ACKNOWLEDGEMENTS

I wish to express my gratitude to Dr. Edward Maly, my supervisor, for his advice and guidance throughout this study and in the preparation of this thesis. Special thanks to Dr. Perry Anderson and Dr. Shelvah D'Apollonia for their assistance in the analysis of the data.

I am grateful to Marie-Michelle de Châteauneuf for her encouragement and for typing this thesis. I would also like to thank my parents and family for their constant support and encouragement.

This project was supported by funds awarded by Concordia University and by the Quebec Ministry of Education.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

In the field of aquatic toxicology and more specifically in the study of the effects of heavy-metal pollution, most of the work has focused on vertebrates such as fish. Fewer studies have considered the effects of heavy metals on freshwater invertebrates. Although the immediate economic reasoning behind this approach is understandable, consideration must be given to the zooplankton community. The latter form an important link in the food chain of higher organisms. The importance of crustaceans such as *Daphnia* as a food source for young and mature fish has been reported by many authors. Studies of the gut content of young fish show from 1 to 95% cladoceran content by volume, with very few less than 10% (Pennak 1978). Finnell and Reed (1969) in a study of the kokanee salmon estimated that *Daphnia pulex* was the prey species most utilized: In 1965, 85% of the total number of organisms ingested by the salmon was *D. pulex*, and this figure increased to 92% in 1966. Brooks and Dodson (1965) attributed the absence of the large cladoceran *Daphnia pulex* in lakes of Connecticut to differential predation by alewives. The importance of cladocerans as a major food item in fish diet is repeatedly found in the literature, and the list is too long to list here.

Daphnia magna as a test organism in toxicity studies

The use of *Daphnia magna* as a test organism in toxicity studies can be traced back to work done by E. Naumann (1934 a, 1934 b) and by B.G. Anderson (1944, 1948). Although *D. Magna* is not as widely distributed in North America as other cladoceran species, studies have shown that its susceptibility does not differ greatly from other species of *Daphnia*.

Canton and Adema (1978) in a comparison of the sensitivity of D. magna with D. pulex and D. cucullata found no difference in their sensitivities. Similarly, Winner and Farrell (1976) concluded that if survival is used as a toxicity index, then D. magna, D. pulex, D. parvula and D. ambigua do not differ in their sensitivity to copper.

There are several advantages in using Daphnia magna as a test organism. First, they are easy and inexpensive to culture, while being large enough to handle without the aid of a miscroscope. Their parthenogenetic mode of reproduction is advantageous for this type of study in that they produce a new brood (average of 10 daphnids) every 48 hours. Hence any age group is readily available. Daphnia magna has a relatively short life span (about 60 days at 20^{o}C), thus the 48 or 96 hour period used in most bioassays represents a larger portion of their life span than in larger organisms such as fish. Studies also reveal that they are much more sensitive to toxicants than fish (Anderson, 1948). Taking into condiseration this last point, it is clear that fish will not remain in areas where its food has disappeared, and thus one will realize the importance of studies involving the cladoceran Daphnia magna.

CADMIUM: It's occurance and uses.

Cadmium is quite rare in nature, the average earth crust content ranges from 0.1 to 0.5 ppm (Lymburner, 1974). Generally, it is found in association with zinc, lead and copper ores. Nearly all of the cadmium production in the world is as a by-product of zinc smelting. Packard (1977) lists the world production of cadmium in 1975 at 15,554 metric tons. Canada ranked fourth with 1,142 m.t. following Russia (2,950 m.t.), Japan (2,688 m.t.) and the United States (1,989 m.t.).

Lake (1979) summarized the concentration of dissolved cadmium in uncontaminated fresh waters which ranged from 0.02 to 9.09 ug/L. In a national survey of several heavy metals in Canadian drinking water supplies, Méranger et al. (1979) found that the cadmium levels were very low. The cadmium levels in treated water from Dawson creek, Kamloops (B.C.), Clarenville, Grandfalls Newfoundland and Rivière du Loup (Que.) were 0.03, 0.01, 0.05, 0.06 and 0.06 ug/L respectively.

The cadmium concentration in contaminated waters depends on the site and its proximity to the emission source. In a study of heavy metal contamination by atmospheric fallout, Vanloon and Beamish (1977) determined the cadmium content of 31 lakes in the Flin Flon area of Manitoba. The range of cadmium concentration varied from 50 ug/L to 0.2 ug/L depending on the proximity of the lakes to the smelter. Clarke (1974) reviewed the chemical characteristics of mine water from eight selected mines in Canada and found cadmium concentrations ranging from 0 to 250 ug/L. In a study of water samples from rivers and lakes in the U.S. in 1970 it was determined that 42% were between 1 and 10 ug/L and 4% in the excess of 10 ug/L. The highest cadmium level was 90 ug/L in the Tennesse river, Whitesburg, Ala. (Durum, Hem and Heidel, 1971 cited in: Friberg et al. 1974).

The major use of cadmium is in the electroplating industry (35%), followed by use in pigments (23%), as a stabilizer for poly-vinylchloride or P.V.C. (16%) and in nickel-cadmium batteries (13%) (Josephson, 1977). Other minor uses of cadmiun include: television picture tube phosphors, curing agent for rubbers, fungicides, solar cells, and for control rods and shields in nuclear reactors (Malin, 1971).

Zinc: It's occurance and uses

Zinc is considerably more abundant in nature than cadmium, the average earth crust content being about 70 ppm. The world production of zinc in 1975 totaled 4.4 million metric tons. Canada was the primary producer with 1.23 million metric tons (Packard, 1977).

In uncontaminated waters zinc levels rarely exceed 10 ug/L (NRC., 1979). Hem (1972) in a study of the occurance of cadmium and zinc in surface and ground waters of the U.S. estimated the median zinc level of 20 ug/L. The highest level of zinc was 1200 ug/L in Turkey creek near Joplin Mo. In the latter study, however, no mention is made of sources of zinc pollution in these waters. Méranger et. al. (1979) also found that the zinc levels rarely exceeded 10 ug/L in raw, treated and distributed water supplies of Canada. Some high levels were recorded in raw water samples of Dawson creek, B.C. (1[illegible] ug/L) and Magog, Que. (330 ug/L). In some samples the levels of zinc in treated and distributed waters exceeded those found in raw water: Kamloops, B.C., Grandfalls Newfoundland and Drummondville, Que. had raw water containing 5, 10 and 15 ug/L of zinc respectively, which increased to 40, 50 and 30 ug/L of zinc, respectively, once the water was treated and distributed. The authors attribute this to the leaching of zinc into the water from galvanized pipes used in the distribution of the water.

Contamination of water by zinc is primairly from smelting and refining operations. Vanloon and Beamish (1977) in their survey of 31 lakes found 7 lakes with an average zinc concentration > 100 ug/L, 3 lakes had concentrations between 51 and 100 ug/L, 8 lakes ranged from 11 to 50 ug/L, and 12 lakes had zinc concentrations less than or equal to 10 ug/L. The highest level of zinc, 8000 ug/L, was found in Ross Lake. A similar study in the U.S. detected zinc levels up to 21,000 ug/L in waters of the Coeur d'Alene River Id. where tailings of zinc ores were deposited (NRC., 1979).

Zinc is widely used in industry for the protection of steel and iron against corrosion (galvanizing). It is also used as an alloying metal with iron, copper, magnesium, aluminum and titanium. Other uses of zinc occur in white paints, enamel, glazes, rubbers, glass, wood preservative and in fungicides (Casarett and Doull, 1975).

Objective of this study

Zinc is omipresent in the environment, and it is almost always accompanied by cadmium. Previous studies have only considered the individual toxicity of such metals to Daphnia magna, even though these metals rarely occur as isolated entities. Taking this into consideration, we decided to examine not only the individual toxicities of zinc and cadmium to D. magna, but also examine their effect when they occur together.

Four sets of experiments were designed in this study. The first set of experiments were conducted in order to determine the acute toxicity of cadmium to Daphnia magna. The second set of experiments were designed to determine the acute toxicity of zinc. The remaining experiments were designed to determine the effect of a mixture of cadmium and zinc. The last two sets differed in the ratio of cadmium and zinc, since the ratio may have an effect on the action of either toxicant in Daphnia.

MATERIALS AND METHODS

Culturing and maintenance of Daphnia magna

The culture of Daphnia magna used in this study was obtained from the Arbor Scientific supply house. The purchase was made in May 1979. Upon reception, the Daphnia were transfered in a container containing the spring water they were shipped in. Over the span of the following week the original culture medium was diluted by a factor of 0.25 per day, using an algal solution (described in the next section). After one week the Daphnia were then transfered into a 430 liter polyethylene tub (Figure 1), which contained the basic culture medium.

Regular maintenance of the Daphnia culture consisted of straining the culture, once every two weeks, with a net in order to remove part of the population to prevent crowding. On a weekly basis fifty liters of the culture medium was replaced with an equivalent amount of algae solution, which served as the main food source for the Daphnia.

The temperature of the culture was maintained at $20^{0}C(\pm 1^{0} C)$, by the use of three (150 watts) thermostatically controlled heaters. The photoperiod of 12 hr. day/12 hr. night was obtained by setting a timer on a series of fluorescent lights (5400 Lux). Over the 18 month period of this study, the main culture was emptied every two months and cleaned to remove organic wastes.

Culturing and maintenance of algae

Agar slants of Chlorella vulgaris were obtained at the same time from Arbor Scientific. Suspended solutions of this algae were initiated by using five liters of Bristol's algae medium, described in Table 1.

Figure 1. Photograph of the tubs used for the *Daphnia* and algae cultures. The left tub is the algae culture and the right tub is the *Daphnia* culture.

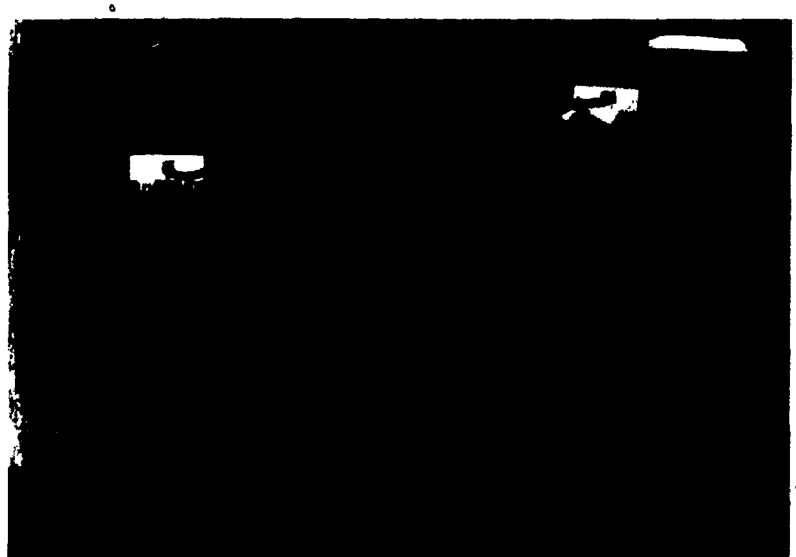


Table 1: Composition of Bristol's algae medium

Six stock solutions, 400 mls. in volume, contained one of the following;

$NaNO_3$	....	10.0 g
$CaCl_2$	....	1.0 g
$MgSO_4 \cdot 7\,H_2O$	....	3.0 g
K_2HPO_4	....	3.0 g
KH_2PO_4	....	7.0 g
NaCl	....	1.0 g

Fifty mls of each of the above solutions were added to 4.7 liters of glass distilled water to make up the medium. (Ward's, 1979).

These concentrated algal solutions were then used to seed a second 430 liter tub, containing charcoal filtered city of Montreal water. The characteristics of this water, which was also later used for bioassays, is listed in Table 2a and 2b. Thermostatically controlled heaters were also used to maintain the temperature at 20°C (± 1°C). The light regime was the same as that for the Daphnia culture. Other algal species (mostly Chlorophyta) were present in low numbers in the culture.

Exposure apparatus

The apparatus used for experiments is illustrated in Figure 2. The exposure chambers consisted of eight 15 L aquaria. Each of these tanks was drained at one end by a standpipe which allowed for a total volume of 14 liters in each tank. These drainage pipes were enclosed by a P.V.C. pipe with holes drilled near the bottom of the tank. The input of toxicant and/or water was placed at the opposite end of the tanks. This set up insured that the water coming in at one end from the top, would be drained from the bottom at the other end.

Twenty-two circular polyethylene containers (Figure 3) were suspended from the top of each of the eight tanks. Each container had a volume of 65 ml. These were cut on two sides and covered with plankton mesh (50 u) to allow for the flow of water through them. In all the experiments one Daphnia was placed in each of these containers. Between each experiment the containers were removed, cleaned and randomly redistributed to the test tanks.

The rate of diluting water was set at 200 ml/min for all the experiments, thus yielding a turnover time of 1.2 hr. The flow rate was maintained constant by using part of a serial diluter (Figure 4).

Table 2a: Characteristics of the dechlorinated city water

Test	Values	Determined by:
pH	6.95 (20°C)	Corning pH meter Model 10
Dissolved Oxygen	8.60 mg/l (20°C)	YSI Model 54 A
Alkalinity	80 mg/l ($CaCO_3$)	Hach colorimetric method
Calcium	100 mg/l ($CaCO_3$)	Hach colorimetric method
Total Hardness	130 mg/l ($CaCO_3$)	Hach colorimetric method
Conductance	300 uohms/cm	Hach meter
Color	0 STD	Hach meter
Turbidity	0 FTU (formazin)	Hach meter

Table 2b: Atomic Absorption analysis of the dechlorinated city water

Metal	Concentration (ug/l)	Determined by:
Cadmium	1.0	graphite furnace
Zinc	n.d.[1]	flame
Nickel	n.d.	graphite furnace
Iron	3.0	graphite furnace
Copper	n.d.	graphite furnace

[1] n.d.: not detectable

Figure 2. Photograph of the exposure apparatus used in this study. Shows: settling tank, head tank, Mariotte bottles, serial diluter, mixing chambers, eight aquaria and the *Daphnia* containers.

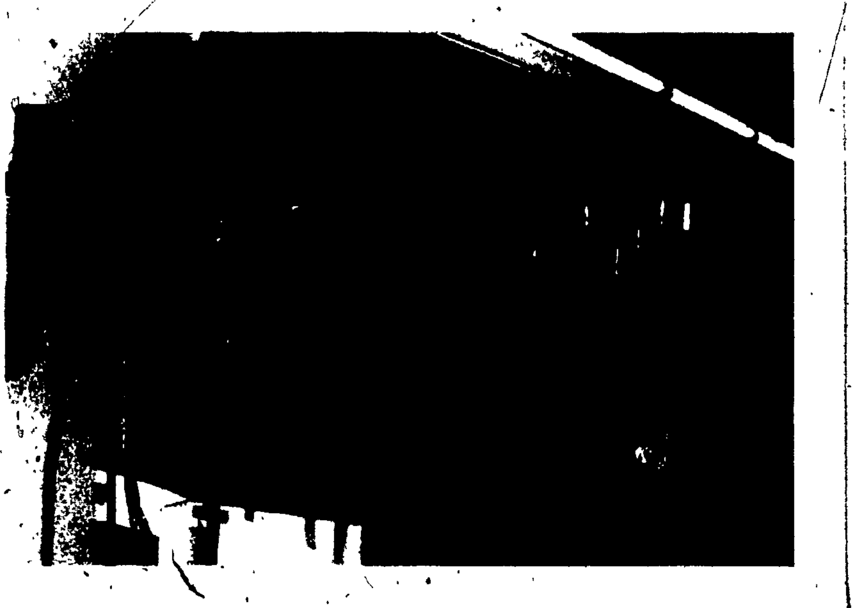


Figure 3. Photograph of the polyethylene *Daphnia* test containers.

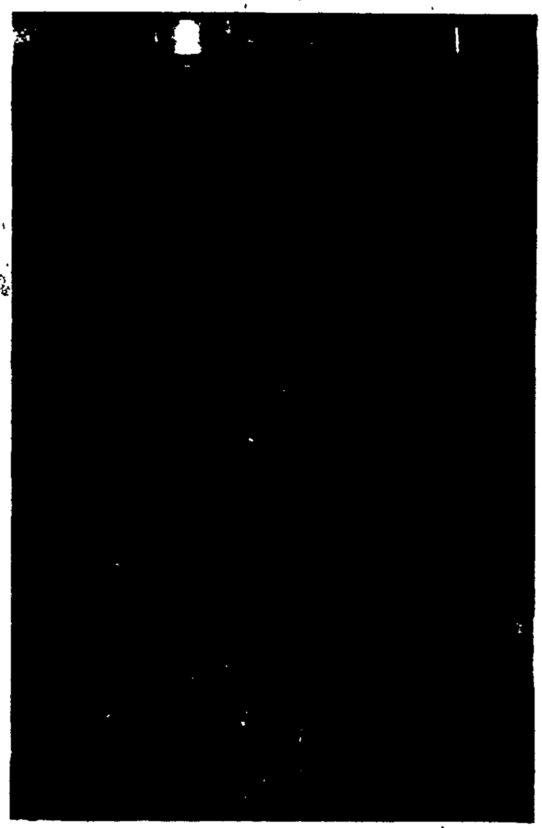


Figure 4. Photograph showing the serial diluter and Mariotte tubing entering the mixing chamber before distribution to the test tanks.

This consisted of a plexiglass box (60 x 10 x 10 cm.) with a series of eight glass faucets, whose deviation from a vertical position towards a horizontal position offered a control of the flow rates. Proper aeration and constant pressure of incoming diluting water was maintained by the use of a settling tank and a head tank. A constant temperature of 20°C was maintained by mixing 11°C dechlorinated water with 27°C dechlorinated water supplied to the lab by the physical plant of the university.

Toxicant concentrations were controlled by using six (18 L) Mariotte bottles (Figure 5). Intramedic polyethylene tubing (I.D. = 0.86 mm; O.D. = 1.27 mm) was used to provide a constant flow rate of 0.3 ml/min from each Mariotte. The toxicant flowing from the Mariotte was introduced, via a funnel system, to the respective mixing chamber, where it was diluted before being distributed to the tanks. Cadmium chloride ($CdCl_2 \cdot 2.5 H_2O$) and Zinc chloride ($ZnCl_2$) were the forms of metals used in this study. Concentrations in the Mariotte bottles were set so that the desired concentration would be obtained after dilution.

Determination of cadmium and zinc concentrations

The determination of metal quantities added to each tank was performed daily throughout the duration of each experiment. The total cadmium was determined by graphite furnace and the total zinc by flame atomic absorption. The conditions used for each determination is shown in Table 3.

A series of five standards were made daily for each metal, and the respective absorbance values were determined. The absorbance values for the zinc were obtained by the integrated value given by the instrument, whereas peak readings from the recording chart were used in the case of

Figure 5. Photograph of the Mariotte bottles used to deliver the toxicant solutions to the exposure tanks.

Table 3: Conditions for atomic absorption spectrophotometric determinations of cadmium and zinc concentrations

	Cadmium	Zinc
Instrument	Perkin-Elmer (Model 503)	Perkin-Elmer (Model 503)
Radiation source	Hollow Cathode Lamp	Hollow Cathode Lamp
Current	4 mA	15 mA
Furnace	HGA-2100 graphite furnace using pyrolysed tubes	4 inch single slot flame burner
Wavelenght	228.8 nm	213.9 nm
Slit width	0.7 nm	0.7 nm
Resonance line	U.V. Range	U.V. Range
Function display	Absorbance	Absorbance
Readout	Peak Mode integration	3 sec. Integration
Fuel	Nitrogen: 30cc/min (Normal flow)	Air : 50 cc/min Acetylene: 22.5 cc/min
Purge system	Automatic High Temperature (2700°C)	
Drying temperature	150°C	
Drying time (HGA)	30 sec	
Charing temperature	250°C	
Charing time (HGA)	20 sec	
Atomizing temperature	2100°C	
Atomizing time (HGA)	8 sec	
Recorder range	10 mV	
Chart speed	5 mm/min	

Table 3; Continued

	Cadmium	Zinc
Sample size	20 uL	5 ml/min (Aspiration rate)
Sensitivity	0.225 ug/L	20 ug/L
Linear Working Range	7.5 ug/L	1000 ug/L

cadmium. A linear regression was performed on the collected readings of each metal, and the resulting regression equation was determined.

A sample of water (100 ml) was obtained daily from the interior of the containers holding the Daphnia. In order to determine the zinc concentration of each tank, the sample was aspirated into the burner and the average of five readings at 3 seconds integration was determined. The samples used for cadmium determination usually had to be diluted with glass distilled water in order to operate within the linear working range of the graphite furnace. The average peak height of four 20 microliter injections was then determined and corrected by the dilution factor. The mean absorbance or peak value was then used to determine the concentration of each tank. The mean concentration of each tank over experimental period was then later used in the treatment of the data.

Culturing of twelve hour old *Daphnia magna*

For all the experiments in this study the Daphnia used were cultured in the same manner. Twelve hours before the start of experiments, 500 gravid females were chosen. These were separated into groups of ten, and placed in 50 ml beakers containing 40 mls of the algal solution. After the twelve hour period, the adult Daphnia were removed using a large bore size pipet, and the remaining juveniles were pooled.

A juvenile was placed in each of the 22 containers in each of the 8 test tanks. The total number of daphnids used for each experiment was 176.

Evaluation of the suitability of the flow-through system for the maintenance of Daphnia magna.

Prior to running the bioassays in a flow-through system, two life tables (Krebs, 1972) were constructed to determine if the system was suitable for the maintenance of Daphnia magna.

Twenty new born Daphnia were randomly picked from the progeny of 25 adult females. These daphnids were divided into two groups; ten for the static system and ten for the flow-through. Both groups were maintained at 20°C and subjected to the same photoperiod (12 hr day/12 hr night). Each group was fed equal amounts of Chlorella each day. The amount of food given was determined by spectrophotometric readings (at $\lambda = 665$ Å for chlorophyll A).

The static system consisted of ten 120 ml polyethylene beakers each containing one daphnid. Half the medium was changed daily during feeding. In the case of the flow-through system, each of ten daphnids was placed in the same containers that were later used in the bioassays.

Daily observations were made and the number of daphnids produced was recorded. Once counted the newly produced young were removed. These observations were continued until the twenty original daphnids had died. Life table statistics were calculated as in Krebs (1972).

Design of bioassay experiments

Four sets of experiments were conducted in this study. In all the experiments, 12 (±12) hour old Daphnia magna were used. The 176 daphnids used in each experiment were randomly distributed to eight test aquaria. In each experiment two of these aquaria, a total of 44 daphnids were used as control. The remaining six tanks, each containing 22 daphnids, were subjected to different toxicant concentrations.

Observations were started at the twenty-fourth hour, (very few deaths were noted before that time period) and were made every third hour (i.e.: 24:00 hr, 27:00 hr, 30:00 hr, ..., 96:00 hr) throughout the test period. Three exceptions to this method were made. In two of the cadmium experiments observations were made either at every half hour or every hour from the start of the experiments. This was necessary because of the high rate of mortality in the assays with high cadmium levels. The third exception, is that one of the zinc experiments was extended over the 96 hour period up to 140 hours.

Mortality was estimated as the lack of movement in the second antenules and internal organs in a five second period of observation. Dead organisms were immediately removed from their containers. The time of death of each individual was recorded.

In the first set of bioassays five experiments were conducted to determine the acute toxicity of cadmium to Daphnia magna. Thirty different cadmium levels ranging from 3.4 ug/L to 31,400 ug/L were tested.

The second set of bioassays consisted of four experiments, designed to determine the acute toxicity of zinc. The concentrations used ranged from 10.6 ug/L to 450.9 ug/L.

The third and fourth sets of experiments were designed to determine the effect of cadmium-zinc mixtures on Daphnia magna. These two sets differed in the ratio of metals present. In the third set, consisting of two experiments, the ratio was set at one to one in terms of potency, as determined from the first two sets of bioassays. Since cadmium was found to be more toxic than zinc, this resulted in a mixture containing more zinc than cadmium in terms of weight. In the fourth experiment equal amounts of cadmium and zinc, in terms of weight, were added.

Treatment of data

To assess the acute toxicity of cadmium and zinc to *Daphnia magna*, the data were analyzed by the dosage-mortality curve (Finney, 1971) and the time-mortality curve (Litchfield, 1949). For each experiment the cumulative percent mortality for each concentration was plotted against time on a probit-log paper. A linear regression was used to determine the best fit line on these points. The resulting regression equation was then used to determine the median effective time (ET50) and the percent mortality in probits for 36, 48, 60, 72, and 96 hours for each concentration. From these results two types of graphs were contructed. The first graph plotted the median effective time against the log of the concentration of the metal. The second graph was the dose-response relationship. To construct this, the probit response, obtained from the regression equation forementioned at 36, 48, 60, 72 and 96 hours was plotted against the log of the metal concentration. A best fit line on these points was obtained by using the statistical package, Statpak, available at the university computer terminal. The expected probits, obtained from the probit analysis routine, were then used to obtain the weighting coefficients and the working probits used in the maximum likelihood estimation of the dose-response curve (Finney, 1971). The calculations of the regression parameters, goodness of fit statistics, fiducial limits and the LC50, were obtained through a fortran program (Weinstein, 1979). The dosage-mortality curves were also tested for similarities in slopes by the use of an F-test (Sokal and Rohlf, 1969).

The assessment of the potencies of the cadmium and zinc mixtures followed the criteria outlined by Anderson and Weber (1975). The various forms of multiple toxicity are classified as, concentration-addition, response-addition, supra-additive synergism, or infra-additive antagonism.

The concentration addition model assumes that the discrete entities of a toxicant mixture act on the same receptor in the test organism. The relative potency of a mixture can be predicted from the discrete dose-response curves of the toxicants. This is accomplished by the derivation of a relative potency factor, which is used in the conversion of the concentration of one toxicant in terms of the other toxicant used. The relative potency factor is calculated as:

$$R_{cz} = \text{Antilog} \left[\frac{Y^* - a_c}{b_c} - \frac{Y^* - a_z}{b_z} \right]$$

where:

- R_{cz} : relative potency factor converting zinc concentration into cadmium equivalent units.
- Y^* : given level of response
- a_c : intercept of cadmium
- a_z : intercept of zinc
- b_c : slope of cadmium
- b_z : slope of zinc

The predicted potency of the toxicant mixture can then be expressed as:

$$Y = a_c + b_c \log (R_{cz} \times C_z + C_c)$$

Contrary to the concentration-addition model, the response-addition model assumes that the discrete entities in a mixture of toxicants act on different receptors to elicit a common response. The relative potency of the mixture can also be predicted from the discrete dose-response curves in terms of the proportion of animals responding. The difference with this model, however, is that it takes into consideration the fact that the tolerances may or may not be correlated. The expected proportion responding when the tolerances are not correlated is expressed as:

$$P_{cz} = P_c + P_z \ (1 - P_c)$$

where: P_{cz} : Proportion responding to a mixture of cadmium and zinc
P_c : proportion responding to cadmium only
P_z : proportion responding to zinc only

If the tolerances are positively correlated then the expected proportion responding is expressed as:

$$P_{cz} = P_c \ ; \text{ if } \ P_c > P_z$$

$$P_{cz} = P_z \ ; \text{ if } \ P_z > P_c$$

Lastly, if the tolerances are negatively correlated then the expected proportion responding is determined as:

$$P_{cz} = P_c + P_z \ ; \text{ if } \ P_c + P_z < 1$$

$$P_{cz} = 1 \ ; \text{ of } \ P_c + P_z \geq 1$$

Supra-additive synergism can be described as observed responses which surpass those estimates predicted by the response-addition and concentration addition models. On the other hand, infra-additive antagonism shows observed responses which fall short of the estimates predicted by the response-addition and concentration addition models. For both of these models, no prediction can be made from the discrete dose-response data.

RESULTS

Life table analysis

The suitability of the flow-through apparatus used in this study for bioassays with Daphnia magna was determined by comparing life table parameters with that of a static system. The cohort life table established for both systems is shown in Table 4.

In order to simplify the calculations the age of the daphnids were subdivided into 3 day class intervals, and the pivotal age ($\bar{x}$) was determined. For both systems the following life table parameters were tabulated: l_x, the number surviving at the start of age interval x; m_x, the mean number of offsprings per female of age x, and e_x the average expectation of life at age x.

The lack of replicates does not allow for a statistical comparison of the two systems. However, there seems to be some advantage in using the flow-through system if we consider that at 53 days all the daphnids in the static system were dead whereas only 50% died in the flow-through one. The flow-through system daphnids produced 1,510 offsprings as compared to 1,014 for the static system daphnids. In addition the calculated intrinsic rate of increase (r) for the flow-through system (0.27/d) was greater than for the static system (0.22/d). These differences may not be great on a short time period, but they may be significant over the span of several generations.

Table 4: Life table statistics of *Daphnia magna* cultured in a flow-through and static system.

		Flow-through system			Static system		
x	$\bar{x}$	l_x	m_x	e_x	l_x	m_x	e_x
1 - 3	2	1.00	0.00	15.30	1.00	0.00	11.40
4 - 6	5	1.00	0.00	14.30	1.00	0.00	10.40
7 - 9	8	1.00	0.00	13.30	1.00	0.00	9.40
10 - 12	11	1.00	7.14	12.30	1.00	5.30	8.40
13 - 15	14	1.00	15.11	11.30	1.00	10.00	7.40
16 - 18	17	0.90	13.50	11.50	1.00	1.30	6.40
19 - 21	20	0.90	9.75	10.50	1.00	0.00	5.40
22 - 24	23	0.90	25.33	9.50	0.80	0.75	11.03
25 - 27	26	0.90	17.00	8.50	0.70	5.57	5.36
28 - 30	29	0.90	27.00	7.50	0.70	16.43	4.36
31 - 33	32	0.80	26.33	7.38	0.60	27.67	4.00
34 - 36	35	0.80	16.75	6.38	0.60	18.33	3.00
37 - 39	38	0.70	36.00	6.21	0.50	42.80	2.50
40 - 42	41	0.60	52.00	6.17	0.40	40.00	2.00
43 - 45	44	0.60	20.00	5.17	0.30	30.33	1.50
46 - 48	47	0.60	22.20	4.17	0.20	0.00	1.00
49 - 51	50	0.50	16.00	3.90	0.10	0.00	2.00
52 - 54	53	0.50	18.00	2.90	0.00	-	-
55 - 57	56	0.30	0.00	3.50			
58 - 60	59	0.20	37.00	4.00			
61 - 63	62	0.20	39.00	3.00			
64 - 66	65	0.10	25.00	4.50			
67 - 69	68	0.10	32.00	3.50			
70 - 72	71	0.10	0.00	2.50			
73 - 75	74	0.10	37.00	1.50			
76 - 78	77	0.10	47.00	0.50			
79 - 81	80	0.00	-	-			

Description of symbols:

- x : 3 day-class interval
- $\bar{x}$: pivotal age (day)
- l_x : number surviving at start of age x
- m_x : mean number of offsprings per female of age x
- e_x : average expectation of life at age x

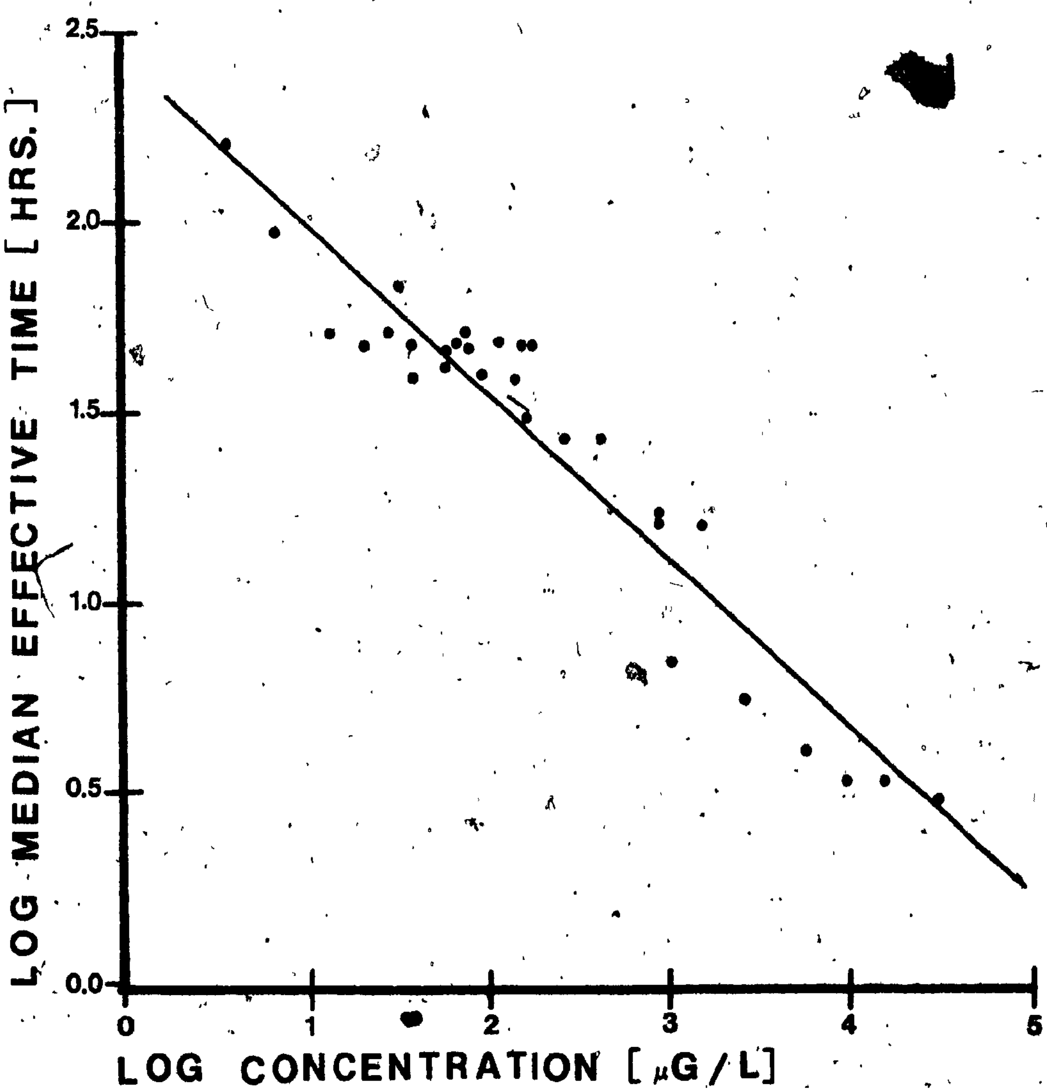
The effects of cadmium on Daphnia magna

The empirical results obtained for the cadmium bioassays are tabulated in Appendix I. For each of the thirty cadmium concentrations assayed, the cumulative number responding at each observation period and the respective percent mortality calculated are listed. The data represent five experiments, in which six different concentrations were tested along with two controls for each. In the total of ten controls, no mortality was observed during the course of the experiments.

The time-mortality curve obtained for cadmium is illustrated in Figure 6. The regression equation of this line was calculated as, $y=2.437 - 0.441x$, with a correlation coefficient of -0.955 (significant at $\alpha = 0.01$), and a goodness of fit test, using chi-square, which reveals that the line adequately represents ($P < 0.05$) the data. The regression parameters and their correlation coefficients used to determine these points have been tabulated in Appendix II. A t-test for the significance of the correlation coefficients (Sokal and Rohlf, 1969) was applied and revealed that 24 out of 30 correlation coefficients were significant at $\alpha = 0.01$, 2 were significant at $\alpha = 0.05$, and 4 were not significant. The latter 4 were among the highest cadmium concentrations in which the rate of mortality allowed for only one degree of freedom in the data.

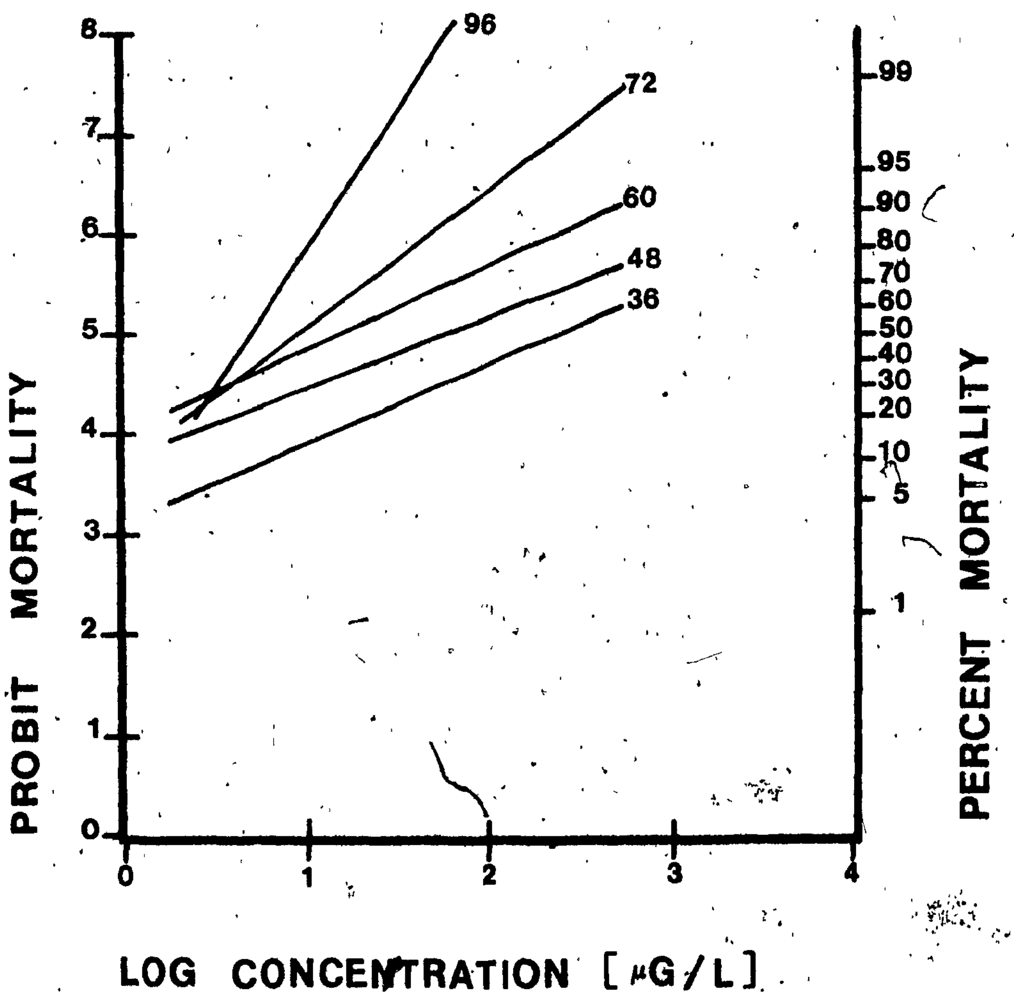
The dosage-mortality curves for cadmium at 36, 48, 60, 72 and 96 hours are illustrated in Figure 7. The regression equation parameters, predicted probit mortality, 95% fiducial limits and empirical probits for these lines are tabulated in Appendix III a. - III e. The goodness of fit test using a chi-square test, shows that all of these lines are an

Figure 6. The time-mortality curve for Daphnia magna exposed to cadmium.



LOG MEDIAN EFFECTIVE TIME [HRS.]
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
LOG CONCENTRATION [µG/L]

Figure 7. The dosage-mortality curves for Daphnia magna exposed to cadmium at 36, 48, 60, 72 and 96 hours.



PROBIT MORTALITY
PERCENT MORTALITY
LOG CONCENTRATION [μG/L]
96
72
60
48
36
0
1
2
3
4
5
6
7
8
99
95
90
80
70
60
50
40
30
20
10
5
1

adequate representation ($P < 0.05$) of the data. The dosage-mortality curves in Figure 7 have the following slopes, 0.831, 0.701, 0.839, 1.351 and 2.801 for the 36, 48, 60, 72 and 96 hour lines respectively. The results for F-test for the equality of slopes are listed in Table 5. The test results for the comparison of the 36 hour vs. 48 hour and the 48 hour vs. 60 hour lines show that these lines have equal slopes at the three α levels tested. The comparison between the 60 hour vs. 72 hour and 72 hour vs. 96 hour lines show different slopes at $\alpha = 0.10$. This difference is further demonstrated by the comparison between the 60 hour line and the 96 hour line which shows different slopes at all α levels tested. The LC50 values, extrapolated from these lines, for 36, 48, 60, 72 and 96 hours are 203.80, 58.16, 15.81, 8.88 and 5.00 ug/L respectively.

The effects of zinc on Daphnia magna

The empirical results for the twenty-three different zinc concentrations used in the second set of bioassays are tabulated in Appendix IV. Listed are the cumulative number of Daphnia responding at each observation period and the respective percent mortality calculated. A total of eight controls were used, two for each of the four experiments conducted. No mortality was observed in the controls during the course of these experiments.

The time-mortality curve for the zinc bioassays is illustrated in Figure 8. The calculated regression equation for this line is; $y = 2.611 - 0.328 x$. The correlation coefficient of -0.454 for this line is significant at $\alpha = 0.05$ and the chi-square for goodness of fit reveals that the regression line adequately ($P < 0.05$) represents the data. Appendix V lists the regression parameters and the correlation

Table 5: Test for the equality of slopes for the cadmium dosage-mortality curves

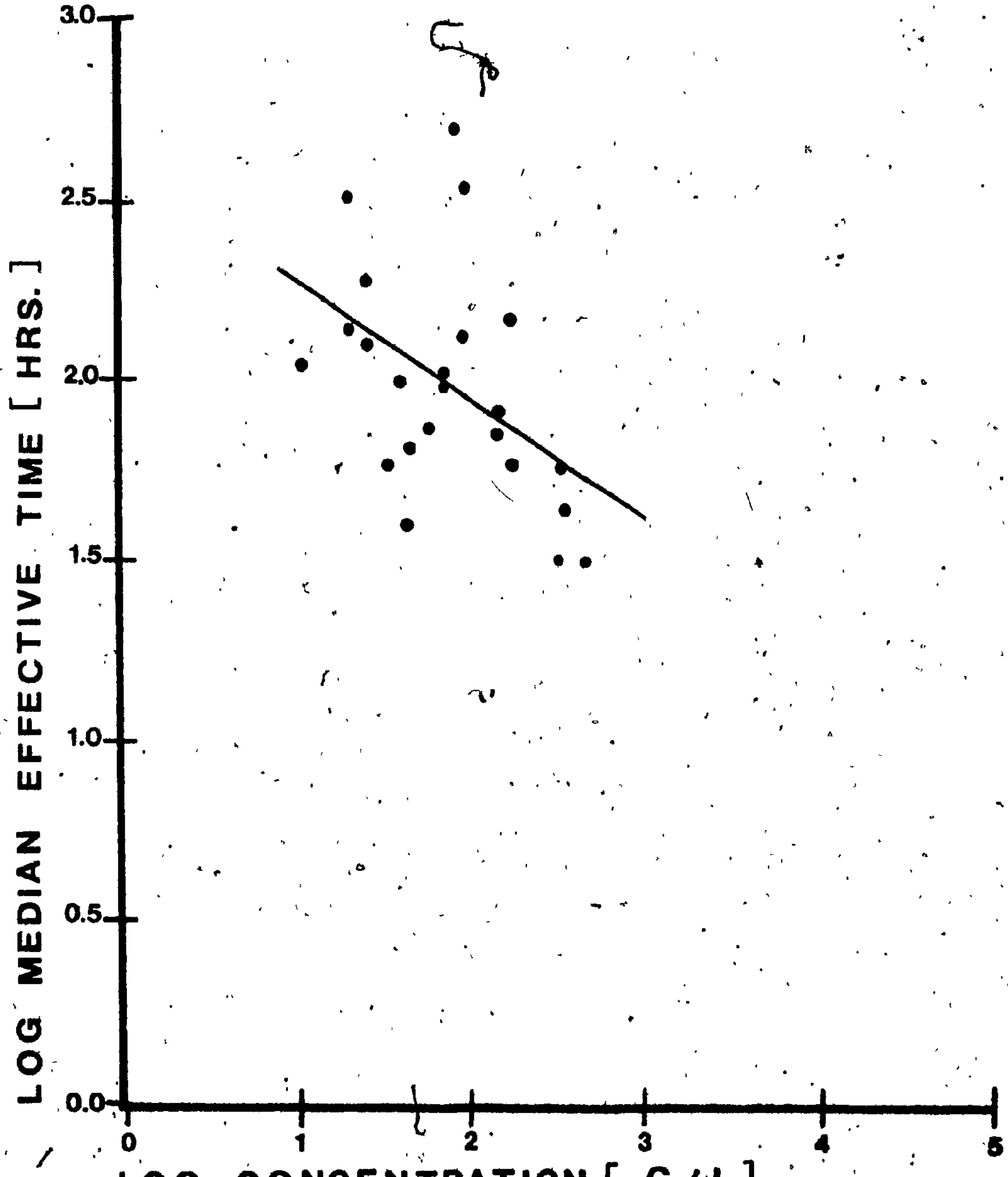
Lines compared	F_s	Degrees of freedom[1] N	D	α - Level	Critical F - value
36 hr. vs. 48 hr.	0.109	1	36	0.001	12.60 *
				0.01	7.31 *
				0.1	2.84 *
48 hr. vs. 60 hr.	0.452	1	32	0.001	13.30 *
				0.01	7.56 *
				0.1	2.88 *
60 hr. vs. 72 hr.	3.870	1	25	0.001	13.90 *
				0.01	7.77 *
				0.1	2.92 **
72 hr. vs. 96 hr.	6.340	1	12	0.001	18.60 *
				0.01	9.33 *
				0.1	3.18 **
60 hr. vs. 96 hr.	20.970	1	17	0.001	15.70 **
				0.01	8.40 **
				0.1	3.03 **

* Slopes are equal

** Slopes are not equal

1. N: numerator
   D: denominator

Figure 8. The time-mortality curve for Daphnia magna exposed to zinc.



3.0
2.5
2.0
1.5
1.0
0.5
0.0
LOG MEDIAN EFFECTIVE TIME [HRS.]
0
1
2
3
4
5
LOG CONCENTRATION [μG / L]

coefficients used in the determination of the median effective time at each concentration. Twenty-one of these correlation coefficients were significant at $\alpha = 0.01$ and the remaining two were significant at $\alpha = 0.05$ according to the t-test.

Illustrated in Figure 9 are the dosage-mortality curves for 36, 48, 60, 72 and 96 hours for zinc. The regression parameters, predicted responses, 95% fiducial limits, and the empirical responses used in the construction of these lines are listed in Appendix VIa. - VIe. The chi-square test for goodness of fit showed that the lines adequately represent the data at $P < 0.005$ for the 36 hour line and at $P < 0.05$ for the 48, 60, 72 and 96 hour lines. The slopes for the 36, 48, 60, 72 and 96 hour dosage-mortality curves are 0.785, 0.656, 0.587, 0.747 and 0.363 respectively. The results for the F-test for equality of slopes, Table 6, reveal that all the slopes are equal at all α levels tested. The LC50 values determined from the 36, 48, 60, 72 and 96 hour lines are 861.06, 708.94, 420.25, 136.10 and 67.91 ug/L.

The effects of cadmium-zinc mixtures on *Daphnia magna*

The effect of two different mixtures of cadmium and zinc on *Daphnia magna* were assayed. In the first two experiments cadmium and zinc were in a ratio of equal potency. The third experiment differed in that the cadmium and zinc were present in equal concentrations.

The empirical results obtained for the cadmium-zinc mixtures, in terms of equal potency, are tabulated in Appendix VIII. The latter lists the actual concentrations of cadmium and zinc, the cumulative number of

Figure 9. The dosage-mortality curves for Daphnia magna exposed to zinc for 36, 48, 60, 72 and 96 hours.

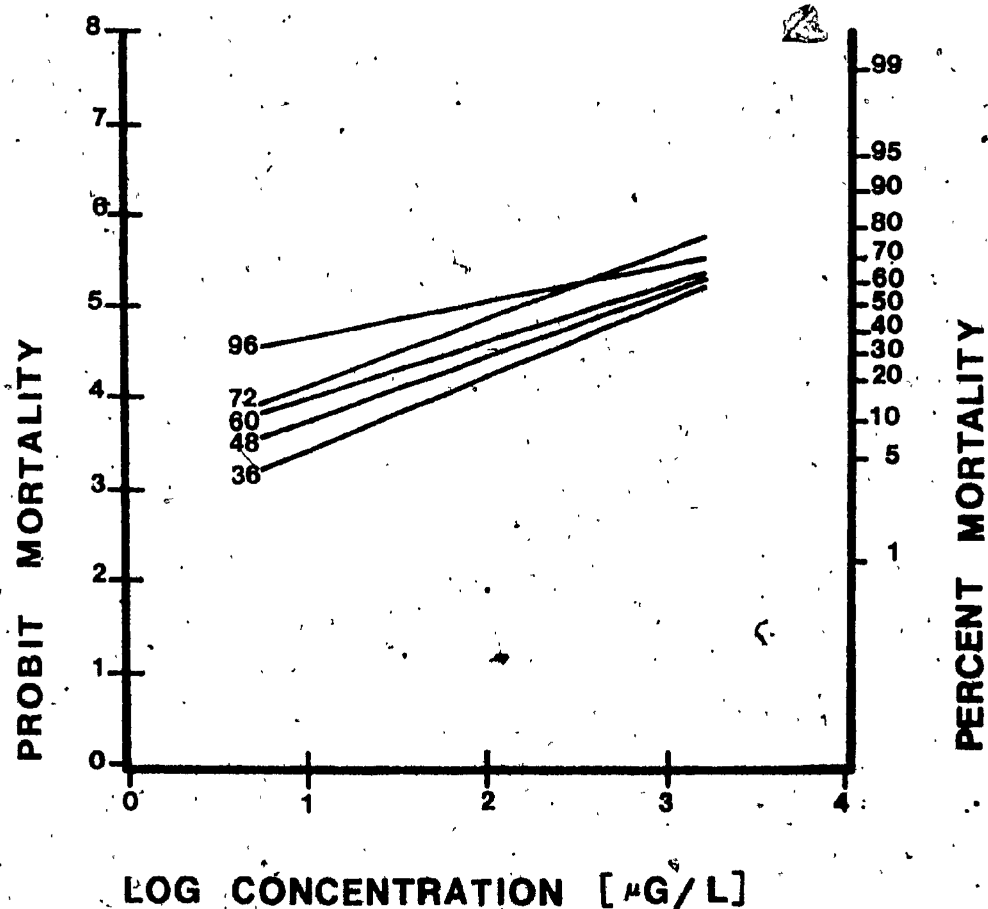

PROBIT MORTALITY
PERCENT MORTALITY
LOG CONCENTRATION [μG/L]
96
72
60
48
36
0
1
2
3
4
5
6
7
8
99
95
90
80
70
60
50
40
30
20
10
5
1

Table 6: Test for the equality of slopes for the zinc dosage-mortality curves

Lines compared	F_s	Degrees of freedom[1] N	Degrees of freedom[1] D	α - level	Critical F - value
36 hr. vs. 48 hr.	0.080	1	37	0.001 0.01 0.1	12.60 * 7.31 * 2.84 *
48 hr. vs. 60 hr.	0.079	1	37	0.001 0.01 0.1	12.60 * 7.31 * 2.84 *
60 hr. vs. 72 hr.	0.193	1	38	0.001 0.01 0.1	12.60 * 7.31 * 2.84 *
72 hr. vs. 96 hr.	0.781	1	36	0.001 0.01 0.1	12.60 * 7.31 * 2.84 *
60 hr. vs. 96 hr.	0.303	1	36	0.001 0.01 0.1	12.60 * 7.31 * 2.84 *

* Slopes are equal

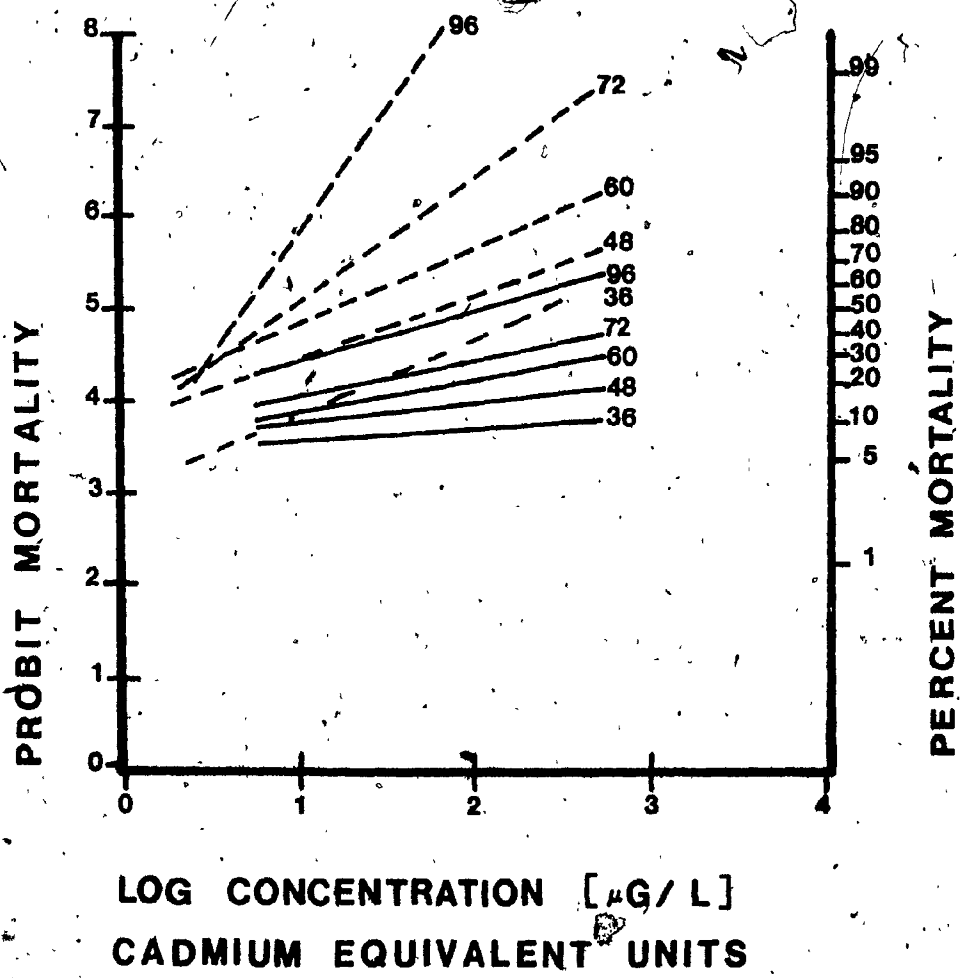
** Slopes are not equal

1. N: numerator
   D: denominator

Daphnia responding at each observation period and the respective cumulative percent mortality. Four controls, two for each experiment, showed no occurrence of mortality during the experiments.

The dosage-mortality curves were constructed by using a relative potency factor to convert the actual zinc concentration into cadmium equivalent units. Calculation of the dosage-mortality curves by using zinc equivalent units showed no significant change in the results , hence only the results based on the cadmium equivalent units are described. The regression lines for the mixture at 36, 48, 60, 72 and 96 hours are shown in Figure 10 as solid lines. The broken lines represent the expected dosage-mortality curves based on the cadmium discrete lines. The data used to draw these lines: regression parameters, predicted mortality, 95% fiducial limits, empirical response and expected response are listed in Appendix VIIIa. - VIIIe. The calculated chi-square values reveal that the lines for 36, 48 and 60 hours are a good fit at $P < 0.05$ whereas the lines for the 72 and 96 hours show a good fit at $P < 0.005$. The dosage-mortality curves for 36, 48, 60, 72 and 96 hours, shown as solid lines in Figure 10, seem to indicate a gradual increase in slope with time, 0.114, 0.236, 0.377, 0.383 and 0.560 respectively. However the results of the comparison of slopes in Table 7 shows that the slopes do not differ at all α levels. A similar comparison was also made to compare the observed versus the expected dosage-mortality lines. Each line was compared to its respective expected line in Table 8. The results show that all the observed dosage-mortality lines differ from their expected lines at $\alpha = 0.10$. It is clear that the dosage-mortality curves for the cadmium-zinc mixture in terms of equal potency ratio falls short from the expected lines. By using a common probit response of 4.5, a comparison of the shift in the concentration which elicits the said response was used to quantify the difference. Quantitatively, the dif-

Figure 10. The dosage-mortality curves for Daphnia magna exposed to cadmium-zinc mixtures in terms of equal potency for 36, 48, 60, 72 and 96 hours. Observed results are represented by solid lines and the expected results are represented by broken lines.



PROBIT MORTALITY
PERCENT MORTALITY
LOG CONCENTRATION [μG/ L]
CADMIUM EQUIVALENT UNITS
0
1
2
3
4
5
6
7
8
99
95
90
80
70
60
50
40
30
20
10
5
1
96
72
60
48
96
36
72
60
48
36

Table 7: Test for the equality of slopes for the cadmium-zinc mixture in terms of equal potency

Lines compared	F_s	Degrees of freedom[1] N	D	α- level	Critical F - value
36 hr. vs 48 hr.	0.076	1	6	0.001	35.50 *
				0.01	13.70 *
				0.1	3.78 *
48 hr. vs. 60 hr.	0.063	1	10	0.001	21.00 *
				0.01	10.00 *
				0.1	3.29 *
60 hr. vs. 72 hr.	0.001	1	13	0.001	17.80 *
				0.01	9.07 *
				0.1	3.14 *
72 hr. vs. 96 hr.	0.159	1	14	0.001	17.10 *
				0.01	8.86 *
				0.1	3.10 *
36 hr. vs. 96 hr.	0.344	1	9	0.001	22.90 *
				0.01	10.60 *
				0.1	3.36 *

* Slopes are equal

** Slopes are not equal

1. N: numerator

D: denominator

Table 8: Test for the equality of slopes between the observed and expected dosage-mortality curves for the cadmium-zinc mixture in terms of equal potency

Lines compared	F_s	Degrees of freedom[1] N	D	α - level	Critical F - value
36 hr. vs. 36 hr. (o) (e)	3.350	1	21	0.001	14.60 *
				0.01	8.02 *
				0.1	2.96 **
48 hr. vs. 48 hr. (o) (e)	3.220	1	21	0.001	14.60 *
				0.01	8.02 *
				0.1	2.96 **
60 hr. vs. 60 hr. (o) (e)	3.210	1	21	0.001	14.60 *
				0.01	8.02 *
				0.1	2.96 **
72 hr. vs. 72 hr. (o) (e)	6.340	1	17	0.001	15.70 *
				0.01	8.40 *
				0.1	3.03 **
96 hr. vs. 96 hr. (o) (e)	4.700	1	9	0.001	22.90 *
				0.01	10.60 *
				0.1	3.36 **

(o) Observed (e) Expected

* Slopes are equal

** Slopes are not equal

1. N: numerator
   D: denominator

ference between the observed and expected lines are 12.3 x 10^6, 1847, 160, 45 and 4 fold decreases in potency for the 36, 48, 60, 72 and 96 hours lines respectively. It is important to note however that this difference is not constant over the whole response range (i.e.: the difference in slopes yeild greater differences at greater response levels).

Appendix IX describes, in a similar fashion to the previous data, the empirical results obtained for the cadmium-zinc mixture in terms of equal concentrations. The regression analysis for 36, 48 and 60 hours revealed that no dosage-mortality curve could be established, since no mortality occured in 36 and 48 hours and that the Daphnia only responded at two concentrations in 60 hours. The regression parameters for the 72 and 96 hours lines have been included in Appendix Xa. - Xb. The lines adequately ($P < 0.05$) represent the data, as shown by the calculated chi-square values. Comparing the observed 72 hour dosage-mortality line with the observed 96 hour lines shows no difference in slopes at all α - levels (Table 9). When these observed lines are compared to their respective expected lines the results show that the 72 hour line differ at $\alpha = 0.01$, whereas the 96 hour lines do not differ. The difference between the observed and expected lines were found to be 100 and 33 fold decreases in the potency for the 72 and 96 hour lines respectively.

Table 9: Test for the equality of slopes for the cadmium-zinc mixture in terms of equal concentration and between observed and expected dosage-mortality curves

Lines compared	F_s	Degrees of freedom[1] N	D	α - level	Critical F - value
72 hr. vs. 96 hr.	0.0469	1	4	0.001	74.10 *
				0.01	21.20 *
				0.1	4.54 *
72 hr. (o) vs. 72 hr. (e)	9.660	1	12	0.001	18.60 *
				0.01	9.33 **
				0.1	3.18 **
96 hr. (o) vs. 96 hr. (e)	2.840	1	4	0.001	74.10 *
				0.01	21.20 *
				0.1	4.54 *

(o) Observed (e) Expected

* Slopes are equal

** Slopes are not equal

1. N: numerator

D: denominator

DISCUSSION

In order to evaluate the effects of hazardous materials on aquatic life under laboratory conditions it is important that several conditions be met. Ideally the organism chosen will be representative of other organisms related to it in terms of general classification and ecological role. In addition, the organism must also be representative in terms of sensitivity to hazardous substances. In this study Daphnia magna was chosen as a representative of the crustacean component of the zooplankton community. Daphnia magna adequately represents other freshwater crustaceans in that it inhabits the open waters of lakes, it plays a major role as a grazer on phytoplankton, and it is an important link in the trophic chain (Brooks and Dodson, 1965). As mentioned in the introduction previous studies have shown Daphnia magna to be sufficiently representative of other cladocerans in terms of sensitivity to toxicants.

Laboratory studies must insure proper maintenance and growth conditions for the test organism in order to successfully detect any deleterious effects caused by the toxicants. Previous studies using Daphnia as a test organism in bioassays have employed a static system (Anderson 1948, Biesinger and Christensen 1972, Baudouin and Scoppa 1974, Winner and Farrell 1976, and Shcherban 1979). The culture mediums used in such systems vary in pH (7.4-9.5), alkalinity (42-119 mg/L), total hardness (45-160 mg/L) and oxygen content (7.4-9.5 mg/L).

There are some disadvantages in using the static system and these are eliminated by use of a flow-through system. With the static system the experimenter must change the assay medium in order to prevent oxygen depletion, plating out of metal toxicants and build up of organic wastes.

The frequency of such changes varies from study to study. Some make the changes daily, some every second day, while others change the assay medium only once a week. These changes require the preperation of fresh assay medium each time, thus increasing the variation in the quality of the medium and its concentration of toxicants. Another disadvantage of this procedure is that it requires a more frequent manipulation of the test organisms, hence inducing an unnecessary stress factor. These problems are eliminated with the use of a flow-through system.

Another important factor to consider in the planning of such a study is the occurance of the toxicants in natural waters and the likelihood that the organism will be in contact with them. In the introduction we have elaborated on the occurance and use of cadmium and zinc. Also presented is the evidence of the contamination of natural waters by these metals. Clearly with such widely used metals it is likely that the zooplankton component of these lakes will be affected by their presence.

Previous studies with Daphnia have only considered the effects of heavy metals as discrete entities. Considering the fact that in general the probability of encountering cadmium and zinc as discrete entities is far less than finding them in association, this study examined not only the effect of these metals as discrete entities but also as components of a mixture.

Suitability of the exposure apparatus

In order to insure that the flow-through apparatus constructed for this study, would support the growth of daphnids, a cohort life table (Krebs, 1972) was constructed and compared to a static system. The results in Table 4 indicate that the daphnids have a slightly greater longevity, offspring production and consequently a greater intrinsic rate of increase in the flow-through system. The intrinsic rates of increase of 0.22/day for the static system and 0.27/day for the flow-through system approximate the values obtained by Hall (1964) for D. galeata mendotae (r = 0.23/day). Of importance is the fact that both systems, the static used to culture the 12 hour old daphnids and the flow-through system used in the bioassays, show no mortality in the first ninty-six hours. The choice of the flow-through system in this study was influenced by the results obtained above and by the fact that there is less manipulation of the animals.

The acute toxicity of cadmium to Daphnia magna

The effect of cadmium on the 12 (± 12) hour old daphnids was assessed by the development of the time-mortality and dosage-mortality curves. The time-mortality curve, Figure 6, shows that no threshold concentration was attained. Concentrations of cadmium as low as 3.4 (± 0.4) ug/L failed to show any threshold effect. Concentrations of cadmium below that level were not tested because the apparatus used did not allow for constant low toxicant concentrations over a prolonged period of time. Our observations show that at 3.4 ug/L (Appendix I) mortality was quite significant with 36 percent mortality after 92 hours. Thus we conclude that the threshold concentration, at which no response is observed, must be much less than 3.4 ug/L. Anderson (1948) found that the threshold concentration of cadmium for Daphnia magna is lower than 2.6 ug/L, whereas Shcherban (1979) found the 72 hour LCo (the threshold concentration at which no mortality is observed in 72 hr) to be less than 0.1 ug/L. In a similar study with Daphnia pulex, Bertram and Hart (1979) found no difference in longevity between the control daphnids and those exposed to 1 ug/L of cadmium.

Dosage-mortality curves are usually estimated for one time period, 48 or 96 hours, but in this study they were determined for 36, 48, 60, 72 and 96 hours (Figure 7). As stated by Bliss (1935, p. 135) "the dosage-mortality curve is primarily descriptive of the variation in susceptibility between the individuals of a population". The slope of the lines are an estimate of $1/\sigma$, the reciprocal of the standard deviation. As determined by the F-test for equality of slopes the dosage-mortality curves for 36, 48 and 60 hours do not differ, indicating that there is no change in the variation of susceptibility in the daphnids. One will note, however, that after the 60 th hour the slopes increase significantly (Table 5), thus indicating a reduction of the variation in susceptibility. In order to explain this effect, one must consider some basic pharmacological principles. To elicit a response, a toxicant must first penetrate the protective barriers of an organism to reach the target site(s). This, of course, is dependent on the quantity of toxicant present, the duration of the exposure, the number of barriers (membranes) before the reaction site is reached and the biophysical affinity of the toxicant for the cell membranes. A prolonged exposure would therefore lead to a greater amount of toxicant at the critical target site(s), causing a greater number of individuals to respond.

The dosage-mortality curves for cadmium (Figure 7) show the expected decrease in the LC50 values with prolonged exposure time. Biesinger and Christensen (1972) found the 48 hour LC50 (18°C) at 65 ug/L for young Daphnia magna. Canton and Adema (1978) also used Daphnia magna at 19°C and determined the 48 hour LC50 at 47 ug/L. In similar studies, Baudouin and Scoppa (1974) using Daphnia hyalina found a 48 hour LC50 (10^{o}C) of 55 ug/L whereas Bertram and Hart (1979) using Daphnia pulex found the 72 hour LC50 (22^{o}C) at 62 ug/L. The 48 hour LC50 (58.16 ug/L) of cadmium determined in this study for juvenile Daphnia magna supports the findings of the forementioned studies.

The acute toxicity of zinc to Daphnia magna

The results obtained for the second set of experiments reveal that Daphnia magna is less sensitive to zinc than to cadmium. These results were to be expected considering that cadmiun is found less frequently than zinc in natural waters, hence the daphnids may have evolved a greater tolerance for zinc than for cadmium.

The time-mortality curve (Figure 8) obtained for zinc shows a greater variability than for cadmium. The empirical data (Appendix IV) clearly shows this variation. For example, at 330.8 ug/L of zinc only 41 percent mortality was observed at 96 hours, whereas 100 percent mortality was observed for the same time period when the daphnids were exposed to 303.7 ug/L of zinc. Considering that the same experimental procedures were used in both the cadmium and zinc experiments it is doubtful that the procedure would be the source of variation. It is possible to concieve that the variation for zinc may be inherent in the daphnid population. The occurence of such variability does not allow for the determination of the threshold concentration. From Appendix IV, we note that 32 percent mortality was observed after 96 hours when the daphnids were exposed to 10.6 ug/L of zinc. Hence the threshold concentration must be well below that level. Anderson (1948) in his 64 hour studies at 25°C with Daphnia magna found that the threshold concentration was considerably lower than 150 ug/L. Shcherban (1979) found that no mortality occured in a 72 hour period when Daphnia magna was exposed to 50 ug/L of zinc at 25°C. Clearly, one can see that variability of sensitivity to zinc is not unique to this study.

This variability is even clearer when the dosage-mortality curves are examined. The dosage-mortality curves for 36, 48, 60, 72 and 96 hours (Figure 9) do not show any difference in slope as did the cadmium lines. The five lines are shown statistically to have slopes which do not differ (Table 6). Thus, there seems to be no change in the variability of susceptibility with time. One may postulate that if given more time, the same effect could be seen, if one assumes that the permeability of zinc is less than for cadmium. This however does not seem to be the case since the results of one experiment which was allowed to continue up to 140 hours showed just as much variability. For example, Appendix IV shows that at 74.5 ug/L of zinc 27 percent of the daphnids had died after 140 hours, whereas when exposed to 45.7 ug/L of zinc 86 percent mortality was observed at the same time period. It would seem that the variability phenomenon observed here, is one that encompasses more than just kinetic parameters, in other words it involves more than the toxicant mechanism of penetration.

Similarly to the cadmium dosage-mortality lines, the zinc lines also show a decrease in the LC50 values with longer periods of exposure (Figure 7). As we noted earlier the variability in the data is further demonstrated when a comparison of the LC50 values from different studies is made. Biesinger and Christensen (1972) determined the 48 hour LC50

(18°C) value at 100 ug/L. Braginskiy and Shcherban (1978) estimate the 24 hour and 72 hour LC50 at 163 ug/L and 12 ug/L respectively. In a similar study Baudouin and Scoppa (1974) used Daphnia hyalina and found zinc to be more toxic than cadmium in that the 48 hour LC50 was 40 ug/L. However, a comparison of their data to that of Biesinger and Christensen (1972) for other metals show relatively lesser differences except for zinc.

The multiple toxicity of cadmium-zinc mixtures to Daphnia magna

All previous investigations concerned with the effects of heavy metals on the cladoceran Daphnia magna have focused on their effects as seperate entities. Yet, it is unusual to find a body of water that is contaminated by a single toxicant. In order to establish water quality criteria, that will protect the ecosystem as a whole one must take into consideration that combinations of toxicants may have potencies which differ from those of individual toxicants. Although, the number of studies dealing with the combined effects of heavy metals on fish are numerous (E.I.F.A.C., 1980), no such studies have considered their effects on Daphnia.

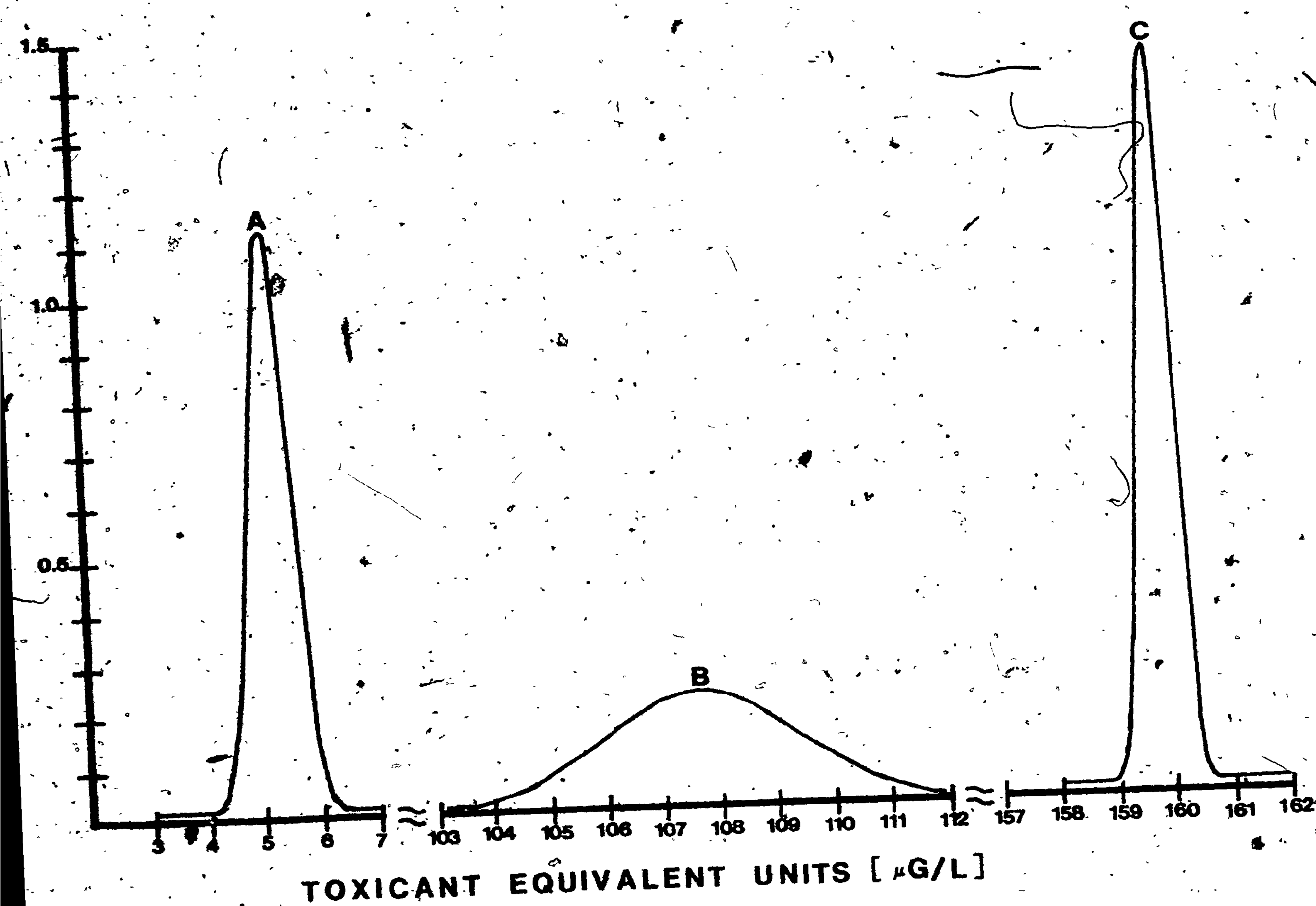
The forementioned lacuna prompted us to investigate the combined effects of cadmium and zinc on Daphnia magna. These two metals were chosen primarily because they are known to occur in association in lakes inhabited by cladocerans.

The potency of the mixture may be dependent on the ratio of the constituent toxicants. Hence, two different mixtures of cadmium and zinc were assayed. The first mixture contained cadmium and zinc in a ratio of equal potency as determined from single toxicant experiments. The dosage-mortality curves for this mixture (Figure 10) show an infra-additive effect. In other words the observed mortalities caused by the mixture were much lower than those predicted by the concentration-addition model. No attempt was made to compare the observed results against the response-addition model, since the predicted response of the latter are higher than those of the concentration-addition model (Anderson, 1980). The slopes of the observed dosage-mortality curves for the mixture are not significantly different at all α - levels (Table 8), thus indicating no change in the variability in susceptibility over the experimental period. On the other hand the observed dosage-mortality curves differed significantly from their respective expected lines (Table 9), which are derived from the concentration-addition model. These results resemble to some extent the results obtained with the discrete zinc lines, however, analysis of the data based on zinc equivalent units revealed the same infra-additive effect.

In the experiments just discussed, cadmium and zinc were added in terms of equal potency and since cadmium was found to be more toxic, this resulted in a greater amount of zinc being present in the mixture. The second type of mixture assayed contained equal amounts of zinc and cadmium. The results obtained (Appendix IX) also clearly show an infra-additive effect.

In order to compare the results of the two mixtures with the expected results obtained from the discrete toxicant line we have opted to represent the data as tolerance distributions (Figure 11). This is possible because the dosage-mortality lines (Appendix Xa -Xb), show a statistically significant good fit and thus describe a normal distribution (Finney, 1971). The 96 hour dosage-mortality curves were chosen as a basis for comparison. The tolerance distributions for these curves were derived by using the LC50 value as the mean (u) and the inverse of the slope as the standard deviation (σ). Once these parameters are determined it is possible to

Figure 11. The 96 hour tolerance distribution of *Daphnia magna* to discrete toxicant (Curve A), Equi-potent toxicant mixture (Curve B), and the Equi-concentration toxicant mixture (Curve C).


1.5
1.0
0.5
A
B
C
3
4
5
6
7
103
104
105
106
107
108
109
110
111
112
157
158
159
160
161
162
TOXICANT EQUIVALENT UNITS [μG/L]

construct the tolerance distribution functions (Blommers and Lindquist, 1960). The ordinate value is obtained by the following expression:

$$y = \frac{1}{\sigma \sqrt{2 \pi}} e^{-(x^2 / 2 \sigma^2)}$$

where:
- y = ordinate value (the value of the function)
- σ = standard deviation (inverse of the slope)
- π = 3.1416
- e = 2.7183
- x = X - u
- X = magnitude of the toxicant equivalent units
- u = mean (LC50 value)

The data used in the derivation of the 96 hour tolerance distribution have been tabulated in Appendecies XIa - XIc. The 96 hour tolerance distributions are illustrated in Figure 11. The abscissa is expressed in terms of toxicant equivalent units instead of x, in order to demonstrate the shift in the means (i.e.: the 96 hour LC50 values). The ordinate values describe the tolerance distribution function which allots for equal areas under the curves, thus taking into account that the curves describe the response of equal number of animals.

Curve A in Figure 11 depicts the 96 hour tolerance distribution of the discrete cadmium dosage-mortality curve. The 96 hour tolerance distributions for the equi-potent and equi-concentration mixtures are represented by curves B and C respectively. The displacement of the B and C curves to the right of curve A, clearly illustrates the infra-additive effect. In other words the LC50 values increase from 5.20 ug/L for the discrete curve to 107.68 ug/L for the equi-potent curve and to 159.73 ug/L for the equi-concentration curve. Considering the fact that the curves circumbscribe equal areas, the height of the curves can be used to describe the variation in the response of the daphnid population. However, for a quantitative analysis of the change in variability, comparison of the standard deviations is commonly accepted. Figure 11 shows that curve B, which contains more zinc than cadmium in terms of weight, shows a greater variability in the response than for the discrete curve (A). Comparing the standard deviations of these curves reveals a five-fold increase in the variability of the response. A similar comparison of the equi-concentration curve (C) with the discrete curve (A) shows an even greater infra-additive effect, but unlike the equi-potent curve, it shows a decrease in the variability in the response. Quantitatively comparing the equi-concentration curve (C) to the discrete curve (A) shows a 1.3 fold decrease in the variation of response, and a 6.4 fold decrease when compared to the equi-potent curve.

From Figure 11 we note that when the toxicant mixture contains more zinc than cadmium in terms of weight (curve B) there is a greater variability in the response as compared to a mixture where the cadmium concentration is increased to equal that of zinc (curve C).

Although the actual mechanism responsible for this phenomenon cannot be deduced from the results presented in this study, one can postulate some reason to account for the results. There is a possibility that the greater variability in the equi-potent curve may be due to the greater amount of zinc relative to cadmium. We have already noted in the analyses of the discrete dosage-mortality curves that a greater variability in the response was found with zinc than with cadmium. Hence, one may postulate that the same effect is occuring here and that by increasing the relative amount of cadmium to equal that of zinc eliminates the variability. As we noted earlier the variability to zinc may be inherent in the daphnid population used or it may be due to variation in the type of zinc species found in the mixture which may have different potencies.

Comparison of the sensitivity of Daphnia magna to cadmiun and zinc with that of other freshwater organisms

Having discussed the results of this study, we find that it is essential that we reflect on their significance by comparing them to studies dealing with other organisms. Even though the experimental parameters vary from study to study, such comparisons are mandatory if quality criteria are to protect communities as a whole. In order to simplify the comparison, we have included Table 10, which lists the toxicity of cadmium and zinc to various vertebrates and invertebrates.

With the exception of the flagfish (Jordanella floridae), all the organisms listed in Table 10 are more sensitive to cadmium than to zinc. The difference in potency, however, between cadmium and zinc varies among species.

The most sensitive temperate freshwater fish to cadmium is the carp (Cyprinus carpio), whereas the most resistant is the perch (Roccus americanus). The tropical flagfish (Jordanella floridae) shows the greatest resistance to cadmium whereas the killifish (Fundulus diaphanus) is the most sensitive. Among the invertebrates, the snail (Amnicola sp.) is most resistant to cadmium and the amphipod (Gammarus sp.) is the most sensitive. In comparison this study shows that Daphnia magna is more sensitive to cadmium (96 hour LC50 $\simeq$ 0.005 mg/L) than any of the organisms listed in Table 10.

Table 10: The toxicity of cadmium and zinc to various vertebrates and invertebrates

Species	Temperature (°C)	Hardness (mg/L $CaCO_3$)	Metal	Duration (hrs)	LC50 (mg/L)	Reference
VERTEBRATES						
Carassius auratus	25	20	Cd	96	2.34	Pickering et al. 1966
	15	20	Zn	96	6.44	Pickering et al. 1966
Cyprinus carpio	28	55	Cd	96	0.24	Rehwoldt et al. 1972
	28	55	Zn	96	7.8	Rehwoldt et al. 1972
Fundulus diaphanus	28	55	Cd	96	0.11	Rehwoldt et al. 1972
	28	55	Zn	96	19.2	Rehwoldt et al. 1972
Jordanella floridae	28	44	Cd	96	2.5	Spehar 1976
	25	45	Zn	96	1.5	Spehar 1976
Lepomis gibbosus	28	55	Cd	96	1.5	Rehwoldt et al. 1972
	28	55	Zn	96	20.1	Rehwoldt et al. 1972
Lepomis macrochirus	25	20	Cd	96	1.94	Pickering et al. 1966
	15	20	Zn	96	5.38	Pickering et al. 1966
Pimephales promelas	25	20	Cd	96	0.63- 1.05	Pickering et al. 1966
	25	50	Zn	96	6.2 -13.7	Mount 1966
Roccus americanus	28	55	Cd	96	8.4	Rehwoldt et al. 1972
	28	55	Zn	96	14.4	Rehwoldt et al. 1972
Roccus saxatilis	28	55	Cd	96	1.11	Rehwoldt et al. 1972
	28	55	Zn	96	6.8	Rehwoldt et al. 1972

Table 10: Continued

Species	Temperature (^oC)	Hardness (mg/L $CaCO_3$)	Metal	Duration (hrs)	LC50 (mg/L)	Reference
INVERTEBRATES						
Amnicola sp.	17	?	Cd	96	8.4	Rehwoldt et al. 1973
	17	?	Zn	96	14.0	Rehwoldt et al. 1963
Asellus aquaticus	30	?	Cd	72	0.21	Braginskiy et al. 1978
	30	?	Zn	72	1.52	Braginskiy et al. 1978
Chironomus sp.	17	50	Cd	96	1.2	Rehwoldt et al. 1973
	17	50	Zn	96	18.2	Rehwoldt et al. 1973
Cloeon dipterum	30	?	Cd	72	0.93	Braginskiy et al. 1978
	30	?	Zn	72	1.33	Braginskiy et al. 1978
Cyclops abyssorum	10	?	Cd	48	3.8	Baudouin et al. 1974
	10	?	Zn	48	5.5	Baudouin et al. 1974
Eudiaptomus padanus	10	?	Cd	48	0.55	Baudouin et al. 1974
	10	?	Zn	48	0.50	Baudouin et al. 1974
Gammarus sp.	17	50	Cd	96	0.07	Rehwoldt et al. 1973
	17	50	Zn	96	8.1	Rehwoldt et al. 1973
Nais sp.	17	50	Cd	96	1.7	Rehwoldt et al. 1973
	17	50	Zn	96	18.4	Rehwoldt et al. 1973

Table 10: Continued

Species	Temperature (^oC)	Hardness (mg/L $CaCO_3$)	Metal	Duration (hrs)	LC50 (mg/L)	Reference
INVERTEBRATES CONTINUED						
Tricoptera sp.	17	50	Cd	96	3.4	Rehwoldt et al. 1973
	17	50	Zn	96	58.1	Rehwoldt et al. 1973
Zygoptera sp.	17	50	Cd	96	8.1	Rehwoldt et al. 1973
	17	50	Zn	96	26.2	Rehwoldt et al. 1973
Daphnia magna	18	45	Cd	48	0.065	Biesinger et al. 1972
	18	45	Zn	48	0.100	Biesinger et al. 1972
Daphnia magna	20	130	Cd	48	0.058	This study
	20	130	Zn	48	0.709	This study
Daphnia hyalina	10	?	Cd	48	0.055	Baudouin et al. 1974
	10	?	Zn	48	0.040	Baudouin et al. 1974

The pumpkinseed (*Lepomis gibbosus*) is the most resistant to zinc, whereas the closely related bluegill (*Lepomis macrochirus*) is the most sensitive to zinc. Although the tropical flagfish (*Jordanella floridae*) was the most resistant to cadmium it is the least resistant to zinc. The killifish (*Fundulus diaphanus*) which showed the greatest sensitivity to cadmium shows the greatest resistance to zinc. The copepod (*Eudiaptomus pandanus*) shows the greatest sensitivity to zinc, whereas, the caddis fly larvae (*Tricoptera* sp.) shows the greatest resistance to zinc. Once again the results obtained in this study reveal that *Daphnia magna* is more sensitive (96 hour LC50 = 0.068 mg/L) to zinc than any of the organisms listed in Table 10.

Studies dealing with the effect of cadmium-zinc mixtures on fish have shown results which vary, in that in some cases the mixture is found to be additive and in other cases to be supra- or infra-additive (E.I.F.A.C., 1980). We were unable to find literature on the effects of cadmium-zinc mixtures on invertebrates. Albeit, comparison to the results obtained for the multiple toxicity of heavy metals to fish, *Daphnia magna* is much more sensitive to such mixtures.

Clearly *Daphnia magna* is much more sensitive to cadmium and zinc than the other organisms listed in Table 10. Although *Daphnia magna* may be representative of other cladocerans, this sensitivity excludes it as a representative of aquatic organisms in general. Yet, if the aim of setting water quality criteria is to protect all the organisms in the aquatic environment, then surely the sensitivity of *Daphnia magna* must be taken into consideration, especially if one considers that the cladoceran community is a primary source of food for numerous fish.

In summary this study has determined: first that the flow-through apparatus is more suitable than the static system for use in bioassays in that it offers a better maintenance of the daphnids; second that *Daphnia magna* is more sensitive to cadmium than to zinc; third that both cadmium-zinc mixtures assayed showed an infra-additive effect; fourth that variation in the ratio of the constituents of the mixture has an effect on the tolerance distributions of the daphnid population; and fifth that in comparison to other freshwater organisms *Daphnia magna* is one of the most sensitive to heavy metals such as cadmium and zinc.

REFERENCES

Anderson, B.G. 1944. The toxicity threshold of various substances found in industrial wastes as determined by the use of Daphnia magna. Sewage Works J. 16 : 1156-1165

-1948. The apparent thresholds of toxicity to Daphnia magna for chlorides of various metals when added to Lake Erie water. Trans. Amer. Fish. Soc. 78 : 96 - 113

Anderson, P.D. & L.J.Weber 1975. The toxicity to aquatic populations of mixtures containing certain heavy metals. Proc. International Conference on Heavy Metals in the Environment. Toronto, Ontario, Canada 933 - 953

Anderson, P.D. 1980. Personal communication.

Baudouin, M.F. & P.Scoppa 1974. Acute toxicity of various metals to freshwater zooplankton. Bull.Environ.Contam. & Toxicol. 12 : 745 - 751

Bertram, P.E. & B.A.Hart 1979. Longevity and reproduction of Daphnia pulex (de Geer) exposed to cadmium contaminated food or water. Environ. Poll. 19 : 295 - 305

Biesinger, K.E. & G.M.Christensen 1972. Effects of various metals on survival, growth, reproduction, and metabolism of Daphnia magna . J.Fish.Res.Bd.Can. 29 : 1691 - 1700

Bliss, G.I. 1935. The calculation of the dosage-mortality curve. Ann. Appl. Biol. 22 : 134 - 167

Bloomers, P. & E.F.Lindquist 1960. Elementary statistical methods in psychology and education. Houghton Mifflin, Boston, 528pp.

Braginskiy, L.P. & E.P.Shcherban 1978. Acute toxicity of heavy metals to aquatic invertebrates at different temperatures. Hydrobiological J. 14 : 78 - 82

Brooks, J.F. & S.I.Dodson 1965. Predation, body size and composition of plankton. Science 15 : 28 - 35

Canton, J.H. & D.M.M.Adema 1978. Reproducibility of short term and reproduction toxicity experiments with Daphnia magna and comparison of the sensitivity of D. magna with D.pulex and D.cucullata in short term experiments. Hydrobiologia 59 : 135 - 140

Casarett, L.J. & J.Doull 1975. Toxicology: the basic science of poisons. Macmillan Inc. N.Y. 768 pp.

Clarke,R.McV. 1974. The effects of effluents from metal mines on aquatic ecosystems: a literature review. Environment Canada Fisheries and Marine Services. Technical Report 488.

E.I.F.A.C. 1980. Report on combined effects on freshwater fish and other aquatic life of mixtures of toxicants in water. IN PRESS.

Finnell,L.M. & E.B.Reed 1969. The diel vertical movement of kokanee salmon, Oncorhynchus nerka, in Granby reservoir, Colorado. Trans. Amer. Fish. Soc. 98 : 245-252

Finney,D.J. 1971. Probit analysis. 3rd ed. Cambridge University Press, London 333pp.

Friberg,L. , M.Piscator , G.F.Nordberg , & T.Kjellstrom 1976. Cadmium in the environment. C.R.C. Press.

Hall,D.J. 1964. An experimental approach to the dynamics of a natural population of Daphnia galeata mendotae . Ecology 45 : 94 - 112

Hem,J.D. 1972. Chemistry and occurence of cadmium and zinc in surface water and ground water. Water Resources Research 8 : 661 - 679

Josephson,J. 1977. Out west, they talk about cadmium. Environ. Sci. & Tech. 11 : 336 - 337

Krebs,C.J. 1972. Ecology : the experimental analysis of distribution and abundance. Harper and Row,N.Y. 694pp.

Lake,P.S. 1979. Accumulation of cadmium in aquatic animals. Chemistry in Australia 46 : 26 - 29

Litchfield,J.F.Jr. 1949. A method for rapid graphic solution of time per cent effect curves. J.Phar.& Exper. Thera. 97 : 399 - 408

Lymburner,D.B. 1974. Environmental contaminants inventory study N^{o} 2: The production, use and distribution of cadmium in Canada. Canada Center for Inland Waters, Inland Waters Directorate Report Series N^{o} 39.

Malin,H.M.Jr. 1971. Metal focus shifts to cadmium. Environ. Sci. & Tech. 5 : 754 - 755.

Méranger,J.C. , K.S.Subramanian , & C.Chalifoux. 1979. A national survey for cadmium, chromium, copper, lead, zinc, calcium, and magnesium in Canadian drinking water supplies. Environ. Sci. & Tech. 13 : 707 - 711

Mount, D.I. 1966. The effect of total hardness and pH on acute toxicity of zinc to fish. Int. J. Air Water Poll. 10 : 49 - 56

National Research Council 1979. " Zinc " N.R.C. Subcommitte on zinc. University Park Press, Baltimore, 471pp.

Naumann, E. 1934a. Uber die toxizitat des Kupersulfats fur Daphnia magna. K.fysiogr. Sallsk. Lung Forh, Bd. 4, S. 41 - 49

-1934b. Uber die Toxizitat des Zinksulfats fur Daphnia magna. K.fysiogr.Sallsk. Lung Forh, 4.S. 123 - 128

Packard, R. 1977. Metal bulletin handbook. 10th ed. Drogher Press, London, 915pp.

Pennak, R.W. 1978. Freshwater invertebrates of the United States. 2nd ed. John Wiley N.Y.

Pickering, D.H. & C.Henderson 1966. The acute toxicity of some heavy metals to different species of warm water fishes. Int. J. Air Water Poll. 10 : 453 - 463

Rehwoldt, R., L.W.Menapage, B.Nerrie, & D.Alessandrello 1972. The effect of increased temperature upon the toxicity of some heavy metal ions. Bull.Environ. Contam.& Toxicol. 8 : 91 - 96

Rehwoldt, R., L.Lasko, C.Shaw, & E.Wirhowski 1973. The acute toxicity of some heavy metal ions towards benthic organisms. Bull. Environ. Contam. & Toxicol. 10 : 291 - 294

Shcherban, E.P. 1979. Toxicity of some heavy metals for Daphnia magna Strauss, as a function of temperature. Hydrobiological J. 40 : 75 - 80

Sokal, R.R. & F.J.Rohlf 1969. Biometry: the priciples and practice of statistics in biological research. W.H.Freeman & Comp. San Fran. 776pp.

Spehar, R.L. 1976 Cadmium and zinc toxicity to flagfish. J.Fish.Res.Bd.Can. 33 : 1939 -1945

Van Loon, J.C. & R.J.Beamish 1977. Heavy metal contamination by atmospheric fallout of several Flin Flon area lakes and the relation to fish populations. J.Fish.Res.Bd.Can. 34 : 899 - 906

Ward's N.S.E. Inc. 1979. Culture of algae in the laboratory. Ward's N.S.E., Rochester,N.Y. 4pp.

Weinstein,N.L. 1978 Multiple toxicity assessment for mixtures of aquatic pollutants. M.Sc. thesis. Concordia University, Montreal,Quebec,Canada. 116pp.

Winner,R.W. & M.P.Farrell 1976. Acute and chronic toxicity of copper to four species of <u>Daphnia</u>. J.Fish.Res.Bd.Can. 33 : 1685 - 1691

Appendix I: Empirical data of cadmium bioassays with *Daphnia magna*

Concentration (ug/L)	Time (hrs)	Number used	Number dead	Cumulative % Mortality
31,400.0 (1200.0)[a]	2.5	22	2	9
	3.0	22	15	68
	3.5	22	16	73
	4.0	22	22	100
15,700.0 (400.0)	3.5	22	11	50
	4.0	22	18	82
	4.5	22	22	100
9,110.0 (253.0)	3.5	22	11	50
	4.0	22	20	91
	4.5	22	22	100
5,680.0 (171.0)	3.5	22	2	9
	4.0	22	8	36
	4.5	22	18	82
	5.0	22	20	91
	5.5	22	22	100
2,560.0 (106.0)	5.5	22	9	41
	6.0	22	17	77
	6.5	22	18	82
	7.0	22	22	100
1,484.0 (433.0)	15.0	22	3	14
	16.0	22	10	45
	17.0	22	13	59
	18.0	22	18	82
	19.0	22	21	95
	21.0	22	22	100
1,020.0 (65.0)	5.0	22	1	5
	5.5	22	3	14
	7.0	22	10	45
	7.5	22	17	77
	8.0	22	18	82
	9.0	22	19	86
	9.5	22	20	91
	10.0	22	22	100

a. Values in parentheses denote +/- standard deviations.

Appendix I: Continued

Concentration (ug/L)	Time (hrs)	Number used	Number dead	Cumulative % Mortality
880.0 (100.0)	13.0	22	1	5
	16.0	22	4	18
	17.0	22	8	36
	18.0	22	12	55
	19.0	22	16	73
	20.0	22	21	95
	21.0	22	22	100
859.0 (122.0)	12.0	22	4	18
	16.0	22	6	27
	17.0	22	7	32
	18.0	22	12	55
	19.0	22	17	77
	20.0	22	20	91
	21.0	22	22	100
421.0 (9.0)	18.0	22	1	5
	19.0	22	2	9
	20.0	22	3	14
	22.0	22	6	27
	23.0	22	8	36
	26.0	22	10	45
	31.0	22	11	50
	32.0	22	13	59
	33.0	22	17	77
	35.0	22	18	82
	36.0	22	22	100
261.0 (0.7)	16.0	22	1	5
	17.0	22	2	9
	19.0	22	3	14
	20.0	22	4	18
	21.0	22	5	23
	24.0	22	7	32
	32.0	22	11	50
	33.0	22	12	55
	35.0	22	13	59
	36.0	22	18	82
	37.0	22	20	91
	39.0	22	21	95
	44.0	22	22	100

Appendix I: Continued

Concentration (ug/L)	Time (hrs)	Number used	Number dead	Cumulative % Mortality
181.0 (34.0)	21.0	22	2	9
	24.0	22	5	23
	27.0	22	7	32
	39.0	22	8	36
	42.0	22	10	45
	45.0	22	12	55
	48.0	22	14	64
162.0 (23.0)	21.0	22	5	23
	24.0	22	8	36
	27.0	22	9	41
	30.0	22	10	45
	33.0	22	11	50
	36.0	22	15	68
	39.0	22	16	73
	48.0	22	18	82
	51.0	22	19	86
	54.0	22	21	95
160.0 (0.5)	29.0	22	1	5
	32.0	22	2	9
	33.0	22	3	14
	36.0	22	4	18
	39.0	22	5	23
	40.0	22	6	27
	42.0	22	8	36
144.0 (19.0)	21.0	22	2	9
	24.0	22	4	18
	42.0	22	7	32
	45.0	22	12	55
	48.0	22	15	68
	51.0	22	17	77
	54.0	22	20	91
118.0 (7.0)	21.0	22	4	18
	39.0	22	5	23
	42.0	22	7	32
	45.0	22	12	55
	48.0	22	14	64
	54.0	22	16	73

Appendix I: Continued

Concentration (ug/L)	Time (hrs)	Number used	Number dead	Cumulative % Mortality
97.0 (17.0)	21.0	22	3	14
	24.0	22	4	18
	27.0	22	6	27
	30.0	22	7	32
	33.0	22	8	36
	36.0	22	10	45
	42.0	22	11	50
	45.0	22	12	55
	48.0	22	14	64
	51.0	22	15	69
86.1 (13.4)	24.0	22	2	9
	27.0	22	3	14
	30.0	22	5	23
	42.0	22	6	27
	48.0	22	9	41
	51.0	22	10	45
	57.0	22	12	55
	60.0	22	14	64
	63.0	22	16	73
	69.0	22	17	77
	72.0	22	19	86
	78.0	22	21	95
	81.0	22	22	100
83.3 (9.3)	21.0	22	1	5
	27.0	22	2	9
	36.0	22	3	14
	39.0	22	4	18
	48.0	22	5	23
	51.0	22	8	36
	54.0	22	11	50
	57.0	22	14	64
	66.0	22	18	82
	69.0	22	19	86
	75.0	22	21	95
	81.0	22	22	100

Appendix I: Continued

Concentration (ug/L)	Time (hrs)	Number used	Number dead	Cumulative % Mortality
76.7 (5.8)	27.0	22	1	5
	30.0	22	4	18
	39.0	22	6	27
	45.0	22	7	32
	51.0	22	10	45
	54.0	22	11	50
	57.0	22	12	55
	60.0	22	14	64
	66.0	22	19	86
	69.0	22	20	91
	72.0	22	21	95
	78.0	22	22	100
61.0 (3.0)	21.0	22	1	5
	24.0	22	3	14
	33.0	22	4	18
	36.0	22	6	27
	42.0	22	9	41
	45.0	22	10	45
	48.0	22	11	50
	51.0	22	13	59
	54.0	22	15	68
57.9 (6.1)	21.0	22	1	5
	36.0	22	5	23
	39.0	22	7	32
	42.0	22	12	55
	45.0	22	15	68
	58.0	22	16	73
	51.0	22	17	77
	57.0	22	19	86
	69.0	22	20	91
	72.0	22	21	95
	78.0	22	22	100

Appendix I: Continued

Concentration (ug/L)	Time (hrs)	Number used	Number dead	Cumulative % Mortality
40.0 (5.6)	21.0	22	3	14
	27.0	22	4	18
	30.0	22	6	27
	33.0	22	7	32
	39.0	22	8	36
	42.0	22	10	41
	45.0	22	12	55
	48.0	22	16	73
	60.0	22	18	82
	66.0	22	19	86
	75.0	22	20	91
	81.0	22	21	95
	90.0	22	22	100
37.5 (3.9)	21.0	22	1	5
	24.0	22	3	14
	27.0	22	4	18
	33.0	22	5	23
	39.0	22	6	27
	42.0	22	7	32
	48.0	22	9	41
	51.0	22	10	45
	60.0	22	11	50
	63.0	22	12	55
	66.0	22	14	64
	75.0	22	17	77
	78.0	22	18	82
	81.0	22	20	91
	84.0	22	21	95
	87.0	22	22	100
32.3 (3.6)	21.0	22	1	5
	24.0	22	2	9
	39.0	22	3	14
	42.0	22	5	23
	45.0	22	6	27
	48.0	22	8	36
	51.0	22	9	41
	54.0	22	10	45
	57.0	22	11	50
	60.0	22	12	55
	66.0	22	13	59
	75.0	22	15	68
	78.0	22	18	82
	81.0	22	20	91

Appendix I: Continued

Concentration (ug/L)	Time (hrs)	Number used	Number dead	Cumulative % Mortality
28.0 (4.5)	24.0	22	1	5
	30.0	22	3	14
	33.0	22	4	18
	42.0	22	6	27
	45.0	22	7	32
	48.0	22	8	[redacted]
	51.0	22	9	41
	54.0	22	10	45
	57.0	22	11	50
	63.0	22	15	68
	69.0	22	16	73
	75.0	22	18	82
	78.0	22	19	86
	81.0	22	22	100
21.9 (3.1)	27.0	22	2	9
	33.0	22	4	18
	36.0	22	5	23
	42.0	22	8	36
	48.0	22	11	50
	51.0	22	12	55
	54.0	22	13	59
	57.0	22	14	64
	60.0	22	15	68
	63.0	22	16	73
	66.0	22	17	77
	69.0	22	18	82
	75.0	22	21	95
	87.0	22	22	100
14.7 (2.0)	21.0	22	2	9
	27.0	22	3	14
	42.0	22	4	18
	45.0	22	8	36
	48.0	22	10	41
	51.0	22	11	50
	54.0	22	12	55
	57.0	22	13	59
	60.0	22	14	64
	69.0	22	15	68
	87.0	22	18	82

Appendix I: Continued

Concentration (ug/L)	Time (hrs)	Number used	Number dead	Cumulative % Mortality
6.5 (1.2)	21.0	22	1	5
	39.0	22	3	14
	54.0	22	4	18
	57.0	22	6	27
	63.0	22	7	32
	69.0	22	8	36
	72.0	22	9	41
	75.0	22	10	45
	90.0	22	11	50
3.4 (0.4)	21.0	22	1	5
	54.0	22	2	9
	66.0	22	4	18
	69.0	22	5	23
	78.0	22	6	27
	87.0	22	7	32
	93.0	22	8	36

Appendix II: <u>Regression parameters for the graphs correlating % Mortality vs. time on probit-log scale for cadmium</u>

Concentration (ug/L)	Log-concentration	Slope	Intercept	Correlation coefficient	Log ET50
31,400.0 (1200.0)[a]	4.497	14.257	- 1.835	0.928-	0.479
15,770.0 (400.0)	4.198	15.167	- 3.190	1.000-	0.540
9,110.0 (253.0)	3.960	1.600	1.000	1.000-	0.540
5,680.0 (171.0)	3.754	17.456	- 5.727	0.986*	0.614
2,560.0 (106.0)	3.408	16.730	- 7.517	0.952-	0.748
1,484.0 (433.0)	3.171	25.088	-25.533	0.979**	1.217
1,020.0 (65.0)	3.009	11.083	- 4.328	0.983**	0.842
880.0 (100.0)	2.944	16.165	-14.970	0.939**	1.235
859.0 (122.0)	2.934	9.185	- 6.215	0.843*	1.221
421.0 (9.0)	2.624	7.727	- 6.154	0.963**	1.443
261.0 (0.7)	2.417	6.974	- 5.043	0.947**	1.440
181.0 (34.0)	2.258	2.162	1.378	0.913**	1.675
162.0 (23.0)	2.210	5.235	- 2.728	0.973**	1.476
160.0 (0.5)	2 204	7.792	- 8.049	0.989**	1.675
144.0 (19.0)	2.158	5.274	- 3.361	0.919**	1.585
118.0 (7.0)	2.072	1.533	2.403	0.847**	1.694
97.0 (17.0)	1.987	3.915	- 1.270	0.995**	1.601
86.1 (13.4)	1.935	4.994	- 3.377	0.950**	1.677
83.3 (9.3)	1.921	5.800	- 4.847	0.929**	1.698
76.7 (5.8)	1.885	6.648	- 6.197	0.946**	1.684
61.0 (3.0)	1.785	4.695	- 2.828	0.979**	1.667
57.9 (6.1)	1.763	6.370	- 5.175	0.977**	1.597
40.0 (5.6)	1.602	4.839	- 2.690	0.982**	1.589
37.5 (3.9)	1.574	4.442	- 2.523	0.950**	1.694
32.3 (3.6)	1.509	4.517	- 2.874	0.953**	1.743
28.0 (4.5)	1.447	5.084	- 3.763	0.985**	1.724

Appendix II: Continued

Concentration (ug/L)	Log concentration	Slope	Intercept	Correlation coefficient	Log ET50
21.9 (3.1)	1.340	5.826	- 4.713	0.977**	1.667
14.7 (2.0)	1.167	3.754	- 1.411	0.961**	1.708
6.5 (1.2)	0.813	3.674	- 0.262	0.976**	1.968
3.4 (0.4)	0.531	1.986	0.600	0.930**	2.215

a. Values in parentheses denote +/- standard deviations

\- Not significant

* Significant at $\alpha = 0.05$

** Significant at $\alpha = 0.01$

Appendix III a.: Regression parameters for 36 hr. dosage-mortality curve for cadmium

Log-concentration concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response
2.62	5.258	5.001	5.515	6.10
2.42	5.092	4.883	5.301	5.60
2.26	4.959	4.784	5.134	4.77
2.21	4.918	4.752	5.084	5.23
2.20	4.909	4.745	5.073	4.09
2.16	4.876	4.719	5.033	4.77
2.07	4.801	4.657	4.945	4.65
1.99	4.735	4.599	4.871	4.77
1.93	4.685	4.553	4.817	4.40
1.92	4.677	4.546	4.808	4.09
1.89	4.652	4.522	4.782	4.25
1.79	4.568	4.437	4.699	4.53
1.76	4.544	4.412	4.676	4.77
1.60	4.411	4.263	4.559	4.77
1.58	4.394	4.243	4.545	4.40
1.51	4.336	4.174	4.498	4.09
1.45	4.286	4.113	4.459	4.09
1.34	4.195	3.999	4.391	4.40
1.17	4.053	3.818	4.288	4.40
0.81	3.754	3.428	4.080	3.90
0.53	3.521	3.119	3.923	3.67

Regression equation parameters are as follows:

Intercept = 3.081 Slope = 0.831

Calculated chi-square value = 31.39 ($P < 0.05$)

Appendix III b.: Regression parameters for 48 hr. dosage-mortality curve for cadmium

Log-concentration (ug/L)	Predicted response	95% fiducial limits Lower	95% fiducial limits Upper	Empirical response
2.26	5.347	5.138	5.556	5.23
2.21	5.312	5.115	5.509	5.91
2.20	5.305	5.110	5.500	4.89
2.16	5.277	5.091	5.463	5.47
2.07	5.214	5.046	5.382	5.11
1.99	5.158	5.003	5.313	5.35
1.93	5.116	4.970	5.262	4.89
1.92	5.109	4.964	5.254	4.77
1.89	5.088	4.947	5.229	4.89
1.79	5.018	4.885	5.151	5.00
1.76	4.997	4.865	5.129	5.23
1.60	4.885	4.749	5.021	5.11
1.58	4.871	4.734	5.008	4.77
1.51	4.822	4.677	4.967	4.65
1.45	4.779	4.626	4.932	4.77
1.34	4.702	4.529	4.875	5.00
1.17	4.583	4.374	4.792	4.77
0.81	4.331	4.031	4.631	4.25
0.53	4.135	3.758	4.512	3.90

Regression equation parameters are as follows:

Intercept = 3.763 **Slope = 0.701**

Calculated chi-square value = 14.45 ($P < 0.05$)

Appendix IV: Continued

Concentration (ug/L)	Time (hrs)	Nunber used	Number dead	Cumulative % Mortality
24.0 (2.5)	27.0	22	1	5
	45.0	22	2	9
	63.0	22	3	14
	72.0	22	4	18
	104.0	22	7	32
	116.0	22	8	36
	140.0	22	9	41
19.0 (9.6)	30.0	22	1	5
	48.0	22	2	9
	78.0	22	3	14
	81.0	22	4	18
18.9 (8.6)	24.0	22	1	5
	42.0	22	2	9
	60.0	22	3	14
	69.0	22	4	18
	75.0	22	5	23
	78.0	22	6	27
	81.0	22	7	32
	96.0	22	11	50
10.6 (10.2)	45.0	22	1	5
	63.0	22	2	9
	66.0	22	3	14
	72.0	22	4	18
	81.0	22	6	27
	84.0	22	7	32

Appendix V: Regression parameters for the graphs correlating % Mortality vs. time on probit-log scale for zinc.

Concentration (ug/L)	Log Concentration	Slope	Intercept	Correlation coefficient	Log ET50
450.9 (24.4)[a]	2.654	10.326	-10.563	0.972**	1.507
330.8 (33.4)	2.520	3.494	-0.783	0.974**	1.655
303.7 (27.5)	2.482	9.660	-9.667	0.969**	1.518
301.7 (19.6)	2.480	3.551	-1.311	0.981**	1.777
164.6 (13.9)	2.216	5.854	-5.372	0.980**	1.772
160.8 (10.5)	2.206	2.637	-1.048	0.984**	2.293
135.1 (29.6)	2.131	3.123	-0.770	0.979**	1.848
127.3 (11.7)	2.105	2.995	-0.717	0.971**	1.909
85.9 (9.4)	1.934	2.277	-0.137	0.995**	2.136
84.7 (9.3)	1.928	0.835	2.579	0.918*	2.588
74.5 (11.5)	1.872	1.054	2.136	0.887*	2.717
66.6 (7.0)	1.823	1.439	1.986	0.963**	2.094
64.2 (2.5)	1.808	2.562	-0.284	0.896**	2.062
52.7 (6.7)	1.722	1.584	2.014	0.983**	1.885
45.7 (6.2)	1.660	2.025	1.634	0.878**	1.662
41.2 (3.8)	1.615	2.513	0.413	0.964**	1.825
36.1 (10.9)	1.558	2.520	-0.050	0.965**	2.004
29.9 (3.9)	1.476	3.201	-0.697	0.962**	1.780
24.5 (5.8)	1.389	2.202	0.369	0.963**	2.103
24.0 (2.5)	1.380	2.022	0.404	0.989**	2.272
19.0 (9.6)	1.279	1.547	1.068	0.976**	2.541
18.9 (8.6)	1.267	2.414	-0.143	0.925**	2.130
10.6 (10.2)	1.025	4.357	-3.938	0.967**	2.051

a. Values in parentheses denote +/- standard deviations.
\- Not significant
* Significant at $\alpha = 0.05$
** Significant at $\alpha = 0.01$

Appendix VI a.: Regression parameters for 36 hr, dosage-mortality curve for zinc

Log-concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response
2.65	4.776	4.501	5.051	5.23
2.52	4.674	4.435	4.913	4.65
2.48	4.643	4.415	4.871	5.23
2.48	4.643	4.415	4.871	4.25
2.22	4.439	4.271	4.607	3.67
2.13	4.368	4.215	4.521	4.25
2.10	4.344	4.195	4.493	3.90
1.93	4.211	4.071	4.351	3.67
1.93	4.211	4.071	4.351	3.90
1.87	4.164	4.021	4.307	3.90
1.82	4.125	3.978	4.272	4.25
1.81	4.117	3.969	4.265	3.30
1.72	4.046	3.886	4.206	4.40
1.66	3.999	3.829	4.169	4.77
1.61	3.960	3.779	4.141	4.25
1.56	3.921	3.729	4.113	3.90
1.48	3.858	3.647	4.069	4.25
1.39	3.787	3.552	4.022	3.90
1.38	3.779	3.542	4.016	3.67
1.28	3.701	3.436	3.966	3.30
1.28	3.701	3.436	3.966	3.30

Regression equation parameters are as follows:

Intercept = 2.696   Slope = 0.785

Calculated chi-square value = 38.72 ($P < 0.005$)

Appendix VI b.: Regression parameters for 48 hr. dosage-mortality curve for zinc

Log-concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response
2.52	4.783	4.496	5.070	5.11
2.48	4.757	4.482	5.032	4.65
2.22	4.586	4.385	4.787	4.53
2.14	4.534	4.353	4.715	4.53
2.10	4.508	4.336	4.680	4.25
1.93	4.396	4.251	4.541	3.90
1.93	4.396	4.251	4.541	4.09
1.87	4.357	4.217	4.497	3.90
1.82	4.324	4.186	4.462	4.40
1.81	4.317	4.179	4.455	3.90
1.72	4.258	4.116	4.400	4.65
1.66	4.219	4.071	4.367	4.89
1.61	4.186	4.030	4.342	4.53
1.56	4.153	3.988	4.318	4.09
1.48	4.101	3.919	4.283	4.65
1.39	4.042	3.838	4.246	4.09
1.38	4.035	3.829	4.241	3.67
1.28	3.970	3.736	4.204	3.67
1.28	3.970	3.736	4.204	3.90
1.03	3.806	3.496	4.116	3.30

Regression equation parameters are as follows:

Intercept = 3.130 Slope = 0.656

Calculated chi-square value = 27.02 ($P < 0.05$)

Appendix VI c.: Regression parameters for 60 hr. dosage-mortality curve for zinc

Log-concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response
2.52	4.939	4.674	5.204	5.60
2.48	4.916	4.662	5.170	4.89
2.22	4.763	4.578	4.948	5.00
2.21	4.757	4.574	4.940	3.67
2.13	4.710	4.545	4.875	4.65
2.10	4.693	4.534	4.852	4.65
1.93	4.593	4.459	4.727	4.09
1.93	4.593	4.459	4.727	4.25
1.87	4.558	4.428	4.688	3.90
1.82	4.528	4.400	4.656	4.25
1.81	4.522	4.394	4.650	4.65
1.72	4.470	4.338	4.602	4.77
1.66	4.434	4.296	4.572	5.11
1.61	4.405	4.261	4.549	4.77
1.56	4.376	4.223	4.529	4.40
1.48	4.329	4.161	4.497	5.00
1.39	4.276	4.087	4.465	4.25
1.38	4.270	4.079	4.461	3.90
1.28	4.211	3.994	4.428	3.90
1.28	4.211	3.994	4.428	4.09
1.03	4.065	3.778	4.352	3.90

Regression equation parameters are as follows:

Intercept — 3.460 ; Slope — 0.587

Calculated chi-square value — 24.75 ($P < 0.05$)

Appendix VI d.: Regression parameters for 72 hr. dosage-mortality curve for zinc

Log-concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response
2.52	5.288	5.024	5.552	6.70
2.48	5.259	5.007	5.511	5.23
2.22	5.064	4.880	5.248	5.60
2.21	5.057	4.875	5.239	3.90
2.13	4.997	4.833	5.161	4.89
2.10	4.975	4.817	5.133	4.89
1.93	4.848	4.716	4.980	4.40
1.93	4.848	4.716	4.980	4.25
1.87	4.803	4.676	4.930	4.09
1.82	4.766	4.641	4.891	4.53
1.81	4.758	4.633	4.883	4.77
1.72	4.691	4.563	4.819	5.00
1.66	4.646	4.513	4.779	5.23
1.61	4.609	4.470	4.748	4.89
1.56	4.571	4.424	4.718	4.65
1.48	4.512	4.350	4.674	5.35
1.39	4.444	4.262	4.626	4.40
1.38	4.437	4.252	4.622	4.09
1.28	4.362	4.153	4.571	3.90
1.28	4.362	4.153	4.571	4.40
1.03	4.175	3.897	4.453	4.25

Regression equation parameters are as follows:

Intercept = 3.406 Slope = 0.747

Calculated chi-square value = 12.60 ($P < 0.05$)

Appendix VI e.: Regression parameters for 96 hr. dosage-mortality curve for zinc

Log-concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response
2.48	5.235	4.932	5.538	5.91
2.21	5.137	4.918	5.356	4.25
2.13	5.108	4.912	5.304	5.23
2.10	5.097	4.909	5.285	5.35
1.93	5.036	4.886	5.186	4.65
1.93	5.036	4.886	5.186	4.53
1.87	5.014	4.874	5.154	4.25
1.82	4.996	4.862	5.130	4.89
1.81	4.992	4.859	5.125	5.11
1.72	4.959	4.830	5.088	5.35
1.66	4.938	4.807	5.069	5.47
1.61	4.919	4.784	5.054	5.23
1.56	4.901	4.759	5.043	5.00
1.48	4.872	4.716	5.028	6.70
1.39	4.840	4.664	5.016	4.77
1.38	4.836	4.657	5.015	4.39
1.28	4.800	4.595	5.005	4.09
1.28	4.800	4.595	5.005	4.77
1.03	4.709	4.428	4.990	4.65

Regression equation parameters are as follows:

Intercept = 4.335 Slope = 0.363

Calculated chi-square value = 25.90 ($P < 0.05$)

Appendix VII: Empirical results for the cadmium-zinc mixture, in terms of equal potency ratio

Concentration[a] (ug/L) cadmium	zinc	Time (hrs)	Number used	Number dead	Cumulative % Mortality
8.5	53.9	46.0	22	1	5
(0.3)[b]	(5.5)[b]	70.0	22	2	9
		79.0	22	3	14
		85.0	22	4	18
		97.0	22	7	32
10.2	50.4	23.0	22	2	9
(3.5)	(19.1)	71.0	22	3	14
		74.0	22	4	18
		77.0	22	6	27
		86.0	22	7	32
		89.0	22	8	36
		92.0	22	9	41
15.5	78.2	28.0	22	1	5
(0.8)	(8.0)	34.0	22	2	9
		55.0	22	3	14
		73.0	22	4	18
		76.0	22	5	23
		79.0	22	7	32
		88.0	22	8	36
		91.0	22	9	41
		94.0	22	11	50
		97.0	22	16	73
16.9	78.1	53.0	22	1	5
(4.7)	(22.0)	80.0	22	2	9
		86.0	22	3	14
		89.0	22	7	32
		92.0	22	8	36
		95.0	22	10	45

a. Concentration denotes total cadmium or total zinc

b. Values in parentheses denote +/- standard deviations.

Appendix VII: Continued

Concentration (ug/L) cadmium	zinc	Time (hrs)	Number used	Number dead	Cumulative % Mortality
24.1	98.1	52.0	22	1	5
(1.4)	(12.8)	97.0	22	2	9
25.7	102.3	80.0	22	1	5
(1.8)	(11.8)	89.0	22	2	9
52.9	120.8	25.0	22	3	14
(4.7)	(13.6)	46.0	22	4	18
		67.0	22	5	23
		97.0	22	8	36
55.6	131.0	71.0	22	1	5
(3.7)	(5.7)	83.0	22	2	9
		89.0	22	3	14
121.3	145.0	73.0	22	1	5
(14.1)	(10.9)	88.0	22	2	9
		97.0	22	4	18
142.7	153.1	62.0	22	1	5
(24.5)	(5.6)	89.0	22	3	14
		92.0	22	4	18
		95.0	22	5	23
275.6	213.0	28.0	22	1	5
(17.7)	(13.2)	34.0	22	2	9
		40.0	22	3	14
		46.0	22	4	18
		52.0	22	7	32
		61.0	22	8	36
		64.0	22	9	41
		67.0	22	12	55
		70.0	22	14	64
		76.0	22	15	68
		82.0	22	16	73
		85.0	22	17	77
		88.0	22	19	86
		91.0	22	20	91
		94.0	22	21	95

Appendix VII: Continued

Concentration (ug/L) cadmium	zinc	Time (hrs)	Number used	Number dead	Cumulative % Mortality
283.8 (17.0)	240.2 (18.0)	38.0	22	1	5
		53.0	22	2	9
		74.0	22	5	23
		77.0	22	6	27
		80.0	22	10	45
		83.0	22	11	50
		86.0	22	14	64
		89.0	22	18	82
		92.0	22	19	86
		95.0	22	20	91

Appendix VIII a.: Regression parameters for 36 hr. dosage-mortality curve for cadmium-zinc mixture in terms of equal potency ratio

Log-cadmium equivalent concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response	Expected response
1.35	3.651	2.772	4.530	3.70	4.20
1.53	3.671	2.950	4.392	3.28	4.35
1.91	3.715	3.125	4.305	4.00	4.67
2.51	3.783	2.749	4.817	3.67	5.17

Regression equation parameters are as follows:

Intercept = 3.497 Slope = 0.114

Calculated chi-square value = 1.75 ($P < 0.05$)

Relative potency factor used = 0.237

Expected response equation: $y = 3.081 + 0.831 \log (0.237\, C_z + C_c)$[a]

a. C_z denotes total zinc concentration and
C_c denotes total cadmium concentration

Appendix VIII b.: Regression parameters for 48 hr. dosage-mortality curve for cadmium-zinc mixture in terms of equal potency ratio

Log cadmium equivalent concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response	Expected response
1.11	3.743	3.197	4.289	2.99	4.55
1.16	3.755	3.232	4.278	3.95	4.58
1.34	3.797	3.348	4.246	3.87	4.70
1.80	3.906	3.554	4.258	4.14	5.04
2.47	4.064	3.526	4.602	4.37	5.49
2.48	4.066	3.523	4.609	3.33	5.50

Regression equation parameters are as follows:

Intercept = 3.481 Slope = 0.236

Calculated chi-square value = 6.39 ($P < 0.05$)

Relative potency factor used = 0.082

Expected response equation: $y = 3.763 + 0.701 \log (0.082\, C_z + C_c)$

Appendix VIII c.: Regression parameters for 60 hr. dosage-mortality curve for cadmium-zinc mixture in terms of equal potency ratio

Log cadmium equivalent concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response	Expected response
1.02	3.827	3.391	4.263	3.59	4.85
1.08	3.849	3.435	4.263	4.15	4.90
1.27	3.921	3.570	4.272	4.23	5.06
1.30	3.932	3.590	4.274	3.29	5.08
1.76	4.106	3.844	4.368	4.26	5.47
2.17	4.260	3.942	4.578	3.18	5.81
2.45	4.366	3.961	4.771	4.84	6.05
2.47	3.373	3.961	4.785	4.26	6.07

Regression equation parameters are as follows:

Intercept = 3.442 Slope = 0.377

Calculated chi-square value = 13.35 ($P < 0.05$).

Relative potency factor used = 0.038

Expected response equation: $y = 3.994 + 0.839 \log (0.038\ C_z + C_c)$

Appendix VIII d.: Regression parameters for 72 hr. dosage-mortality curve for cadmium-zinc mixture in terms of equal potency ratio

Log cadmium equivalent concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response	Expected response
1.08	4.061	3.677	4.445	3.92	5.18
1.13	4.080	3.713	4.447	4.32	5.25
1.31	4.149	3.839	4.459	4.49	5.49
1.34	4.160	3.858	4.462	3.94	5.53
1.78	4.329	4.104	4.554	4.38	6.12
2.12	4.459	4.200	4.718	3.19	6.58
2.18	4.482	4.210	4.754	3.66	6.66
2.46	4.589	4.235	4.943	5.27	7.04
2.46	4.589	4.235	4.943	4.78	7.04

Regression equation parameters are as follows:

Intercept = 3.647 Slope = 0.383

Calculated chi-square value = 21.92 ($P < 0.005$)

Relative potency factor used = 0.065

Expected response equation: $y = 3.719 + 1.351 \log (0.065\ C_z + C_c)$

Appendix VIII e.: Regression parameters for 96 hr, dosage-mortality curve for cadmium-zinc mixture in terms of equal potency ratio

Log cadmium equivalent concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response	Expected response
0.97	4.405	4.050	4.760	4.35	5.72
1.04	4.444	4.110	4.778	4.61	5.91
1.22	4.545	4.260	4.830	4.95	6.42
1.26	4.568	4.292	4.844	4.61	6.53
1.74	4.836	4.625	5.047	4.59	7.87
2.09	5.032	4.783	5.281	4.00	+ ∞ *
2.16	5.072	4.808	5.336	4.17	+ ∞
2.45	5.234	4.894	5.574	6.65	+ ∞
2.46	5.240	4.897	5.583	5.77	+ ∞

Regression equation parameters are as follows:

Intercept = 3.862 Slope = 0.560

Calculated chi-square value = 39.07 ($P < 0.005$)

Relative potency factor used = 0.015

Expected response equation: $y = 3.00 + 2.801 \log (0.015\ C_z + C_c)$

* + ∞ denote 100% mortality is the expected response

Appendix IX: Empirical results for the cadmium-zinc mixture in terms of equal concentrations

Concentration[a] (ug/L) cadmium	zinc	Time (hrs)	Number used	Number dead	Cumulative % Mortality
64.1 (3.2)[b]	51.7 (4.7)[b]	85.0	22	1	5
110.0 (19.0)	98.9 (16.5)	85.0	22	1	5
152.5 (15.0)	152.1 (23.0)	70.0	22	1	5
		85.0	22	2	9
		88.0	22	5	23
		91.0	22	9	41
		94.0	22	12	55
323.2 (20.2)	273.2 (12.7)	22.0	22	1	5
		52.0	22	2	9
		58.0	22	3	14
		61.0	22	4	18
		70.0	22	6	27
		76.0	22	7	32
		79.0	22	9	41
		82.0	22	11	50
		85.0	22	15	68
		88.0	22	16	73
		91.0	22	17	77
		94.0	22	18	82
294.5 (18.6)	297.6 (13.0)	73.0	22	2	9
		76.0	22	3	14
		79.0	22	5	23
		88.0	22	11	50
		91.0	22	14	64
		94.0	22	17	77

a. Concentration denotes total cadmium or total zinc
b. Values in parentheses denote +/- standard deviations

Appendix IX: Continued

Concentration (ug/L) cadmium	zinc	Time (hrs)	Number used	Number dead	Cumulative % Mortality
428.6 (24.3)	370.4 (17.0)	43.0	22	1	5
		58.0	22	2	9
		61.0	22	3	14
		64.0	22	4	18
		67.0	22	6	27
		70.0	22	8	36
		73.0	22	9	41
		76.0	22	11	50
		79.0	22	12	55
		82.0	22	15	68
		85.0	22	18	82
		88.0	22	19	86
		91.0	22	21	95

Appendix X a.: Regression parameters for 72 hr. dosage-mortality curve for cadmium-zinc mixture in terms of equal concentration

Log cadmium equivalent concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response	Expected response
2.21	2.665	1.759	3.571	2.74	4.49
2.50	4.105	3.636	4.574	2.86	4.62
2.53	4.254	3.771	4.737	4.69	4.60
2.66	4.900	4.225	5.575	4.90	4.66

Regression equation parameters are as follows:

Intercept = -8.310   Slope = 4.966

Calculated chi-square value = 7.02 ($P < 0.05$)

Relative potency factor used = 0.065

Expected response equation: $y = 3.647 + 0.383 \log (0.065\ C_z + C_c)^a$

a. C_z denotes total zinc concentration and

C_c denotes total cadmium concentration

Appendix X b.: Regression parameters for 96 hr. dosage-mortality curve for cadmium-zinc mixture in terms of equal concentrations

Log cadmium equivalent concentration (ug/L)	Predicted response	95% fiducial limits Lower	Upper	Empirical response	Expected response
2.19	4.952	4.125	5.779	4.98	+ ∞ *
2.48	5.983	5.413	6.553	5.99	+ ∞
2.51	6.090	5.476	6.704	5.94	+ ∞
2.64	6.552	5.660	7.444	6.73	+ ∞

Regression equation parameters are as follows:

Intercept = -2.833 Slope = 3.555

Calculated chi-square calue = 0.38 ($P < 0.05$)

Relative potency factor used = 0.015

Expected response equation: $y = 3.00 + 2.801 \log (0.015\ C_z + C_c)$

* + ∞ denotes 100% mortality is the expected response.

Appendix XI a: Derivation of the 96 hour tolerance distribution for the discrete dosage-mortality line

Parameters used to determine the tolerance distribution are:

LC50 = u = 5.20

Slope = 2.801

α = 1/2.801 = 0.357

y = (1.117) (2.7183)$^{-(x^2/0.255)}$

x = X - u

X	x	y
4.3	-0.9	0.047
4.4	-0.8	0.091
4.5	-0.7	0.164
4.6	-0.6	0.272
4.7	-0.5	0.419
4.8	-0.4	0.596
4.9	-0.3	0.785
5.0	-0.2	0.955
5.1	-0.1	1.074
5.2	0.0	1.117
5.3	0.1	1.074
5.4	0.2	0.955
5.5	0.3	0.785
5.6	0.4	0.596
5.7	0.5	0.419
5.8	0.6	0.272
5.9	0.7	0.164
6.0	0.8	0.091
6.1	0.9	0.047

Appendix XI b: Derivation of the 96 hour tolerance distribution of the equipotent dosage-mortality line

Paramaters used to determine the tolerance distribution are:

LC50 = u = 107.68 ug/L

Slope = 0.560

α = 1/.560 = 1.786

$y = (0.223)(2.7183)^{-(x^2/6.380)}$

$x = X - u$

X	x	y
103.68	-4.0	0.018
104.68	-3.0	0.050
105.68	-2.0	0.119
106.78	-0.9	0.196
106.88	-0.8	0.202
106.98	-0.7	0.207
107.08	-0.6	0.211
107.18	-0.5	0.214
107.28	-0.4	0.217
107.38	-0.3	0.220
107.48	-0.2	0.222
107.58	-0.1	0.223
107.68	0.0	0.223
107.78	0.1	0.223
107.88	0.2	0.222
107.98	0.3	0.220
108.08	0.4	0.217
108.18	0.5	0.214
108.28	0.6	0.211
108.38	0.7	0.207
108.48	0.8	0.202
108.58	0.9	0.196
109.68	2.0	0.119
110.68	3.0	0.050
111.68	4.0	0.018