THE EFFECT OF SUBLETHAL CONCENTRATIONS OF SELECTED TOXICANTS ON THE NEGATIVE PHOTOTACTIC RESPONSE OF *DUGESIA TIGRINA*

by

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Dugesia tigrina, 13 X

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INTRODUCTION

The growing concern for a healthful environment for man and other organisms has led to the development of air and water standards in order to preserve and/or improve it. This has taken the form of governmental regulation and restriction on the disposal of substances into the air, freshwater, and the oceans by various industries. How restrictive these standards should be is a problem. Should all of a "polluting" substance be eliminated or should a given amount be allowed in the environment and should that amount be related to the water quality, e.g., the hardness or pH of the water?

In considering this issue we should not lose sight of the fact that certain substances, e.g., heavy metals, which may be toxic in high concentrations also may be essential for the proper functioning of an organism's body in low or trace amounts (Prosser, 1973). The only known essential metal in human nutrition fifty years ago was iron. Today, we know that, among others, zinc, nickel, cadmium, copper, and chromium are also essential. This need applies to other organisms as well (Prosser, 1973). Not only do we need to know what metals are essential, but also, the nature of their biological roles (Vallee, 1971).

For freshwater it has been the policy to allow a

given amount of pollutant to be returned to the environment. The criteria were set by EPA (under directions from Congress) according to the quality of the receiving water (National Academy of Sciences, 1972). Public Law 92-500 states that point source effluent standards must be completely met by July 1, 1983. This means that by 1983 there are maximum amounts of polluting substances that may be in an effluent discharged into a freshwater source by an industry or municipality. These standards may be altered in a given area in order to maintain the water quality of the region.

Most of the work on determining 'safe' levels of toxicants has involved using fish as the test organism in developing application factors. "The application factor provides a way of predicting a safe level which is not known from a median lethal concentration which is known. The LC_{50} is multiplied by an application factor...to obtain a concentration which presumably has no sublethal or chronic effects" (Sprague, 1971). Eaton (1973) reviewed this work on application factors and emphasized that "we do not know how well these ideas might apply to the estimation of 'safe' levels for various groups of aquatic invertebrates."

To date a great deal of work has been done to develop methods of detecting when harmful substances, i.e., pollutants or toxicants, are released to the environment. In general acute tests (96 hours or less) are conducted to determine the toxicity of various compounds (heavy metals,

detergents, pesticides, oils, and other organic compounds) to organisms in the environment. Different species are used: birds, rats, and mice for gaseous substances; marine invertebrates and fish for pollutants in the ocean; fish and a few invertebrate species when testing freshwater for pollutants.

The effects a toxicant may have on fish are as follows (Stephan and Mount, 1973):

> "Direct effects are those caused only by a direct action of the toxic agent on fish. Indirect effects are those caused by an action of the toxic agent on something other than fish which, in turn, causes an effect on fish....Induced effects are those brought about by a direct action of the toxic agent on fish but which can only occur in the presence of another agent."

All of these effects could be noted in the way an animal reacts to pollutants in its environment, i.e., its survival, growth, reproduction, and metabolism.

In the early 60's a few investigators began to consider what effects sublethal concentrations of toxicants might have on the environment. Crandall and Goodnight (1963) noted histological changes in the body tissues of guppies exposed to low sublethal concentrations of several toxicants (lead, zinc, and sodium pentachlorophenate). Sprague (1971) reviewed the literature on the work concerning sublethal effects of toxicants on aquatic life. He cautioned researchers to be sure that changes to or within an organism were "ecologically meaningful, i.e., whether

they reduce the chances of the animal to be successful in its environment".

There are several lines of research that could be conducted on the effects of sublethal concentrations of toxicants. One approach is to study the effects chronic exposure to a toxicant has on the survival, growth, reproduction, and metabolism of the test organism. If a fish is used, this takes considerable time because fish have long generation times in relation to the persistence of the pollutant in the water. Another approach might be to study the long term effects of toxicants on aquatic organisms that are exposed to the toxicant for only a short time. Such a brief exposure could result in the annihilation of a species in a given area long after the pollutant is gone from that area (LaRoche, 1972). A third approach is to consider the effect a sublethal concentration of a toxicant has on the behavior of an organism. Changes in behavior may be so drastic, such as interruptions in mating or escape behavior, that the species can no longer survive in that particular habitat. Or they may be more subtle, such as alterations in predation rates, so that the population or community structure is affected.

The purpose of this study was to determine what effect sublethal concentrations of selected toxicants might have on the negative phototactic response of the planarian, Dugesia tigrina. This response tends to keep the animal in

darker areas of its environment where there is less likli-hood of exposure to direct sunlight and/or ultraviolet light. It has been shown that even short exposure to the above rays is fatal to planarians (Hyman, 1951).

LITERATURE REVIEW

Behavioral Studies on Planarians

Dugesia tigrina has a well-developed nervous system composed of paired ganglia in the head region, the brain, and two ventral longitudinal nerve cords connected by transverse nerves. In the anterior region the muscle and neural tissues are very closely connected (Corning and Kelly, 1973). There are numerous peripheral nerves to various sensory 'organs' and muscles in the rest of the body. Removal of the head does not affect coordinated movements in planarians. This tends to indicate that the brain serves as a sensory relay station receiving stimuli and transmitting the information to other parts of the body.

Planarians have sensory cells for perception of their environment. Tactile receptors are located over the entire surface of the body; there are a greater number of them along the lateral margins of the body and on the auricles. Also in the auricular region of the head are ciliated pits or grooves of epithelial tissue specialized for the detection of dissolved substances in the water. For body orientation and detection of gravity, planarians have statocysts located near the brain.

Planarians show a general body sensitivity to light but the exact structures involved have not been identified (Hyman, 1951). When these structures are stimulated, the animal responds with klinokinetic movements (undirected

movements in response to the intensity of a stimulus) to avoid the light (Hyman, 1951). Nonimage-forming eyes containing photoreceptive cells that function in light-dark discrimination are located in the head region.

Gliding movements of planarians are produced by the beating of cilia over the mucous secretion or slime laid down by the ventral tissues of the animals. The slime lubricates and smooths the substratum for the animal (Hyman, 1951).

Planarians are basically stimulus-response systems and their responses to light, currents, and chemicals are stereotyped. Dugesia tigrina exhibits a negative phototaxis. Even with both eyes removed the animal is stimulated by an increase in light intensity, but can no longer orient away from the source of light as before. McConnell (1967) found that reactions to light decreased when any planarian slime was present; no explanation was given for this decreased response. Planarians also respond with respect to "odor" or chemical stimuli.

A planarian moving in the periphery of an area with light or chemical stimulation present will use klinotaxis as its sampling method - successive sampling of the environment with bending from side to side. Then ... "as it draws nearer the source the gradient steepens, its behavior may change to tropotaxis and it will cease to bend from side to side" (Carthy, 1971).

Learning studies using planarians have presented some controversy (Corning and Kelly, 1973). The controversy developed when Thompson and McConnell (1955) reported classical conditioning of D. dorotocephala using light and shock as the conditioned stimulus and the unconditioned stimulus, respectively.

Halas and his associates tried unsuccessfully to duplicate Thompson's and McConnell's results using different techniques and a different species (Halas, et al., 1961, 1962; James and Halas, 1964). From this work they concluded that planarians could not be classically conditioned. Several other researchers (Bennett and Calvin, 1964; Brown, 1967) also failed to show conditioning in planaria.

Jensen (1965) used these negative studies to attack the work of McConnell and others. He tried to " 'explain' away all planarian learning demonstrations" (Corning and Kelly, 1973). Most of his objections were refuted by data already available. The rebuttal came when several investigators showed that learning did occur in planarians. Jacobson (1967) used classical conditioning to show learning and Wells (1967) used a van Oye maze which serves as housing and test chamber to show simple conditioning. Since that time planarians have demonstrated habituation, classical conditioning, differential classical conditioning, avoidance learning, and operant training (Corning and Kelly, 1973).

Toxicity Studies

The toxic effects of metals on aquatic life have been studied for a large part of this century. Heavy metals are found in mining wastes, brine from oil wells, and in effluents from chemical and metal-processing plants (Doudoroff and Katz, 1953). The toxicity studies on heavy metals have been reviewed several times (Doudoroff and Katz, 1953; Skidmore, 1964; Bryan, 1971; McKim et al., 1975).

Jones (1937, 1940) used a planarian, Polycelis nigra, in acute bioassays using heavy metals. He found that the toxicity of a metal was related to its solution pressure - the more positive the solution pressure the more toxic a metal would be because it would tend to combine with chemicals in protoplasm more easily. Jones' order of toxicity for a select number of the 18 metals used is: mercury > silver > copper > cadmium > zinc > nickel > chromium > cobalt > lead. The heavy metals were just as toxic to Polycelis nigra as to fish (Jones, 1940).

For marine organisms in general it has been determined that the order of toxicity of heavy metals is: mercury > silver > copper > cadmium > zinc > lead > chromium > nickel > cobalt (Bryan, 1971). The lists compiled by Jones and Bryan differ in the ordering of the last four metals. It may be that the lower solution pressures produce variability in toxicity depending on the water quality.

Anderson (1944) found that the heavy metals were

just as toxic or more toxic to Daphnia magna as to fish. Dowden and Bennett (1965) used several invertebrates, frog eggs, and three species of fish to determine the toxicity of 86 chemicals to aquatic life. Patrick et al. (1968) showed that zinc and hexavalent chromium were more toxic to the snail, Physa heterostropha, than to the bluegill when tested under the same conditions. Sullivan (1973) found that zinc, cadmium, and hexavalent chromium were more toxic, that copper was just as toxic, and that lead was less toxic to the rotifer, Philodina sp., than to the bluegill.

Studies on Daphnia magna have shown that heavy metals affect the survival, growth, reproduction, and metabolism of the animal under acute and chronic exposure (Biesinger and Christensen, 1972). Thorp and Lake (1974) used five freshwater invertebrates in their studies on cadmium and zinc. They found that the species tested had a wide range of sensitivities to cadmium. In combination with zinc the effects were only additive.

The addition of organic compounds to the aquatic environment has had varied results. The use of alkyl benzene sulfonate (ABS) in detergents has been curbed since its deleterious effects were discovered. Laboratory studies on the effects of ABS on mayfly, nymphs, Hydropsychidae larvae, crayfish, freshwater shrimp, and sowbugs showed that in general the number of animals was greatly reduced depending on the concentration of the toxicant (Surber and Thatcher,

1963).

Eisler et al. (1972) used three species of marine fish and eight marine invertebrate species to determine the toxicity of nitrilotriacetic acid (NTA) and NTA-containing detergents. They found NTA to be non-lethal at 20 °/oo salinity except at very high concentrations. NTA acted as a chelating agent in decreasing the toxicity of mercury and cadmium.

Holden (1972) reviewed the effects of various pesticides on aquatic life. In general the insecticides were more toxic than the herbicides. "Only diquat and dalapon are approved for use in fish-bearing waters in Britain, neither being particularly toxic to invertebrates" (Holden, 1972).

LaRoche et al. (1970) used two invertebrate species and one fish species to study the toxicity of oils and oil dispersants to marine life. They determined that crude oil is less toxic than refined oil. Tarzwell (1971) reviewed the literature on the toxicity of oil and oil dispersant mixtures to aquatic life. "Adverse effects of oil and its components in water may be of several kinds, among which are an adverse effect on some vital function..., the biological concentration of potential carcinogenic compounds in the flesh, or a change of chemotactic reaction to normal activity and development" (Tarzwell, 1971).

Sublethal Effects of Toxicants on Aquatic Life

Even though the importance of the effects of sublethal concentrations of toxicants has been known for several years (Cairns, 1966; Warner, 1967) very little work has been done concerning the behavioral changes that occur when animals are exposed to these sublethal concentrations. Lemke and Mount (1963) found that the growth rate of bluegills was reduced in 13 ppm ABS with the least effects at the lower concentrations. They found that ABS did not affect swimming performance and concluded that the toxicant did not affect organs involved in swimming endurance tests. At 3 ppm, juvenile bluegills showed no mortality in 30 days, and no apparent effects on swimming, growth, or the normal histology of the body tissues.

Cairns (1966) discussed the effects sublethal concentrations of toxicants on organisms in the aquatic environment. These included physiological changes, embryonic development, histological changes, disruptions in normal behavior patterns, and changes in community structure. He found that exposure to ABS reduced spawning in the American killifishes. The population structure of guppies was affected by the insecticide, dieldrin.

Foster et al. (1966) found that flagfish in various concentrations of ABS captured tubificid worms and spat them out repeatedly. At the highest concentration used, some worms were uneaten, while at lower concentrations the worms

were consumed in a progressively shorter time as the amount of toxicant decreased. They attributed this reaction to a destruction of the taste mechanism.

LaRoche (1972) used the killifish for studies on the long range effects of short-term exposure to zinc, cadmium, and copper. He concluded that even short-term exposures could drastically affect the killifish. Pickering (1974) found that nickel levels at or below 0.38 mg/l do not affect the survival, growth, and reproduction of fathead minnows.

Warner (1967) briefly reviewed some work done by Kaminski on the use of various behavioral responses of an oligachaete, a mollusc, an isopod, and a fish in order to provide a "multidimensional picture of toxicant effects". Warner advocated using simultaneous behavior parameters in fish to measure toxicant effects. One conclusion he drew was that a "quantified behavioral change is the most sensitive indicator yet developed of a toxicant induced change in living systems".

Sprague (1971) reviewed the work done on measuring sublethal toxicity. He covered some of the recent trends and discussed the usefulness of such studies in relation to "anti-pollution efforts". Arthur and Leonard (1970) subjected two species of snails and an amphipod to six weeks of copper stress and measured effects on survival, growth, feeding, and reproduction of the animals. They concluded that the species used were more sensitive than fish to copper

stress.

MacInnes and Thuberg (1973) found that mud snails exposed to sublethal concentrations of certain toxicants showed altered oxygen consumption rates and exhibited "distressed or retracted behavior". Very low dichromate concentrations had no effect on survival of Daphnia, but there may have been an increase in oxygen consumption (Sherr and Armitage, 1973).

Parsons (1972) reported that the activity (crawling or attachment to the substrate) of the winkle, Littorina, was inhibited by refinery effluent whether it was "attached to or detached from its substrate". The animals recovered when transferred to sea water.

Kittredge et al. (1974) used behavioral responses of a crab and an oyster as indicators of oil pollution. They found that the water soluble fraction of crude oil completely inhibited the response of the male crab to sex pheromone secreted by the female. In oysters the cilia on the gills altered their beating rate when exposed to naphthalene; oral groove (food) transport increased while pumping rate (oxygen) decreased to about 10% of the control rate.

MATERIALS AND METHODS

Physical Surroundings

Because it has been determined that planarians do not respond to red wavelengths (Jenkins, 1967), all experiments were conducted in a dark room with a red light (600 millimicrons, 1.4 microwatts maximum intensity as determined by an Isco Model SR Spectroradiometer) as the source of background illumination. A Tempscribe (Bacharach Instrument Company, Pittsburgh) was used to continuously record room temperature (accurate to 1.0 C).

Dilution Water

A soft dilution water (modified from Cairns and Sheier, 1957) was used for all of the experiments. The dilution water was prepared from glass-distilled water and A.S.C. grade chemicals. Each of the first five chemicals (Table 1) was measured from a concentrated (200X) stock solution directly into the distilled water. The last three chemicals were weighed out directly and added to the distilled water. The volume prepared each time was 20 liters. The dilution water was bubbled with CO_2 until the calcium and magnesium salts were dissolved. It was then bubbled with air at least 12 hours to raise the pH and oxygenate the water.

The desired characteristics of the water were a pH of 7.5, an alkalinity of 35 ppm $CaCO_3$, and a total hardness of 40 ppm $CaCO_3$. The dilution water was analyzed before each experiment for these characteristics (Table 2). Total

Table 1. Chemical composition of the soft dilution water.*

Compound	Concentration (g/l)
$NaHCO_3$	2.30×10^{-2}
NH_4NO_3	3.60×10^{-3}
KCl	1.46×10^{-2}
H_2SiO_3	1.00×10^{-3}
Fe citrate	2.00×10^{-5}
$CaCO_3$	2.00×10^{-2}
$CaSO_4$	3.44×10^{-2}
$MgSO_4 \cdot 7H_2O$	2.46×10^{-2}

* See text for preparation of the dilution water.

Table 2. Chemical analyses of dilution water.

Experiment	pH units	Alkalinity (ppm $CaCO_3$)	Total hardness (ppm $CaCO_3$)
Zinc	7.1 - 7.9	34 - 38	37.6
Nickel	7.1 - 7.8	32 - 36	28.9
Cadmium	7.1 - 7.9	34 - 38	38.1
Copper	7.1 - 7.8	33 - 36	31.6
Chromium	7.1 - 7.9	34 - 37	33.1
Diquat	7.1 - 7.9	34 - 38	37.3
ABS	7.1 - 7.9	34 - 39	40.4

hardness of the dilution water was obtained by calculation (APHA, 1971) after measuring the amount of calcium and magnesium present with a Unicam SP 90 atomic absorption spectrophotometer. The variations in hardness were caused by undissolved calcium and magnesium salts.

Animals

Dugesia tigrina were purchased from Carolina Biological Supply Company (Burlington, North Carolina). Approximately 130 animals were placed in a 14 (W) x 24 (L) x 16 (H) cm all-glass aquarium with one liter of the soft dilution water.

The aquarium was covered and aerated. The animals were kept in total darkness at 23 ± 1 C for at least five days before an experiment was conducted to maximize the response to light. The animals were not fed during holding or experimentation because it has been shown that feeding can alter the toxicity of toxicants to organisms (Sprague, 1973).

Preparation of Test Solutions

A freshly prepared solution (100 ppm of metal or organic toxicant) of each toxicant was diluted to obtain the concentrations required in all experiments. The toxicants used were: zinc, nickel, cadmium, copper, chromium, diquat, and alkyl benzene sulfonate (ABS).

LC_{50} Studies

A 96-hour LC_{50} for each toxicant was obtained before

determining what sublethal concentrations to use in the light response studies. Concentrations used in these studies were chosen arbitrarily after preliminary work indicated the range of toxicity (Table 3).

Test chambers for the acute bioassays were 10.5 cm soft glass culture dishes covered with plastic wrap to reduce evaporation. Five animals were placed in each dish along with 50 ml of the appropriate toxicant concentration. Tests were conducted in duplicate. The number of animals living at the end of 24, 48 and 96 hours was determined by touching the animals to see if they moved. Observations were made to see if animals responded to probing only and to see if head regression occurred in the animals. The LC_{50} values were determined by graphical interpolation of the data (APHA, 1971).

Light Response Studies

The light response studies were conducted using sublethal concentrations of the seven toxicants. The amount of metal used was verified by using an atomic absorption spectrophotometer (Table 4).

Animals were divided into 6 groups of 15 animals each and held in dilution water or toxicant solution 24, 48, or 96 hours before being tested in a given solution (Table 5). Groups 1, 2, and 3 were controls for groups 4, 5, and 6 respectively.

Test chambers for each group were constructed from

Table 3. Toxicant salt and concentrations used for LC_{50} studies.

Toxicant	mg of cpd/l for 100 ppm solution of toxicant	concentrations used in ppm of metal or toxicant
Zinc as $ZnSO_4 \cdot 7H_2O$	440.0	21.90, 15.46, 9.72, 5.0*, 0
Nickel as $NiSO_4 \cdot 6H_2O$	477.7	20.80, 11.24, 5.64, 2.00, 1.06, 0.64, 0
Cadmium as $3CdSO_4 \cdot 8H_2O$	228.2	108.00, 11.80, 5.33, 2.70, 2.10, 1.71, 1.10, 0.53, 0
Copper as $CuSO_4 \cdot 5H_2O$	392.8	10.00, 9.36, 1.59, 1.12, 1.00, 0
Chromium as $K_2Cr_2O_7$	282.8	9.36, 5.42, 2.24, 1.35, 0.67, 0
Diquat as diquat dibromide (239.7g cation/liter)	493.0**	100*, 50*, 10*, 5*, 2*, 1*, 0
Alkyl benzene sulfonate (48.7% ABS)	205.3	100*, 15*, 10*, 5*, 1*, 0

* calculated; obtained by serial dilution
** liquid weight

Table 4. Concentrations of toxicants used for light response studies.

Toxicant	Sublethal concentrations in ppm of toxicant
Zinc	2.04, 1.48, 1.00, 0.44
Nickel	1.5*, 1.0*, 0.5*
Cadmium	0.94, 0.66, 0.32
Copper	0.60, 0.50, 0.20
Chromium	1.59, 1.25, 0.53
Diquat	3.0*, 1.0*
ABS	3.0*, 1.0*

* Calculated; obtained by serial dilution.

Table 5. Experimental conditions under which animals in each group were held and tested for light response studies.

Group	Holding Solution	Test Solution
1	dilution water	same dilution water
2	dilution water	fresh dilution water
3	dilution water	fresh toxicant water
4	toxicant solution	same toxicant solution
5	toxicant solution	fresh toxicant solution
6	toxicant solution	fresh dilution water

38 mm o.d. acrylic tubing split lengthwise then cut to appropriate lengths (Figure 1). The ends were small flat pieces of plexiglass attached by silicone sealer. The light source was a 50 amp tungsten flashlight bulb (450 + millimicrons, 0.25 microwatts maximum intensity) connected to house current via a transformer. The light source was placed 0.6 cm above the bottom of the test chamber which produced a 1.5 cm circle of light. An exposure of 8 minutes resulted in a maximum change of 0.5 C in the water temperature in the test chamber. There are no reported studies to indicate whether this slight change in temperature of the water would affect crawling speed in planarians.

A distance of 5 cm from the center of the light was marked off on each end of the test chamber to serve as a guide for the distance each planarian would be required to travel (Figure 1). This distance was chosen after a series of preliminary tests showed that the animals wandered aimlessly at distances greater than 5 cm away from the light. It appears that 5 cm is the maximum distance of penetration of light rays from reflection and refraction of the incident rays of light.

For each group, five animals were placed in 50 ml of the appropriate holding solution in a 10.5 cm culture dish covered with plastic wrap to reduce evaporation. At the time of testing there were sometimes only 3 or 4 animals in a culture dish. Cannibalism could account for the disappear-

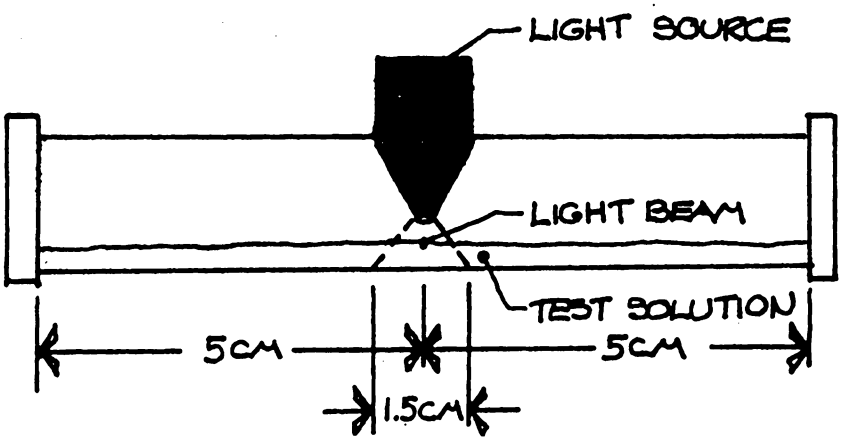

Figure 1. Test chamber for light response studies.

ance of the animals. Different animals were used for each time period (24, 48, and 96 hours).

On the day of testing, an animal was transferred with a camel's hair brush to a test chamber containing 3.5 ml of the appropriate solution (Table 5). The light source was turned on and the time required for the planarian to move 5 cm away from the center of the light was recorded. If an animal stayed in the 1.5 cm circle of light for 3 minutes, it was recorded as no response. If an animal took longer than 5 minutes to travel 5 cm, it was recorded as no response. These two criteria of no response were arbitrarily chosen after several preliminary experiments showed that if an animal had not responded within these time limits it was not going to respond at all.

After an animal was tested it was placed in a test tube with members of its own group for that toxicant concentration and time period for later analyses. The test chamber was cleaned with a Kimwipe to remove any slime present, because it has been shown that slime makes it easier for an animal to move on a surface (Jenkins, 1967) and because it has been shown that the response to light is reduced in the presence of slime (McConnell, 1967). No reason was given for this reduced response to light in the presence of slime. Another 3.5 ml of the test solution were added to the test chamber and the next animal was tested.

A random number table was used to determine the order

for the testing of the groups. Animals within a group were randomly selected by reaching into the culture dish and withdrawing an animal.

For the metals used samples of the holding solution were saved to be analyzed for the amount of metal remaining in solution. The culture dishes were emptied; then 5 ml of 0.2 N HNO_3 were added to each and allowed to sit for 10 minutes to dissolve any metal that might be present in the slime left by the animals or that had plated out onto the glass. The acid was placed in test tubes; each dish was rinsed with 5 ml of distilled water which was then added to the proper tube of acid. The samples were analyzed to determine the concentration of metal present in the slime.

For analyses of the data, a square root transformation was used to normalize the data (Sokal and Rohlf, 1969). A t-test was used for comparing a treatment group with its control. A two-way anova for each time was used to check for effects of concentration and for group-toxicant interaction. A three-way anova was used to check for effects of time on toxicity of the substances used (Sokal and Rohlf, 1969).

RESULTS AND DISCUSSION

LC_{50} Studies

Analyses of the solutions used in this series of experiments showed that the calcium and magnesium levels remained relatively constant over 96 hours (Table 6). The amount of metal present changed over time (Table 7). These changes could be due to: a) plating out of the metal onto the surface of the glass container; b) metal ions sticking to slime secreted by the planarians; c) metal ions being incorporated into the slime before it is secreted; or d) metal ions becoming part of the animal's body.

A plating out experiment showed: a) 15% of the zinc and 50% of the copper plated out; b) for nickel, cadmium, and chromium the amount of metal was relatively constant (within the limits of error for the machine).

Metal sticking to the secreted slime is a reasonable explanation because it has been shown that the mucus secreted by the gills of fish tends to bind with heavy metals that are present in the environment. This mucus is then sloughed off and it settles to the bottom of the stream or holding chamber; later some of the metal is released back into the environment (Carpenter, 1927). This may be the case with zinc in the present experiment (Table 7). Even subtracting 15 percent for plating, the zinc levels in the toxicant solution decreased drastically over 48 hours, but had increased by the end of 96 hours. The level of copper (Table 7) decreased drastically

Table 6. Amount of calcium and magnesium in dilution water for LC_{50} studies.

Experiment	Time							
	0 hour conc.(ppm)		24 hours conc.(ppm)		48 hours conc.(ppm)		96 hours conc.(ppm)	
	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg
I (zinc)	12.3	2.5	12.6	2.5	14.1	2.7	14.1	2.6
II (nickel)	8.0	2.5	8.9	2.7	9.4	2.6	9.1	2.7
III (cadmium)	11.8	2.8	12.7	2.7	13.0	2.7	14.5	2.8
IV (copper)	9.1	2.0	9.7	2.0	9.6	2.1	11.1	2.3
V (chromium)	12.0	2.2	11.8	2.4	13.1	2.7	*	*
VI (diquat)	11.3	2.4	10.6	2.5	*	*	11.7	3.1
VII (ABS)	12.0	2.9	11.1	2.9	12.0	2.9	10.7	2.7

* No reading.

Table 7. Amount of metal (ppm) remaining in solution for LC_{50} studies.

Metal	0 hour Concentration	24 hour Concentration	48 hour Concentration	96 hour Concentration
Zinc	21.90	14.50	13.20	15.00
	15.46	9.64	*	*
	9.72	6.42	5.86	6.68
	---	4.24	4.14	4.20
	0.00	0.00	0.12	0.12
Nickel	20.80	18.18	*	*
	11.24	10.12	*	*
	5.64	5.48	5.82	*
	2.00	2.28	2.18	2.44
	1.06	1.08	1.20	1.28
	0.64	0.60	0.66	0.88
	0.00	0.00	0.00	0.00
Cadmium	108.00	114.68	*	*
	11.80	13.78	11.13	*
	5.33	6.48	6.30	6.04
	2.70	3.13	3.27	3.23
	2.10	2.43	2.57	2.90
	1.71	1.64	1.69	1.73
	1.10	1.12	1.10	1.13
	0.53	0.67	0.53	0.61
	0.00	0.00	0.00	0.00
Copper	10.00	1.35	1.88	1.29
	9.36	1.12	1.53	1.88
	1.59	0.50	0.20	1.12
	1.12	0.50	0.70	0.50
	1.00	0.30	0.50	0.30
	0.04	0.08	0.20	0.00
Chromium	9.36	10.38	17.88	17.40
	5.42	5.19	5.66	9.70
	2.24	3.30	3.30	5.33
	1.35	1.32	1.87	2.16
	0.67	0.88	0.83	1.21
	0.00	0.00	0.00	0.00

* No animals surviving.

within 24 hours, but did not change significantly after that. Plating out could not account for all of this reduction. (See a later discussion on metals in slime).

Generally in the nickel and cadmium experiments the amounts of metal present at all time periods were not significantly different and the variations in concentrations could be within the error range of the atomic absorption spectrophotometer. For the chromium experiment the amount of metal present at all time periods were significantly higher than the introduced amounts. One possible explanation is leaching of chromium from the culture dishes used as holding chambers. However, this is unlikely because analyses of the control solutions from all time periods showed that chromium was not present. Another possibility is that under chromium stress the animals lose some of their body chromium to the environment. This may be the case considering that there is no other apparent source for the chromium (See later discussion).

No attempt was made to analyze the two organic compounds, diquat and ABS, during the experiments. The percent survival over time was used to determine LC_{50} values for each toxicant (Table 8). After 96 hours the order of toxicants, from most toxic to least toxic, was: copper > cadmium > nickel > zinc > ABS > chromium >> diquat. Results for the metals compare favorably with the results obtained by Jones (1940) for the planarian, Polycelis nigra. His order of toxicity

Table 8. LC_{50} values for toxicants in ppm toxicant.

Toxicant	Time 24 hours	48 hours	96 hours
Zinc	7.10	5.48	5.48
Nickel	7.70	3.30	2.55
Cadmium	23.30	6.75	2.20
Copper	10.00	9.36	1.77
Chromium	*	*	7.30
Diquat	*	*	18.30
ABS	8.17	7.00	7.00

* More than 50% survival.

was: copper > cadmium > zinc > nickel > chromium. The same order of toxicity was obtained for the amphipod, Gammarus pulex (Jones, 1937).

Biesinger and Christensen (1972) found the order of toxicity of the above heavy metals to Daphnia magna to be the same as that of Polycelis nigra. For the rotifer, Philodina acuticornis, "the most to least toxic of the metals (in ppm) tested in soft water were: cadmium, copper > zinc > chromium > nickel" (Sullivan, 1973). Considering the work of other researchers, it appears that heavy metals are just as toxic to Dugesia tigrina as to other invertebrates and that this species could be used as a test animal for acute bioassays with heavy metals.

Light Response Studies

In these studies for some of the toxicants, it was necessary to use very low sublethal concentrations because the animals, even though alive, would only respond when touched with a glass rod (as in 2.00 ppm nickel, 1.71 ppm cadmium, and 5.0 ppm ABS) or because regression of the head region occurred (as in 4.03 ppm zinc, 1.23 ppm cadmium, and 5.0 ppm diquat).

Analyses of the holding solutions showed that the relative amounts of calcium and magnesium present remained constant over 96 hours. For all of the experiments there was essentially no heavy metal present in the controls (Table 9). The concentration of heavy metal in the holding solutions of

Table 9. Average amount of metal (ppm) remaining in holding solution for groups 4, 5 and 6* as compared to their controls.

Metal	Concentration	Time 24 hours	48 hours	96 hours
Zinc	0.00	0.03	0.03	0.015
	0.44	0.30	0.29	0.31
	1.00	0.63	0.71	0.77
	1.48	1.06	1.26	1.09
	2.04	1.16	1.01	1.30
Nickel	a	a	a	a
Cadmium	0.00	0.0	0.0	0.0
	0.32	0.32	0.31	0.31
	0.66	0.65	0.65	0.65
	0.94	0.91	0.92	0.88
Copper	0.00	0.003	0.003	0.003
	0.20	0.12	0.16	0.13
	0.50	0.15	0.13	0.17
	0.60	0.66	0.37	0.18
Chromium	0.00	0.0	0.0	0.0
	0.53	0.57	0.59	0.69
	1.25	1.05	1.12	1.56
	1.59	2.05	1.93	1.80

* For explanation of groups refer to Table 5.
a Did not have nickel lamp for AA.

the experimental groups varied over time (Table 9). For zinc and copper the reduced amount of metal remaining in solution is not all accounted for by plating out of the metal. The concentration of metal appears to be related to the amount found in the slime. The amount of cadmium present stayed relatively constant over 96 hours.

Control groups for chromium showed that no chromium leached from the culture dishes. However, by the end of 96 hours the amount of chromium present was higher than the introduced amounts (30% greater at 0.53 ppm, 25% greater at 1.25 ppm, and 13% greater at 1.59 ppm). This increase supports the idea that under chromium stress Dugesia tigrina actually loses some of its body chromium to the environment. Chromium is found in high concentrations in nucleic acids (National Academy of Science, 1974). A breakdown in the cellular structure of part of the animal could release the chromium in the nucleic acids. It appears there is actual tissue damage under chromium stress.

Analyses of the slime showed quantitatively that the heavy metals were concentrated in the slime secreted by the animals under stress (Table 10). For the control groups (1, 2, and 3) no heavy metals were present in the slime except for the zinc experiments where the amount varied from 0.17 ppm to 0.21 ppm. This amount is very small when compared to the amount of zinc in the slime for the experimental groups (4, 5, and 6). The zinc could have leached from the

Table 10. Average amount of metal (ppm) in slime for groups 4, 5 and 6* as compared to their controls.

Metal	Concentration	Time 24 hours	48 hours	96 hours
Zinc	0.00	.18	.17	.21
	0.44	4.52	5.59	4.71
	1.00	4.21	4.80	6.37
	1.48	4.97	1.35	4.51
	2.04	10.55	9.02	11.48
Nickel		a	a	a
Cadmium	0.00	0	0	0
	0.32	0.30	0.46	0.53
	0.66	0.36	0.46	0.58
	0.94	0.39	0.59	0.60
Copper	0.00	0	0	0
	0.20	0.16	0.18	0.13
	0.50	0.74	0.80	0.72
	0.60	1.16	1.44	1.35
Chromium	0.00	0	0	0
	0.53	0.09	0.11	0.09
	1.25	0.22	0.17	0.25
	1.59	0.16	0.26	0.29

* For explanation of groups refer to Table 5.
a Did not have nickel lamp for AA.

soft glass culture dishes used as holding chambers. Or it may be that zinc is a normal constituent of the slime secreted by Dugesia tigrina.

The experimental groups showed a definite concentration of heavy metals in the slime. Whether this was a simple binding or metal and slime or whether the animals actually incorporated the metal ions into their slime is unknown. Since the amount of metal present changed over time for zinc and copper, it was probably a coagulation of the slime by the heavy metal. The slowly increasing amount of cadmium present over time suggests that this metal was actually incorporated into the slime when it was produced. Friberg et al. (1974) reported that dietary factors affected the absorption of cadmium in vertebrates, and a calcium deficiency or a low protein intake increased the uptake of cadmium. It is possible that cadmium was selectively absorbed by the animals as a substitute for calcium and then incorporated into the molecular structure of the slime. There was no way to deduce this because it is not even known if there is a change in the amount of slime produced let alone if substitutions occur at the molecular level.

For the light response studies it was found that 4.4% of all the animals would not respond to light. Of these no responses 6.8% were in group 1, 10.8% were in group 2, 10.8% were in group 3 (animals acutely exposed to toxicant), 23.0% were in group 4, 25.6% were in group 5, and 23.0% were

in group 6. Groups 1, 2, and 3 were not significantly different from each other but were significantly different from groups 4, 5, and 6. It appears that exposure to a toxicant over time results in damage to the animals so that they cannot crawl away from a beam of light.

If all groups were pooled for a toxicant, the percent of no response was: 3.9% for the zinc experiments, 2.7% for the nickel experiments, 10.6% for the cadmium experiments, 4.1% for the copper experiments, 4.0% for the chromium experiments, 3.4% for the diquat experiments, and 1.2% for the ABS experiments. The number of no responses were not significantly different for zinc, nickel, copper, chromium, and diquat. The number of no responses for cadmium and for ABS were significantly different from each other and from the other toxicants.

All animals in the two lower concentrations of zinc for all three time periods responded to the light. It appears that they are able to handle these concentrations of zinc so that their response to light is not inhibited. All animals in nickel and diquat for 24 hours responded to the light. It appears that the animals are able to cope with a short exposure to the toxicants but are less able to cope with longer exposures. All animals exposed to ABS for 48 and 96 hours responded to the light. It appears that the ABS is inactivated in some way so that all of the animals would at least respond to the light. For some of the toxicants

time of exposure to the toxicant affected the animals so that they could not move away from a beam of light.

For the crawling speeds of the animals, the time required to travel 5 cm was converted to crawling speed in cm/sec x 10^{-2}.

The mean crawling speed for each group was computed along with standard error and confidence intervals (Figures 2 to 8). The results give an overall view of what happened to the animals under stress.

The value for control group 1 is a composite for all c-1s across concentrations for a given time period and serves as a control for group 4; the value for control group 2 (c-2) is a composite of all c-2s across concentrations for a given time period and serves as a control for group 5; the value for control group 3 (c-3) is a composite of all c-3s across concentrations for a given time period and serves as a control for group 6. A priori, it was thought that these controls would differ significantly from their respective treatment groups at different concentrations and time periods. Also, a significant difference between group 2 and group 6 would tend to indicate that the toxicant was actually affecting the animal in some way instead of the effect being due to fresh dilution water alone. A t-test was the statistical test used (Table 11).

For zinc the only significant difference in groups occurred in 48 hours between groups 3 and 6 at the highest

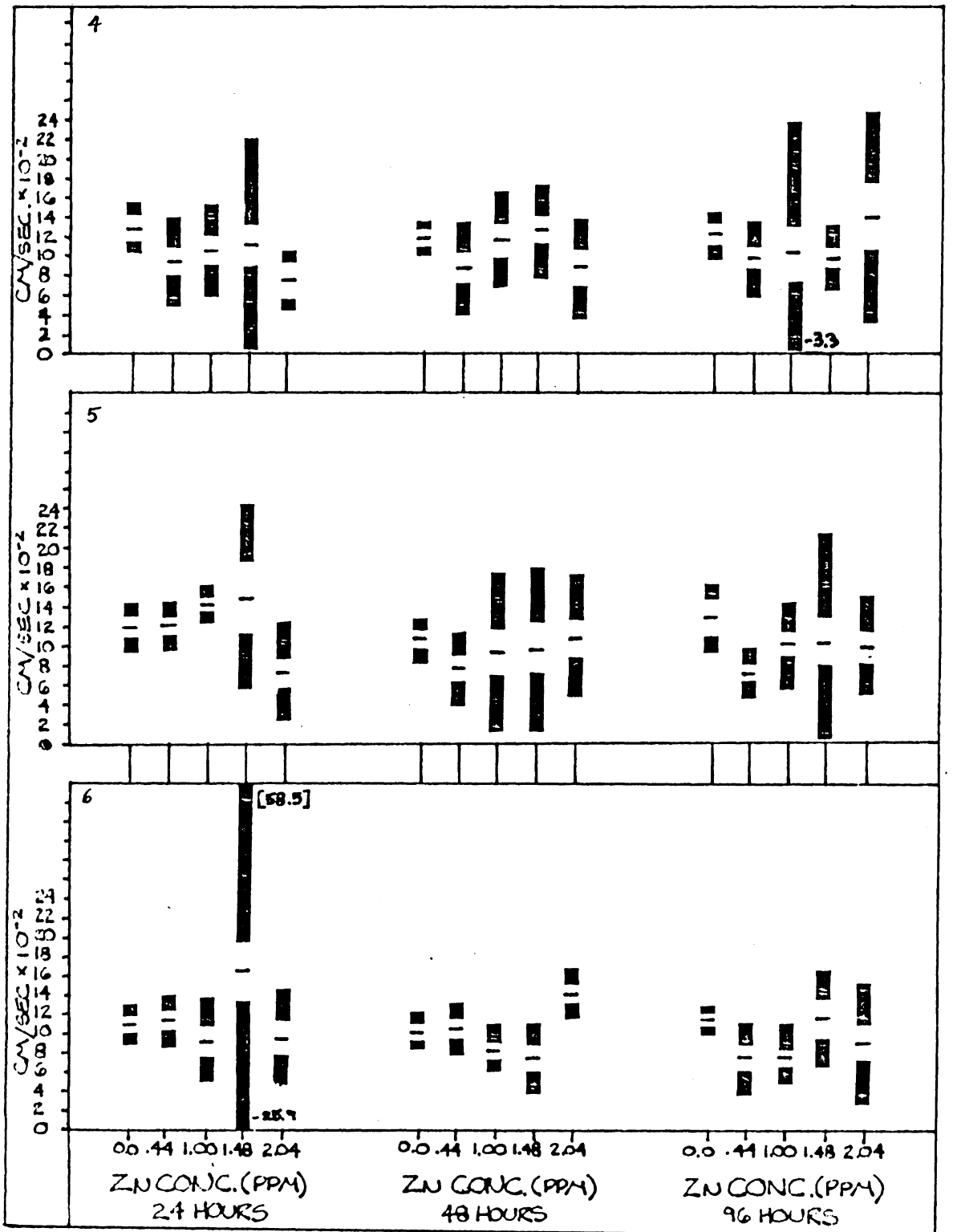

Figure 2. Mean crawling speeds for groups 4, 5, and 6 under zinc stress as compared to their controls with standard error (I I) and confidence intervals ( ) shown.

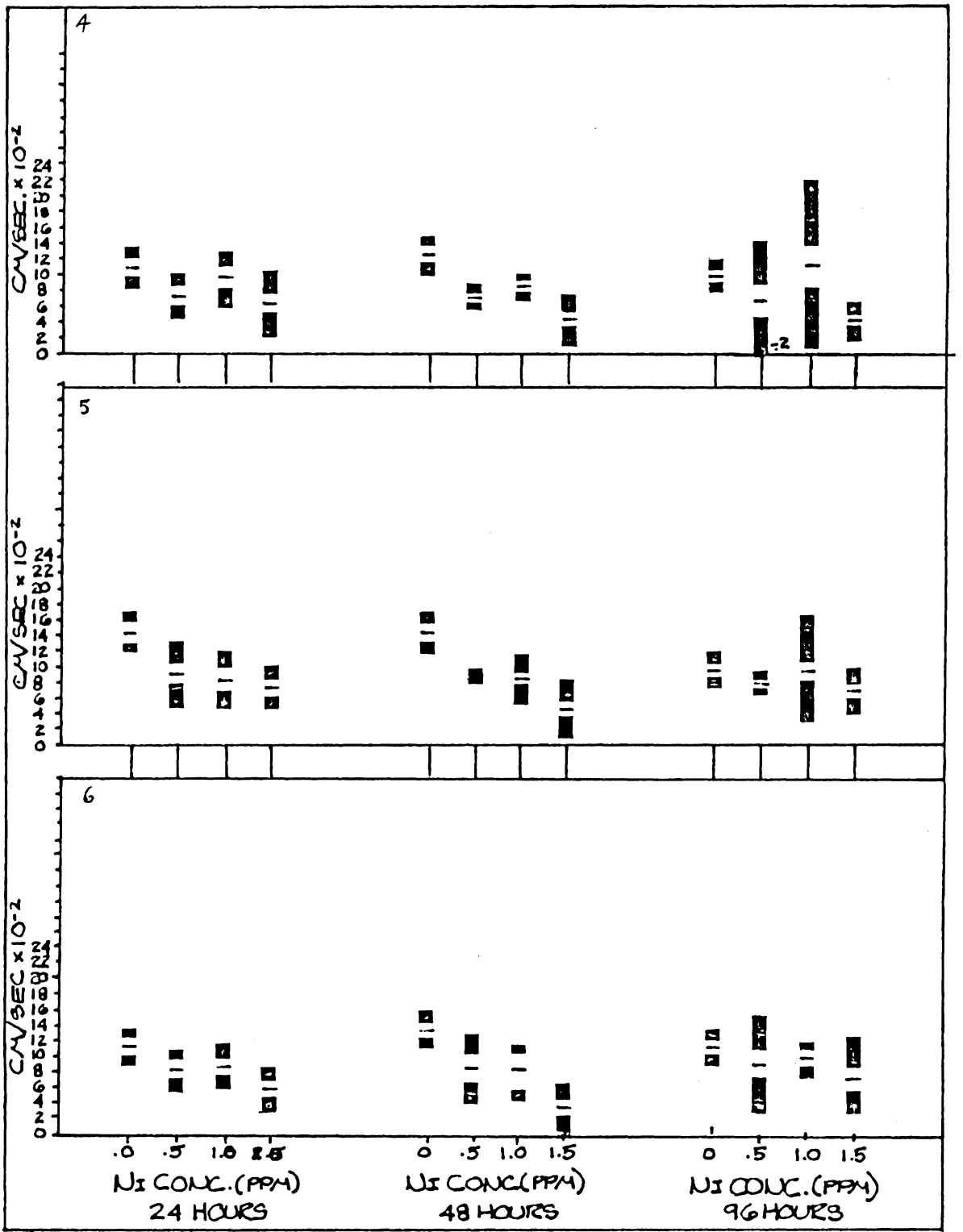

Figure 5. Mean crawling speeds for groups 4, 5, and 6 under nickel stress as compared to their controls with standard error (| |) and confidence intervals (▬▬) shown.

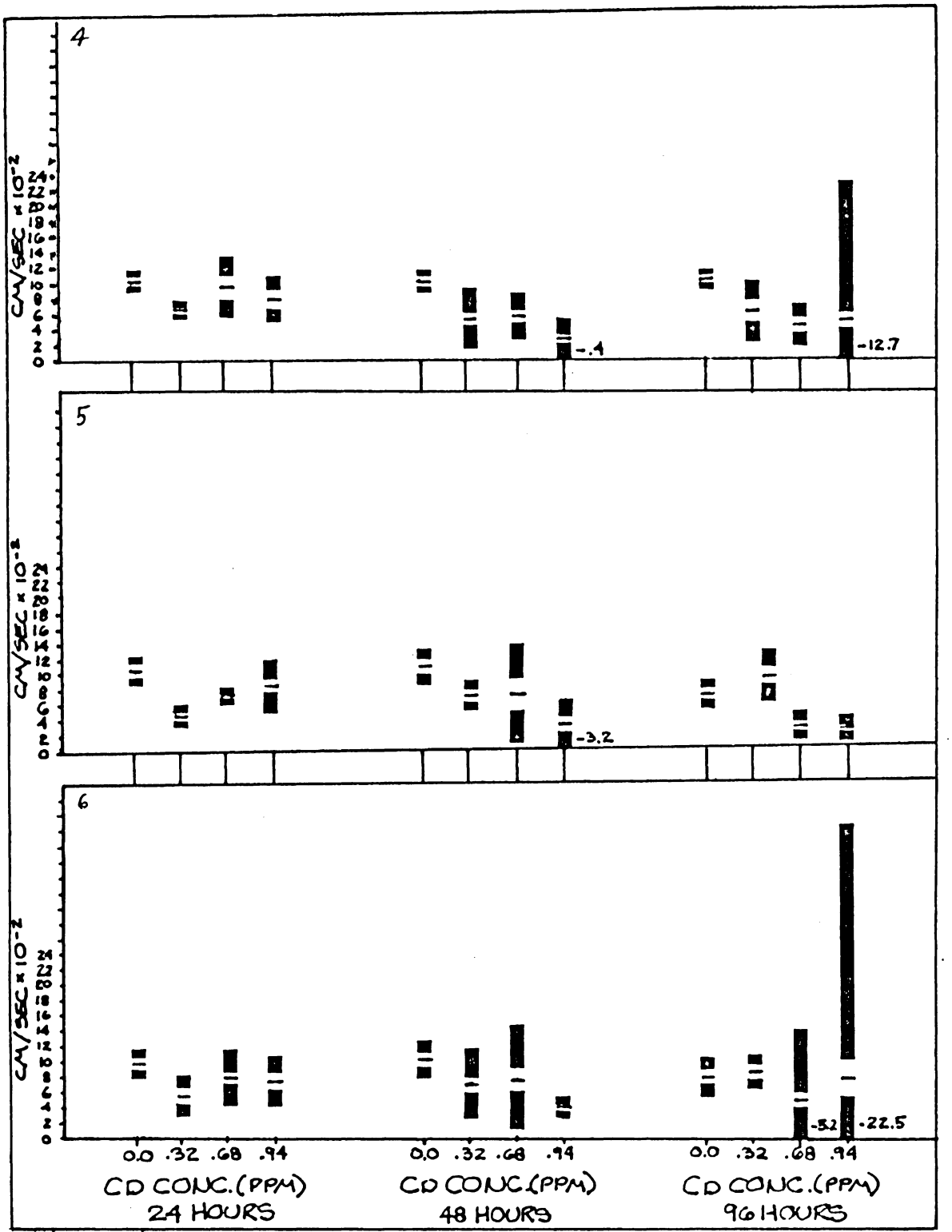

Figure 4. Mean crawling speeds for groups 4, 5, and 6 under cadmium stress as compared to their controls with standard error (| |) and confidence intervals (▬) shown.

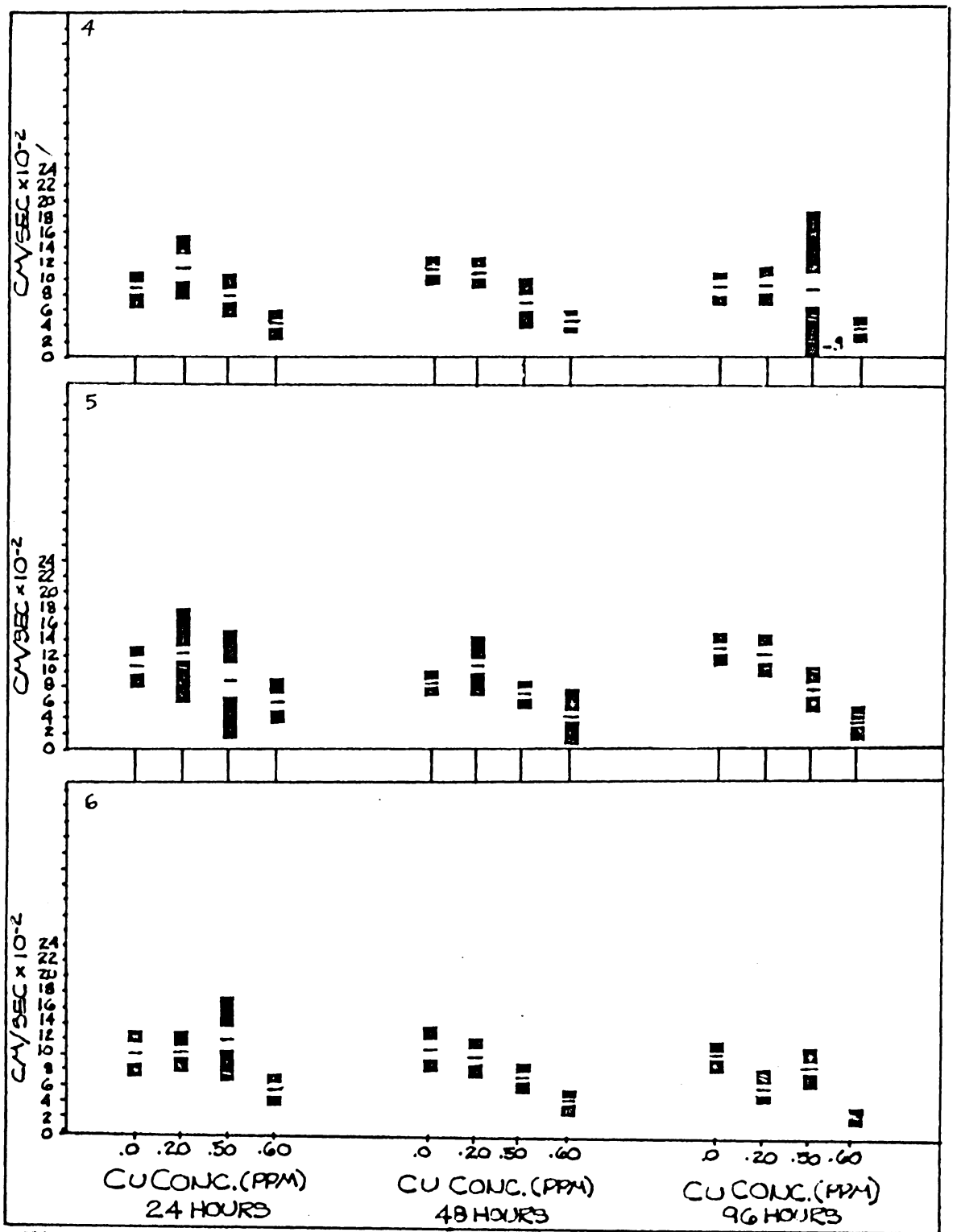

Figure 5. Mean crawling speeds for groups 4, 5, and 6 under copper stress as compared to their controls with standard error (I I) and confidence intervals (▬) shown.

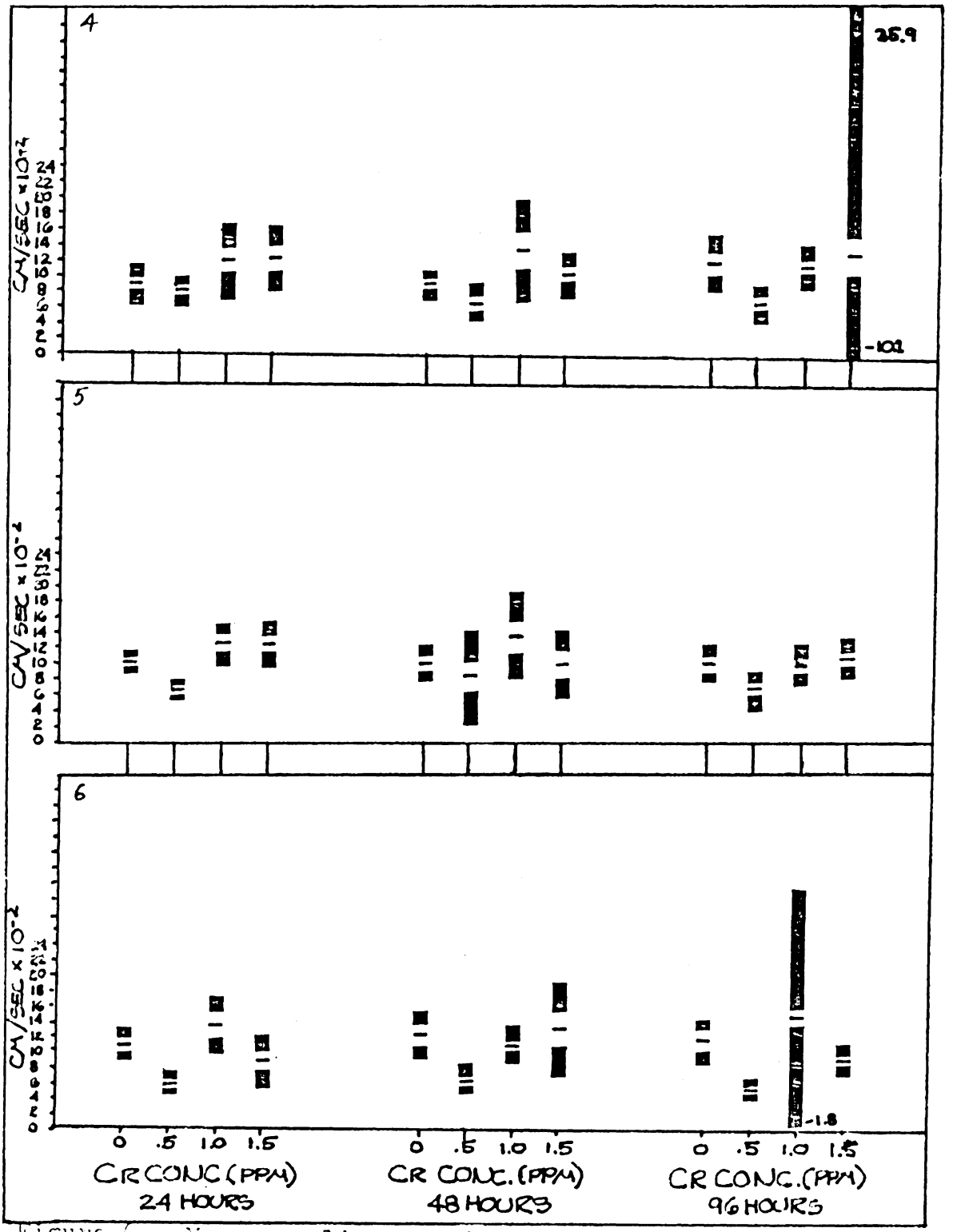

Figure 6. Mean crawling speeds for groups 4, 5, and 6 under chromium stress as compared to their controls with standard error (| |) and confidence intervals (▬) shown.

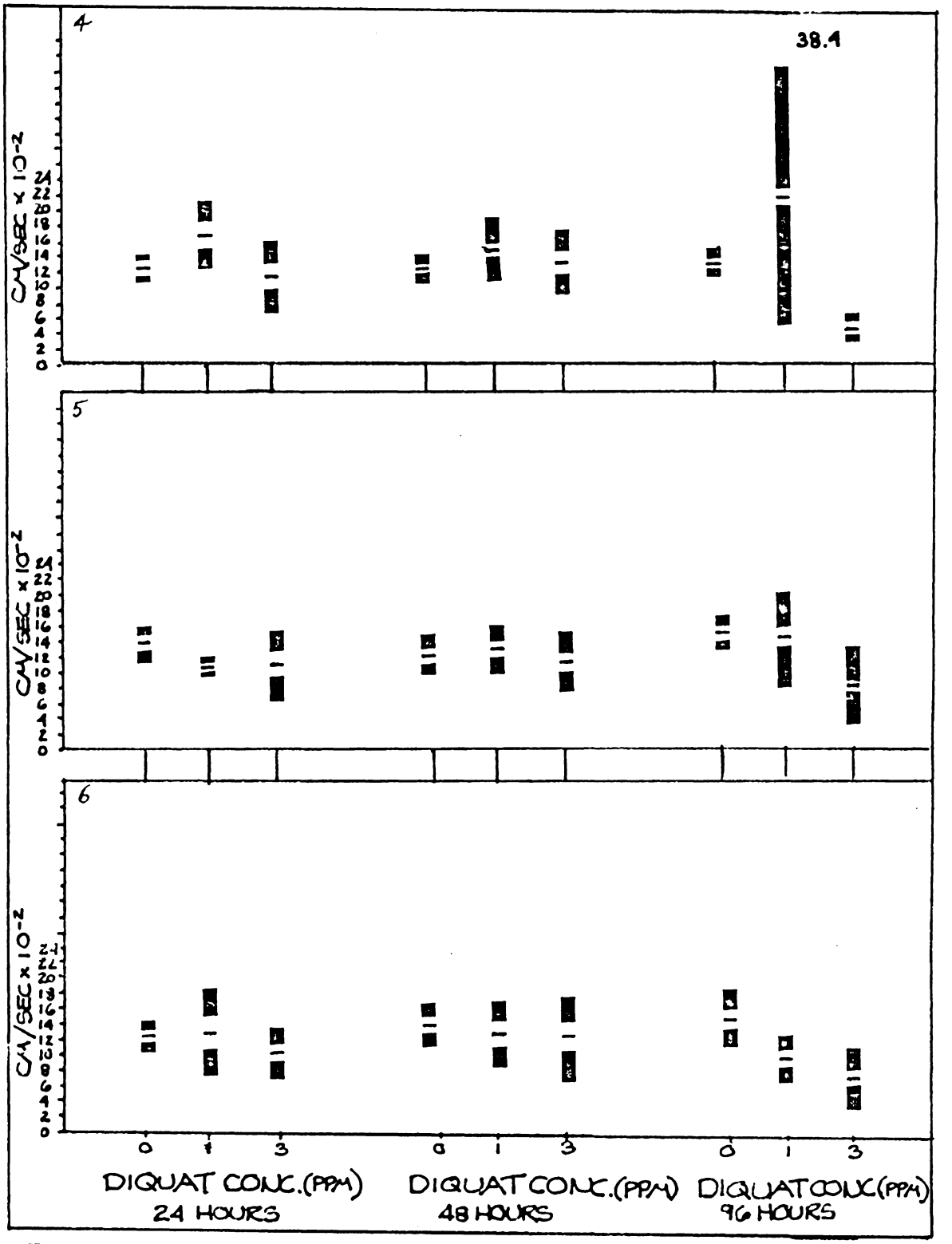

Figure 7. Mean crawling speeds for groups 4, 5, and 6 under diquat stress as compared to their controls with standard error (I I) and confidence intervals (▬) shown.

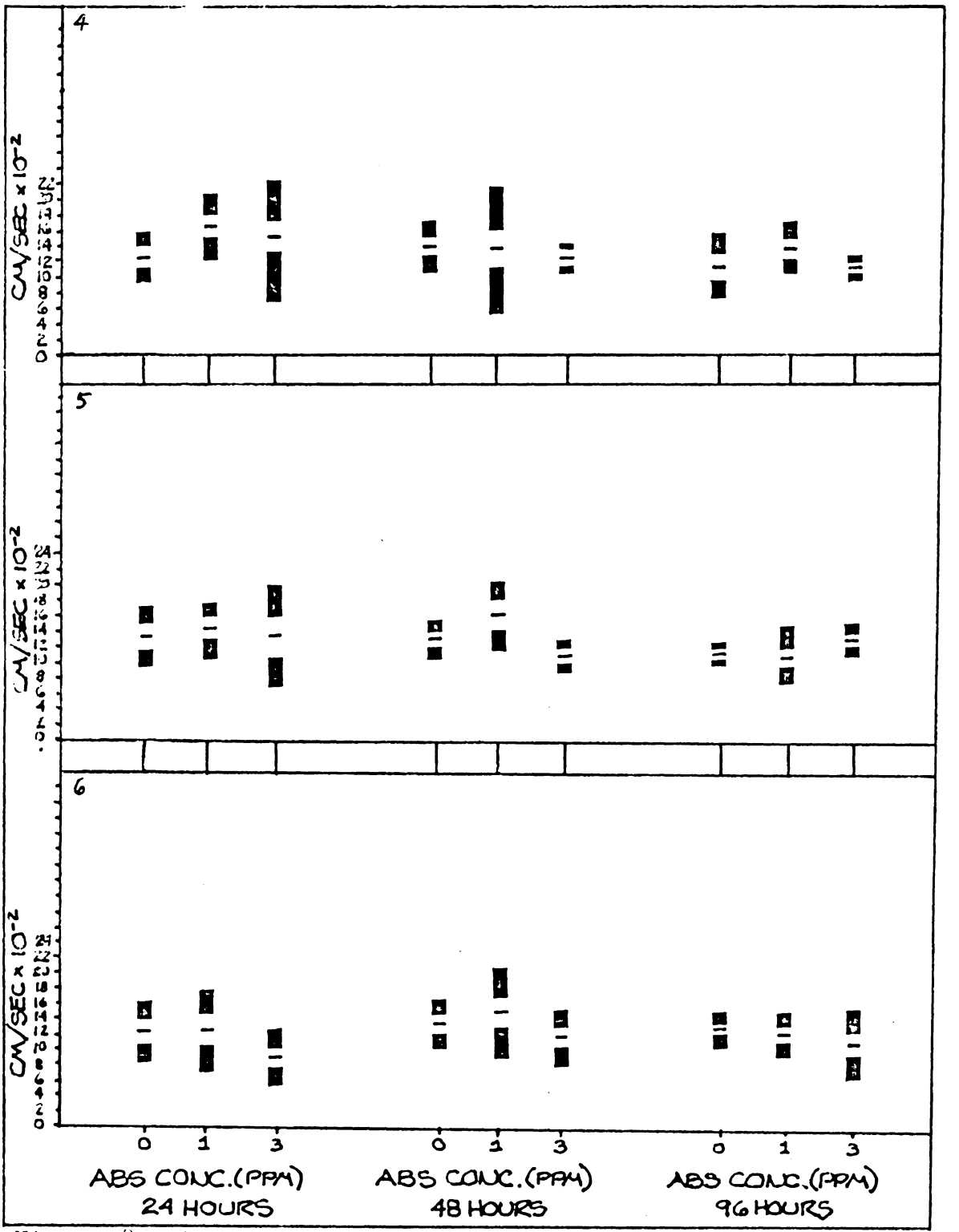

Figure 7. Mean crawling speeds for groups 4, 5, and 6 under ABS stress as compared to their controls with standard error (| |) and confidence intervals (▬) shown.

Table 11. t-values for *a priori* tests between groups at the different toxicant levels and time periods.

Toxicant and concentration	*a priori* test	24 hours	48 hours	96 hours
Zn 2.04 ppm	3 & 6		2.11*	
Ni 0.5 ppm	1 & 4		2.33*	
	2 & 5		2.47*	
	2 & 6	2.68*		
1.0 ppm	2 & 5	2.69*	2.41*	
	3 & 6		2.59*	
	2 & 6	2.38*	2.57*	
1.5 ppm	1 & 4		3.23**	2.62*
	2 & 5	2.99**	3.54*	
	3 & 6	2.63*	4.28***	
	2 & 6	3.78**	4.10***	
Cd 0.32 ppm	1 & 4	3.22**	3.77**	
	2 & 5	3.59**		
	3 & 6	3.54**		
	2 & 6	3.22**		
0.66 ppm	1 & 4		3.45**	4.45**
	2 & 5	2.18*		
0.94 ppm	1 & 4		5.03***	
	2 & 5		3.16**	2.74*
	3 & 6		2.65*	
	2 & 6		3.24**	
Cu 0.20 ppm	3 & 6			2.78*
	2 & 6			3.37**
0.50 ppm	2 & 5			3.35**
0.60 ppm	1 & 4	2.16*	3.15**	2.70*
	2 & 5		2.67*	4.94***
	3 & 6		2.94**	5.04***
	2 & 6			5.09***
Cr 0.50 ppm	2 & 5	2.76*		
	3 & 6	3.25**	2.33*	3.09**
	2 & 6	3.13**	2.094*	3.03**
Diquat 1 ppm	2 & 6			3.01*
3 ppm	1 & 4			5.60***
	2 & 5			4.90**
	3 & 6			2.53*
	2 & 6			3.98**

* 0.05 ** 0.01 *** P<0.001

concentration (2.04 ppm) (Table 11). The animals under stress moved faster than the control group. Zinc seemed to be tied up in the slime so that it did not have very much effect on the animals. The animals appear to acclimate to the zinc still in solution.

Under nickel stress the most significant differences were noted at the end of 48 hours. The higher the stress the slower the animals in a given treatment group moved. These differences were more pronounced at the highest concentration (1.5 ppm) of nickel.

Cadmium stress at the lowest concentration (0.32 ppm) exerted its effect within 24 hours. The crawling speeds were greatly reduced in all treatment groups at that concentration. At the highest concentration (0.94 ppm) of cadmium the difference did not appear until after 48 hours. Again the crawling speeds were reduced under stress.

Significant differences under copper stress occurred after 96 hours. The highest concentration (0.60 ppm) had the greatest effect on crawling speeds; they were greatly reduced.

Chromium stress produced differences between groups 3 and 6, and 2 and 6 at the lowest concentration (0.53 ppm) only; but this occurred at all time periods. The crawling speeds were much slower than the control groups.

Under diquat stress the higher concentration (3.0 ppm) produced significant differences between groups after 96 hours. Again the speeds were slower for the treatment groups

than for the controls.

For ABS there was no significant differences between groups at any of the time periods.

From these a priori tests it can be seen that sublethal concentrations of toxicants affect the crawling speed of Dugesia tigrina. If a toxicant affected the animals then the crawling speeds were always slower for the treatment groups than for the control crawling speeds.

One explanation for this reduced crawling speed could be that the muscles of the animal were affected in some way so it could not move as fast. This is a definite possibility because in some cases an animal gave whole body contractions apparently in an effort to increase its rate of moving, but this did not appear to help. The control groups appeared to glide along effortlessly.

Another explanation for the slower crawling speeds in treatment groups could be that the toxicant affected the cilia used in gliding over the mucus laid down by the animal, because Naitoh (1966) found that sublethal concentrations of nickel stopped the beating of cilia in Paramecium. It appears that sublethal concentrations of the metals decreased the frequency of beating of the cilia. In some cases the rate was so reduced that whole body contractions were used to "escape" the light.

A third explanation is that something happened to the slime. There may not have been as much produced by the

animals under stress as by the controls. Or there may have been changes in the quality of the slime so that the "smoothness" of the slime was affected. There have been no studies on slime to show if either of these explanations are valid. Other explanations are an effect on receptor sensitivity or a slowing of neural transmission. There have been no reported studies of this kind.

Any one of the above possibilities is feasible in explaining the slower movement of the treatment groups. A combination of the above factors could produce the effect.

All data for each toxicant for each time period were analyzed using a 2-way anova (Table 12). It can be seen that in general differences among groups occurred, differences among toxicant concentrations existed, and there was a group-toxicant interaction.

The a priori t-tests indicated the differences between groups. For the differences among concentrations of a toxicant a Duncan multiple-range test was used on the appropriate time period for the toxicants involved (Table 13). As can be seen there were significant differences between the lowest sublethal concentration and the highest sublethal concentration used. For zinc, cadmium, and chromium the crawling speeds were slowest in the lowest concentrations used. Under nickel, copper, diquat, and ABS stress the crawling speeds were slowest in the highest sublethal concentrations used. These opposing findings indicate that the mode of action of the

Table 12. F values for 2-way anova.

Experiment		24 hours	48 hours	96 hours
I	Group	1.258 ns	0.834 ns	2.150 ns
	Zn	0.333 ns	0.520 ns	6.797 ***
	Group-Zn	1.821*	1.129 ns	1.736 ns
II	Group	6.476***	13.908***	1.851 ns
	Ni	0.696 ns	7.663**	2.292 ns
	Group-Ni	1.269 ns	1.620 ns	1.169 ns
III	Group	8.809***	23.201***	10.027***
	Cd	13.302***	6.051**	2.757 ns
	Group-Cd	1.044 ns	6.188***	5.694***
IV	Group	2.813*	9.926***	14.955***
	Cu	58.232***	42.379***	41.809***
	Group-Cu	2.768**	1.696 ns	2.097*
V	Group	1.897 ns	0.899 ns	1.792 ns
	Cr	47.140***	30.832***	53.540***
	Group-Cr	1.036 ns	1.330 ns	1.811 ns
VI	Group	1.068 ns	1.086 ns	6.469***
	Diquat	6.912*	7.720**	30.174***
	Group-Diquat	0.647 ns	0.312 ns	9.143***
VII	Group	1.685 ns	0.180 ns	0.689 ns
	ABS	13.447***	17.728***	15.807***
	Group-ABS	0.684 ns	0.716 ns	6.755***

* - 0.05
** - 0.01
*** - $P<0.001$

Table 13. Results of Duncan's multi-range test on levels of toxicants for each time period where there was significance according to the 2-way anova.

Toxicant	Time	Ranked transformed data in cm/sec with concentrations (ppm) shown
Zinc	96 hours	.44 1.00 1.48 2.04 .2968 .2972 .3426 .3479
Nickel	48 hours	1.5* 0.5* 1.0* .2885 .3161 .3422
Cadmium	24 hours	.32 .66 .94 .2565 .2948 .3089
	48 hours	.32 .94 .66 .2606 .2812 .2999
Copper	24 hours	.6 .2 .5 .2215 .3211 .3317
	48 hours	.6 .5 .2 .2458 .3029 .3299
	96 hours	.6 .5 .2 .2350 .3159 .3275
Chromium	24 hours	.53 1.59 1.25 .2567 .3325 .3463
	48 hours	.53 1.59 1.25 .2608 .3429 .3483
	96 hours	.53 1.25 1.59 .2494 .3472 .3557
Diquat	24 hours	3* 1* .3352 .3671

Table 13. (continued)

Toxicant	Time	Ranked transformed data in cm/sec with concentrations (ppm) shown	
		3*	1*
Diquat	48 hours	.3388	.3700
		3*	1*
	96 hours	.3253	.3650
		3*	1*
ABS	24 hours	.3285	.3799
		3*	1*
	48 hours	.3366	.3883
		3*	1*
	96 hours	.3282	.3622

* Calculated; obtained by serial dilution.

two groups of toxicants differed suggesting different systems in the animal's body were affected by the toxicants. More work needs to be done to determine the modes of action of these toxicants.

If a group-toxicant interaction occurred (Table 12) it was represented graphically (Figures 9 to 15). For zinc in 24 hours (Figure 9) the lower two concentrations appear to have the same effect on the animals. The two higher concentrations differed from each other and from the two lower concentrations. There was no interaction for the other time periods.

Under cadmium stress for 48 hours (Figure 10) the two lower concentrations were significantly different from the highest concentration. After 96 hours in cadmium (Figure 11) the metal in the lower concentrations appeared to affect movement differently than did the two higher concentrations.

Copper stress at 24 hours and 96 hours (Figures 12 and 13) had similar effects at 0.5 and 0.6 ppm, but differed from the effects at 0.2 ppm. Animals in sublethal concentrations of diquat differed after 96 hours (Figure 14) indicating that treatment affected the animals. The same was true for ABS (Figure 15).

All data for each toxicant were analyzed using a 3-way anova (Table 14). It can be seen that time was a factor for only one toxicant - cadmium. The longer the exposure to

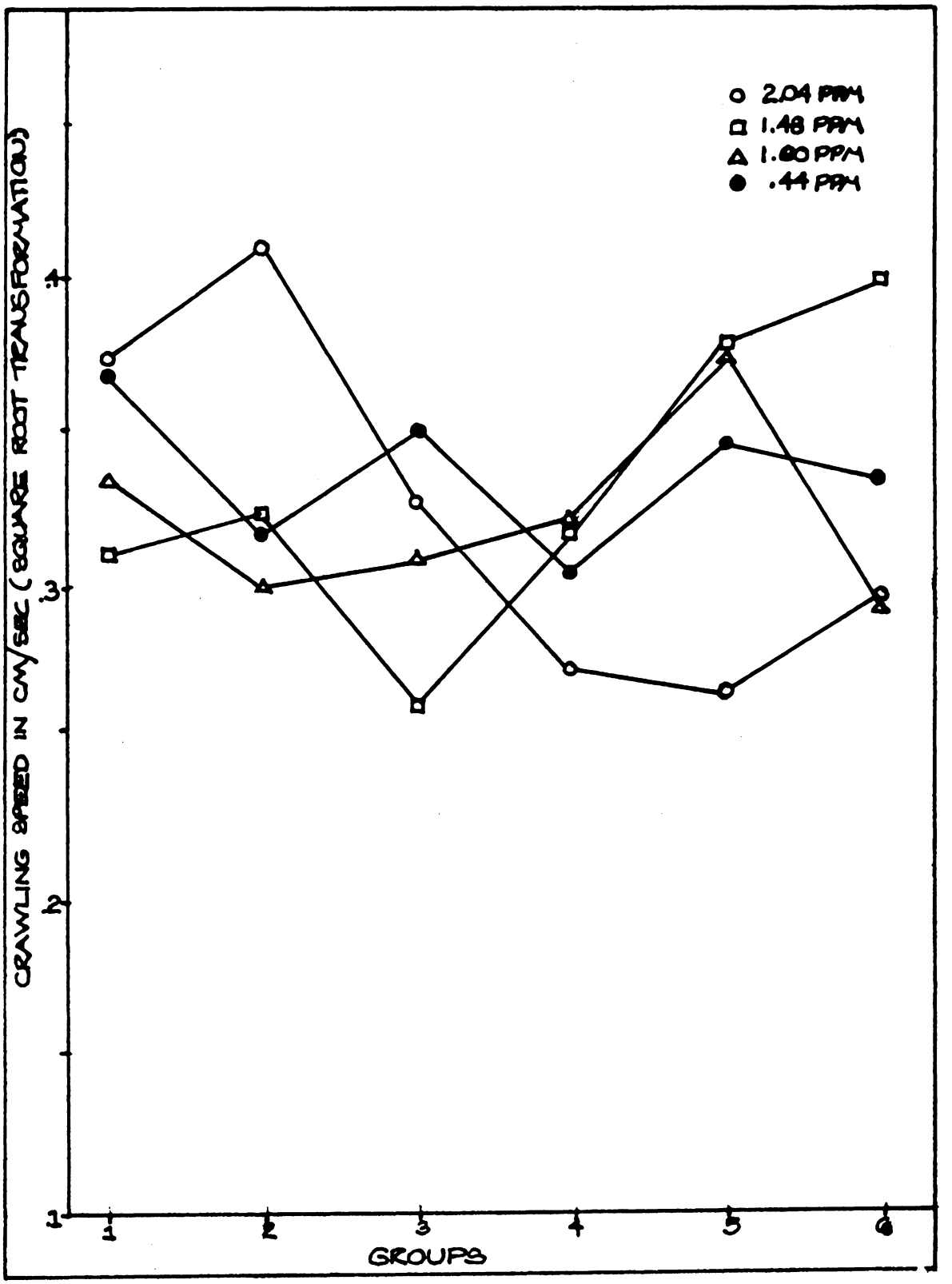

Figure 9. Group-zinc interaction for 24 hours.

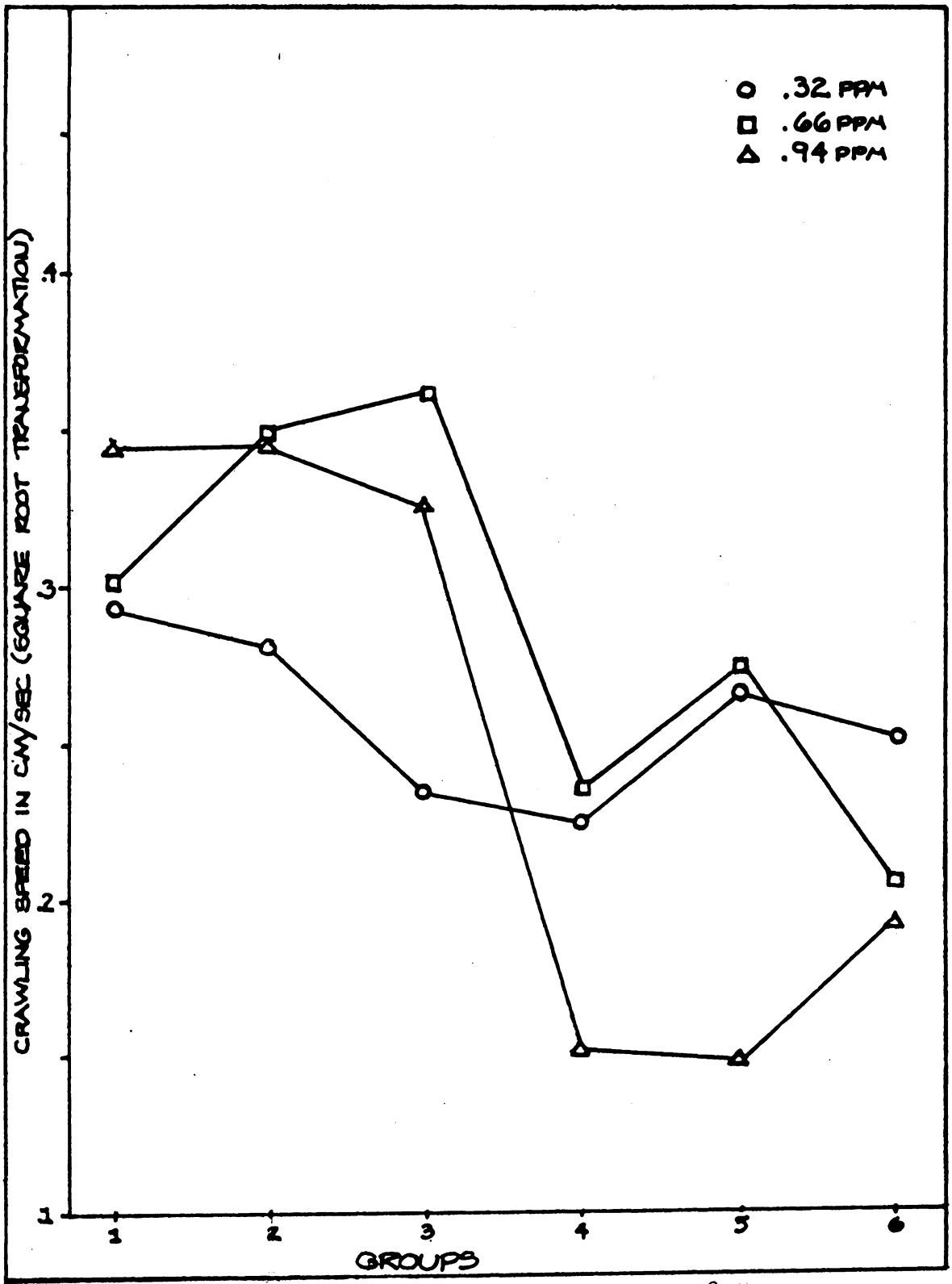

Figure 10. Group-cadmium interaction for 48 hours.

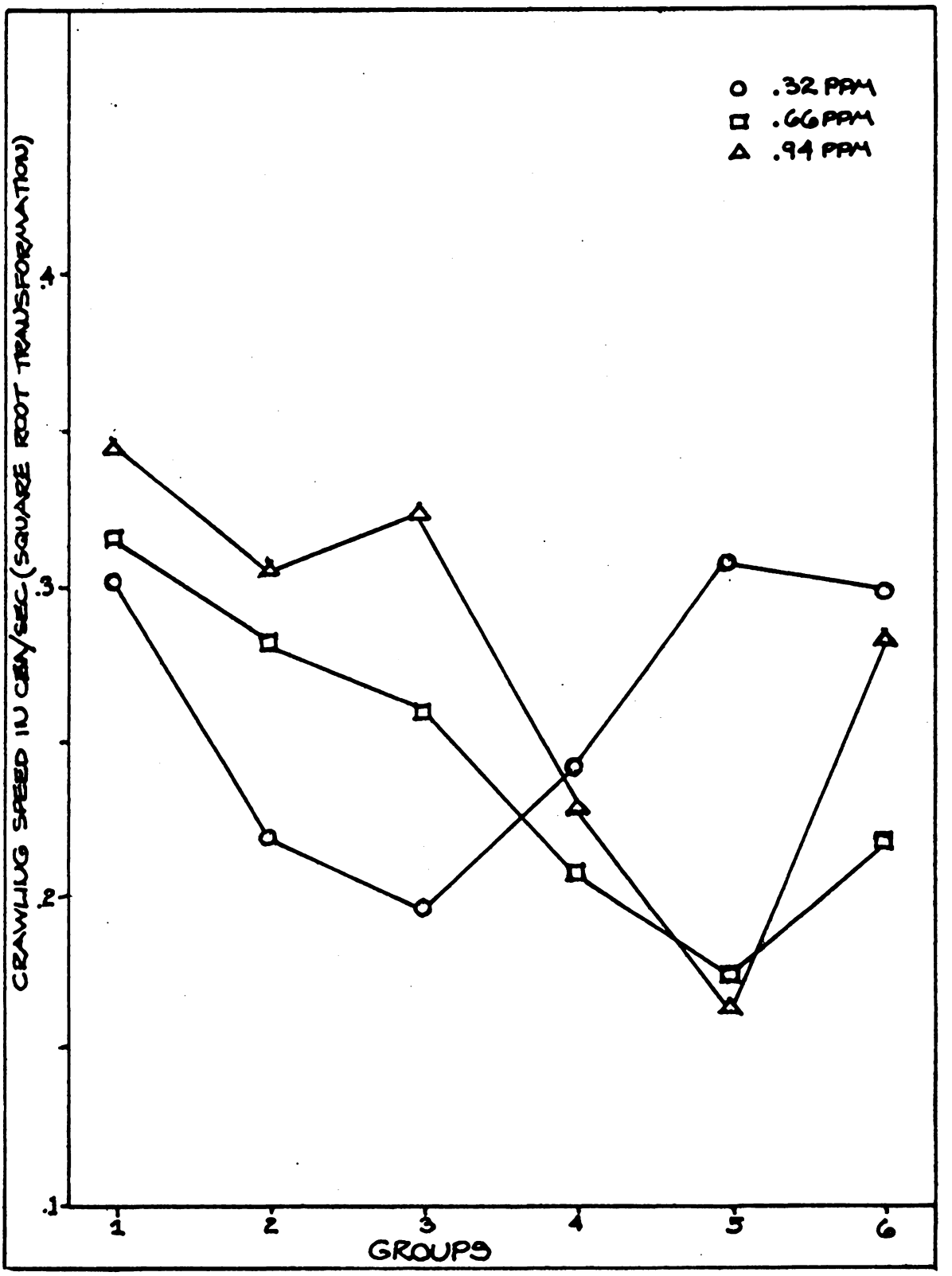

Figure 11. Group-cadmium interaction for 96 hours.

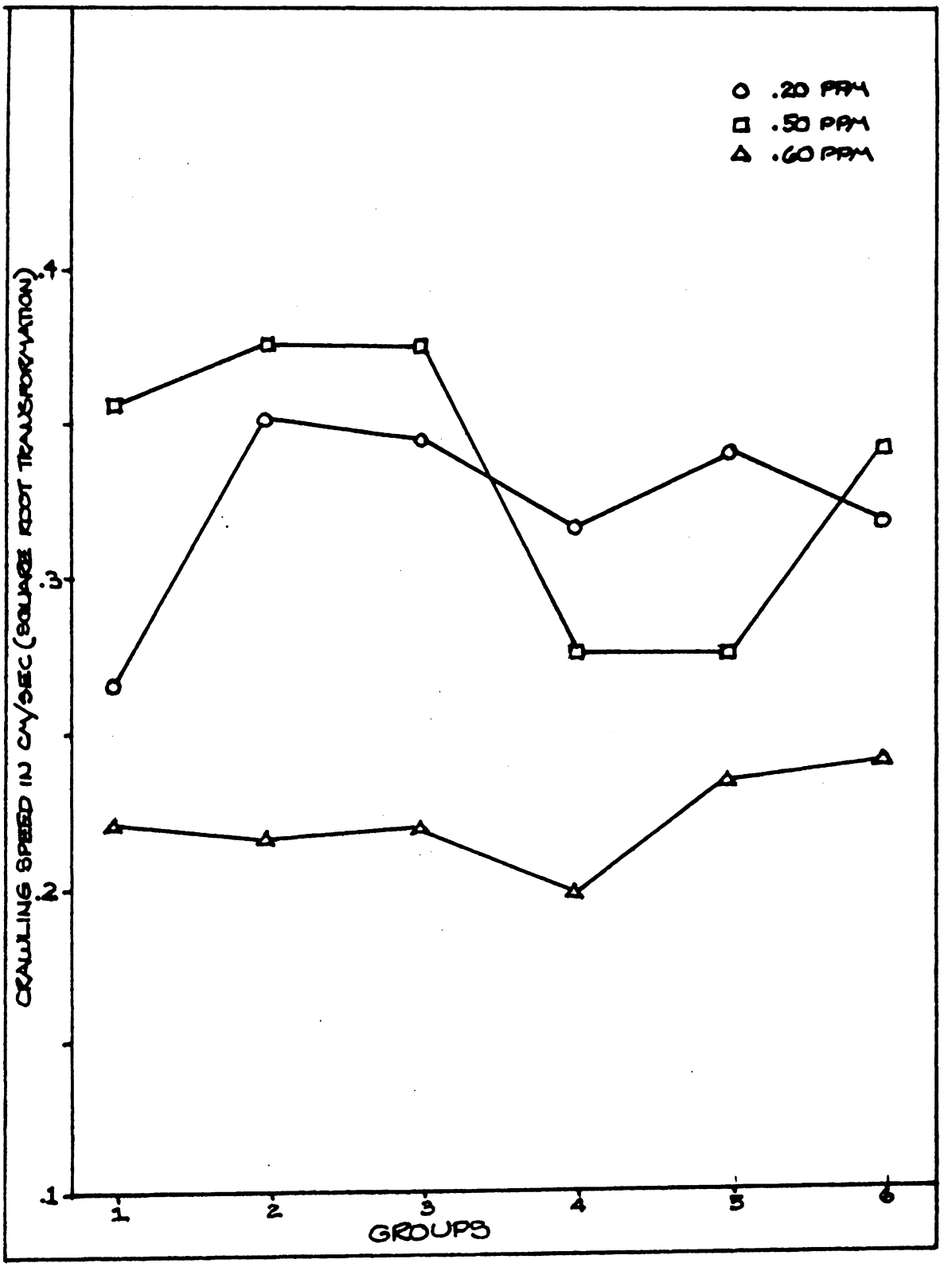

Figure 12. Group-copper interaction for 24 hours.

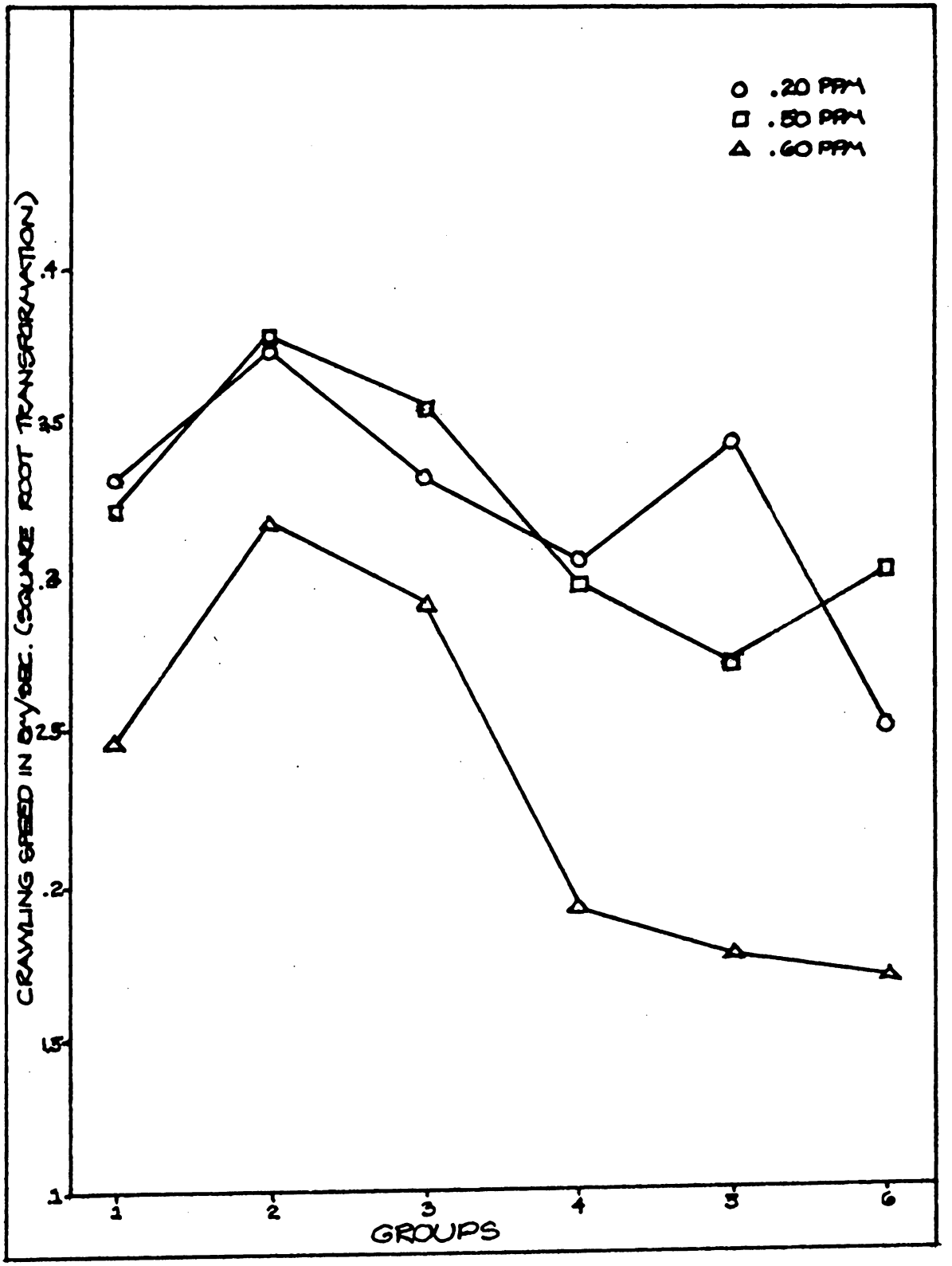

Figure 13. Group-copper interaction for 96 hours.

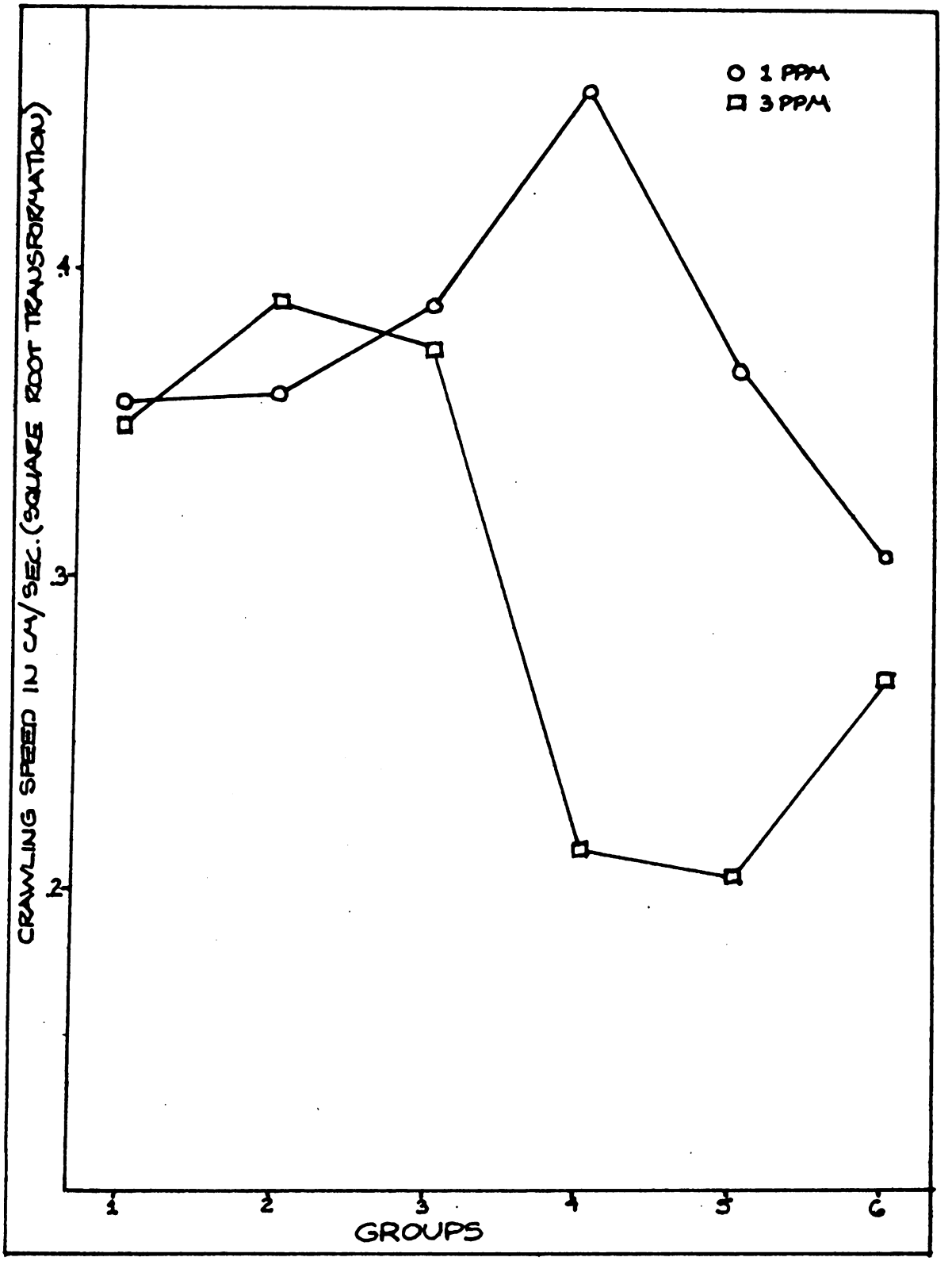

Figure 14. Group-diquat interaction for 96 hours.

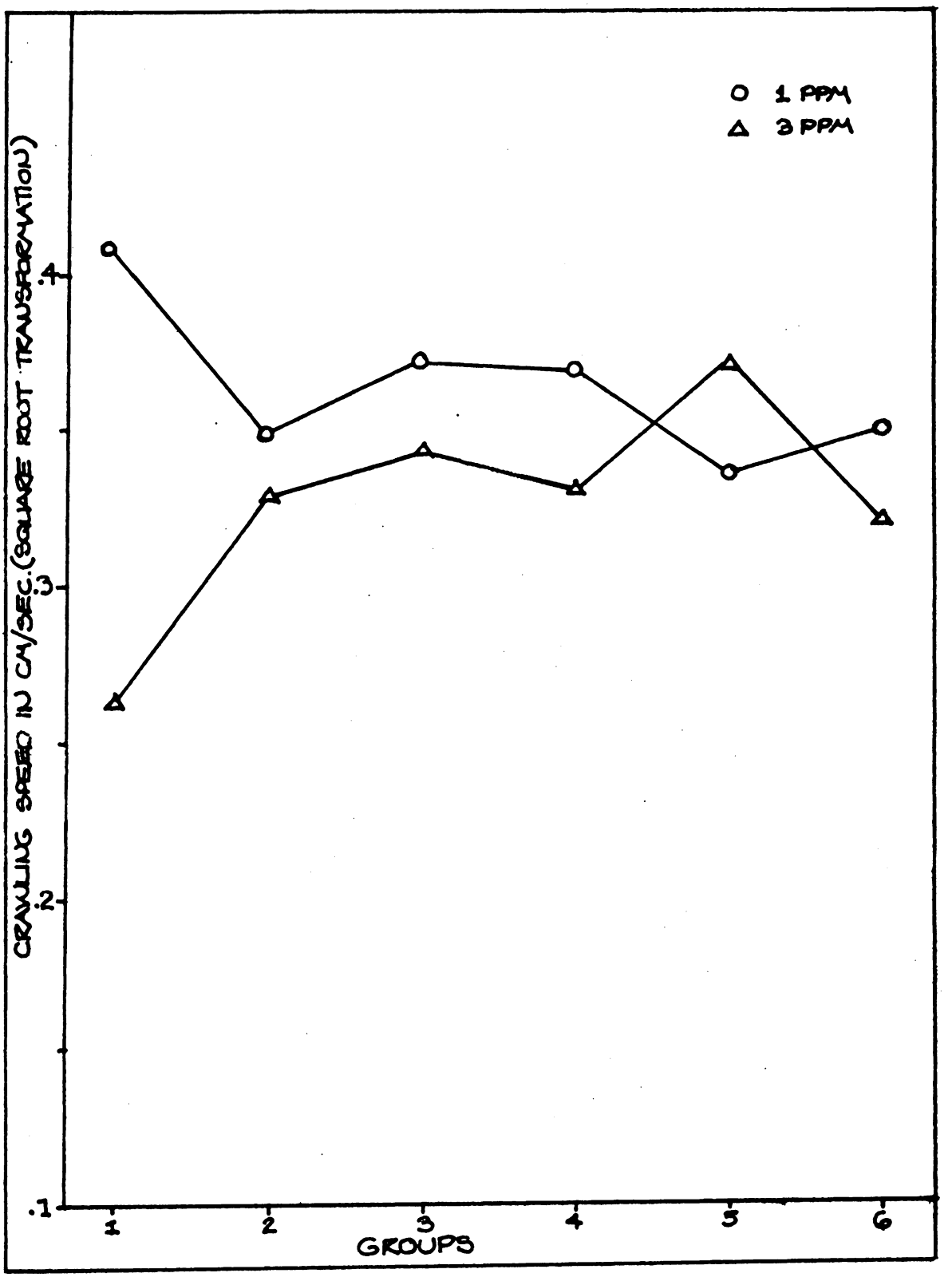

Figure 15. Group-ABS interaction for 96 hours.

Table 14. F values for 3-way anova.

Experiment		F values
I	Group	2.425*
	Zn	1.883 ns
	Time	1.559 ns
	Group-Zn	1.477 ns
	Group-Time	0.737 ns
	Zn-Time	2.144*
II	Group	17.565***
	Ni	7.577***
	Time	1.192 ns
	Group-Ni	2.101*
	Group-Time	1.855 ns
	Ni-Time	1.018 ns
III	Group	24.530***
	Cd	4.606*
	Time	5.552**
	Group-Cd	4.641***
	Group-Time	3.24***
	Cd-Time	4.533**
IV	Group	15.145***
	Cu	129.463***
	Time	0.014 ns

Table 14. (continued)

Experiment		F values
IV	Group-Cu	2.966**
	Group-Time	4.590***
	Cu-Time	2.832*
V	Group	2.133 ns
	Cr	118.735***
	Time	0.746 ns
	Group-Cr	1.015 ns
	Group-Time	1.126 ns
	Cr-Time	0.676 ns
VI	Group	4.442**
	Diquat	27.586***
	Time	1.539 ns
	Group-Diquat	2.430*
	Group-Time	1.749 ns
	Diquat-Time	0.911 ns
VII	Group	1.044 ns
	ABS	44.055***
	Time	1.809 ns
	Group-ABS	2.971*
	Group-Time	0.980 ns
	ABS-Time	0.801 ns

* 0.05 ** 0.01 *** P<0.001

cadmium the slower the animals moved.

If a group-time interaction occurred (Table 14), it was represented graphically (Figures 16 and 17). For cadmium (Figure 16) exposure to the toxicant appeared to slow the crawling speed of the animal except for group 6. After 96 hours exposure to cadmium animals which were placed in fresh dilution water moved faster than those which were exposed for 24 or 48 hours and then placed in fresh dilution water. This suggests that the effects of chronic exposure to cadmium on crawling speed are temporary. Removal of the animal from the toxicant to freshwater results in an immediate increase in crawling speed.

Exposure to copper (Figure 17) slowed down the animals. If an animal was placed in fresh dilution water within 24 hours (group 6) it appeared to be stimulated to faster movement. Longer exposure to copper resulted in slower crawling speeds at 48 and 96 hours. Copper appears to have a biologically significant effect on crawling speed after 96 hours exposure.

If concentration-time interactions occurred (Table 14), they were represented graphically (Figures 18 to 20). For zinc (Figure 18) the effects of concentration on crawling speed at 24 and 48 hours were variable and the responses showed an inverse relationship. After 96 hours exposure the two lower concentrations resulted in the slowest crawling speeds while there appeared to be a marked increase in crawling speed

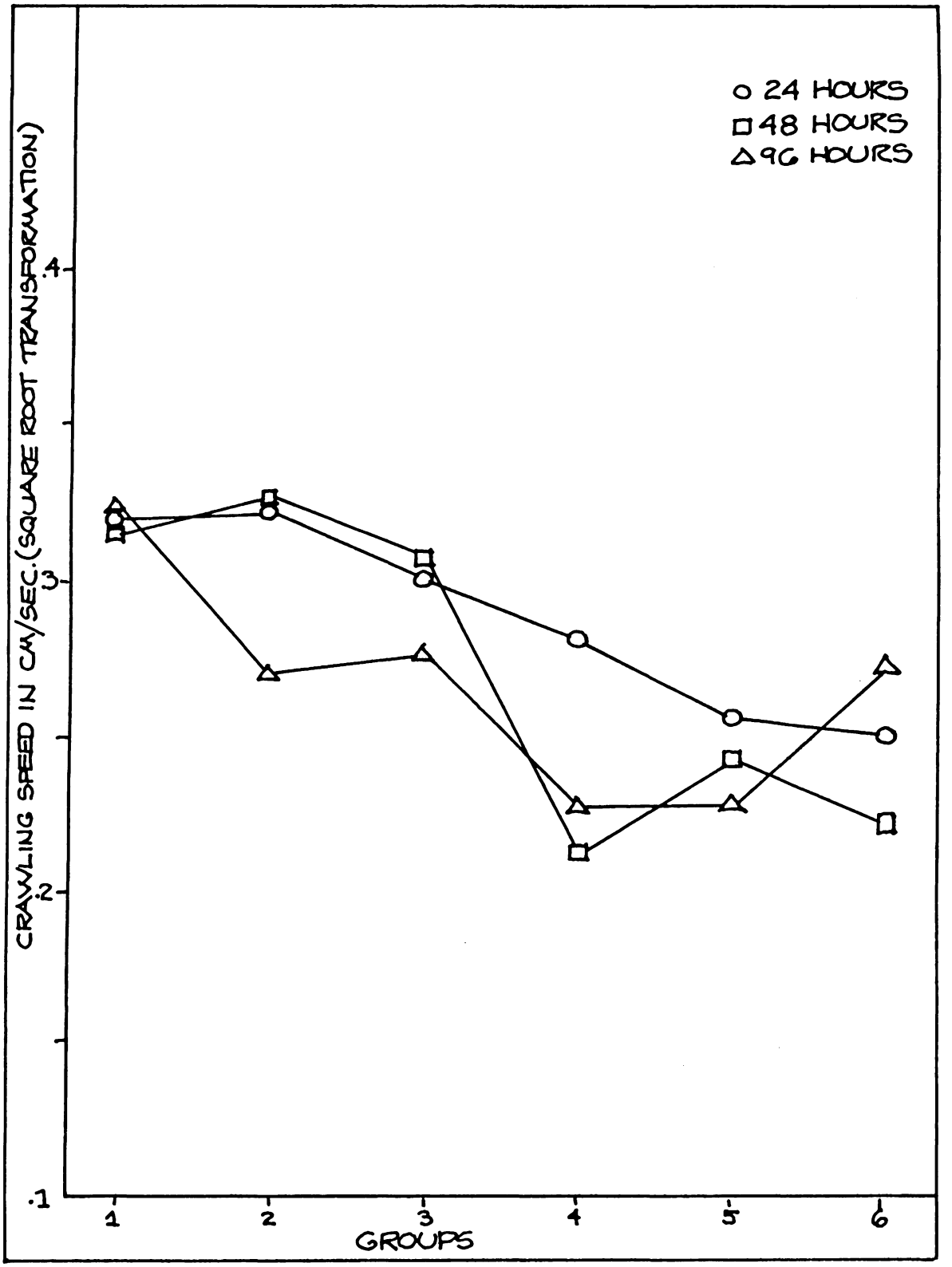

Figure 16. Group-time interaction under cadmium stress.

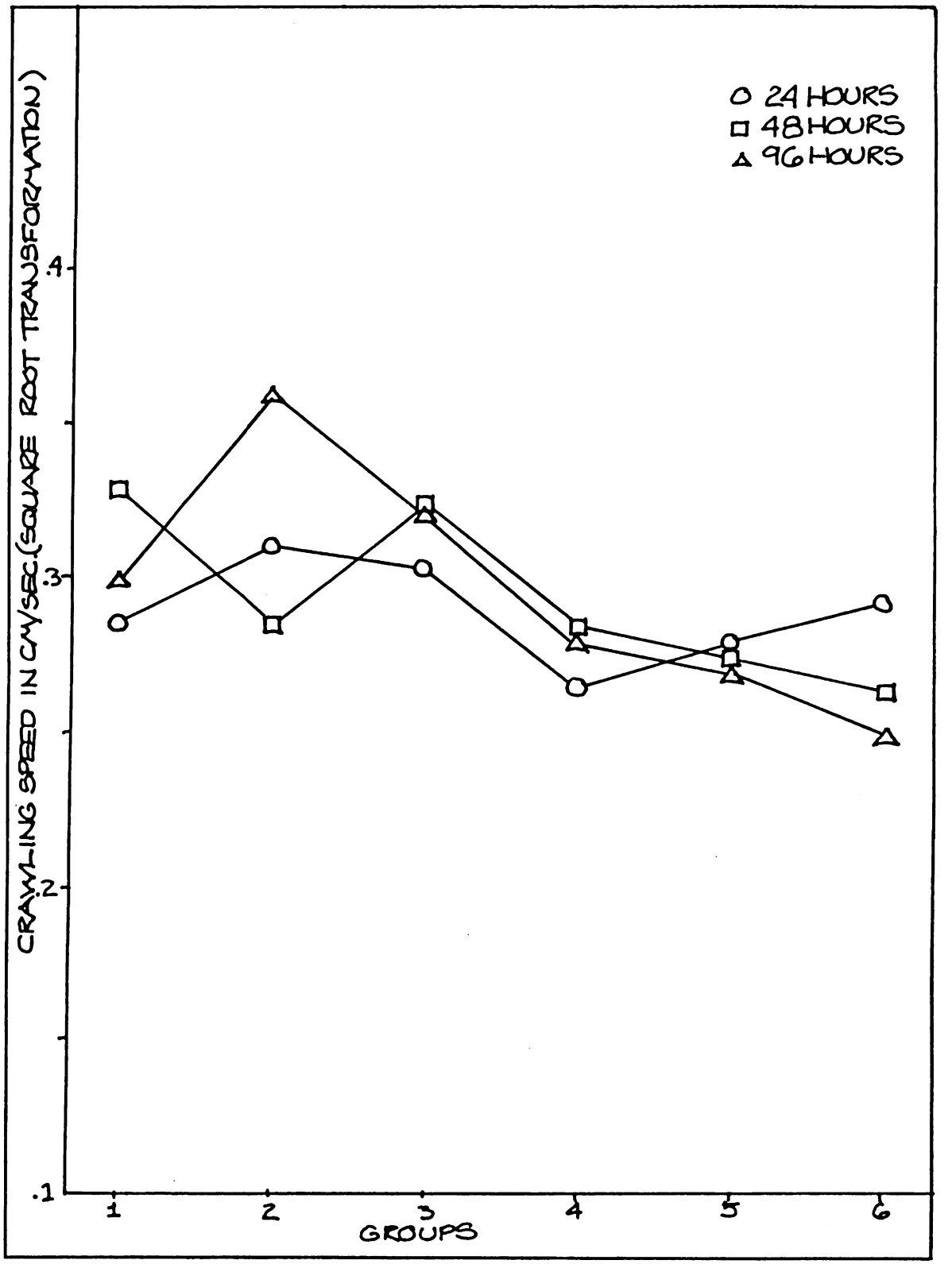

Figure 17. Group-time interaction under [illegible].

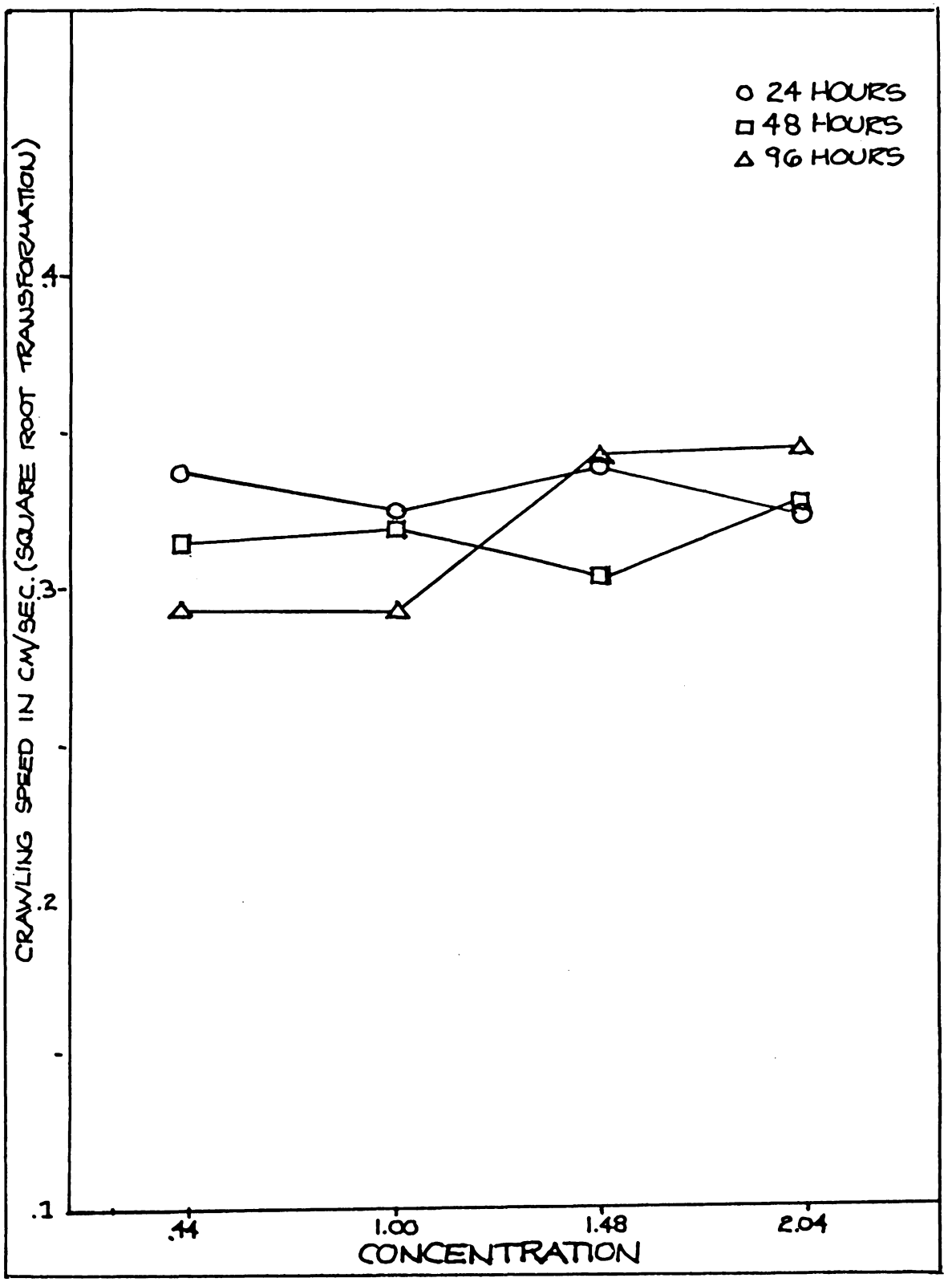

Figure 11. Concentration-time interaction on zinc stress.

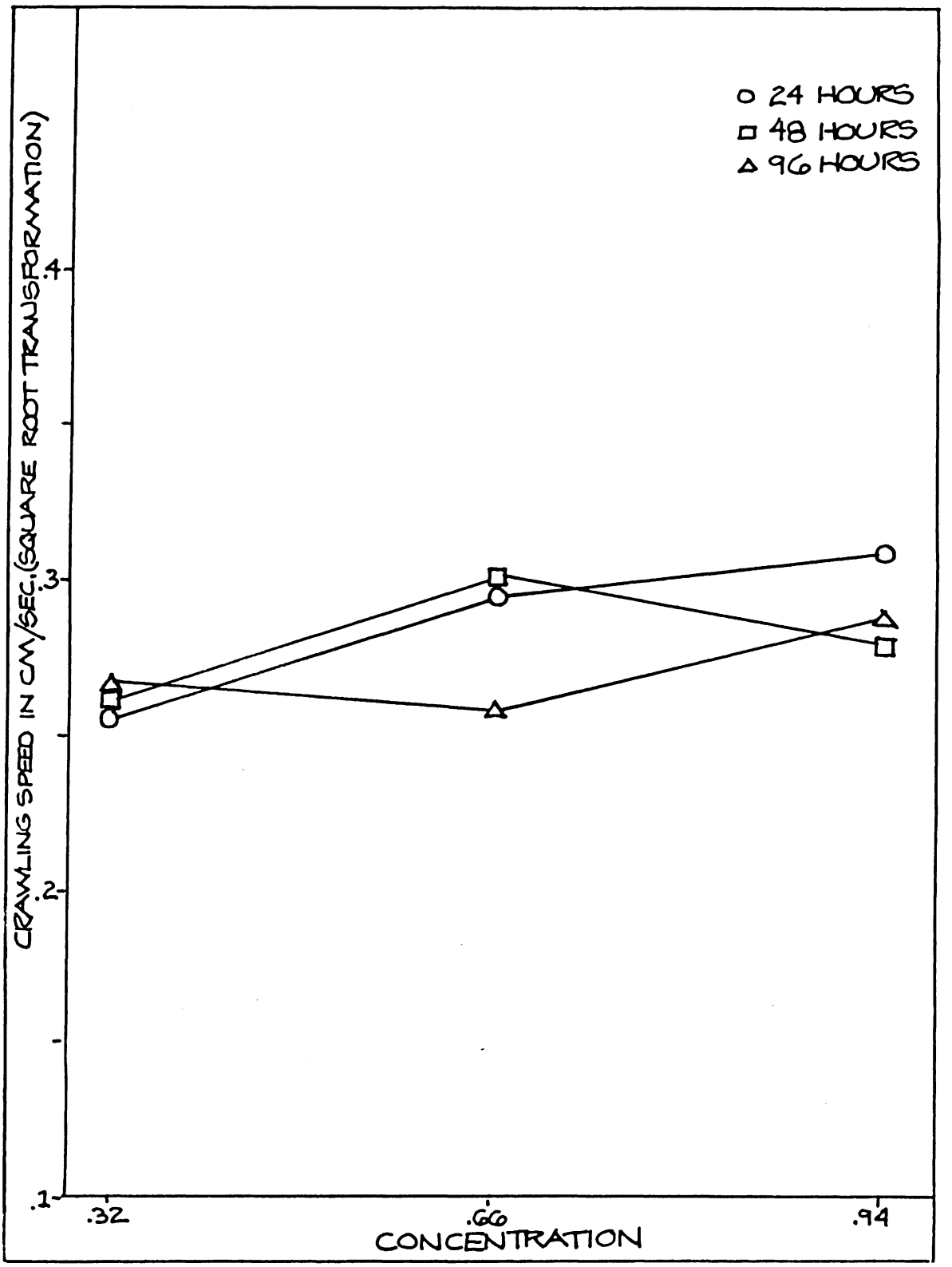

Figure 10. Concentration-time interactions under cadmium stress.

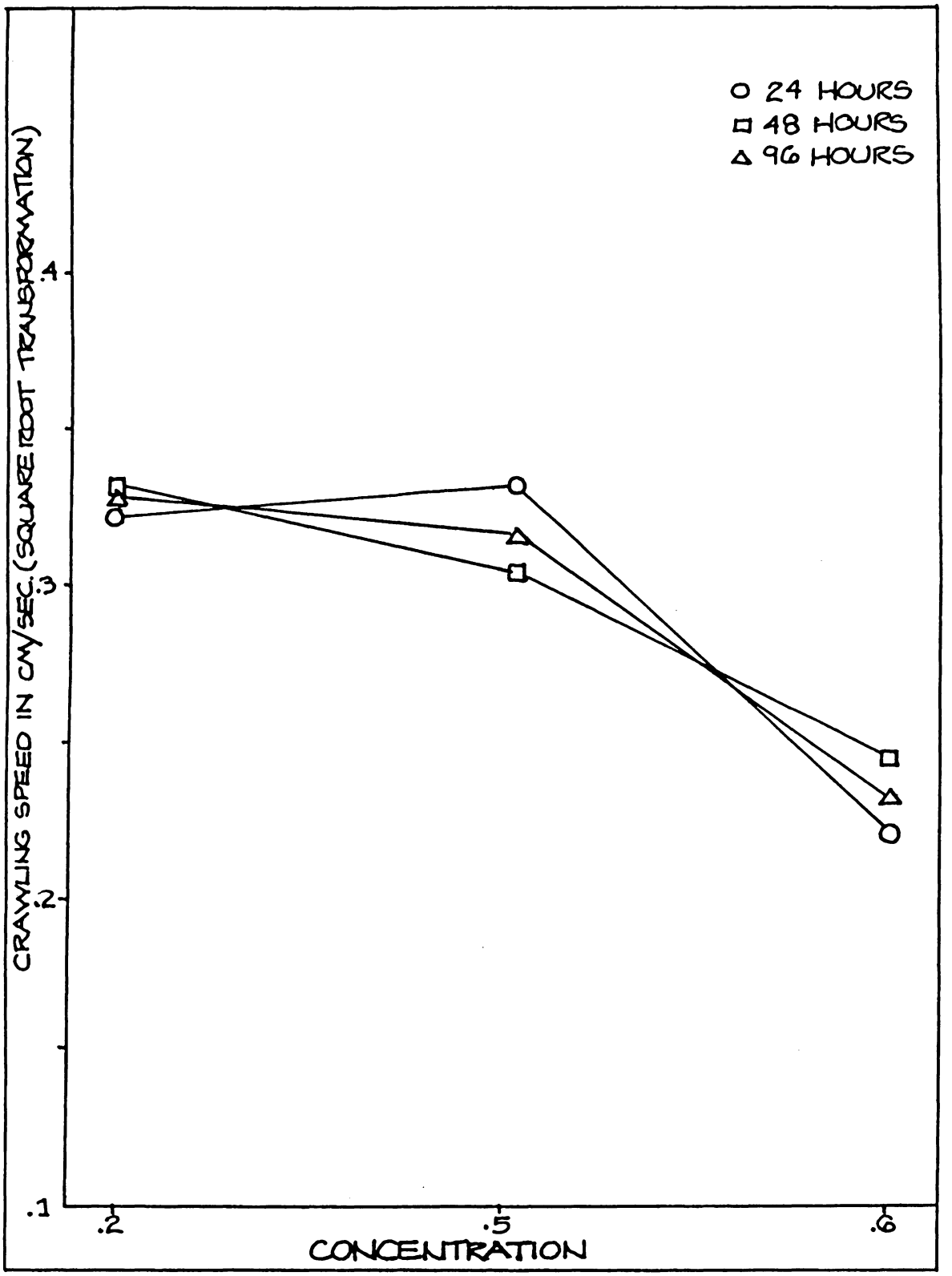

Figure 20. Concentration-time interaction under copper stress.

at the two higher concentrations.

For cadmium (Figure 19) exposure to the lowest concentration of the toxicant resulted in slow crawling speeds for all time periods. At the two higher concentrations cadmium appeared to have a stimulatory effect except for 0.66 ppm after 96 hours. There is no apparent biological explanation for these results.

After exposure to copper (Figure 20) the crawling speeds of the animals were slowed except after 24 hours exposure to 0.5 ppm copper which appeared to have a slight stimulatory effect on the animals.

All of the data from the light response studies show that treatment with sublethal concentrations of toxicants affects the negative phototactic response of Dugesia tigrina as measured by the crawling speed away from a beam of light. The exact mechanism for this change is unknown. The modes of action of the toxicants may be different depending on the concentrations of the toxicants used.

Although all of these studies were conducted in total darkness which is not normal in natural conditions, it is thought that similar reductions in crawling speed would occur in nature.

The significance to the animal of reduced crawling speed should not be underemphasized. This could be a definite handicap in getting away from a predator. The slower crawling speed might increase the animal's exposure to

ultraviolet rays and to direct sunlight. This exposure could be fatal because it has been shown that the above rays may cause death after a short time (Hyman, 1951). Planarians are typically bottom-dwellers found on a hard substrate under rocks and gravel. Their mucous secretions assist in capturing prey and rendering it helpless (Hyman, 1951). If toxicants reduce the amount of slime produced the food-capturing process could be greatly hampered. The animals would starve and eventually die from lack of food.

CONCLUSIONS

1. Dugesia tigrina may be used as a test organism in bio-assays - acute or behavioral.
2. Sublethal concentrations of several toxicants such as heavy metals and organic compounds have a marked effect on the negative phototactic response of Dugesia tigrina as measured by their rate of movement away from light.
3. The exact mechanisms for reduced crawling speeds in sub-lethal concentrations of toxicants is unknown. Further work needs to be done to elucidate the mechanisms involved.
4. Further work needs to be done to find out if heavy metals are bound to the slime produced by the planarians or incorporated into the molecular structure of the slime.

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THE EFFECT OF SUBLETHAL CONCENTRATIONS OF SELECTED TOXICANTS ON THE NEGATIVE PHOTOTACTIC RESPONSE OF *DUGESIA TIGRINA*

by

Carolyn Lea See

(ABSTRACT)

Acute bioassays were conducted on the planarian, *Dugesia tigrina*, to determine the toxicity of zinc, nickel, cadmium, copper, chromium, diquat, and ABS. The order of toxicity from most toxic to least toxic was: copper cadmium nickel zinc ABS chromium diquat.

From these LC_{50} values sublethal concentrations were arbitrarily chosen for use in the light response studies. After acclimation to total darkness, crawling speed away from a beam of light was measured in the controls and the experimentals at 24, 48, and 96 hours.

There were significant differences between the lowest sublethal concentrations and the highest sublethal concentration used in these experiments. For zinc, cadmium, and chromium the crawling speeds were slowest in the lowest concentration used. Under nickel, copper, diquat, and ABS stress, the slowest crawling speeds were found in the highest sublethal concentration used. This suggests that different systems in the animal's body were affected by the toxicants.

It appears that sublethal concentrations of these

toxicants have a marked effect on the negative phototactic response of *D. tigrina* as measured by the crawling speed away from a beam of light. The exact mechanism for reduced crawling speeds is unknown.

Speculation on the fate of the toxicant as it relates to the animal is offered.