for 16% of population; BC50, behavioral change concentration for 50% of population; BC16, behavioral change concentration for 16% of population.

3. Key Words: Nematode, *Caenorhabditis elegans*, behavior, neurotoxicity, mammal.

Toxicology and Industrial Health, 6:3/4, pp. 425–440

ISSN: 0748-2337

chemicals with TLVs (as established by the American Conference of Governmental Industrial Hygienists) have, as one of their bases, direct nervous system effects (Anger, 1984). Neurotoxic disorders are listed by the National Institute for Occupational Safety and Health among ten leading causes of work-related disease and injury (NIOSH, 1988). Neurobehavioral toxicology has been suggested as an endpoint in evaluating a chemical's general health and/or safety risk (Buckholtz and Panem, 1986). In addition, several environmental regulations require evaluation of chemicals' behavioral effects (e.g., 1977 Amended Clean Air Act, Section 103 f(1); 1976 Toxic Substance Control Act, Section 4 (b) (2) (A)). Unfortunately, the usual methods of testing behavioral or neurotoxicity are quite expensive. Consequently, it would be useful to have a rapid, inexpensive test as an initial tier to screen to establish priorities for more involved tests.

As an indicator of neurotoxicity, behavior is believed to be more sensitive than morphology (Norton, 1978; Tilson and Cabe, 1978). However, behavioral endpoints may be difficult to study and particularly difficult to reproduce because of the diverse factors that influence the behavior of higher animals. The authors postulated that the problems of reproducibility of a behavioral assay could be reduced by employing a relatively simple animal with a restricted repertoire of behavior and a computer tracking system to objectively quantitate the movements of a large number of animals.

Although animals vary greatly in many ways, the nervous system shares many common features: the signal used is electrical; it is conducted by neurons; the signal is transmitted between cells at synapses that are nearly identical across a wide range of taxons; and, similar membrane channels and enzymes exist at the molecular level. These features are the same for animals ranging from nematodes (round worms) to humans. The wide variation in behavioral abilities is due to differences in the number of components rather than differences in the properties of individual components. The range of complexity is from approximately 300 neurons in nematodes to roughly 1 trillion in humans.

Nematodes are among the simplest animals with a centralized nervous system. The species *Caenorhabditis elegans* has become a common experimental organism, with more than 800 papers published on it. It is easy to culture and has a short life cycle, maturing within 72 hours of hatching at 20°C. It is small (about 1mm in length), permitting electron microscopic examination of many tissues simultaneously. Furthermore, its tissues usually include only one or two cell types, so the effects of toxicants can be readily studied at the ultrastructural level (Popham and Webster, 1982). In addition, genetically uniform stocks can be easily maintained by natural inbreeding and indefinite freezing in liquid nitrogen.

The neuroanatomy of nematodes has been studied in detail and many features are known. For example, *C. elegans* has 302 neurons that have been fully characterized by serial-section electron microscopy, resulting in a complete descrip-

tion of their connectivity (White et al., 1986). In this aspect, no other animal has been described in such complete detail. Like humans, nematodes have neuromuscular junctions involving acetylcholine as a neurotransmitter. Other neurotransmitters that nematodes have in common with humans include serotonin, norepinephrine, gamma amino butyric acid (GABA), epinephrine, and dopamine (Horvitz et al., 1982, Stretton et al., 1978, Sulston et al., 1975). In addition, it is the only species to have the embryonic cell lineage completely described (Sulston, et al., 1983).

The behavior of the nematode has also been well studied (Dusenbery, 1980a; Chalfie and White, 1988). *C. elegans* has been found to continuously feed when food is available and to move with a wave-like motion. The worm's behavior consists primarily of changes in rate of movement or direction of movement. Dusenbery (1985 a,b) has developed a computer tracking system that can monitor and quantify these parameters.

This study reports the findings of the initial evaluation of the use of *C. elegans* and a computer tracking system as a potential neurobehavioral toxicity screening test. Six substances were chosen for testing: four known human neurotoxins and two chemicals not believed to be neurotoxins. The four known neurotoxins included two metals (mercury and lead) and two organophosphate pesticides (malathion and vapona). The two non-neurotoxins were copper and beryllium (Pounds, 1985). Preliminary findings with mercury and copper were reported previously (Williams and Dusenbery, 1987).

MATERIALS AND METHODS

Materials

Caenorhabditis elegans var. Bristol (strain N2) was used. The testing with mercury was performed in 10 cm Petri dishes filled with 30 ml of nematode growth medium (NGM) (Brenner, 1974). Due to concern about lead precipitating with phosphate, subsequent testing with the other chemicals was conducted without the potassium phosphate buffer normally used in the NGM. Potassium phosphate ($K_xH_xPO_4$, pH 6.0) was replaced with equal molar KCl (K-plates). The testing was performed using plates that had a lawn of *Escherichia coli* strain OP50 as a food source. All cultures were grown in constant temperature incubators at 20°C.

With the metals, reagent-grade salts from Fisher Scientific were used in this study. The water soluble salts of the four metals were $HgCl_2$; $BeSO_4 \cdot 4H_2O$; $CuCl_2 \cdot 2H_2O$; and $Pb(NO_3)_2$. Commercial pesticide products from Prentiss Drug and Chemical Company were used: a 20% vapona (2,2-dichlorovinyl dimethyl phosphate) product, Prentox Vapon 20% Emulsifiable Concentrate; and a 57% malathion product, Prentox 5-lb Malathion Spray. Both pesticide products were an emulsion that permitted dispersion of the chemicals in water.

Behavioral changes were recorded by an RCA close-circuit television (CCTV) camera (Model TC2000) linked to an IBM AT microcomputer via an Imaging Technology camera-interface (Fig. 1). Software had been written to simultaneously track several hundred nematodes and report their rate of locomotion and frequency of change in direction in real time (Dusenbery, 1985 a,b; Pline and Dusenbery, 1987). The analog signal from the CCTV camera is converted to a digital signal and stored in a frame memory together with signals from the computer indicating the recorded position of the individuals being tracked. Then, the signal is converted back to an analog signal and sent to a separate monitor to allow for visualization of the tracking process. The position of each individual is determined once per second.

In order to focus the small subjects, a macro lens of 25mm focal length was used. This was focused down to a field of about 7cm × 7cm. For this system, it is critical for the subject to have high contrast with respect to the background. Consequently, a dark-field illumination system was employed that allows only scattered light from the (translucent) nematodes to enter the camera. This ar-

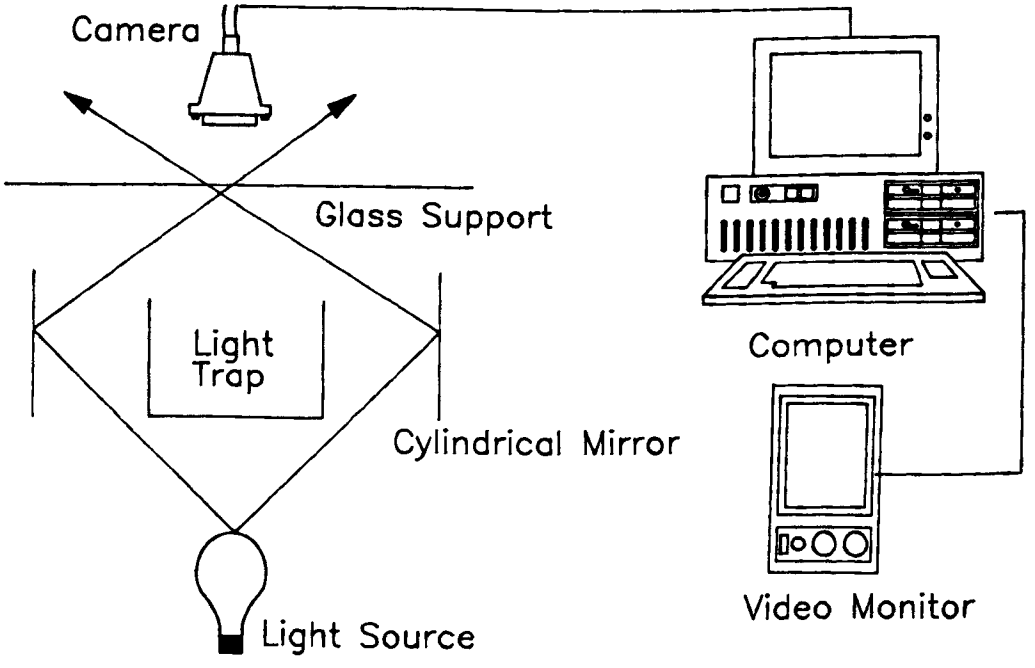

FIGURE 1. Schematic of the computer tracking system. The glass plate with a pool of 1% agar containing *C. elegans* is placed on the glass support. By means of the dark-field illumination arrangement, the computer tracks up to 300 nematodes and provides data on their rate of locomotion and any change in direction (reversals). The nematodes can be viewed on the video monitor.

rangement results in an image in which the nematode is bright against a black background.

Experimental Design

A 0.4ml aliquot of a prepared solution of the chemical in water was spread over the 10cm-diameter Petri dish containing the agar with a lawn of OP50. The concentrations of all the solutions were within their water solubility range (Windholz, 1976). Concurrent water vehicle control plates were established. The plates were placed (overnight) in a dessicator over a saturated solution of $CaCl_2$ to remove the excess moisture. The chemicals were found to diffuse evenly into the agar within a few hours.

The survival studies were conducted by transferring (on the tip of a hypodermic needle) 7 young adults (3- to 4-day-old) *C. elegans* to the plates (Williams and Dusenbery, 1988). The cultures were maintained in the 20°C incubator for 24-hours. After this exposure period, the plates were observed under a dissecting microscope to determine survival. Death was scored as a total lack of movement and lack of response to probing with a needle. If the nematodes were not found on the plate they were assumed to be dead and decomposed beyond recognition. With standard cultures, dead nematodes are rarely found on plates. The testing was usually repeated at least 10 times (70 individuals) for each concentration.

Larger populations of *C. elegans* were used for the behavioral studies. These populations can easily be obtained by using synchronized cultures (Cox et al., 1981) of 3- to 4-day-old adult worms. The worms were washed from the synchronized culture plates and transferred to the exposed and vehicle control plates in a 0.3% NaCl plus 25 mM potassium phosphate buffer of pH 6.0. Approximately 300 to 400 worms were added per plate. These cultures were also maintained in the 20°C incubator for 24 hours.

After this exposure period, the plates were removed from the incubator and the worms were washed from the Petri dishes with the same solution described previously. The worms were rinsed several times to prevent inclusion of any bacterial or agar debris. The transfer procedure takes about 90 minutes. Once the worms were clean, they were transferred in 100μl of solution to a thin layer of agar on a glass plate 10cm × 20cm. The agar pool was formed with approximately 6ml of 1% agar with the same salt concentration as the rinsing solution.

After the worms had dispersed onto the 1% agar, the slide was inverted and placed above another glass sheet under the video camera for tracking. Between the glass sheet and the agar is a 5mm-wide air gap. A stream of hydrated air is continuously pumped through this gap in order to prevent drying of the agar. With each tracking experiment, a threshold light intensity is chosen to best discriminate nematodes from background. An average distance and average

number of changes in direction (reversals) per subject tracked are reported. The testing was repeated with 6 to 10 replicates per concentration.

Data Evaluation

In the survival studies, for each concentration an overall average was derived and the dose-response relationship was plotted. Using a computer program (Tallarida and Murray, 1981) and the methods of Litchfield and Wilcoxon (1949), the LC16 and LC50 with 95% confidence limits were calculated (Table 1).

With the behavioral studies, the software program provided an overall average distance of movement per second for all the nematodes on the slide that were being tracked (usually between 150 to 200 but up to 300 simultaneously). Each tracking was conducted for about a 5-minute period and was repeated 3 times per plate. The average of the 3 observations was used as the rate of movement for the animals on that slide. The parallel vehicle control cultures were treated in the same way. For each day, a ratio was derived for the distance moved for each population of an exposure concentration to a chemical versus the distance moved for the control cultures, and an overall average ratio was determined for each exposure concentration.

This approach was straightforward for all the chemicals except mercury. With mercury, the lowest concentrations that produced hyperactivity equal to 16% and 50% of the peak value for the hyperactivity were used as the BC16 and BC50.

RESULTS

Figures 2 through 7 graphically summarize the results of this study. In each figure, the rate of locomotion of the exposed nematodes as a percent of the parallel vehicle controls is plotted as a function of chemical concentration. For comparison purposes, the survival rates are also plotted as a function of chemical

TABLE 1
Levels for Lethality and Behavioral Changes (mg/1)

Chemical	LC16	LC50		BC16	BC50	
Copper	54	170	(89–325)	47	141	(80–249)
Beryllium	13	50	(41–63)	5	15	(10–22)
Lead	211	421	(267–662)	22	115	(57–231)
Malathion	61	195	(135–281)	7	45	(23–86)
Vapona	0.23	0.51	(0.27–0.97)	0.01	0.18	(0.08–0.40)
Mercury	5	16	(10–26)	0.68	0.80	

Note: The ranges shown in the parentheses are a 95% confidence limit = $S^{2.77/n}$, where S = (LC84/LC50 + LC50/LC16)/2 or (BC84/BC50 + BC50/BC16)/2 (Litchfield and Wilcoxon, 1949). The Hg BC50 and BC16 were derived directly from the plot of the exposed movement shown in Fig. 7.

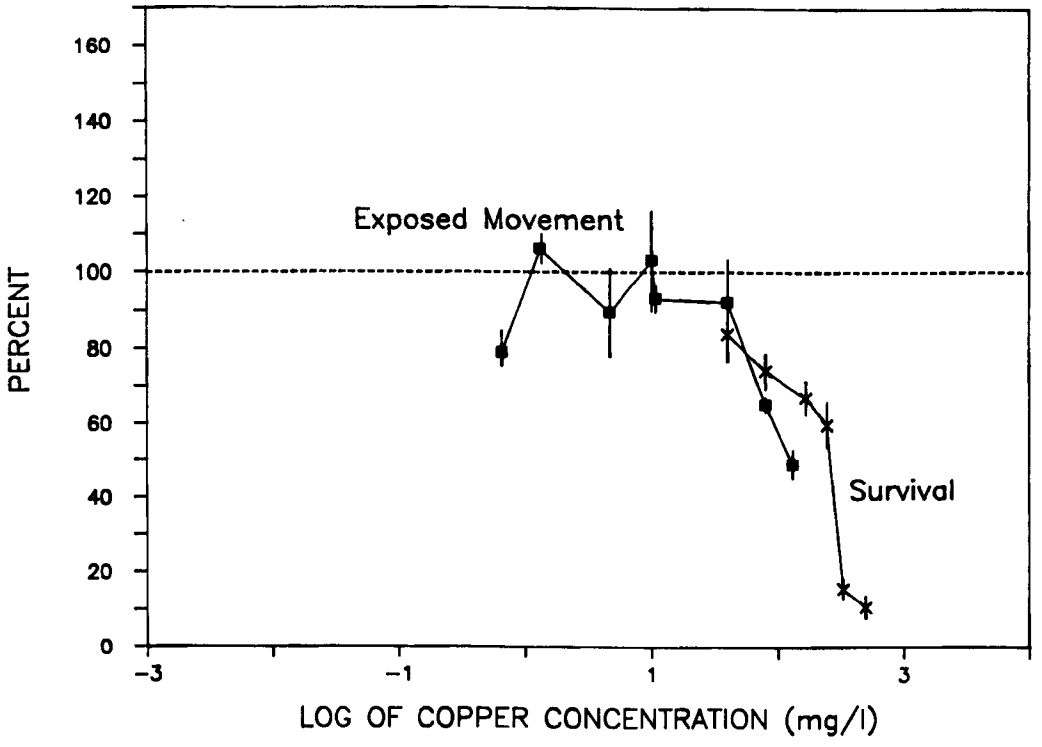

FIGURE 2. Behavioral changes and survival rates for *C. elegans* exposed to copper ($CuCl_2 \cdot 2H_2O$). The rate of movement of the exposed nematodes varies little from the controls until concentrations reach lethal levels. Each point is the mean of 6 to 10 experiments, ±SE.

concentration. Each data point is the average of 6 to 10 separate experiments. Table I provides the derived LC16, LC50, BC16, and BC50 for each chemical.

With all six chemicals, it was found that once the exposure concentration reached levels causing significant lethality, movement and survival decreased more or less in parallel (Figs. 2–7). This response is what one would expect. That is, as chemical concentrations reach the lethal range, the decrease in rate of movement corresponds to nematodes becoming generally debilitated (i.e., overt signs of intoxication). Thus, for neurotoxicity to be demonstrated by a substance, a behavioral change must be observed at a concentration below the lethal range for the particular chemical. It is postulated that the more specifically neurotoxic a chemical is, the greater the difference in concentration between the two types of effects.

With exposures to copper ($CuCl_2 \cdot H_2O$), there appears to be no behavioral changes in *C. elegans* until the copper concentrations reach the lethal levels (Fig. 2). Once the copper concentration is in the lethal range, the rate of movement

of the surviving animals drops quickly. This result is consistent with the low neurotoxicity of copper.

The other supposed non-neurotoxin that was tested, beryllium ($BeSO_4 \cdot 4H_2O$), showed less clear results (Fig. 3). At low concentrations, there appears to be little difference between survival and behavior. However, above about 80% survival, the rate of movement of animals exposed to beryllium drops much more rapidly than the rate of survival. From a qualitative evaluation of the plot of the data, the response to beryllium appears to be intermediate or indeterminate.

With lead ($Pb(NO_3)_2$), malathion, and vapona, *C. elegans* had the response expected of a neurotoxin. With all three chemicals, locomotion was significantly reduced at concentrations well below any concentration that caused a reduction in survival (Figs. 4–6). A gradual decline of movement was sustained over the entire range tested.

Caenorhabditis elegans exposed to mercury ($HgCl_2$) demonstrated a totally different response (Fig. 7). At mercury exposure levels between 0.4 and 1mg/l,

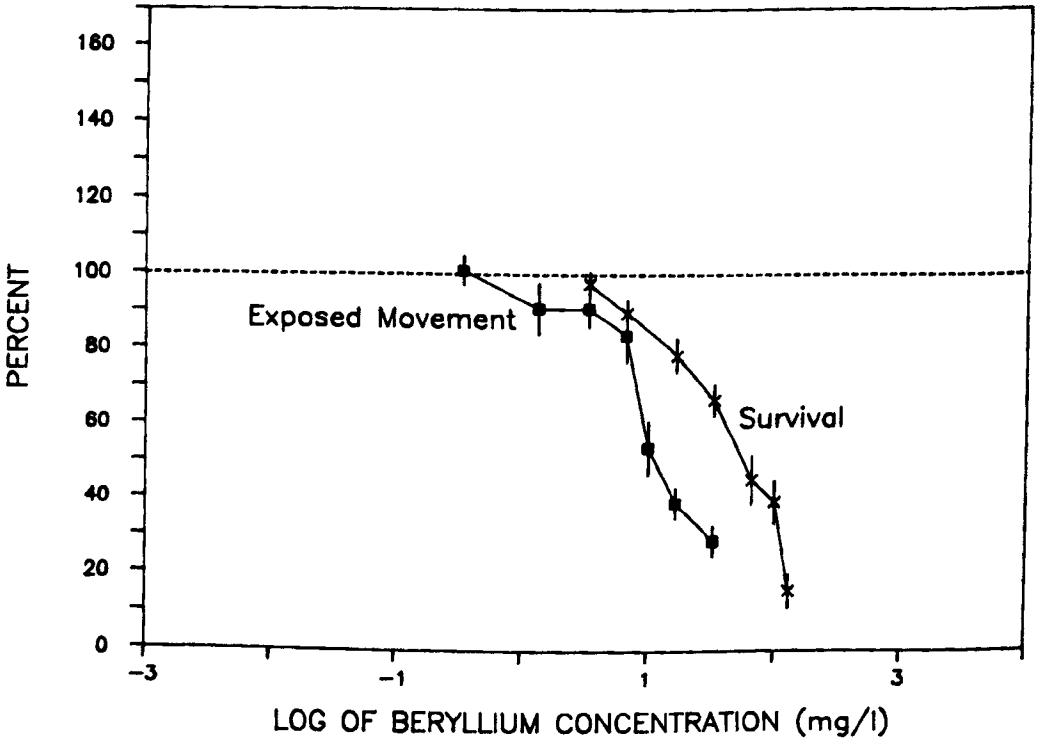

FIGURE 3. Behavioral changes and survival rates for *C. elegans* exposed to beryllium ($BeSO_4 \cdot 4H_2O$). At low concentrations, there appears to be little difference between survival and behavior. As the concentrations increase, the behavioral changes are more dramatic. Each point is the mean of 6 to 10 tests, ±SE.

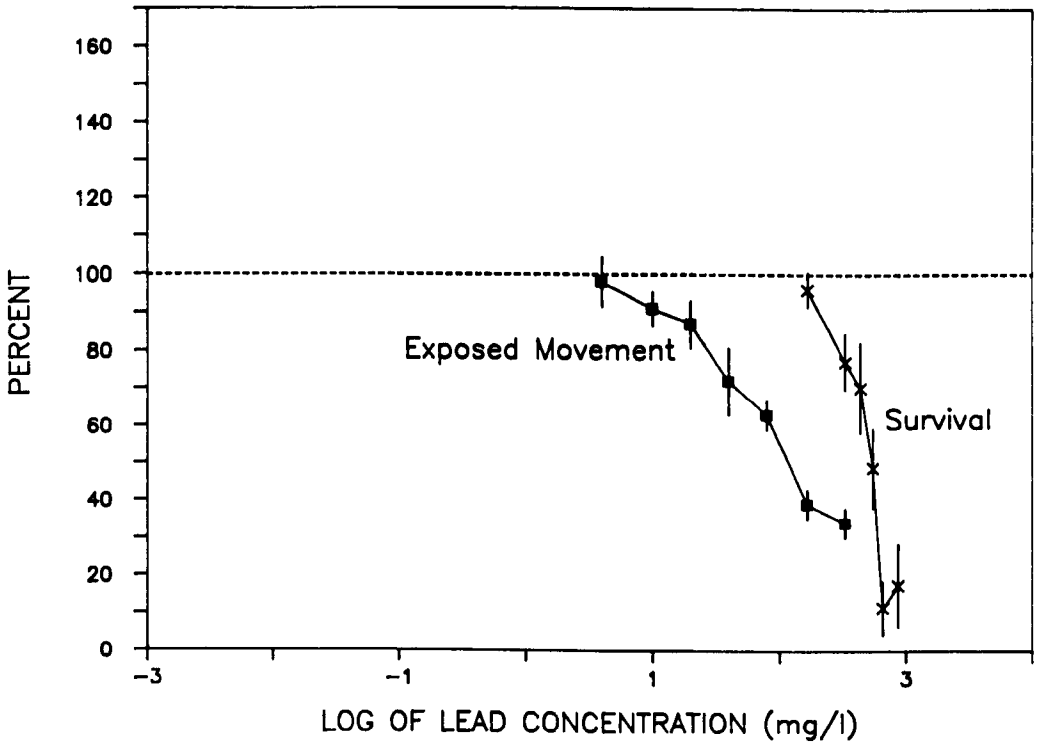

FIGURE 4. Behavioral changes and survival rates for *C. elegans* exposed to lead ($Pb(NO_3)_2$). Locomotion is significantly reduced at concentrations well below lethal levels. Each point is the mean of 6 to 10 experiments, ±SE.

the animals exhibited a steep increase in activity that peaked at a rate about 50% above that of the parallel vehicle controls. The movement activity remained elevated until exposure levels reached about 10mg/l. At this point, the concentrations were well into the lethal range and the rate of movement dropped sharply.

It is postulated that this hyperactivity with mercury exposure represents a behavioral effect analogous to the behavioral change observed in humans upon exposure to mercury. Such changes include personality alterations, tremors, loss of sleep, and general hyperactivity (Friberg, 1977). As with humans, these types of effects in the nematodes are seen at exposure levels below lethal concentrations.

DISCUSSION

Of the six plots (Figs. 2–7), those for lead, malathion, vapona, and mercury are consistent with significant neurotoxicity and that of copper is not. The results from the beryllium exposures are unclear.

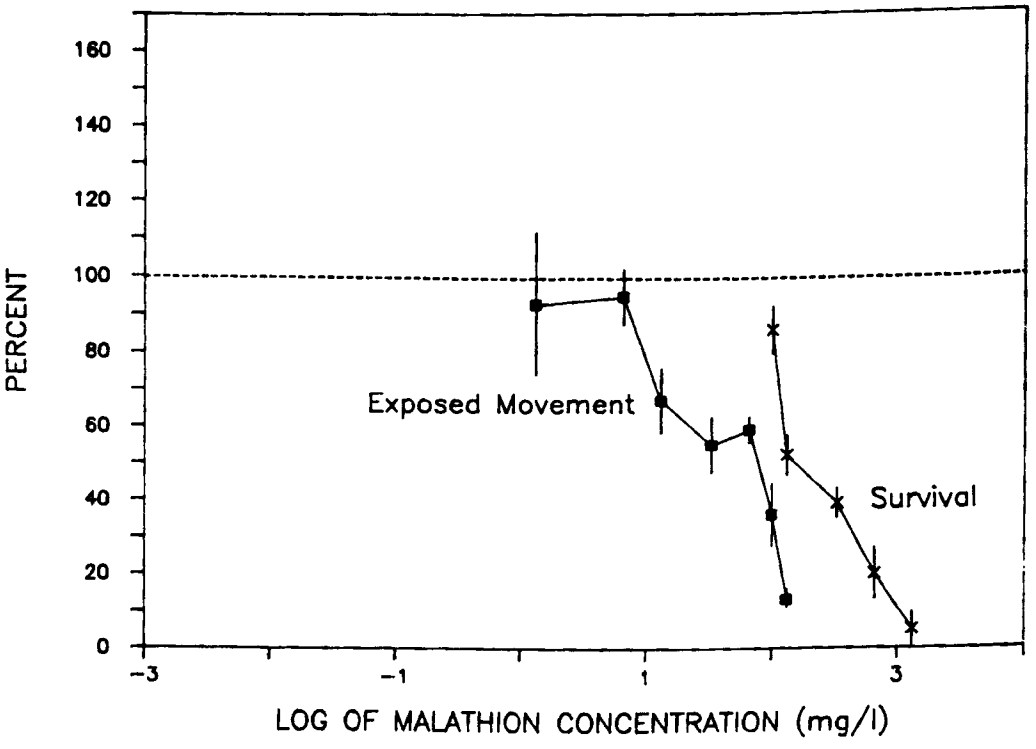

FIGURE 5. Behavioral changes and survival rates for *C. elegans* exposed to a malathion emulsion. Locomotion was found to be reduced at levels below the lethal range. Each point is the mean of 6 to 10 experiments, ±SE.

If this approach is to have practical utility, it would be useful to have an index of neurotoxicity. Several different indices have been calculated for evaluation. The simplest approach is to calculate a neurotoxicological index analogous to a margin of safety that is often used for determining the safety of drugs (James, 1985). That is, directly compare the LC50 to the BC50. These calculations have been performed (Table 2). In all cases the LC50 is greater than the BC50. Copper clearly has the lowest ratio. However, the beryllium ratio is similar to lead and, on this basis, could be classified as a neurotoxin.

As previously discussed, once the exposure concentrations enter the lethal range, locomotion and survival both decrease. Consequently, the two dose-response curves can overlap. For this reason, it may prove more appropriate to use a different ratio such as the BC50 compared to the LC16 (the lowest end of the lethality curve used in the probit transformation to calculate an LC50). Alternatively, since the shapes of the behavioral and survival curves may differ, the margin may best be described by the difference at the first significant point of each curve (i.e., BC16 and LC16). Using either of these two comparisons (Table

2), the four known neurotoxins clearly have higher ratios than does either copper or beryllium. In the case of the known neurotoxins, the LC16 is greater than the BC50; however, with copper and beryllium, the BC50 is greater than the LC16.

If further testing upholds the observed relationship between the LC16 and BC50, it may be possible to derive a practical neurotoxicity index without determining the complete concentration range of behavioral responses, which requires much more time and effort than determining the survival curve. For such an approach, the first step would be to determine a survival range (Williams and Dusenbery, 1988). Following the calculation of the LC16, experiments using the computer tracking system could be performed at only the LC16. An index could be calculated by dividing the rate of movement of the vehicle control cultures by the rate of movement of the cultures exposed to the LC16. This value would (then) be multiplied by 0.84. In cases where the movement closely followed the survival curve (e.g., copper), the index would be close to 1. However, with exposures where the movement was significantly altered from the vehicle controls at the

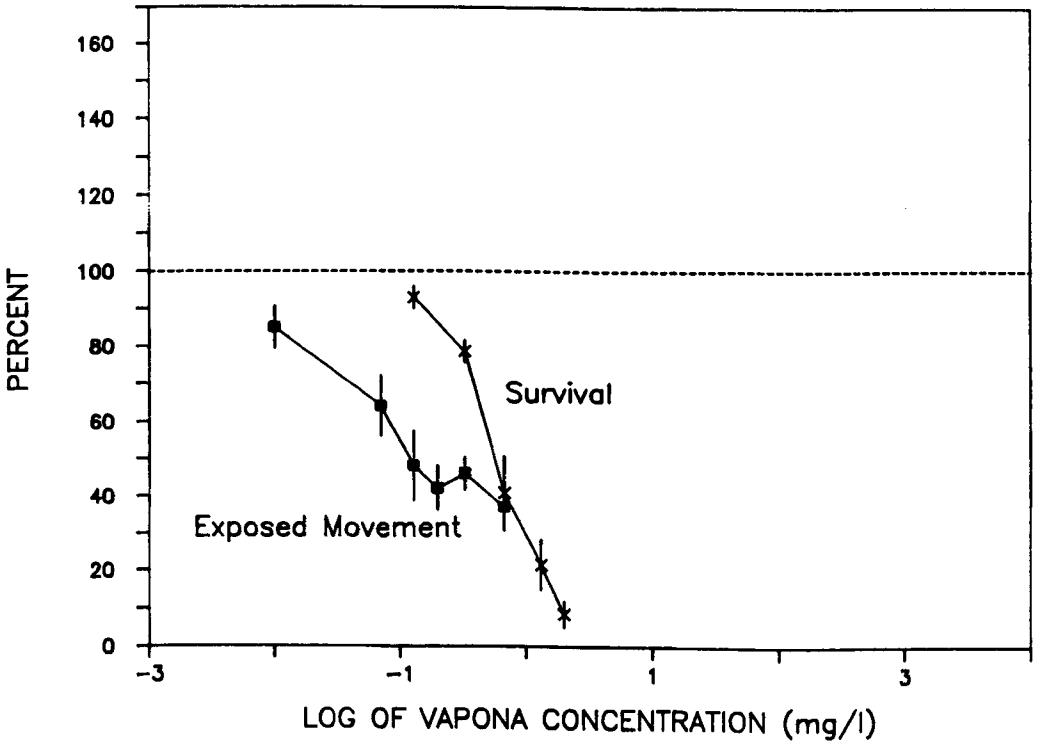

FIGURE 6. Behavioral changes and survival rates for *C. elegans* exposed to a vapona emulsion. Locomotion was found to be reduced at levels below the lethal range. Each point is the mean of 6 to 10 experiments, ±SE.

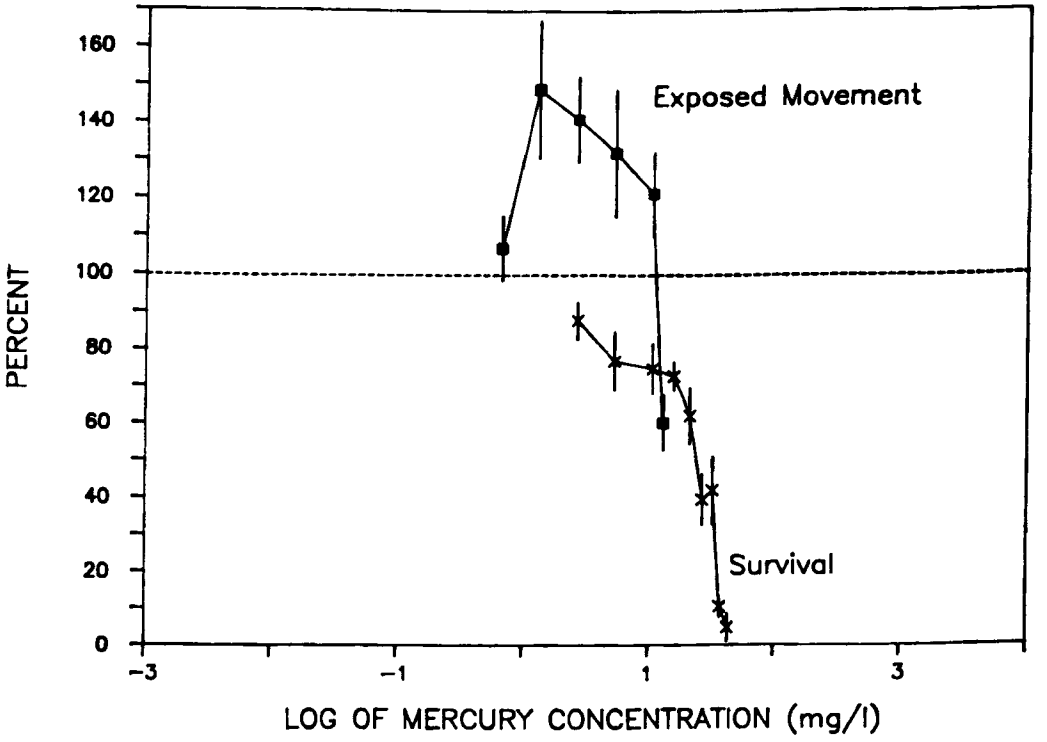

FIGURE 7. Behavioral changes and survival rates for *C. elegans* exposed to mercury ($HgCl_2$). At low exposures, hyperactivity is observed. As the concentrations increase, movement begins to drop in parallel with survival. Each point is the mean of 6 to 10 experiments, ±SE.

LC16 concentration, the value would vary considerably from 1 (Table 2). With typical neurotoxins that reduce activity (e.g., lead, malathion, vapona) the practical index would be greater than 1. With chemicals that have hyperactivity (e.g., mercury), the practical index would be less than 1.

Although much additional testing is needed on a wider range of chemicals, the present data suggest this procedure may be useful in screening for neurotoxins. It appears that exposures of *C. elegans* to neurotoxins result in behavioral changes at concentrations below the significant lethality range. Once the lethality range is entered, the behavioral changes become less useful as an indicator of neurotoxicity. So, as a working hypothesis, it is proposed that in using this procedure, if the LC16 is greater than the BC50, the substance has a greater likelihood of being neurotoxic than if the BC50 is greater than the LC16.

In addition to testing more chemicals (e.g., more organics), the evaluation of other behavioral changes in *C. elegans* is planned. This will include measuring behavioral responses of the nematode to sensory stimuli after exposure to various

TABLE 2
Comparison of *C. elegans* Lethality Levels to Levels for Changes in Behavior

	Neurotoxicological Indices			
Chemical	**LC16/BC50**	**LC16/BC16**	**LC50/BC50**	**Practical Index**
Copper	0.38	1.15	1.21	1.02
Beryllium	0.87	2.60	3.33	1.50
Lead	1.83	9.59	3.66	2.23
Malathion	1.36	8.71	27.86	1.45
Vapona	1.28	23.00	51.00	2.00
Mercury	6.25	7.35	20.00	0.63

Note: The Practical Index is equal to 0.84 multiplied by the rate of movement of the vehicle control animals divided by the rate of movement of the animals exposed to a concentration equal to the LC16. In this approach, the index will equal 1 if movement directly follows survival.

toxicants. Previous studies have reported the normal behavior of *C. elegans* to controlled chemical stimulation (Dusenbery, 1980b,c; Dusenbery, 1985a,b). It is thought that measurements of this more complex behavior may prove more sensitive as an indicator of neurotoxicity than unstimulated movement.

Another advantage of using *C. elegans*, is that it is the animal in which it is easiest to isolate mutations. In the long run, this makes toxicity testing with this organism attractive because mechanisms of toxicity could be studied by isolation of mutants with altered sensitivity to a toxicant, or by comparing toxicity among strains differing in genes with known functions in certain classes of toxicological response. For example, mutants resistant to cholinesterse inhibitors have been isolated (Rand and Russell, 1984) and could be used to confirm particular mechanisms of neurotoxicity.

A previous study has used a computerized video quantification system for behavioral toxicology (Miller et al., 1982). That research evaluated the behavior of an aquatic organism (a barnacle, *Balanus improvisus*) for adverse ecological implications. *C. elegans*, however, is easier to work with and is a genetically uniform organism which has been studied extensively. The computerized system that those investigators used required substantial data processing following the data collection. This included a manual quantitization via frame-by-frame analysis that was a lengthy and tedious process (Greaves and Wilson, 1980). In contrast, the system used in the present study is a real-time method that provides immediate results.

Goss and Sabourin (1985) reported 2 neurobehavioral studies using alternative species, one used either a crustacean (*Carcinus maenus*) or a mollusc (*Aplysia californicus*). The other approach used a type of flat worm (*Dugesia spp.*). Both approaches were relatively inexpensive (ranging from $1000 to $2150) and both took about 14 days to perform. With the crustacean and mollusc, avoidance,

escape reflexes, and success of a conditioned response were the endpoints. With the flat worm, motor behavior and pharynx protusions were evaluated. Both types of testing were performed by visual observations. Also, none of these species has the extensive basic biological information (including behavior) that is known for *C. elegans*. This fact, coupled with the computer tracking method, may prove *C. elegans* to be a more appropriate model for neurotoxicity.

The National Research Council (1977, 1984) has published reference protocols for neurobehavioral-toxicity tests. These protocols suggest such testing contain studies on function (both conditioned and unconditioned behavior) and morphology (neuropathology). For unconditioned behavior, the protocols recommend measuring spontaneous motor activity (SMA). For conditioned behavior, they suggest that some type of schedule-controlled response be used. For morphologic changes in the nervous system, the tissue must be prepared (following the exposure) and microscopically evaluated for changes. With all tests, dose-response data should be generated and the neurobehavioral responses should be observed below other types of toxicity. The testing in the study reported here closely followed the requirements of the protocol for evaluating SMA.

Behavioral screening tests using mammals have been proposed (Reiter et al., 1981). These methods, however, are expensive and highly labor intensive. Considering the increasing costs of animal handling and the rising social concerns over the use of mammals in toxicological studies (Holden, 1986), a test such as described in this report should be useful as the first tier for neurotoxicity screening. In using this system, it is estimated that with an initial investment of less than $15,000 for equipment, the total cost (including overhead, supplies, and labor) for the testing of a chemical for both survival and behavioral effects is less than $1,000. If the practical neurotoxicity index (i.e., behavioral changes at the LC16 concentration) can be performed, the total cost for the testing would be less than $400.

ACKNOWLEDGEMENTS

This work was supported in part by Grant SOH (AHR) 1 RO3 OHO2095 from the National Institute for Occupational Safety and Health.

REFERENCES

ANGER, K. (1984). Neurobehavioral testing of chemicals: impact on recommended standards. Neurobehav. Toxicol. Teratol. **6**:147–153.

BRENNER, S. (1974). The genetics of *Caenorhabditis elegans*. Genetics. **77**:71–94.

BUCKHOLTZ, N. and PANEM, S. (1986). Regulation and evolving science: neurobehavioral toxicology. Neurobehav. Toxicol. Teratol. **8**:89–96.

CHALFIE, M. and WHITE J. (1988). The nervous system. In: The Nematode *Caenorhabditis elegans* (W. Woods, ed.). Cold Spring Harbor Laboratory.

COX, G., KUSCH, M. and EDGAR, R. (1981). Cuticle of *Caenorhabditis elegans*: its isolation and partial characterization. J. Cell Biol. **90**:7–17.

DUSENBERY, D. (1980a). Behavior of free-living nematodes. In Nematodes as Model Biological Systems (B. Zuckerman, ed.). Academic Press, New York, NY.

DUSENBERY, D. (1980b). Responses of the Nematode *Caenorhabditis elegans* to controlled chemical stimulation. J. Comp. Physiol. **136**:327–331.

DUSENBERY, D. (1980c). Appetitive response of the nematode *Caenorhabditis elegans* to oxygen. J. Comp. Physiol. **136**:333–336.

DUSENBERY, D. (1985a). Using a microcomputer and video camera to simultaneously track 25 animals. Comp. Biol. Med. **15**:169–175.

DUSENBERY, D. (1985b). Video camera-computer tracking of nematode *Caenorhabditis elegans* to record behavioral responses. J. Chem. Ecol. **1**:1239–1247.

FRIBERG, L. (1977). Toxicology of Metals, Volume II. USEPA, Research Triangle Park, N.C.

GOSS, L. and SABOURIN, T. (1985). Utilization of alternative species for toxicity testing: an overview. J. Appl. Toxicol. **5**:193–219.

GREAVES, J. and WILSON, R. (1980). Development of an Interactive System to Study Sub-Lethal Effects of Pollutants on the Behavior of Organisms. USEPA, Narragansett, R.I.

HOLDEN, C. (1986). A pivotal year for lab animal welfare. Science. **232**:147–150.

HORVITZ, M., CHALFIE, M., TRENT, C. and EVANS, P. (1982). Serotonin and octopamine in nematode *Caenorhabditis elegans*. Science **216**:1012–1014.

JAMES, R. (1985). General principles in toxicology. In: Industrial Toxicology: Safety and Health Practices in the Workplace (P. Williams and J. Burson, eds). Van Nostrand Reinhold, New York.

LITCHFIELD, J. and WILCOXON, F. (1949). A simplified method of evaluating dose-effect experiments. J. Pharmacol Exp. Ther. **96**:99–115.

MILLER, D., LANG, W., GREAVES, J. and WILSON, R. (1982). Investigations in aquatic behavioral toxicology using a computerized video quantification system. In: Aquatic Toxicology and Hazard Assessment (J. Peterson, R. Foster, and W. Bishop, eds.). American Society for Testing Materials, Washington, D.C.

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH. (1988). Proposed National Strategies for the Prevention of Leading Work-related Diseases and Injuries, Part 2. Association of Schools of Public Health, Washington, D.C.

NATIONAL RESEARCH COUNCIL. (1977). Principles and Procedures for Evaluating the Toxicity of Household Substances. National Academy of Sciences, Washington, D.C.

NATIONAL RESEARCH COUNCIL. (1984). Toxicity Testing: Strategies to Determine Needs and Priorities. National Academy Press, Washington, D.C.

NORTON, S. (1978). Is behavior or morphology a more sensitive indicator of central nervous system toxicity? Environ. Hlth. Persp. **26**:21–27.

PLINE, M. and DUSENBERY, D. (1987). Responses of plant-parasitic nematode (*Meloidogyne incognita*) to carbon dioxide determined by video camera-computer tracking. J. Chem. Ecol. **13**:873–888.

POPHAM, J. and WEBSTER, J. (1982). Ultrastructural changes in *Caenorhabditis elegans* (nematoda) caused by toxic levels of mercury and silver. Ecotoxicol. Environ. Safety. **6**:183–189.

POUNDS, J. (1985). The toxic effects of metals. In: Industrial Toxicology: Safety and Health Applications in the Workplace (P. Williams and J. Burson, eds.). Van Nostrand Reinhold, New York.

REITER, L., MACPHAIL, R., RUPPERT, P. and ECKERMAN, D. (1981). Animal models of toxicity: some comparative data on the sensitivity of behavioral tests. Proc. 11th Conf. Environ. Toxicol. 11–23.

RAND, J. and RUSSELL, R. (1984). Choline acetyltransferase-deficient mutants of the nematode *Caenorhabditis elegans*. Genetics. **106**:227–248.

STRETTON, A., FISHPOOL, R., SOUTHGATE, M., DONMOYER, J., WALROND, J., MOSES, J. and KASS, I. (1978). Structure and physiological activity of the motoneurones of the nematode *Ascaris*. Proc. Natl. Acad. Sci. USA **75**:3495–3497.

SULSTON, J., DEW, M. and BRENNER, S. (1975). Dopaminergic neurons in the nematode *Caenorhabditis elegans*. J. Comp. Neur. **163**:215–226.

TALLARIDA, R. and MURRAY, R. (1981). Manual of Pharmacologic Calculations. Springer-Verlag, New York.

TILSON, H. and CABE, P. (1978). Strategy for the assessment of neurobehavioral consequences of environmental factors. Environ. Hlth. Persp. **26**:287–299.

WHITE, J., SOUTHGATE, E., THOMPSON, J. and BRENNER, S. (1986). The structure of the nervous system of the nematode *Caenorhabditis elegans*. Phil. Trans. R. Soc. Lond. **314B**:1–340.

WILLIAMS, P. and DUSENBERY, D. (1987). Screening test for neurotoxins using *Caenorhabditis elegans*. In: Model Systems in Neurotoxicology: Alternative Approaches to Animal Testing (A. Shahar and A. Goldberg, eds.). Alan R. Liss, New York.

WILLIAMS P. and DUSENBERY, D. (1988). Using the nematode *Caenorhabditis elegans* to predict mammalian acute lethality to metallic salts. Toxicol. Ind. Hlth. **4**:469–478.

WINDHOLZ, M. (1976). The Merck Index, 9th Ed. Merck & Co., Rahway, NJ.