Prediction of Daphnia magna LC50 on Heavy Metal Containing Samples

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ABSTRACT/ This study assessed the contribution of heavy metals to total toxicity as well as the presence other toxic compounds before and after adding the chemical P to concurrently conducted bioassay tests of Daphnia magna and P. phosphoreum. The following conclusions were drawn from this study : With excessive EDTA dosage, a toxicity reduction in Microtox would occur due to a metal-complex being formed. Microtox was far less sensitive than D. magna to heavy metal toxicity, but extended exposure time and aged reagent could increase the sensitivity.

1. Introduction

Bacterial bioassay has been used and evaluated in numerous comparative studies in recent years. These studies have compared the response of marine luminescent bacteria *Photobacterium phosphoreum* to that of many aquatic organisms, but have focused primarily upon the freshwater cladoceran, *Daphnia magna*. When the toxin sensitivity of luminescent bacteria and *Daphnia magna* are compared, one generally finds satisfactory agreement between these two bioassay methods if pure, singular compounds are being tested. But while single toxin correlations are well documented, no reasonable correlations have been developed between bacterial toxicity EC50 values and the LC50 values obtained from traditional bioassay tests on complex wastewater and heavy metals-containing samples. When compared to traditional bioassay results (Qureshi et al, 1982), the bacterial test, although fast, tends to be oversensitive for some pollutants (chloroform, phenol, etc) but more often quite subsensitive to others (heavy metals, free ammonia, etc).

Being able to determine whether a given sample contains a heavy metal and how much the toxicity of the heavy metal contributes to total toxicity is essential to establishing the

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correlation between two bioassay. If the amount of heavy metal can be reduced to the level where it no longer exhibits toxic effects on the sample, any subsequent toxicity can clearly be contributed to something other than heavy metal concentration alone.

A method of adding EDTA (ethlenediaminetetraacetic acid) a metal-chelating, compound, to wastewater effluent samples possessing toxic heavy metal concentrations has been proposed and tested(Mount, 1988). This series study assessed the contribution of heavy metals to total toxicity as well as the presence other toxic compounds before and after adding the chemical to concurrently conducted bioassay tests of Daphnia magna and P. phosphoreum.

The purpose of the present study is, first, to explore possible toxicity reduction after adding EDTA to the sample using P. phosphoreum test. The second purpose is to use such information to assess the type of sample and to develop a better understanding of the characteristics inherent to the samples. The third purpose is to improve the correlation between the two bioassay tests when heavy metals are a major toxicant by suggesting procedural modifications which would enhance the accuracy, sensitivity and usefulness of the bacterial test.

2. Background

2.1 Characteristics of Heavy Metal Toxicity and Reduction by EDTA

Since these metals react chemically or are absorbed by particulate matter to some degree when dissolved or suspended in water, the active concentration to which the animal is exposed and, in many cases, the chemical form of the metal are not clearly defined. Small changes in dissolved oxygen and CO2 content, PH, alk .nity, and hardness of the test water after both the aqueous chemistry of the toxicant and the animal's physiological response to that toxicant(Jones, 1939).

Recent investigations of toxicity in the presence of added complexing agents indicate that formation of either inorganic or organic complexes greatly reduces metal toxicity. Nishikawa(1969) has observed that the reduction in toxicity is related to the stability constants of the metal complexes formed. Also, Biesinger (1973) observed that zinc and copper solutions with an excess of the strong chelating agents EDTA or NTA are much less toxic to fish and aquatic invertebrates.

In similar studies, the metal-chelating compounds, EDTA and NTA(nitilotriacetic acid) reduce tissue accumulation and toxicity of heavy metals in fish(Muramoto, 1980a) and crustaceans (Podolski, 1979). There are two possible mechanisms by which complexing agents are reduced:either there is reduced uptake of the complexes metal the metal complexes are taken up but are rapidly excreted and do not accumulate (Part, 1984).

2.2 Comparison of Heavy metals Toxicity on Daphnia magna and P. phosphoreum

Although, for individual compounds, the ratio of the susceptibility of the Microtox system and of the other tests is inconsistent, the mean relative susceptibility differs from the sensitivity of other only a factor 7 maximally. Based on the toxicity of all chemicals tested, the daphnids, geometrically average a factor 2.97 as sensitive as the Microtox system (Zwart, 1983)

In Table 1 is shown the sensitivity for the two species in various literatures.

Table 1. Summary of Heavy Metals Toxicity

Description of samples	D.magna[a] mg/L	P.phosphoriem[b] mg/L	Source
Zn	0.1		(Biesinger 1972)
		2.5 (5 min.)	(Bulich 1981)
Cu	0.06		(Biesinger 1972)
		0.8 (15 min.)	(Dorward 1982)
Ag	0.06	0.5 (15 min.)	(In this study)
Cd	0.2		(Mount 1983)
		17 (15 min.)	(Hinwood 1987)

[a] 48-h TC_{50} concentration in mg/L
[b] EC_5 mg/L, exposure time in parenthesis

The above data clearly indicates that P. phosphoriem has much less sentivity (about 10 times) to pure metal toxicity then that of P. magna.

2.3 Microtox System for P. phosphoreum Bioassay

Several marine bacteria are able to give off some light after asequence of biochemical reactions. Taking advantage of this ability, an inhibitive test of light production, the Microtox Toxicity System was used because of S its relatively good sensitivity, simplicity, and convenience due to the marketing of a freeze-dried Photobactereum strain ready for use. A reduction of light intensity in each suspension after the addition of the sample indicates the presence of toxic substances. Light loss should be normalized to correct for phase shift in the blanks. However, in many instances metal salt solutions could not be adjusted to pH 6.7, the recommended valuefor optimum sensitivity the Microtox assay without precipitation (Beckman, 1985). Since alteration of sample pH may cause precipitation of certain toxicant creating problems in data interpretation, the pH of the sample shoule be considered as part of the assay (Qureshi et al, 1984).

2.4 Increasing the Sensitivity of P. phosphoreu

It has been reported that acute toxicity results obtained utilizing a Microtox System are typically lower than those derived from the standard biomonitoring techniques. A plausible explanation is that aquatic organisms accumulate or concentrate toxins within cell tissue, but can not be measured or simulated in the bacteria used for toxicity assessment in the Microtox apparatus.

Likewise, some chemicals require longer exposure time to manifest their full toxic response and to reach a maximum in the inhibition process. The optimal exposure time for each compound depends mainly on the chemical nature of compound itself. For most inorganic substances the toxic effect increases with time, whereas the highest toxicity observed with organic contaminants occurs within minutes of exposure and decreases with longer exposure time (Ribo, J. M., 1985). Also, by extending exposure time to 30-45 minutes, even lower levels of metal toxicant can be detected (Zwart, 1983). Qureshi presented results obtained from the assay of several toxicant using freshly (0.5 hour) reconstituted and aged (4 hour) bacterial reagents. In general, a trend of increasing sensitivity of bacterial reagent with increasing age after reconstitution was observed for most of the metal ions.

3. Experimental Methods

3.1 Biomonitoring Tests

The daphnids to be used for the bioassay test were subcultured from an already existing stock. The brood stock is maintained in dilution water (reconstituted hard water) at 25±1°C, using approximately 10 adults per 1000 ml borosilicate beaker, under a 16-h light and 8-h dark photoperiod. The adults were fed a combination diet of yeast, trout chow, and alfalfa (YFA).

Only daphnids less than 24 hours old were used in the tests. All the samples were unaerated due to the daphnids' characteristic low requirement for dissolved oxygen. The young daphnids were transferred in groups of 10 per 100 ml sample of each test solution. With four concentrations and a control constituting one test, most tests were conducted in triplicate per concentrations.

The number of dead organisms were recorded after 48-h, but when convenient and feasible observations were also made as close as possible to 1, 2, and 24 hours. For each data set, the daily LC50 values and their corresponding 95% confidence intervals were estimated with the aid of a LC50 computer program using statistical methods (US EPA, 1988).

3.2 Heavy Metals Elimination by EDTA

American Chemical Society reagent-grade chemicals were used for all testing. They are :$ZnSO_4$, $PbSO_4$, $AgNO_3$, $CuSO_4$, $HgCl_2$, and $CdCl_2$ for each individual toxicity test as pure compound. Each Microtox toxicity test conducted within 0.5 - 0.75 hour and 3.5 - 4.0 hours of opening the fresh bacterial reagent with the differnt exposure times of 5, 15, 30, and 45 minutes. Different EDTA concentrations were added to each compound until a toxicity reduction took place in order to determine an optimum EDTA dosage. Fresh stock solutions were prepared in distilled water for each experiment. The purpose of using the EDTA was not so much to quantify heavy metal toxicity portion of total toxicity but to make rough measurement of how much the heavy metal contributed to total toxicity since the chelating compound may have absorbed other toxic compounds.

4. Results

4.1 Effect Toxicity on Exposure Time and Aged Reagent

The relationship between exposure time and aged reagent toxicity as determined with the Microtox for a variety of chemicals is presented Table 2. These methods were explored by obtaining EC50 values at more than one exposure time(at 5, 15, 30, and 45 min) using the Microtox test. Table 2 and Figure 1 show that, with longer exposure and the use of aged reagent, a lower detectable concentration can be obtained when using metal as the predominant toxicant.

Table 2. Response Toxicity of Metals in the Microtox

Toxic Compound	Compound Used	Time after reconstitution(hr)	EC_{50} Conc.[a], mg/L min.			
			5	15	30	45
Cu^{2+}	$CuSO_4\ 5H_2O$	0.5	5.2	3.2	1	0.5
		4.0	3.0	2.1	0.3	0.3
Zn^{2+}	$ZnSO_4\ 7H_2O$	0.5	31	19	6	5
		4.0	20	10	3.1	2.1
Ag^{+1}	$AgNO_3$	0.5	1.0	0.8	0.5	0.4
		4.0	0.8	0.5	0.3	0.3

[a]Concentration causing 50% decrease in light reduction at 15°C.

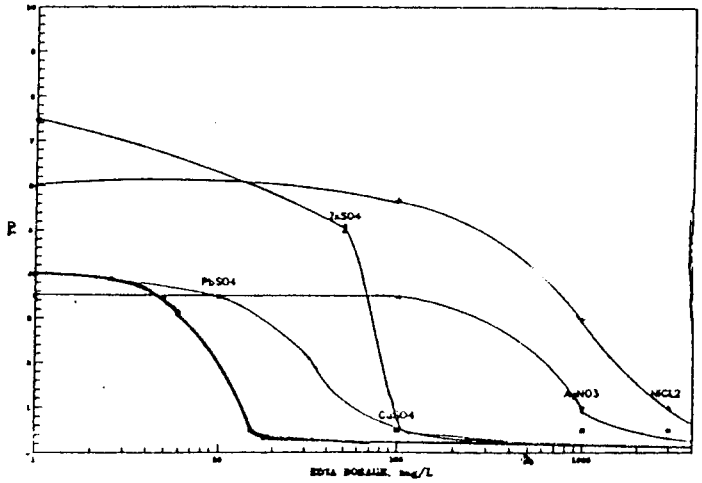

Fig. 1 Metal Toxicity Reduction in Microtox

4. 2. Toxicity Reduction by EDTA

Table 3 presented the optimum EDTA dosage for each metal compound. Figure 1 also presented their toxicity reductions for each in different samples.

5. Analysis

As shown in Table 2, the sensitivity of the metal compound toxicity increases as longer exposure time (from 5 minutes up to 45 minutes) and time after reconstitution from (0. 5 to 4 hour) are given. These operational modified test methods gave total 40 - 70% lower the detectable concentration limit of most metal toxicity on Microtox (increase sensitivity). For example, as the sensitivity of silver increases from 5. 2 mg/l of EC50 to 0. 3, it will exhibit a much toxicity comparaly closer to D. magna (0. 06 mg/L).

Table 3 indicates that each metal has a different detectable limit for the Microtox test; thus, the optimum EDTA dosage is dependent on the metal used. Because the EDTA itself was nontoxic in Microtox, addition of excessive EDTA did not increase toxicity. However, these results differ from those of the previous study in that the metal complex toxicity with D. magna increased with excessive EDTA dosage.

6. Conclusions

The following conclusions can be drawn from this study:

6. 1 With excessive EDTA dosage (about 3000-4000 mg/L in the heavy metal

Table 3. Toxicity Reduction

Compound	Concentration used, mg/L	Toxicity TU[a]	Optimum EDTA mg/L	Post EDTA, TU
$CuSO_4$	10	4	15	<1
$AgNO_3$	3	3.5	1000	1
$ZnSO_4$	100	7.5	100	<1
NiC_{12}	22900	6.0	2000	2
$PbSo_4$	100	4	100	<1

[a]Toxic Unit = 100/dilution of volume percent

containing samples), a toxicity reduction in Microtox will occur due to a metal-complex being formed.

6. 2 Microtox is far less sensitive than D. magna to heavy metal toxicity, but extended exposure time (up to 45 minutes) and use of aged reagent (up to 4 hours) can increase the sensitivity.

6. 3 If a toxicity reduction occurs after adding the EDTA in the sample for Microtox test, the sample contains metals and its toxicity on D. magna can be predicted to be 5-10 fold the Microtox reading.

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