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Toxicity Assessment of Hanford Site Wastes By Bacterial Bioluminescence

Prepared for the U.S. Department of Energy
Office of Environmental Restoration
and Waste Management

Westinghouse Hanford Company Richland, Washington

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LIST OF FIGURES

LIST OF TABLES

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TOXICITY ASSESSMENT OF HANFORD SITE WASTE BY BACTERIAL BIOLUMINESCENCE

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INTRODUCTION

Waste toxicity assessment is an essential part of the Waste Management Program at the Hanford Site located near Richland, Washington. Toxicity data bases, models for predicting toxicity, and methods for rapid evaluation of the biological toxicities of the complex radioactive wastes stored at the Hanford Site are lacking or nonexistent. To effectively handle and treat these wastes, the degree of toxicity of the chemical species they contain must be known.

Hanford Site radioactive wastes are currently stored onsite in double-shell carbon steel tanks, as either an insoluble sludge or a salt fraction made up of saltcake and salt solution. This report addresses the contribution of the nonradioactive inorganic components of low-level wastes to toxicity.

METHOD

Simulated waste mixtures, designed to mimic the expected compositional range of the inorganic components of low-level radioactive wastes, were prepared. The mixtures were composed mostly of sodium nitrate, sodium nitrite, sodium aluminate, and sodium hydroxide. Table 1 shows the concentration range of these components in the mixtures. Because the current strategy for disposal of low-level radioactive wastes involves immobilization of the waste by grouting, test grout specimens were also prepared from these waste mixtures. The dry solid materials that were used to form grout were blast-furnace slag, fly ash, and Portland cement (Table 2).

Potential environmental hazards posed by grouts are largely unknown. To determine whether the current grout technology is adequate in controlling toxicant and pollutant releases for regulatory compliance, regulated metals were deliberately added to the simulated waste mixtures. Table 3 lists the regulated metals and their concentrations in the waste mixtures.

The U.S. Environmental Protection Agency and the State of Washington recognize a 96-h fish acute toxicity test (ASTM 1988) as an indication of the acute toxicity of chemical substances and effluents subject to environmental regulation. However, this test is quite expensive and public resistance to large-scale fish testing may preclude the generation of data bases on lethal toxicity. Therefore, use of surrogate organisms whose response can be related to that of fish for toxicity studies is desirable. Inhibition of

Table 1. Components of Simulated Hanford Site Wastes.

COMPONENT	CONCENTRATION RANGE, M
Aluminate	0.058 - 0.783
Nitrite	0.131 - 1.385
Nitrate	0.223 - 2.357
Phosphate	0.000 - 0.305
Hydroxide	0.078 - 1.240
Carbonate	0.062 - 0.702

Table 2. Dry Materials for Grout Production.

•Blast Furnace Slag
•Fly Ash
•Portland Cement

Table 3. Regulated Metals In Simulated Hanford Site Wastes.

METAL	CONCENTRATION (mg/L)
Silver (Ag)	5.5
Arsenic (As)	5.5
Barium (Ba)	110.0
Cadmium (Cd)	1.1
Chromium (Cr)	1258.0
Mercury (Hg)	0.2
Lead (Pb)	5.5
Selenium (Se)	1.1

bioluminescence of a marine bacterium, Photobacter phosphoreum (called Microtox[1] in this study), is a cost-effective prescreening procedure for assessing toxicities of chemicals. This test is rapid, reproducible, and inexpensive compared to the 96-h fish acute toxicity test.

Bioassays using Microtox were performed on 38 waste mixtures and their grout extracts. Grout extracts were obtained by shaking 1.0 g of ground grout in 1.0 L of distilled deionized water for 10-min followed by filtration to remove the insoluble solids. Bioassays were conducted as described by the manufacturer of the Microtox analyzer. Briefly, the method utilizes a suspension of the bacteria at a concentration of approximately one million microorganisms. The suspension is then challenged by the addition of the test solution. A photometer measures the light output of the bacteria before and after a 5-min exposure to the test solution. A series of concentrations of the test solution (nominal dilutions of the test solution) was tested to obtain the median effective concentration that produces 50% inhibition of bioluminescence (EC50). Data reduction uses a "gamma" function in place of percent light decrease. The "gamma" function is defined as the ratio of the corrected light loss to the light remaining of the bacteria (Table 4). Toxicity of the species of interest is then measured in terms of its EC50 value. Graphical determinations of EC50 values were performed by plotting the gamma data (logarithmic scale) against the concentrations (logarithmic scale) of the test solutions. The concentration at gamma equals one is the EC50 value. Typical examples of this method of calculating EC50 value are shown in Figures 1, 2, and 3. From these figures, it appears that lead nitrate produced the most adverse effect (EC50, 1.977 mg/L) on the bacteria compared to chromium (EC50, 86.3 mg/L) and barium nitrate (EC50, 654.5 mg/L).

Table 4. EC50/Gamma Determination.

EC50	=	Effective Concentration of Toxicant inhibiting luminescence of bacteria by 50%.
	=	Concentration of Toxicant at Gamma equals 1.
Gamma	=	Light Loss (Corr)/Light Remaining.

RESULTS AND DISCUSSION

Comparison of the observed responses of Microtox to regulated metals and the metals' established permissible maximum concentration level (MCL) in nonhazardous wastes is presented in Figure 4. The graph strongly suggests that Microtox can provide a first estimate of the toxicity of metals. Its use in toxicity assessment of Hanford Site wastes may result in the elimination of relatively innocuous wastes from further testing, saving time and money. An

[1]Microtox is a registered trademark of the Microbics Corporation, Carlsbad, California.

Figure 1. Toxicant: Lead Nitrate EC50 Determination.

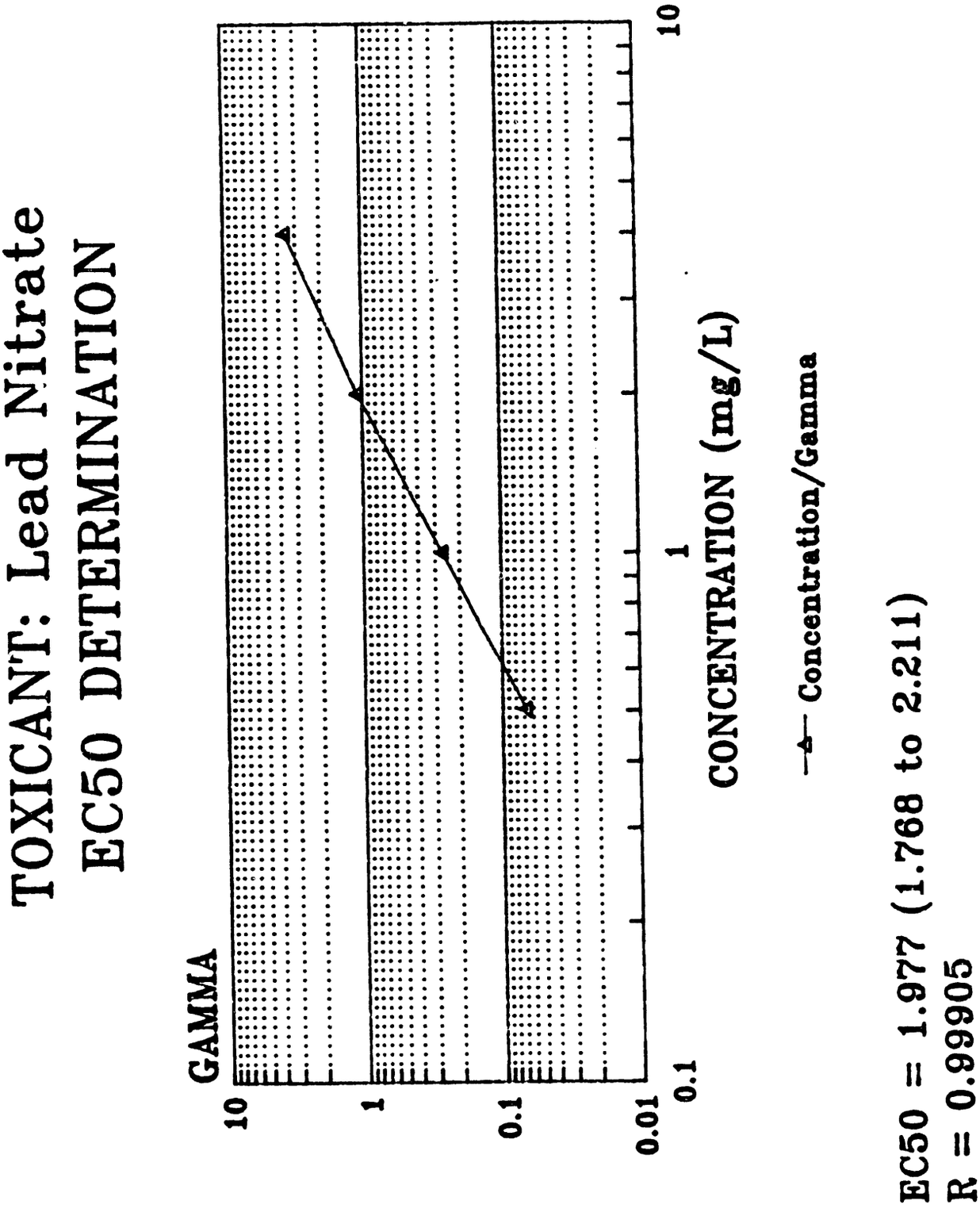


Figure 2. Toxicant: Chromium EC50 Determination.

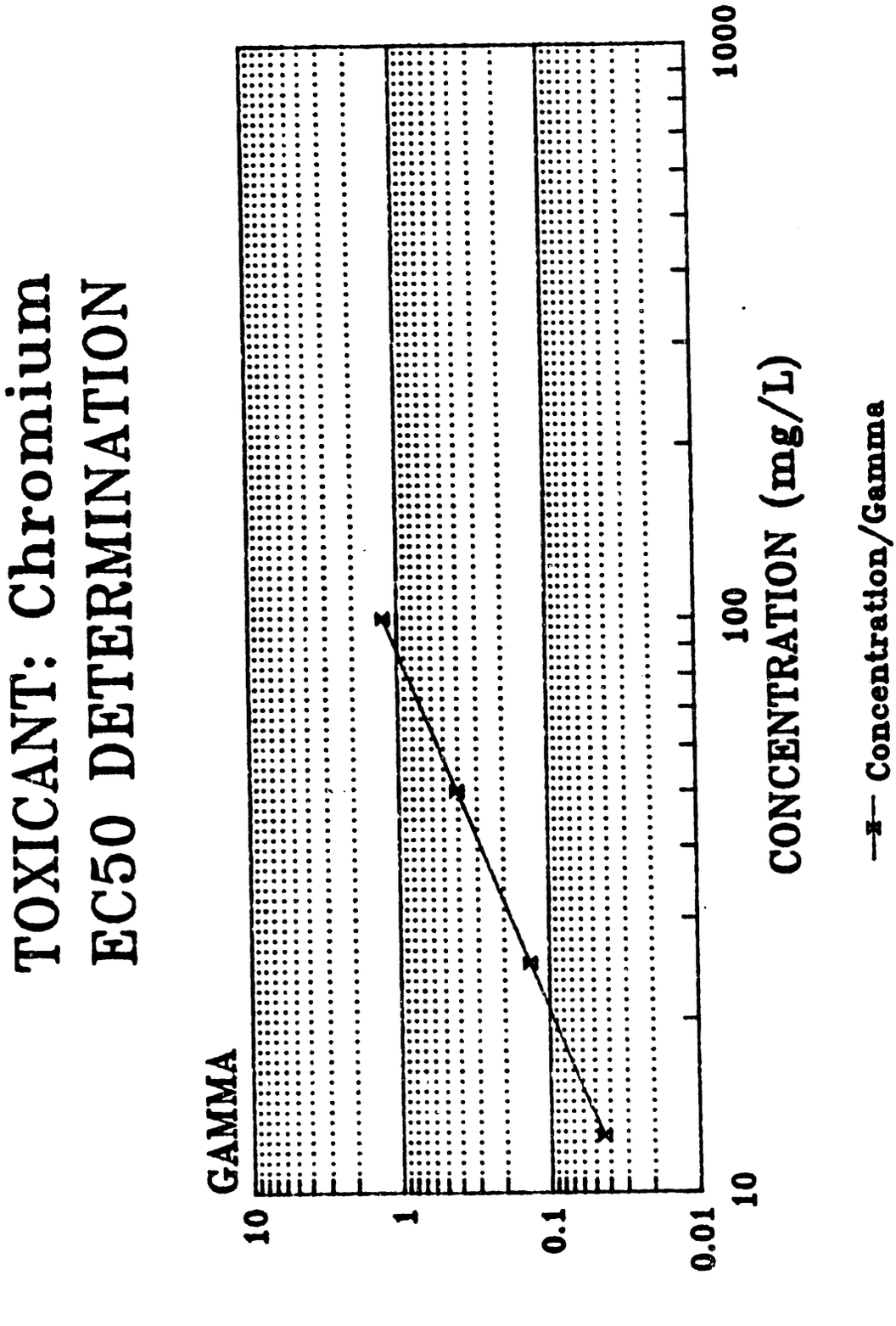


Figure 3. Toxicant: Barium Nitrate EC50 Determination.

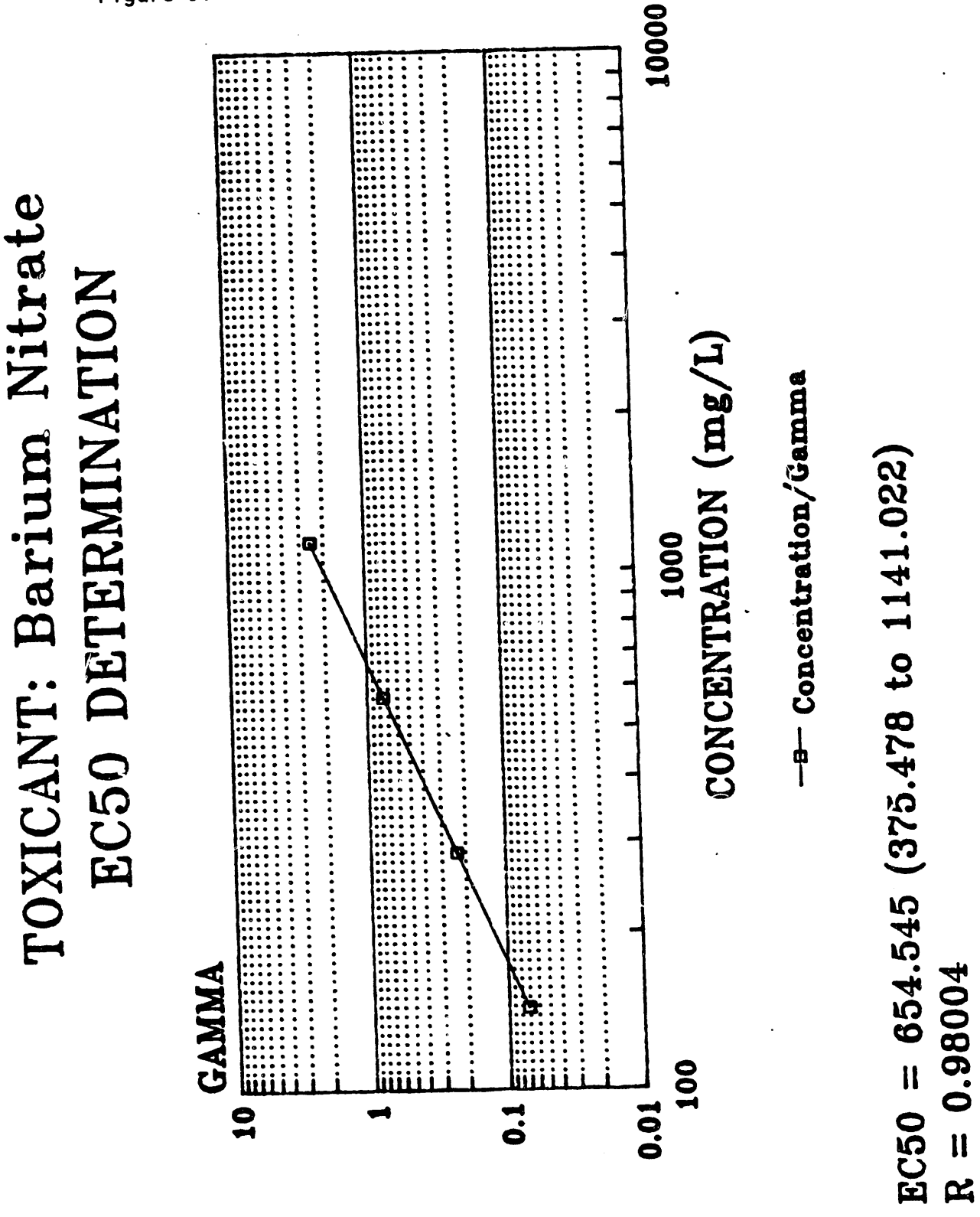


Figure 4. Toxic Response.

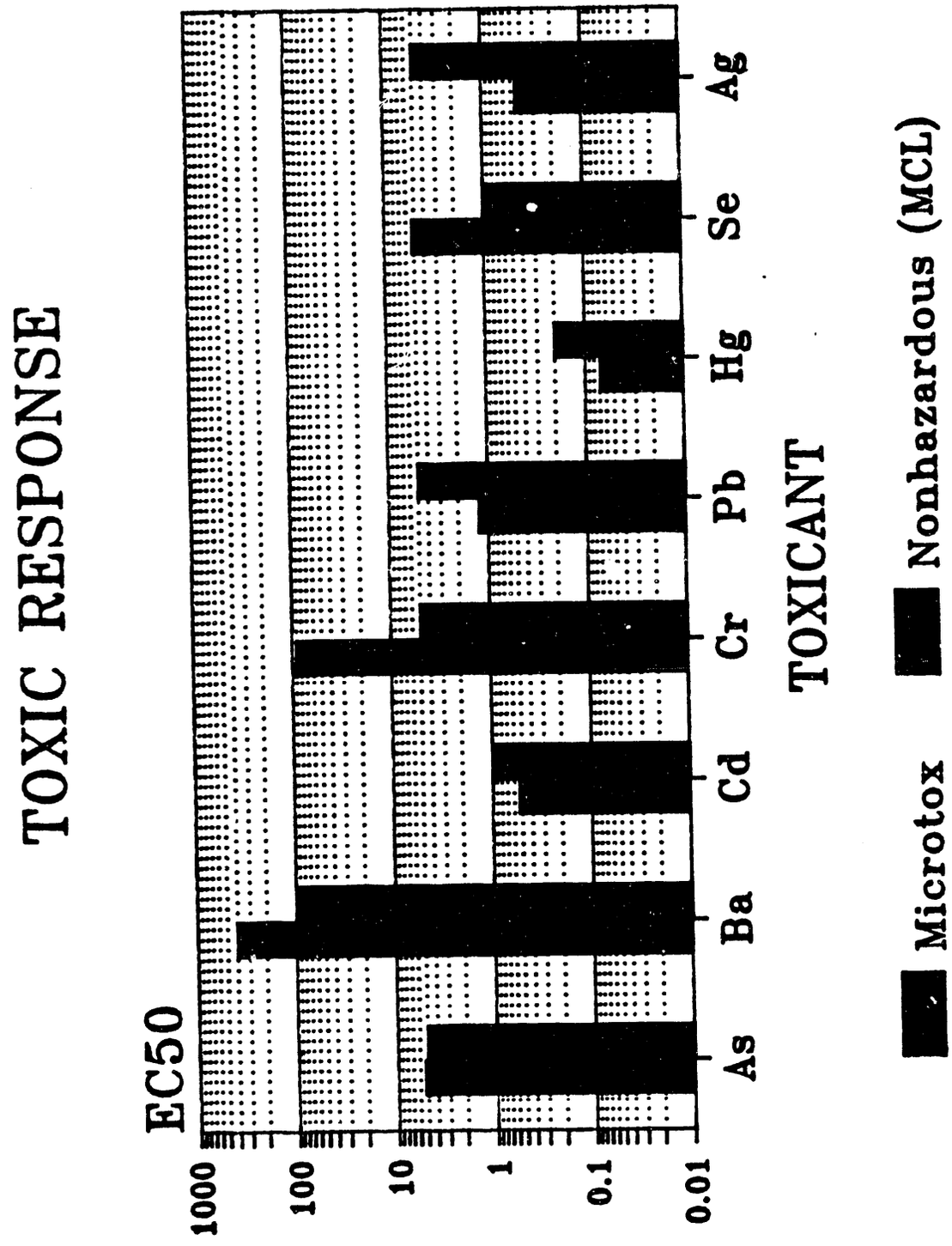


illustration of the relative toxicities of the trace inorganic components of the simulated wastes is depicted in Figure 5. The graph indicates that mercuric chloride is the most toxic while sodium nitrate is the least toxic among the components of the wastes.

Successful toxicant identification requires a complete understanding of dose-response curves, including the influence of the solution matrix and synergistic or antagonistic interactions among toxicants. For example, the effect of two chemicals given simultaneously will produce a response that may be simply additive of their individual responses or may be greater or less than that expected by addition of their individual responses. Study of these interactions often leads to a better understanding of the mechanism of the action of the chemicals involved.

The terms used to describe toxicologic interactions are listed in Table 5. These descriptors are nonquantitative and are often used ambiguously. A simple formula for determining the additive toxicity of mixtures of chemicals is given in Table 6. If the sum of the biological activities of the mixture (S) equals one, the toxicity is additive. The effect is synergistic if this sum is less than one and antagonistic if it is greater than one. It should be noted that the smaller the EC50 value of a species of interest, the greater its toxicity.

Applications of the formula in the interpretation of toxic responses of Microtox to components of Hanford Site wastes are depicted in Figures 6, 7, and 8. Figure 6 displays the responses of Microtox to doses consisting of one EC50 of copper nitrate (EC50, 0.72 mg/L) and one EC50 value of other compounds. As can be seen, the mixture containing copper (Cu) and arsenic (As) [S, 0.67] produces synergism while that of Cu and barium (Ba) [S, 2.47] exhibits antagonism. The latter effect is desirable because this will reduce the toxicity of the mixture. The effects of adding As, lead (Pb), mercury (Hg), or Ba to a mixture consisting of one EC50 each of Cu and zinc (Zn) [S, 0.94] are illustrated in Figure 7. The addition of As enhances synergism [S, 0.79] while antagonism results with the addition of Pb [S, 1.21], Hg [S, 1.55], or Ba [S, 1.59].

The metals common to all mixtures in Figure 8 are Cu, Zn, nickel (Ni), and As with a combined S value of 0.95. Silver (Ag) when added to this mixture raises the S value to 1.125. Substitution of Ag with chromium (Cr), Pb, or Hg produces very little effect on the toxicity of the mixture. It seems that the combination containing mercuric chloride is the least toxic among these combinations. The most plausible reason for this observation may be the slight precipitation of Hg as indicated by the appearance of turbidity, rendering the resulting mixture slightly innocuous to the bacteria.

The variation in the sensitivity patterns of three taxonomic species to the chemicals in the simulated wastes is summarized in Tables 7 and 8 and graphically depicted in Figure 9. The lethal concentration producing 50%

Figure 5. Components of Hanford Site Waste Microtox EC50 (5-minute).

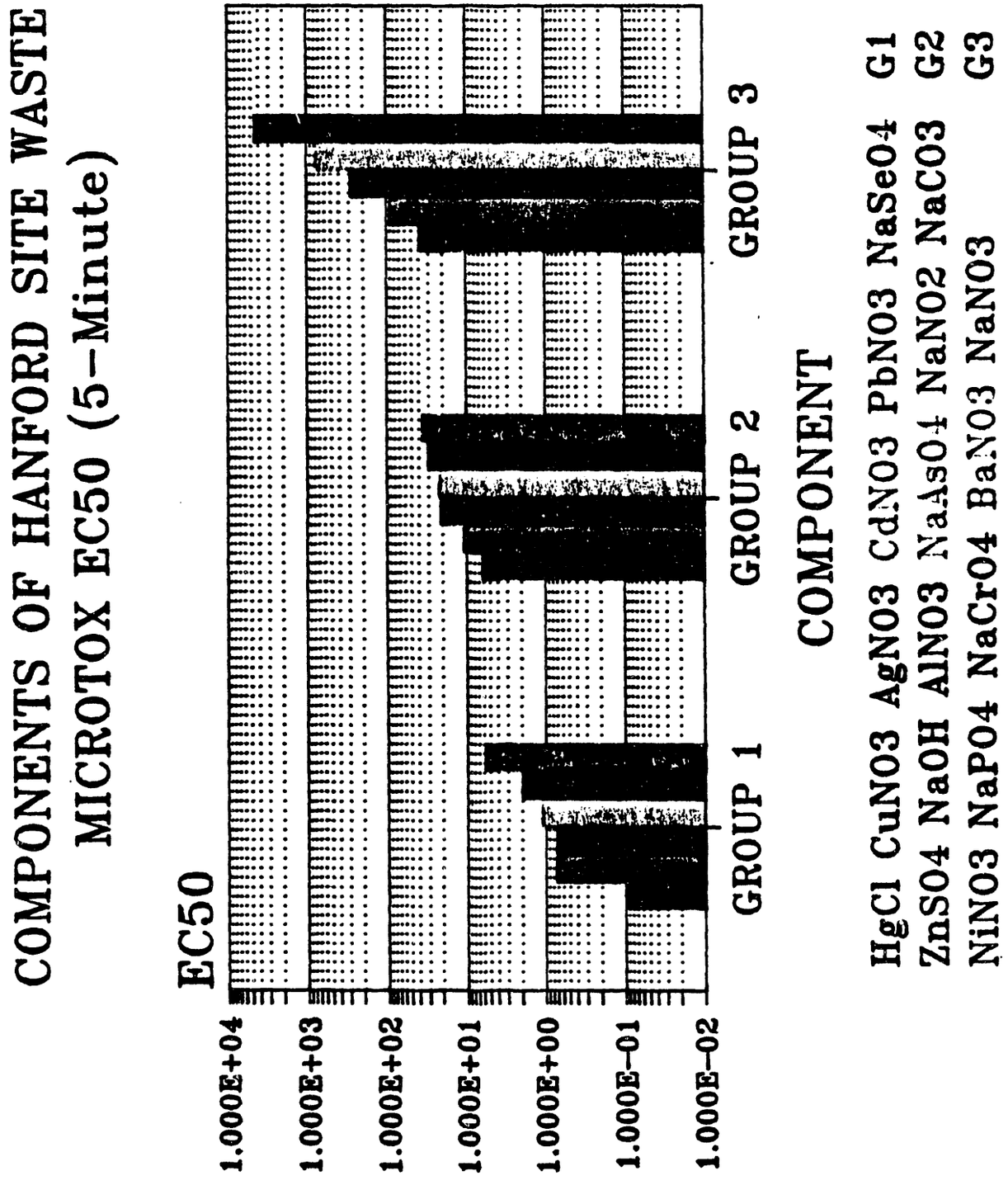


Table 5. Toxicant Chemical Interactions.

CHEMICAL INTERACTIONS	
Additive:	2 + 3 = 5
Synergistic:	2 + 3 = 20
Potentiation:	2 + 0 = 10
Antagonism:	4 + 6 = 8
	4 ÷ 0 = 1

Table 6. Biological Activity Toxicity Equation.

$S = Am/Ai + Bm/Bi$
If $S = 1$, Additive Toxicity $S < 1$, Greater Than Additive Toxicity $S > 1$, Less Than Additive Toxicity
S = Sum of Biological Activity
Ai = Toxicity of Species A
Am = Toxicity of Mixture Containing A
Bi = Toxicity of Species B
Bm = Toxicity of Mixture Containing B

Figure 6. EC50 of Copper 1 EC50 Cu: 1 EC50 Toxicant.

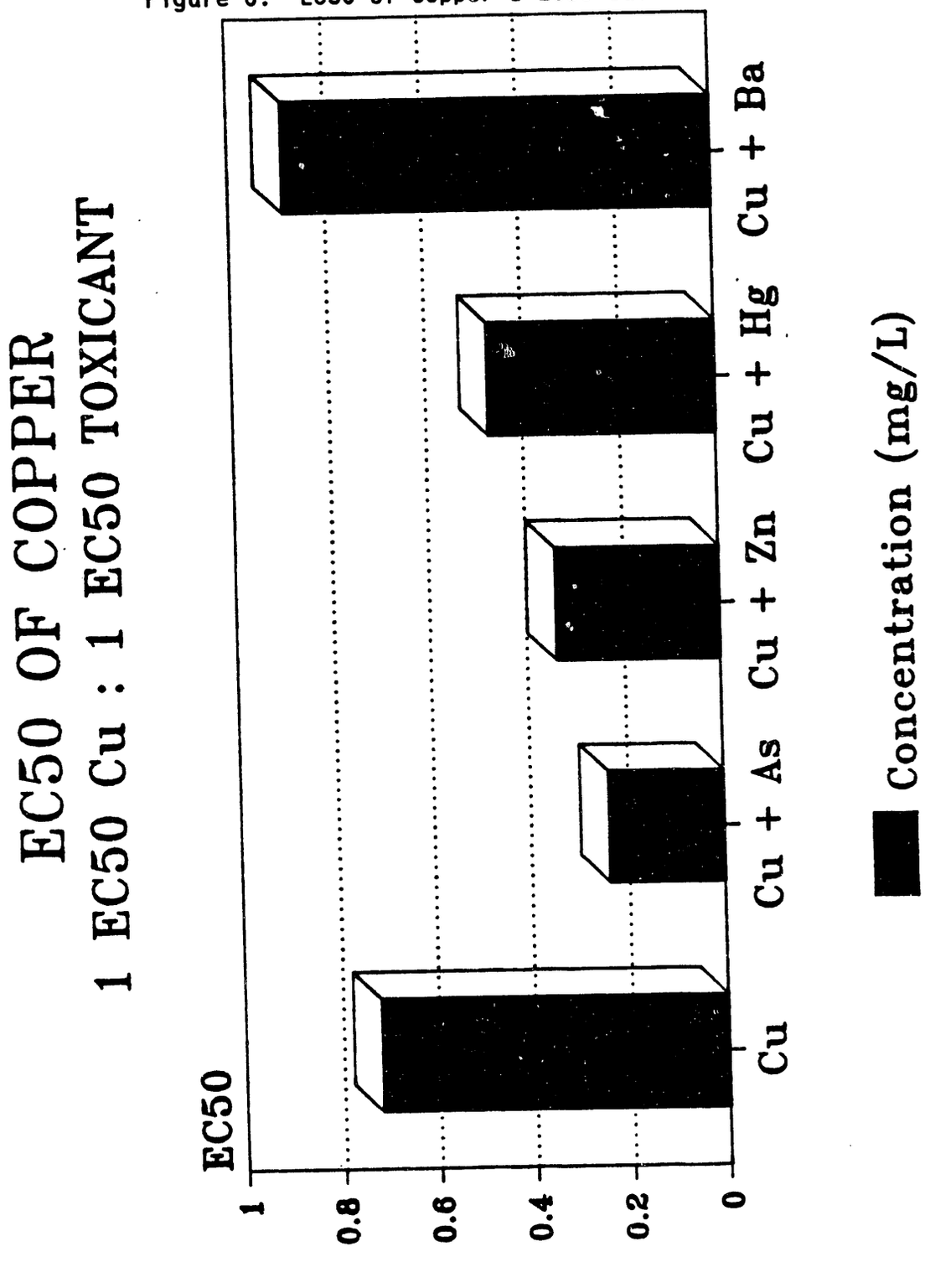


Figure 7. EC50 of Copper 1 : 1 : 1 EC50 Toxicant.

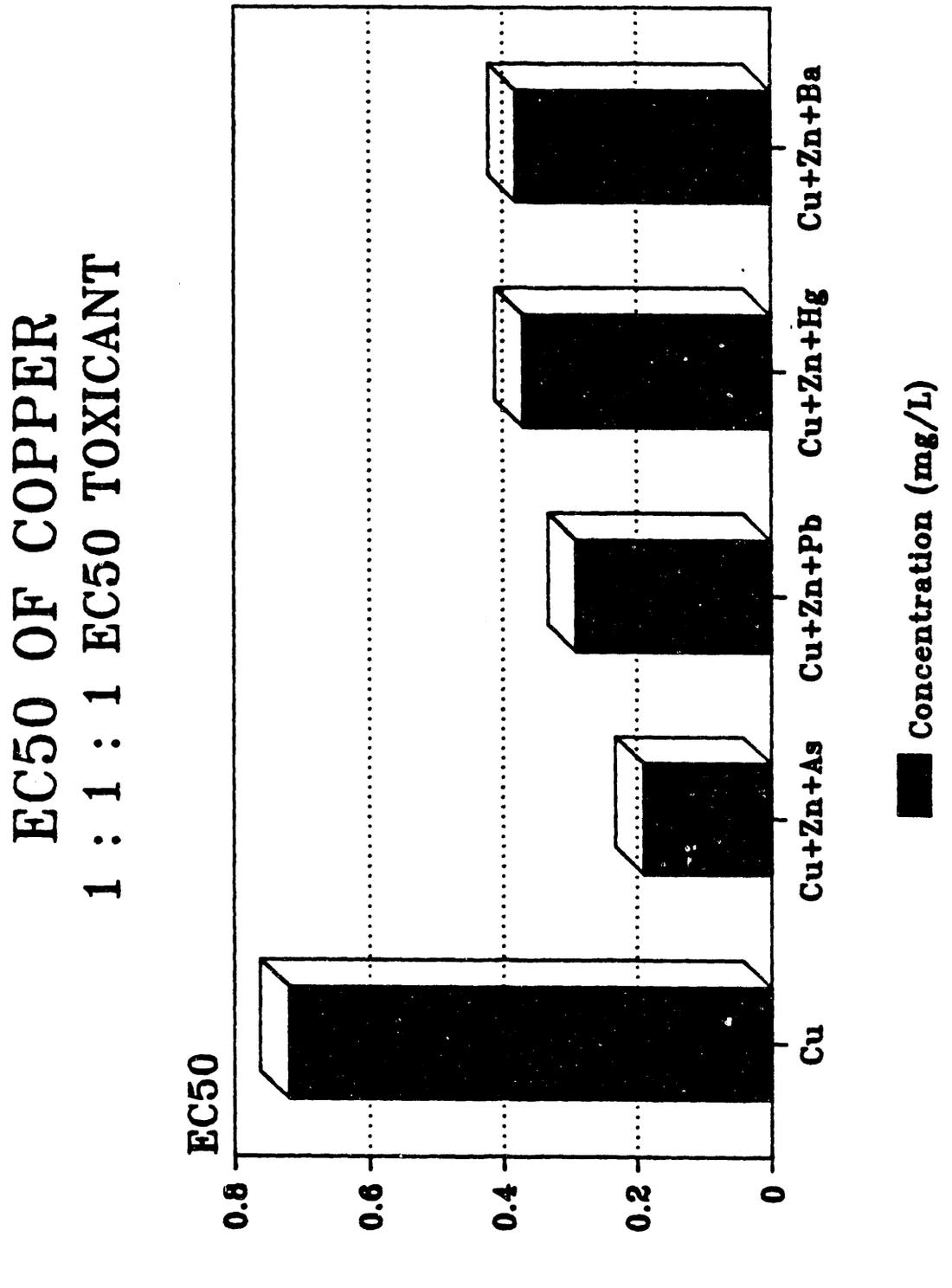


Figure 8. EC50 of Copper 1 : 1 : 1 : 1 : 1 EC50 Toxicant.

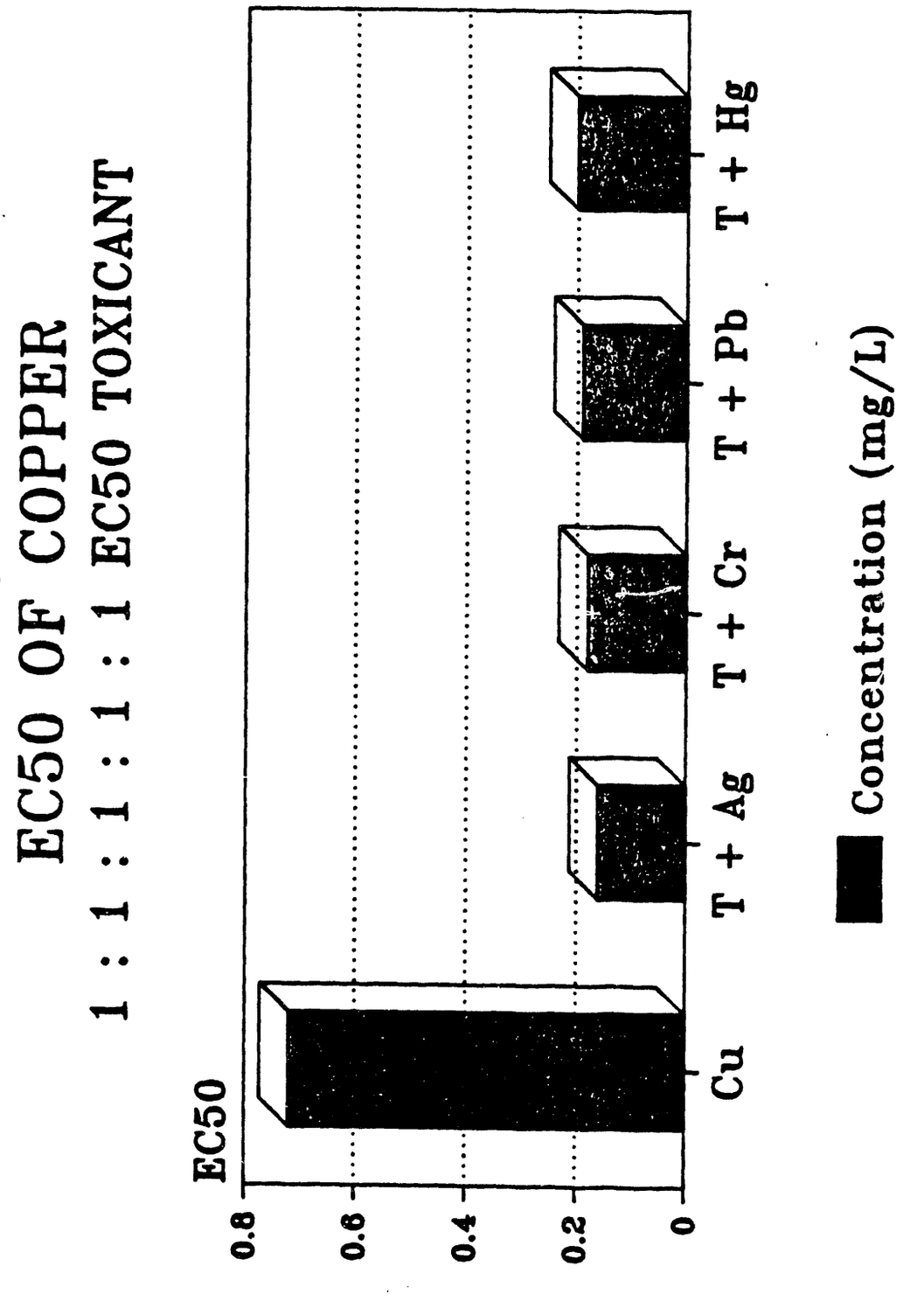


Table 7. Bioassay Comparison Microtox vs Rainbow Trout.

TOXICANT	MICROTOX 5-Min EC50	RAINBOW TROUT 96-h LC50
Hg	0.068	0.21
Cu	0.19	0.24
Cd	0.54	0.80
Zn	1.44	2.20
Se	5.27	50.00
As	5.34	43.00
Ni	7.95	27.30
Cr	91.71	115.85
Ba	403.70	262.80

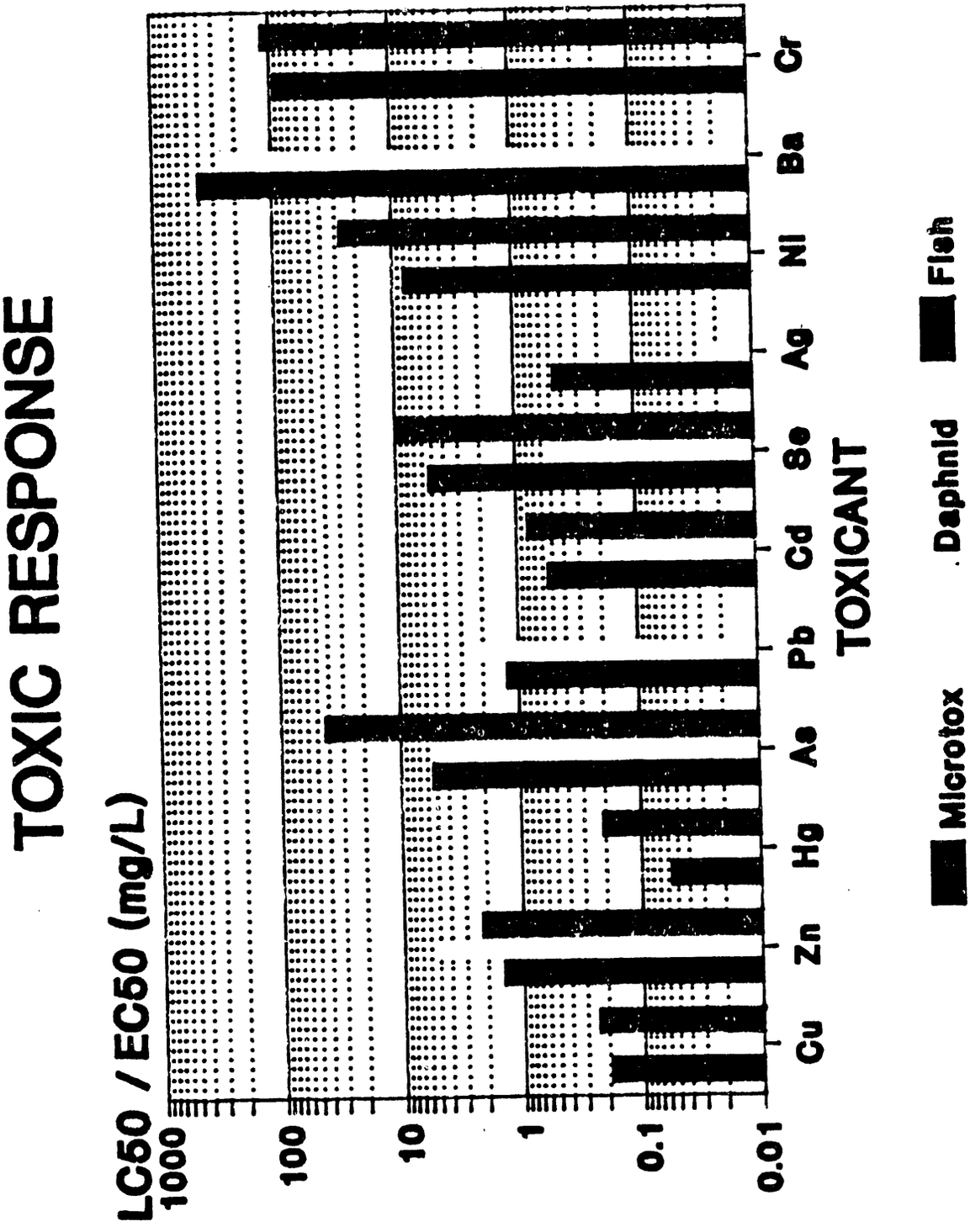
Rainbow trout literature values

Table 8. Bioassay Comparison Microtox vs Daphnid.

TOXICANT	MICROTOX 5-Min EC50	DAPHNID 48-h LC50
Hg	0.068	0.03
Cu	0.19	0.02
Ag	0.46	0.02
Cd	0.54	0.16
Pb	1.24	2.31
Zn	1.44	5.10
Se	5.27	0.55
As	5.34	5.40

Daphnid literature values

Figure 9. Toxic Response.



death (LC50) values for rainbow trout (Salmo gairdneri) and daphnid (Daphnia magna) are those reported in the literature (Qureshi et al. 1980; Sloof et al. 1983; Micromedex[2] 1990) using a 96-h acute toxicity test for fish and a 48-h acute toxicity for daphnids. Correlation plots of Microtox with rainbow trout and daphnia are indicated in Figures 10 and 11, respectively. The correlation equations generated by these plots are given in Table 9. A significant correlation exists when the slope and intercept of the regression curve are not very different from 1.0 and 0.0, respectively. Additionally, the square of the correlation coefficient for the regression must be greater than 0.6. In general, the agreement in the EC50 values of Microtox with those of rainbow trout is very good with a correlation coefficient of 0.97. Greater variation is exhibited between the responses of Microtox and daphnids with a correlation coeffiecient of 0.48.

The observed EC50 values of some of the simulated waste mixtures are listed in Table 10. This table shows that the Microtox's response to these wastes is strongly influenced by pH. For example, waste 5a with pH of 10.5, was adjusted with nitric acid to form waste 5b with a pH of 8.7, which tremendously reduced the toxicity of the waste. This is not surprising because Microtox thrives best at a pH of 6.7. The pH of a solution is a powerful tool for detecting cationic, anionic, and un-ionized species in a waste. Evidently, the neutralization of the waste with nitric acid converted some of the aluminate species to un-ionized aluminum hydroxide, causing coprecipitation of some of the heavy metals with it.

The responses of Microtox and fish to grout extracts are tabulated in Table 11. As can be seen, grout extract #13 induced the most adverse reaction to rainbow trout and Microtox. An examination of the composition of the waste mixture used in the preparation of this grout reveals that it is rich in nitrites and nitrates. Although nitrates are considered nontoxic to fish, nitrites are toxic to aquatic organisms because the nitrite species impairs the ability of the blood to transport oxygen. Apparently, nitrite is responsible for the observed increase in mortality.

CONCLUSION

The data generated to date in this continuing evaluation of rapid screening techniques for toxicity indicate that the luminescent bacteria, Photobacter phosphoreum, commonly called Microtox, can be used to assess the toxicity of Hanford Site Wastes.

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[2]Micromedex is a registered trademark of Micromedex Incorporated, Denver, Colorado.

Figure 10. Microtox vs Rainbow Trout.

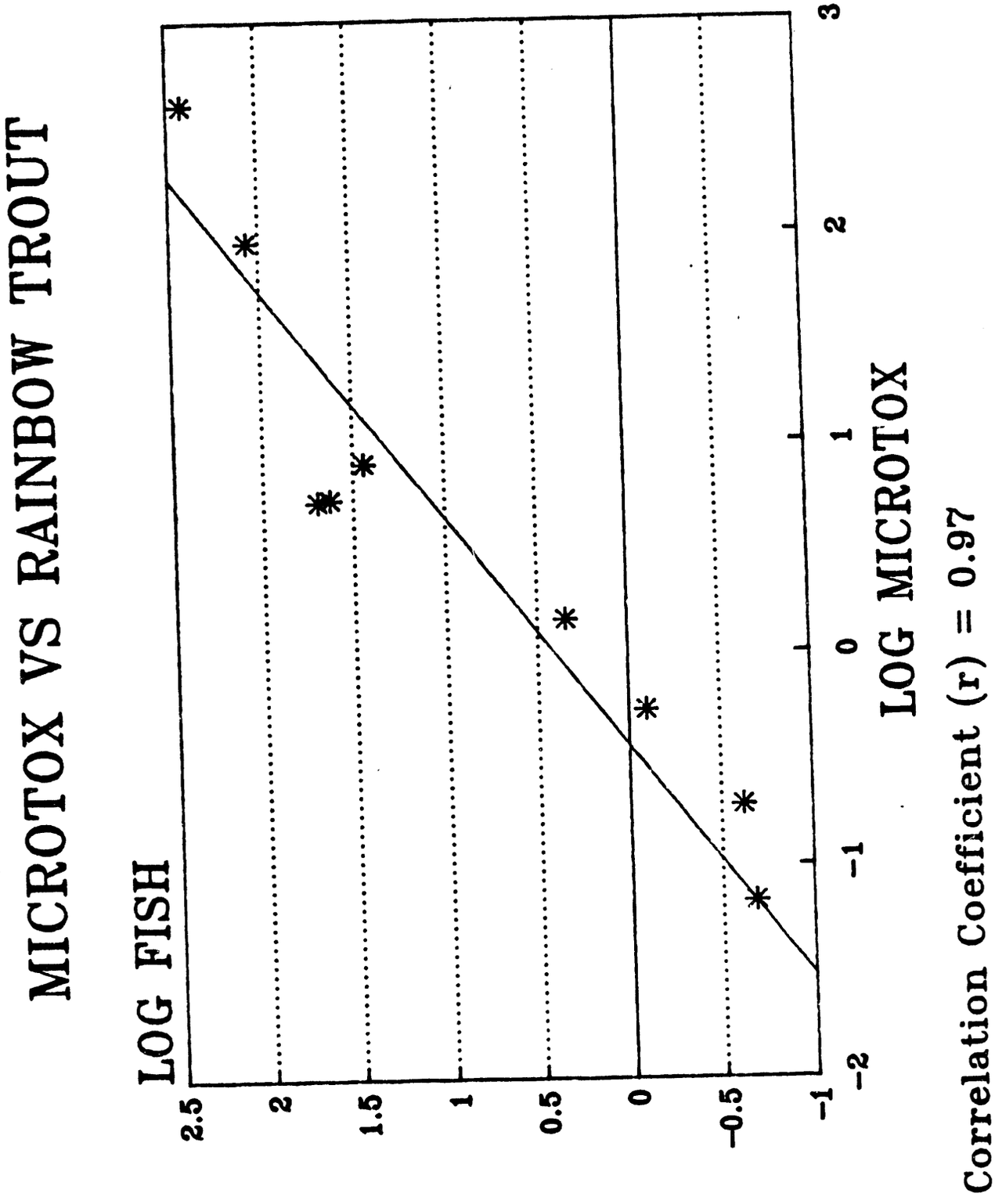

Figure 11. Microtox vs Daphnid.

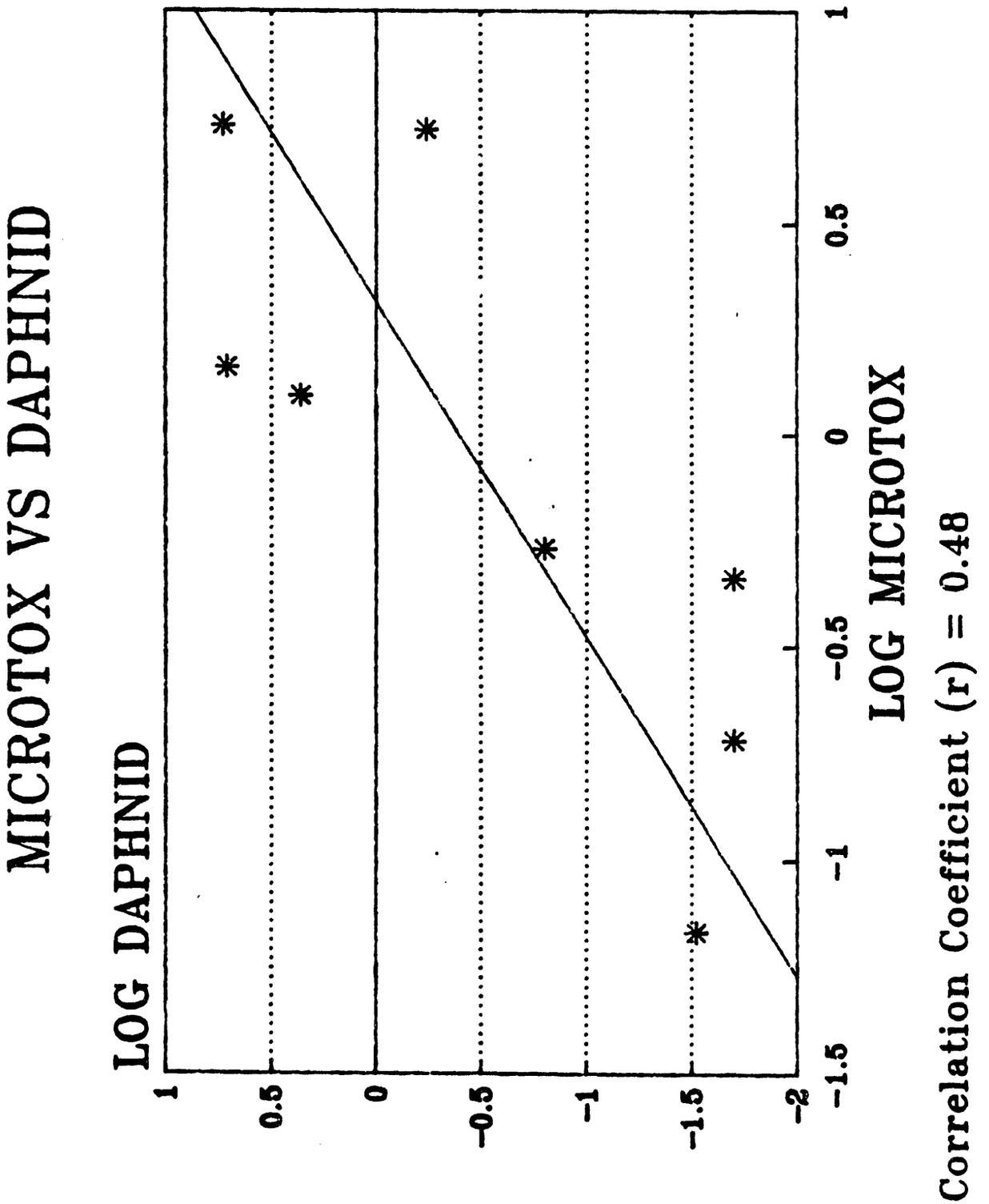

Table 9. Correlation Equations Microtox vs Fish/Daphnid.

Log LC50 = 0.33390 + 0.91379 Log EC50 Microtox
Log LC50 Daphnid = 0.40325 + 1.24696 Log EC50 Microtox

Table 10. EC50 of Some Simulated Hanford Site Wastes.

Waste ID	pH	EC50 (Microtox)
1	14	149
2	13.8	175
3	13.4	270
4	12.7	369
5a	10.5	321
5b	8.7*	9129

*Waste was neutralized with HNO_3

Table 11. Responses of Fish and Microtox to Grout Extracts.

Extract ID	EC50 (Microtox) mg/L	Fish Mortality Rate (n/N)
Grout #3	758	3/30
Grout #13	680	9/30
Grout #20	690	0/30
Grout #34	882	6/30

n/N = # Dead animals/Total # animals

ACKNOWLEDGMENT

James Douglas provided the computer system and initial expertise to run the Microbics Corporation software. Rex Gray prepared the graphical illustrations for this work.

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