United States
Environmental Protection
Agency

Environmental Research
Laboratory
Duluth MN 55804

National Effluent Toxicity
Assessment Center

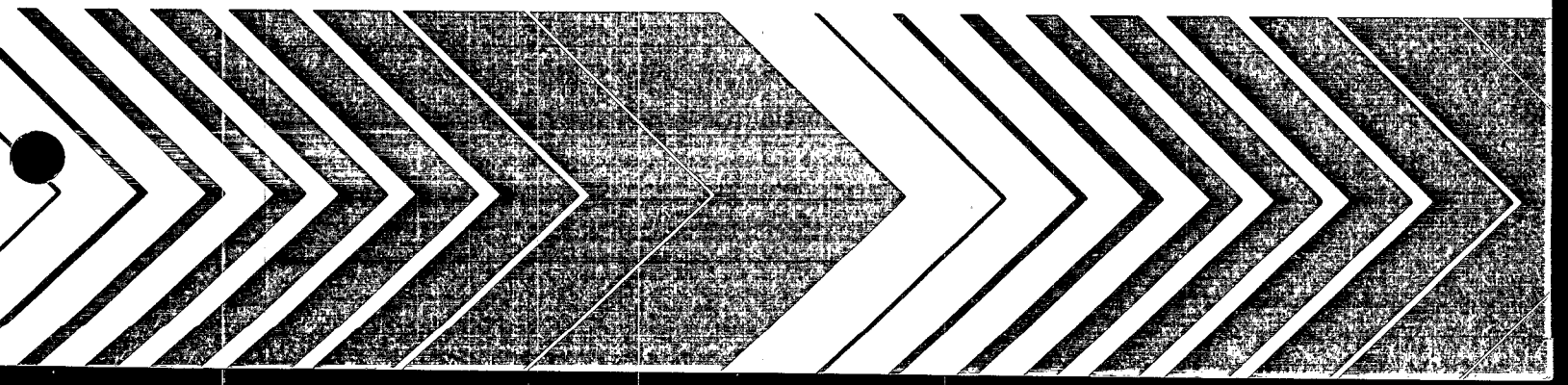
Research and Development

EPA/600/3-88/036 Feb. 1989

EPA

Methods for Aquatic Toxicity Identification Evaluations

Phase III Toxicity Confirmation Procedures



EPA/600/3-88/036
February 1989

Methods for Aquatic Toxicity Identification Evaluations

Phase III Toxicity Confirmation Procedures

Donald I. Mount
U.S. Environmental Protection Agency
Environmental Research Laboratory
Office of Research and Development
Duluth, Minnesota 55804

National Effluent Toxicity
Assessment Center
Technical Report 04-88

Notice

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Foreword

This document, Phase III, describes procedures for confirming that suspected toxicants are the true cause of toxicity. These procedures are applicable and necessary whether or not Phases I and II have been used to identify the suspected toxicants.

There are two major reasons to require confirmation procedures. First, the effluent manipulations used in Phases I and II will, for some effluents, create artifacts that may lead to erroneous conclusions about the toxicants. In Phase III, manipulations of the effluent are absent or minimal and artifacts are far less likely to occur. Sometimes, toxicants will be suspected through other approaches which are not themselves definitive. In either case, confirmation is necessary.

The second reason stems from the definite probability that the substances causing toxicity change from sample to sample, from season to season or some other periodicity. Since toxicity is a generic measurement, just measuring toxicity will not reveal such variability. Phase III procedures will reveal the presence of variable causative agents. Obviously, this crucial information is essential so that remedial action may be taken to remove toxicity.

Confirmation, whether using these procedures or others, should always be completed because the risk is too great to avoid this step. Especially for discharges with little control over the influent or for discharge operations that are very large or complex, the probability that different constituents will cause toxicity over time is great.

Unlike Phases I and II, most of the approaches in Phase III are equally applicable to chronic as well as acute toxicity. Effluent manipulation is minimal, and additives, for the most part, are not used; therefore, chronic tests can be done.

A section is also included for guidance when the treatability approach (EPA, 1988A; EPA, 1988B), rather than the toxicant identification approach, is taken. The treatability approach requires confirmation as much as or more so than the toxicant identification approach.

The reader should refer to Phase I for Quality Assurance/Quality Control (QA/QC), Health and Safety, Facilities and Equipment, Dilution Water, Sampling and Testing.

Abstract

Various procedures are described that provide evidence that the suspected toxicants in effluents are the actual toxicants. These procedures include: correlation, symptoms, relative sensitivity, spiking, mass balance, and miscellaneous procedures.

Contents

		Page
Foreword		iii
Abstract		iv
Contents		v
Figures		vi
Tables		vii
Acknowledgments		viii
1.	Introduction	1-1
2.	Correlation Approach	2-1
3.	Symptom Approach	3-1
4.	Species Sensitivity Approach	4-1
5.	Spiking Approach	5-1
6.	Mass Balance Approach	6-1
7.	Deletion Approach	7-1
8.	Miscellaneous Approaches.	8-1
9.	Conclusions	9-1
10.	When the Treatability Approach Has Been Used	10-1
11.	References	11-1

Figures

Number		Page
2-1.	Correlation of whole effluent toxicity and one suspect toxicant for a POTW effluent. . .	2-2
2-2.	Toxicity contribution for one of two toxicants in a POTW effluent	2-3
2-3.	Toxicity contribution from two toxicants for a POTW effluent	2-4

Tables

Number		Page
6-1.	Comparison of Whole Effluent and Methanol Fraction Toxic Units	6-1

Acknowledgments

I want to thank the staff of NETAC for their contributions to the preparation of this document. Endless hours of technical discussion, generation of data, suggestion for improvement, manuscript review and final editing and typing were all necessary and useful inputs to produce this document. As for Phase I and Phase II, the administrative and financial support of Nelson Thomas and Rick Brandes were crucial.

Section 1
Introduction

The final confirmation phase consists of a group of steps intended to confirm the suspected cause of toxicity. It will usually follow work completed in Phase I and II (Mount and Anderson-Carnahan, 1988A and 1988B; hereafter referred to as Phase I and Phase II), but the boundary may be indistinct. Phase III procedures should also follow after the toxicants have been identified by other means. Rarely does one step or test conclusively prove the cause of toxicity. Rather, all practical approaches are used to provide the "weight of the evidence" that the cause of toxicity has been identified. Described below are various approaches that are often useful in providing that "weight of evidence." These consist of correlation, observation of symptoms, relative sensitivity, spiking, mass balance estimates and miscellaneous adjustments of water quality.

The final confirmation is needed not only to provide data to prove that the suspected toxicants are the cause of toxicity in a series of samples, but perhaps more importantly, to assure that the cause of toxicity is consistent from sample to sample over time. When remedial action involves treatment changes, one must be certain that toxicity from specific toxicants is consistently present and that the suspected toxicants account for all the toxicity. Treatment changes will not necessarily result in removal of everything to an acceptable concentration. If toxicity is caused by a variety of toxicants being added at varying intervals, the remedial actions that are practical may differ from those needed when toxicity is caused by the addition of the same constituents consistently.

There is a strong tendency to shorten or eliminate this final confirmation because by the time it has been reached, an enormous amount of testing may have been done, the investigators are convinced of the cause of toxicity and the final confirmation seems redundant. To skimp at this stage is to invite disaster! One cannot expect to fractionate and otherwise change a complex mixture (that most effluents are) and not produce artifacts or come to some false conclusions about the toxicants.

Not all approaches will be applicable to every effluent, and certainly, in time, additional ones will be developed. They need not be done in a particular order, but some, especially the correlation, require substantial calendar time to complete and should be initiated at the beginning stages. Judgment must be made as to how many Phase III approaches should be used and how many samples for each should be completed. How completely this phase is done will determine the authenticity of the outcome. Certainly the confidence needed is dependent, at least in part, on the significance of the decision that will be based on the results. For example, if a suspected toxicant can be removed by pretreatment or by a process substitution, a higher degree of uncertainty may be acceptable than if an expensive treatment plant is to be built. Such considerations are subjective and cannot be reduced to a single recommended number of samples.

In Phases I and II, the permissibility of "rounding corners" on methods and protocols to reduce cost and allow more testing was discussed. This is all reversed in this phase, because here the definitive data that constitute the basis for decisions are generated. In Phase III, the best test procedures should be followed paying careful attention to test conditions, replicates, quality of test animals, representativeness of the effluent samples tested and strict QA/QC analytical procedures including blanks and recovery. Analytical measurements must be clearly definitive for the identity of the toxicant as well as for the concentration measurement. Sometimes, small differences in toxicity must be detected and if so, concentration intervals should be smaller to better detect small differences. All of the data from Phases I and II are preliminary relative to Phase III. However, since the phases merge from one to another, stricter QA/QC should begin in Phases I and II as soon as the toxicants are suspected, so that the data can be used in Phase III.

The following approaches have been useful in previous toxicity identification evaluations (TIEs) in our laboratory. They need not be done in any particular sequence, and the list for possible approaches will get larger as experience is gained.

Some techniques used in Phase III require keen observations and extensive or broad knowledge of both chemistry and toxicology. Above all, an ability to

synthesize bits of evidence in a logical sequence is essential. Our work shows that the more experienced the investigator, the higher the success rate. This work cannot be done successfully in a compartmentalized fashion where workers do not interact *daily* and certainly cannot be successful without the personal involvement of a well-trained staff.

Section 2
Correlation Approach

The purpose of this approach is to show whether there is a consistent relationship between the concentration of the suspected toxicant(s) and the effluent toxicity. For the correlation approach to be useful, toxicity of the effluent must be sufficiently variable to provide an adequate range of LC50s over which to do the regression analysis. In our experience, an effluent with insufficient variability has not been encountered. The LC50 data versus toxicant concentration must be transformed to give a linear relationship for regression analyses. More importantly, if there is more than one toxicant and each has a *different* toxicity, the concentration of each must be adjusted for the different toxicity before they can be summed. The easiest way to prepare such plots is to convert all the data to toxic units (TUs). Effluent TUs are obtained by dividing 100% by the LC50 in percent of the effluent. The toxicant concentration is converted to TUs by dividing the measured toxicant concentration by the LC50 of the toxicant. If more than one toxicant is present, the concentration of each one is divided by the respective LC50 and the TUs can then be summed (cf., discussion below on additivity).

Figure 2-1 is an example of the regression from an effluent from a publicly owned treatment work (POTW) in which the suspected toxicant was diazinon. The independent variable (x-axis) is the TUs of diazinon and the dependent variable (y-axis) is the TUs of effluent toxicity. The solid line is the observed regression line obtained from the data points, and the dashed line is the expected or theoretical regression line. We know that if there is 1.0 TU of the toxicant in 100% effluent, then the effluent should have 1.0 TU (LC50 = 100%). Likewise, for 2.0 TU of toxicant the effluent TU should be 2.0, et cetera. Thus, the expected line has a slope of one and an intercept of zero. In Figure 2-1, the intercept (0.19) is not significantly different from zero and the slope is very close to 1 (1.05). The r^2 value is 0.63 which, while not high, indicates that the majority of the effluent toxicity is explained by the concentration of the toxicant. In a small data set such as this one is, one value that had about 5.0 effluent TUs lowers the r^2 value substantially.

Figure 2-2 is a similar plot for another POTW in which diazinon was also the suspect toxicant. For these data, the slope is 1.38, the intercept is 1.24 and the r^2 value is only 0.15, all indicating a very poor fit. The r^2 value being so low means a large amount of scatter and, therefore, little can be inferred from the slope and the intercept.

Based on this analysis, we returned to Phase I and II procedures and discovered that two other organophosphates, chlorfenvinphos (CVP) and malathion, were present at measurable concentrations, and CVP was present at toxic concentrations. A new correlation was begun, measuring all three chemicals. CVP and diazinon have nearly identical LC50 values for the species used in this TIE. Malathion is about one-fourth as toxic; therefore, malathion did not contribute much toxicity because of its low concentration and its being less toxic.

In addition to slope and intercept, some judgment of the scatter about the regression line must be made. This can be done statistically, but when the sample size is large, the scatter can be very large and yet not negate the relationship. A suggested approach to avoid the effect of sample size on the significance of scatter is to set a lower limit on r^2. This value (often expressed as percent) provides the measure of how much of the observed effluent toxicity is correlated to the measured toxicant. It is not dependent on choosing the correct LC50 value of the toxicant. The specific choice of the minimum value of r^2 should be made based upon the consequences of the decision. One must recognize that experimental error makes an r^2 value greater than 0.8 or 0.85 difficult to obtain. Therefore, where minimal chance of an incorrect decision is needed, an r^2 value of nearly 0.8 might be used. Where an increased risk of an incorrect decision (i.e., a lesser amount of the toxicity accounted for) is acceptable, a lower value such as 0.6 might be used.

Since less than 1 TU cannot be measured in whole effluent, such values are, of necessity, excluded from the regression. However, when the TUs based on chemical analyses are less than 1.0 TU and effluent LC50 values are less than 1.0 TU, the data support the validity of the regression. In this effluent there was another toxicant present in a different fraction that was not identified and was not always

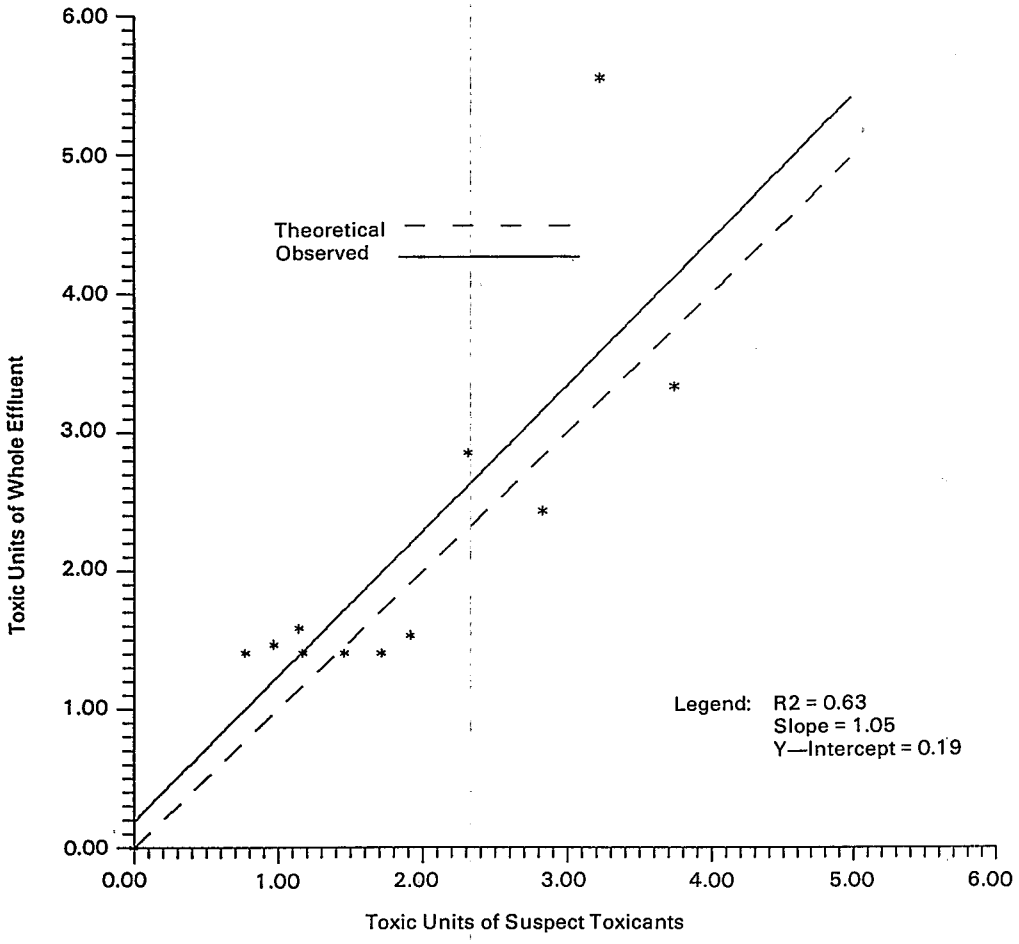


Figure 2-1. Correlation of whole effluent toxicity and one suspect toxicant for a POTW effluent.

measurable. This would explain part of the deviation from the expected values.

Depending on the specific type of variability, correlation may be more definitive when two or more toxicants are present. For example, suppose three toxicants are involved, as in Figure 2-3. If each toxicant has the same LC50 and each is strictly additive with the ratio of their concentrations remaining the same, the slope will be the expected but the intercept will be positive if all are not identified. If the relative amounts (ratios) of each toxicant vary from sample to sample, the slope and intercept will be different from the expected if only one toxicant is identified.

If the toxicity of one of the toxicants is substantially different, *and* if the ratios of the three toxicants vary from sample to sample, *then* the slope, intercept, and r^2 value will all be different from expected if all are not identified. Much can be learned from studying the interrelationship of slope, intercept, and the r^2 value. For example, a high r^2 value and an intercept near zero with a slope larger than 1 can be caused by using an LC50 for the toxicant that is too large.

If the toxicant concentrations vary over a larger range, a significant correlation will be easier to demonstrate than for a narrower range. Great care must be taken to understand the interactions of the toxicants, i.e., whether they are additive, and if so, whether they are additive at different ratios of one to another. If they are not additive, then the exact ratio found in each sample must be tested in order to know the expected toxicity.

Experience has shown that there is a strong tendency to unconsciously assume that toxicity is always caused by the same constituents. If this assumption creeps into the data interpretation but is false, some very erroneous conclusions may be reached. That is why other steps (given below) in the confirmation must accompany the correlation because they help to reveal any changes in the toxicant(s).

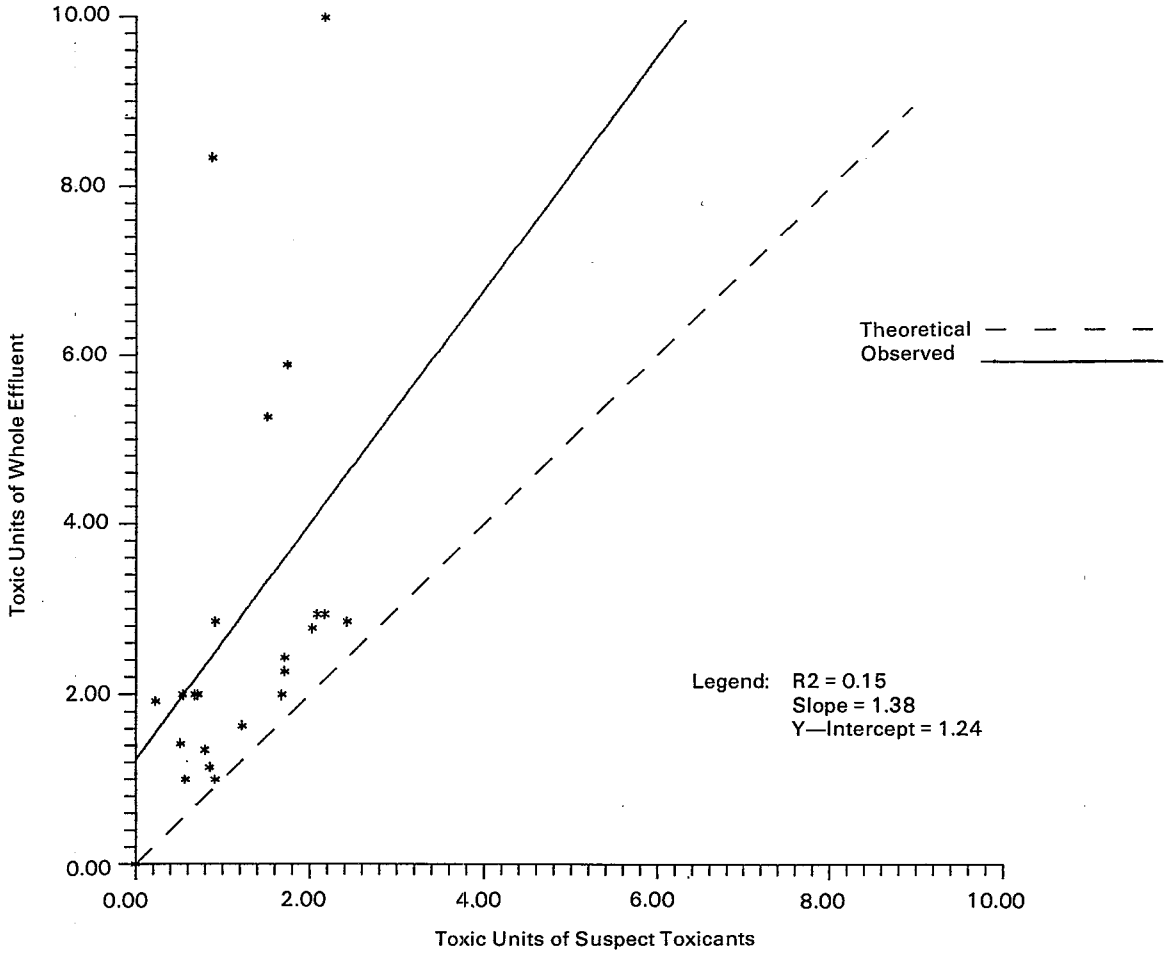


Figure 2-2. Toxicity contribution for one of two toxicants in a POTW effluent .

A difficult problem to be solved is to determine the LC50 of the suspected toxicants in the effluent. Rarely can one remove the toxicants from the effluent and be sure that nothing else has changed or been removed. Therefore, one cannot directly determine the LC50 of the toxicant *in the effluent*. Characteristics such as pH and hardness are readily recognized as affecting ammonia and metal toxicity, respectively. With non-polar organics, suspended solids and total organic carbon may affect toxicity even more. For these reasons, measuring an LC50 in a dilution water may give quite different results than would be obtained if the test were to be conducted on an effluent.

When the LC50s of effluent samples vary widely, a particularly troublesome complication occurs regarding the effect of these common effluent characteristics on toxicity that takes place. If the LC50 is high (e.g., 80% effluent) then the test concentrations which determine the LC50 (e.g., 75% and 100% effluent) will exhibit characteristics similar to 100% effluent. But if the LC50 is 5%, then these characteristics will more closely resemble those of the dilution water.

Changes in pH may also cause problems, particularly since most TIE tests will be done under static rather than in flow-through conditions. Effluent pH, especially of POTWs, usually will rise 1.0-1.5 pH units (e.g., from 7.2-8.4) when the effluent stands in test vessels exposed to air. This pH change would approximately triple the sample toxicity caused by ammonia. We have a large data base for ammonia and we know just what characteristics to be concerned about; for many other compounds we do not have such a large data base. Therefore, great care must be exercised to avoid misjudging the true toxicity of suspected toxicants in the effluent.

Total organic carbon (TOC) and suspended solids (SS) may have great effects on organic chemical toxicity. Effluents are likely to be very complex and variable and the role of TOC and SS may be quite different than in surface water. For example, SS may be composed largely of bacterial cells in one effluent

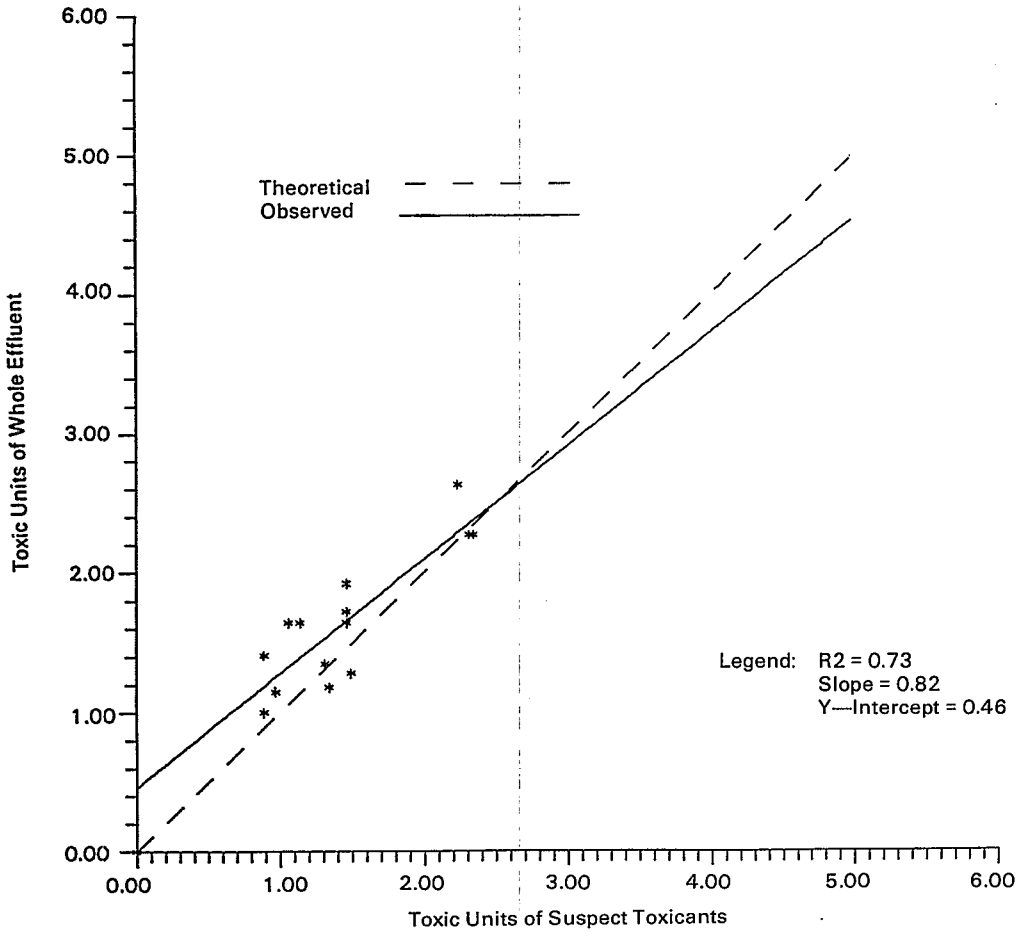


Figure 2-3. Toxicity contribution from two toxicants for a POTW effluent.

sample and largely of clay from storm runoff in another. The rate of equilibrium for adsorption of toxicants to solids as well as the equilibrium itself may be different because of the different organic content. Whether sorption takes place only on the surface or internally in the solids as well, may affect toxicity. Sometimes TOC may be largely dissolved and at other times it may occur in the form of fat droplets, oil films, or as a coating on SS. Any of these forms may have a different effect on the toxicity of an organic chemical.

These factors impact heavily on toxicity measurements. We cannot just measure SS, TOC, or dissolved organic carbon (DOC) and correct for them as we might do for hardness or pH. These require a different approach. When there are two toxicants, one affected by pH and hardness and the other affected by SS and TOC, the complexity may seem overwhelming.

An approach we have not yet tried, but which seems promising, is to collect sets of samples, each collected during a short period (e.g., 24 hours) and do a separate correlation for each set. The basis of this suggestion is that effluent characteristics that affect toxicity would likely be more similar during short time periods than during long time periods. Our experience suggests that POTW effluents vary sufficiently in toxicity over short time periods to provide a useful range. The down side of this approach is that the analytical and toxicological error of measurement may make obtaining a high r^2 value very difficult on the small number of samples. The statistics would have to be carefully developed to use this approach. Simply testing the individual slopes and intercepts separately, probably would not work.

Correlation should be accompanied by other Phase III approaches, described below, because sometimes additional toxicants will occur (especially in POTW effluents). When this occurs and one recognizes that it has occurred, these samples can be omitted from the correlation or their toxicity can be appropriately corrected before use in the correlation.

Section 3
Symptom Approach

Different chemicals may produce similar or very different symptoms. Probably no symptom of intoxication is unique to only one chemical. Therefore, while similar symptoms observed between two samples means the toxicants could be the same, different symptoms means the toxicants are definitely different in the two samples. By observing the symptoms displayed by the test organisms in the effluent and comparing them to the symptoms displayed by test organisms exposed to the suspected toxicants, failure to display the same symptoms means the suspected toxicants are probably not the true ones.

Written descriptions of symptoms are usually not helpful. Behavior is difficult to put into words so that a clear image of behavior is obtained. Compendiums of symptoms are not available for aquatic organisms. The best approach is to expose organisms to the known (or suspected) toxicant(s) and observe how the organisms react. By the time of the final confirmation, toxicity tests with the suspected toxicants will have been conducted using pure compounds. If so, symptoms should already have been observed. A prudent investigator will note the symptoms seen during earlier testing because they can be very helpful in later work as well.

The intensity of exposure can change the symptoms. One must compare symptoms at concentrations that require about the same period of onset. This is most easily done by using exposure concentrations that are some multiple of the LC50. In this way both the unknown (effluent) and the known toxicants (pure compound) can be set at the same toxicity level. This does not mean that observations of the organisms should be delayed until the normal length of the test has elapsed. With some toxicants, the test organisms will show distinctive symptoms soon after the exposure begins, whereas later, symptoms are often more generalized and less helpful. For other toxicants, a sequence of different symptom types are displayed by the test organism over the exposure period and the sequence may be more definitive for a given chemical than the individual symptoms. In few cases will the symptoms be unique enough to specifically identify the toxicant, but symptoms different from those caused by the pure suspected toxicant are convincing evidence that the suspected toxicant is not the true one.

A second caution regarding mixtures of several toxicants. Mixtures of toxicants can produce symptoms in test animals different from the symptoms of the individual toxicants composing the mixture. When more than one toxicant is involved, one must not only include all the toxicants, but include them in the same ratio as the whole effluent. Often the toxicant of the mixture at the highest concentration relative to its LC50 will cause most of the symptoms. Just as for single toxicants, the mixture concentration causing the same endpoint in a similar exposure period should be compared. Spiking effluent with the suspected toxicants and comparing the spiked and unspiked sample, both near their LC50 concentrations, is a good approach.

Symptoms may be quite different among different species of organisms; therefore the use of two or more species provides increased definitiveness in the observations. For both species, one must compare symptoms at concentrations that are equitoxic. The greater the difference in sensitivity, the more important this becomes. The concentration in mg/L is unimportant; the important consideration is that equitoxic concentrations are compared. Suppose, for example, species A and B have LC50 values for a suspected toxicant of 1 and 80 mg/L. Then concentrations of 2 and 160 mg/L might be used to compare symptoms of species A and B, respectively. If the onset of symptoms is rapid, then perhaps 1.25 and 100 mg/L (1.25XLC50) should be tried. Since symptoms vary with the exposure intensity, using various multiples of the LC50 (i.e., 0.5, 1, 2) can add additional confirmation, if the same set of symptoms are seen in both series. If more than one toxicant is involved, and the ratio of the two species' LC50 values for toxicant A is markedly different than for toxicant B, C, D, ..., then the definitiveness of using symptoms is even greater.

Time-to-mortality at equitoxic concentrations can be used as a "symptom" type of test. Some chemicals cause mortality quickly and some cause mortality slowly. If for two effluent samples, one kills quickly and the other kills very slowly, the toxicants are probably not the same.

Section 4
Species Sensitivity Approach

In addition to a comparison of symptoms displayed by species, the LC50 values can be compared for the effluent of concern and the suspected toxicants, using species of different sensitivities. If the suspected toxicants are the true ones, the LC50 values of effluent samples with different toxicity to one species will have the same ratio as for a second species of different sensitivity. And further, the ratio for each species should be the same as for known concentrations of the pure toxicant. The same ratio of LC50 values for two species implies the same toxicant in both samples of effluent. Obtaining the same effluent toxicity ratio among various effluent samples for each species as is obtained by exposure to comparable concentrations of known toxicants, implies that the suspected toxicants are the actual ones present. However, if other effluent characteristics affect toxicity and they vary, the ratios could also be affected.

The common notion that goldfish are resistant and trout are sensitive is very misleading and should be discarded. Many species are more sensitive to certain groups of toxicants than trout. Of course, there are generalizations that can be made. For example, sunfish (Centrarchids), frequently are much more resistant to metals than goldfish, minnows, and daphnids. Daphnids tend to be more resistant to chlorinated hydrocarbon insecticides than fish and more sensitive to organophosphate insecticides. These differences must always be verified for the suspected toxicants; generalities can only be used as an initial guide to species selection. Differences of 10-100X are easy to find in some chemical groups and difficult to find in others. If several toxicants are involved, interpreting the results and designing the ancillary experiments is more difficult. If successful, the power of the result is much greater than for a single toxicant. The difference in sensitivity between *Ceriodaphnia* and fathead minnows has, on several occasions, revealed either a change in the toxicants present in a series of effluent samples, or the presence of other toxicants in addition to the ones suspected.

Comparison of sensitivity among species has another very important use. Some species may evidence toxicity from an effluent constituent that the TIE test species did not. If this happens, then the above comparison will be confused, but at least there will be a warning. To decipher this possibility, one will need to revert back to Phase II and even to Phase I to characterize the additional toxicant and identify it with the new species. A second Phase III may be needed for this toxicant and species. It is important not to assume that the resident species have the same sensitivity as the TIE test species. For freshwater discharges to saltwater, this is critical, because Phases I and II are most easily done on freshwater organisms because the effluent is freshwater. But the concern is for marine organisms and so their protection cannot be assumed (cf., Section 8, Phase I).

Section 5
Spiking Approach

In spiking experiments, the suspected toxicants are increased in concentration in the effluent sample and toxicity is measured to see whether toxicity is increased in proportion to the increase in concentration. While not conclusive, if increased toxicity is proportional to an increase in concentration, considerable confidence is gained about the true toxicants. Two principles form the basis for this added confidence. To get a proportional increase in toxicity from the addition of the suspected toxicant when it is in fact not the true toxicant, both the true and suspected toxicants would have to have very similar toxicity and would have to be strictly additive. The probability of both of these coinciding is small.

Removing the suspected toxicants from the effluent without removing other constituents or in some way altering the effluent is usually not possible. The inability to do this makes the task of establishing the true toxicity of the suspected toxicants in the effluent difficult. For many toxicants, effluent characteristics, such as TOC, SS, or hardness, affect the toxicity of a given concentration. Some characteristics, such as hardness, can be duplicated in a dilution water, but certainly not TOC or SS because there are many types of TOC and SS, and these generic measurements do not distinguish between them. For example, effluent TOC will usually occur as both dissolved and suspended. In POTW effluents, the source of the TOC is likely to be largely from biologic sources, both plant and animal. For POTWs, bacteria are likely to be a large component. If there have been recent storms, oil from runoff might be high. Simulating TOC from such variable sources is next to impossible. TOC is not solely the result of organic chemicals. For suspended solids, shape, porosity, surface-to-volume ratio, charge and organic content, all, or any, will change sorption, and none of these is measured by the standard methods for measuring suspended solids.

In a simple system, such as reconstituted soft water, one can expect for most chemicals that doubling the concentration will double the toxicity, at least in the LC50 range. If solubility is being approached or there are effects from water characteristics such as SS, then the toxicity may not double or conceivably could more than double. For example, if a chemical of high log P is largely sorbed on solids, doubling the total concentration may more than double the toxicity because the added chemical is in solution. Equilibrium may not become estab-lished during the test period and especially not before the test organisms are added.

If several toxicants are involved, then their interaction (additivity, independent action, synergism) must be measured or otherwise included. Since ratios may be as important as concentration, the best way to spike when multiple toxicants are involved is to increase each toxicant by the same multiple of the LC50, e.g., by doubling each. In this way the ratios of the toxicities remain constant. When two or more toxicants are not additive, getting predicted results is very strong evidence. Interpreting spiking data may require a very high level of competence in both toxicology and chemistry; otherwise the data could be very misleading. Using more than one species of differing sensitivity is effective in adding confidence to the results.

When matrix effects are complicated, other types of spiking can be done to reduce the effects of the effluent matrix characteristics. If a method exists for removing the toxicants from the effluent, such as the SPE procedures, the extracts or fractions can be spiked in addition to spiking effluent, using the same principles as described for effluents. The advantage in this approach is that matrix characteristics such as SS and TOC will be absent or much reduced and will not affect spiking experiments as much. The disadvantage is that proof that the extracts or fractions contain the true effluent toxicants must be generated. Some approaches for doing this are given in the next section (Section 6). The use of this approach is especially applicable to fractions from the SPE or the HPLC fractions for non-polar organics. In these, the constituents are separated from much of the TOC, SS and hardness, so that spiked additions are more likely to be strictly additive than is true in the whole effluent. Suggestions and precautions about ratios and all other previously discussed concerns apply here as well. Spiking fractions, however, does not provide the same confidence about the cause of toxicity in the effluent as spiking the effluent directly provides. The mass balance

United States
Environmental Protection
Agency

Center for Environmental Research
Information
Cincinnati OH 45268

BULK RATE
POSTAGE & FEES PAID
EPA
PERMIT No. G-35

Official Business
Penalty for Private Use, $300

Please make all necessary changes on the above label, detach or copy, and return to the address in the upper left-hand corner.

If you do not wish to receive these reports CHECK HERE □; detach, or copy this cover, and return to the address in the upper left-hand corner.

EPA/600/3-88/036