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REVIEW OF THE MICROTOX TOXICITY TEST

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Review of the Microtox Toxicity Test

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EXECUTIVE SUMMARY

This review compares the sensitivity of Microtox with other organisms used in toxicity tests (including fathead minnow, rainbow trout, oyster embryos, *Daphnia* and *Chlorella pyrenoidosa*). It also describes the potential applications, advantages and limitations of the test.

Microtox was found to have a sensitivity comparable with other test organisms, for many pure and complex test substances. Microtox sensitivity and the correlation with other tests increased and data variability decreased for many industrial effluents, as their complexity and toxicity increased. However, Microtox was relatively insensitive to certain simple organics such as ammonia, chloroform, cyanide and effluents or leachates containing a high proportion of insecticides, herbicides, inorganics, or highly lipophillic contaminants.

Although Microtox is often less sensitive than higher organism toxicity tests, Microtox EC_{50} values and acute LC_{50} values for higher test species do not normally differ by more than one order of magnitude. Therefore a "tiered approach" is recommended whereby Microtox is used to rank effluent toxicity and identify priority effluents. Additional more costly tests can then be limited to priority effluents and those to which Microtox is known to be relatively insensitive. For complex effluents Microtox can be calibrated against more sensitive tests, and then used routinely to monitor the compliance of effluents with derived toxicity-based consent conditions.

KEY WORDS

Review, Microtox, Toxicity tests

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1. INTRODUCTION

The Microtox toxicity test was first described by Bulich (1979) and utilises the marine bacterium *Photobacterium phosphoreum* to assess the toxicity of aquatic pollutants. The test is supplied as a commercial standardised package by the Microbics Corporation (Microbics 1982) and the use of freeze dried bacteria allows tests to be conducted without extensive preparation or pre-planning.

Photobacterium sp. emit light as a natural by-product of respiration and this luminescent response can easily be quantified by a sensitive photometer. When exposed to a toxicant, the change in the amount of light emitted by the bacteria is proportional to the toxicant's ability to inhibit metabolism, which in turn gives an indication of its toxicity. This simple and rapid test provides an indication of test substance toxicity after only a 5-30 minute exposure period, whilst other acute toxicity tests of comparable sensitivity typically require exposure periods of between 24 and 96 hours. The Microtox test has become widely used since it is simple, rapid and inexpensive, and can be carried out with minimal laboratory facilities.

Microtox test results are reported in the literature as Median Effective concentrations (EC_{50}s), which are defined as the concentration of a toxicant which results in a 50% reduction in bacterial light output after a given exposure period relative to the control. The lower the EC_{50} for a test substance, the greater the toxicity of the toxicant.

Bacterial bioluminenscence is usually measured at three different exposure times, thereby resulting in three different toxicity values: the 5 min EC_{50}, 15 min EC_{50} and 30 min EC_{50}.

The data reduction method takes into account the time-dependence of the response by introducing the Blank-Ratio (BR) as a correction factor of the actual percentage of light reduction. Instead of using the simple percentage

reduction in light emission, the gamma function (Γ) is computed, which is the ratio of light lost to the light remaining:

$$\Gamma[c]_t = \frac{BR_t[c]_t - I[c]_o}{I[c]_t} + \frac{BR_t \cdot I[c]_o}{I[c]_t} - 1$$

where $BR_t = \frac{I[o]_t}{I[o]_o}$ and

$I[o]$ and $I[o]_t$ refer to the blank reading (control cuvette) at times o and t;
$I[c]_o$ and $I[c]_t$ refer to the reading of a cuvette containing sample concentrations c at times o and t; and
BR_t refers to the blank ratio for the readings at time t.

The dose/response curve (Γ vs c) is a classical sigmoid-shaped curve from which the concentration for Γ=1 (50% reduction in light output) can be determined. Logarithmic transformation of the values, plotting log gamma against log concentration, results in a straight line from which the EC_{50} values can be determined with greater accuracy (Figure 1.1). This method also allows extrapolation to other values, such as EC_{10} which is frequently used as a threshold value for the toxic effect of particular compounds. Linear regression analysis of the data allows the most appropriate straight line to be drawn through the data and the correlation coefficient (r) provides an indication of the quality of fit. Thus the operation results in a calculated value for the EC_{50}, rather than an interpretation of data.

The sensitivity of the Microtox test is dependent on a number of factors including:

1. salinity;
2. pH;
3. temperature; and
4. time, in terms of both the duration of the test and the age of the reconstituted bacterial suspension.

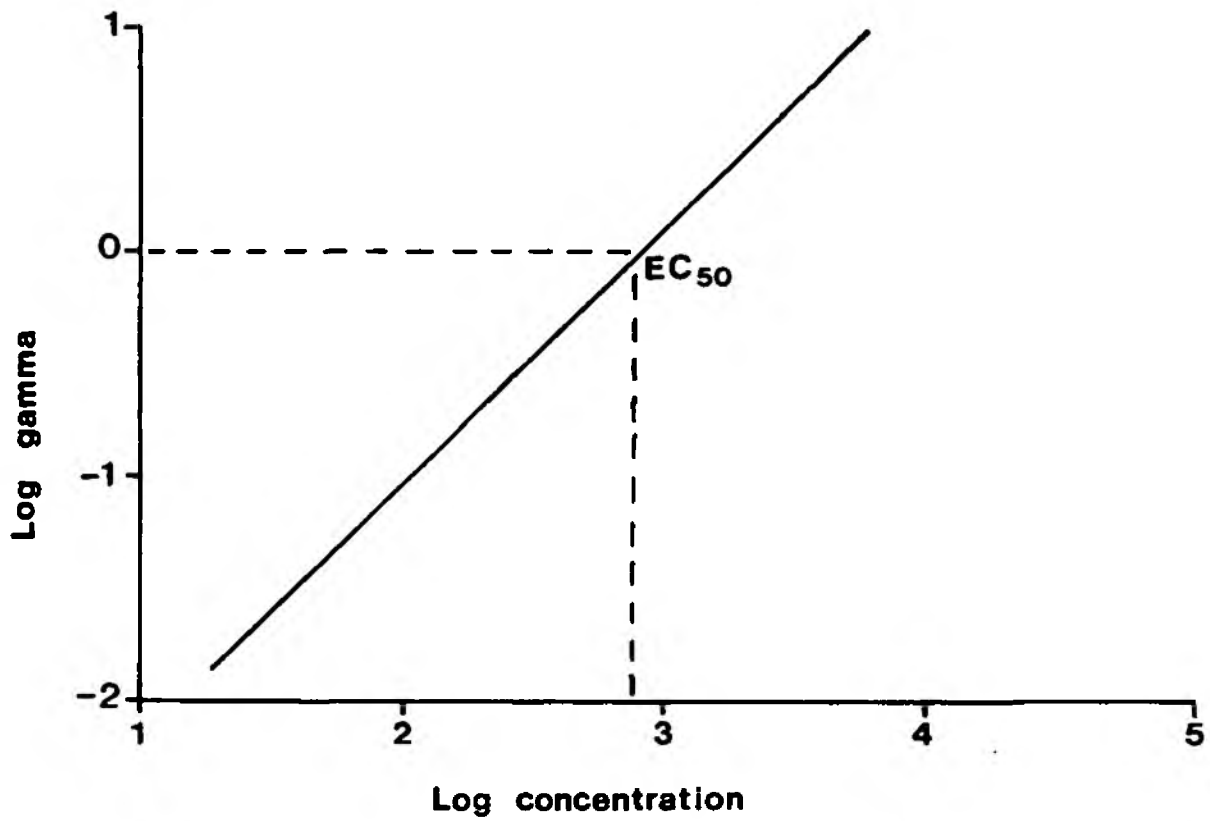

Figure 1.1 Relationship between log gamma and log concentration for the Microtox test

The optimum test conditions for the assay are given below in Table 1.1 and are the result of numerous studies (reviewed by Ribo 1984).

Table 1.1. Optimum test conditions for the Microtox assay

Parameter	Optimum value or range
Saline concentration (g/l)	20
pH	6-8
Temperature (°C)	15 (10-25)
Duration of test (min)	5-30
Age of reconstituted bacteria (hr)	0.25-5

Most acidic and alkaline effluents are effectively buffered by the receiving waters and so toxicity tests should account for this by avoiding apparent toxicity associated with extreme pH values. The pH of test solutions should be corrected to the optimum range using sodium hydroxide for acidic samples or hydrochloric acid for alkaline samples. However, pH changes could result in changes in the availability of free metal ions which may be translated into a toxicity difference.

The time of exposure of *Photobacterium* to given pollutants can have a marked effect on the response of the Microtox test, and three different reaction types, shown in Figure 1.2, have been identified (Ribo 1984). A first class (Type I) of compounds (e.g. phenols) result in an immediate response with a distinct end point at the 50% reduction of light output. In contrast, another class (Type II) of chemicals including heavy metals, shows a slow response with the result that longer exposure time will give increasing toxicity values. Finally, the third class (Type III) of substances, such as mixtures, shows an intermediate response with an asymptotic approach to its maximum toxicity. Longer exposure times have no or limited influence on the final value.

The principal aim of this report is to review the sensitivity of Microtox to pure substances and complex effluents in relation to that of other test organisms. The potential applications, advantages and limitations of the test are also described.

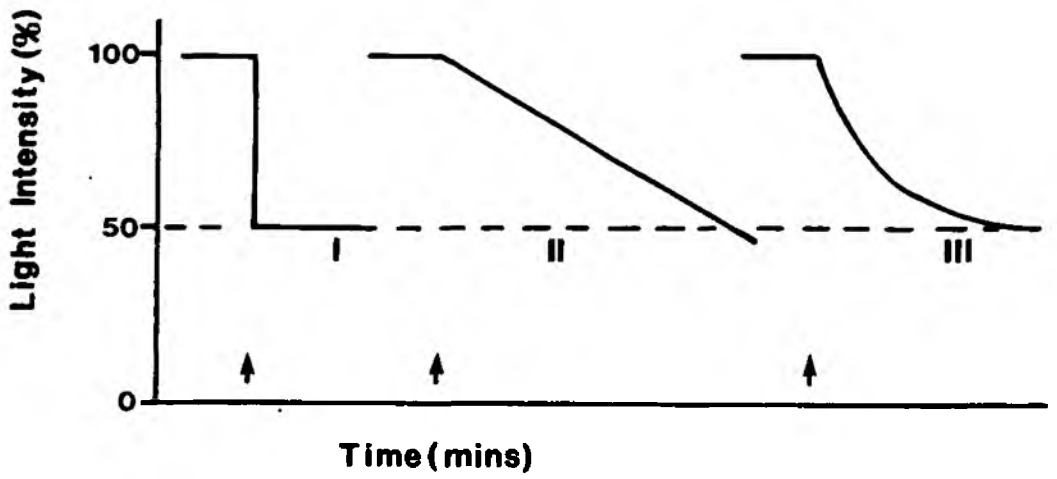

Figure 1.2 *Photobacterium phosphoreum*: Toxic effect-time dependance for three different reaction types:
I - Immediate response, e.g. phenol
II - Slow and constant response, e.g. heavy metals
III - Hyperbolic time/response curve, e.g. mixtures
- Exposure starting time

2. APPLICATIONS

The Microtox toxicity test was originally developed to fulfil the need for a rapid, reproducible and relatively inexpensive test to screen the toxicity of test substances for which no toxicity data were available. Although this still remains one of its primary applications, several others have since been developed and documented. The following section describes the main applications of the test for aquatic pollution assessment, as well as briefly discussing those in different fields, to illustrate its diversity as a test system.

2.1 Acute toxicity screening

The Microtox test, because of its simplicity and reproducibility, is well suited to screening the toxicity of new or previously untested pure compounds and complex effluents. Acute toxicity screening has been carried out on a wide variety of aquatic pollutants including wastewaters (Pols 1988; Casseri *et al* 1983), fossil-fuel process waters (Lebsack *et al* 1981), drilling muds (Halmo and Hagen 1985; Strosher 1984), mycotoxins (Yates and Porter 1982), leachates (Baker 1985), hazardous wastes (Symons and Sims 1988), pesticides (Chang *et al* 1981; Somsundaram *et al* 1990) and numerous other chemicals (Kaiser and Ribo 1988). By using the Microtox test to determine which compounds or effluents represent major risks to the aquatic environment, higher level toxicity tests using algae, invertebrates and fish, which are both costly and time consuming, can be limited to those samples which warrant further analysis.

2.2 Aquatic environmental monitoring

The Microtox test has been used to monitor the quality of the aquatic environment by sampling discharged effluents and also monitoring receiving waters above and below discharges (Vasseur 1989). The test has been used in compliance monitoring for effluent discharges to receiving waters and monitoring the efficiency of toxicity reduction evaluations (TREs) for problem effluents (Hansen 1987, Botts *et al* 1987, Hill 1987). Qureshi *et al* (1982) described the use of Microtox to identify the most toxic fractions of

petrochemical process water. The test has also been used to follow changes in inherent toxicity of receiving waters following remedial action (Peake and MacLean 1983).

Microtox can be used to trace toxicity in sewers and identify those industrial contributors whose effluent streams are highly toxic (Rowlen *et al* 1983; Slattery 1983; 1984). Consequently, pollution incidents can often be traced back to their original source (Durkin *et al* 1987) without time-consuming qualitative chemical analysis. The regular or continuous monitoring of incoming sewage to the system also allows plant operators to be alerted to highly toxic incoming waters and thereby protect activated sludge organisms from toxic stress (McGrath 1988, Logue *et al* 1989).

Parameters such as pH, retention time and activated carbon addition rates have significant impacts on effluent toxicity. Neiheisel *et al* (1983) described how, by monitoring the influent, primary and secondary effluent samples, Microtox can be used as a convenient method for monitoring waste water treatment efficiency.

2.3 Sediment analysis

Sediments are a major repository for persistent aquatic contaminants and there is increasing interest by regulatory agencies worldwide in the development of simple, robust tests for assessing sediment toxicity.

In sediments, pollutants adsorbed to particulate matter can become re-dissolved in the sediment pore water through equilibrium partitioning (Geisy *et al* 1990) and the potential toxicity of sediments can be assessed by three basic test approaches (Chapman 1988):

1. whole intact sediment tests;
2. tests using elutriates and/or pore water; and
3. tests with sediment extracts, whereby a chemical extraction procedure is used to separate chemical components from the sediment, after which the toxicity of each individual component is measured.

However, the measurement of the toxicity of a particular sediment by these three methods may lead to equivocal results. Indeed, the significance and comparability of these three methods still remain to be fully assessed (Chapman 1988; Geisy *et al* 1988, 1990).

A number of studies have been reported in which the Microtox test was used to assess the toxicity of both freshwater (Baker and Griffiths 1985, Ross and Henebry 1989) and marine elutriates (Ankley *et al* 1989). The test has also been successfully applied to the measurement of sediment pore water samples. Since sediment pore water samples are often difficult to obtain, the small volumes required for the Microtox assay mean the test can be easily applied.

The Microtox test has been used to assess the toxicity of sediment extracts (Schiewe *et al* 1985, Dutka and Kwan 1988, Ture and Heyward 1990, Williams *et al* 1986). However, solvent extraction is often environmentally unrealistic, in that it causes normally non-bioavailable compounds to become available to aquatic organisms, possibly resulting in erroneously high toxicity results. Nevertheless it is useful for mapping persistent organics which may not be present in aqueous extracts. Metals and highly acidic or basic organic compounds, which are not often efficiently extracted, add further complications in the assessment of sediment toxicity (Chapman 1988).

The Microtox test is generally less sensitive to leachates, sediment extracts, elutriates and whole sediments compared with toxicity tests using higher organisms (Section 3.6). However, the ranking order of toxic sediments with Microtox is similar to that reported for other tests.

A new method has recently been developed in which the Microtox reagent is exposed directly to untreated sediment (Brouwer *et al* 1990). The advantage of measuring the toxicity of entire sediment samples is coupled with the fact that the sediment-contact bioassay appears to be more sensitive to hydrophobic contaminants, such as polychlorinated biphenyls, when compared to the standard elutriate test. However, at present there are insufficient data on which to assess the utility of this method relative to other commonly used sediment toxicity tests.

2.4 Other applications

This section briefly outlines some of the other diverse applications that have been developed and documented. Microtox has been used to study:

1. the toxicity resulting from the selective mixing of toxic metal ions (Qureshi *et al* 1983, Sellers and Ram 1985);

2. the residual bacteriostatic properties of selected metal-working fluid preservatives (Mallak and Brunker 1983);

3. optimum land loading rates of applied organic wastes to avoid inhibition of normal biodegradation processes.

Several medical applications of the test have been developed, such as measuring the toxicity of saline extracts (Bulich 1986) and mould toxins (Yates and Porter 1982). The effect of radiation on the bacteria has also been studied with the aim of developing a biological dosimeter (Mantel *et al* 1983). Other studies have investigated the use of Microtox, as an alternative to commonly used *in vitro* and *in vivo* methods, to assess the toxicity of medical products (Barton 1987; Burton *et al* 1986; Burton and Dabbah 1989) and irritants (Bulich *et al* 1990). In many cases, Microtox was more sensitive than *in vivo* methods and of comparable, if not higher, sensitivity to *in vitro* methods. The potential of a bioluminescence test, using a dark strain of *Photobacterium* as an alternative method for mutagenicity testing has also been studied (Ulitzur 1986). The extent to which genotoxins induced luminescence in dark strain mutants of luminescent bacteria was assessed, and the test was considered to be a simple, sensitive and cheap alternative to the AMES test for prescreening carcinogens.

3. SENSITIVTY OF MICROTOX TO TEST SUBSTANCES AND CORRELATION WITH OTHER TOXICITY TESTS

3.1 Relative sensitivity of Microtox to test substances

This section compares the sensitivity of the Microtox test for a range of aquatic pollutants with that of acute lethality tests using rainbow trout (*Oncorhynchus mykiss*), fathead minnow (*Pimephales promelas*) and the water flea (*Daphnia magna*). The sensitivity of the Microtox test is also compared with that of the oyster embryo (*Crassostrea gigas*) development test and the algal (*Chlorella pyrenoidosa*) growth inhibition test where data are available. The data on the sensitivity of Microtox compared to *Daphnia*, rainbow trout and fathead minnow have recently been assessed by Munkittrick *et al* (1990), although the data in that review have been supplemented here with additional information.

Comparative studies of sensitivity reported in the literature often do not provide an adequate description of the species of fish, the duration of exposure or the direct source of the original data (Bulich *et al* 1981; Chang *et al* 1981; Bulich 1982, Curtis *et al* 1982). Bulich *et al* (1981) reported the response of Microtox to 20 chemical compounds compared with the response of "fish" lethality tests. The "average value" quoted included a wide range of values for a wide variety of species with tests of varying duration. The inclusion of non-standard LC_{50}s using exotic species or unorthodox exposure regimes alters the "average LC_{50}" and in some cases can lead to a misleading relationship (Munkittrick *et al* 1990). This problem is further complicated by studies which have summarised data into a single number to illustrate a Microtox sensitivity relative to other test species, which when taken out of context is meaningless. For example, after studying the effect of 15 compounds on Microtox and fish lethality assays, De Zwart and Sloof (1983) concluded that fish lethality assays were 1.15 times more sensitive than the Microtox test. However, this number was derived from an average relative sensitivity of four species of fish, *Oncoryhnchus mykiss* (2.04), *Pimephales promelas* (1.99), *Poecilia reticulata* (0.66) and *Oryzias latipes* (0.65). Of course, the value of

1.15 may be a gross overestimation or underestimation of the relative sensitivity of certain compounds to particular species of fish.

It should be emphasised that all measures of sensitivity are directly related to both the compound and the test organism. The data presented in Appendix A, therefore, evaluate the comparability of data based on individual chemicals for those species most commonly used in toxicity testing. This approach has also been undertaken for complex wastes and industrial effluents, oil refinery wastes, municipal wastes and sediment extracts, although for these classes of pollutants there has generally been no identification of the constituents.

3.1.1 Organic chemicals

An extensive review of Microtox EC_{50} data for over 500 organic chemicals has been compiled by Kaiser and Ribo (1988). However, this compilation has been recalculated and re-organised in Table A.1, since the original table presented data as molar concentrations and arranged the information on the basis of chemical formula.

The sensitivity of Microtox to specific chemical classes, which have common structural components and/or a common mode of toxic action has been studied using quantitative structure-activity relationships (QSARs). In these mathematical equations, Microtox toxicity data are related to structural descriptors of the chemical class and highlight compounds of markedly greater toxicity in the given class. They also allow predictions to be made of the potential toxicity of new or previously untested chemicals appropriate to the class.

Relationships have been derived for:

1. chlorinated anilines, benzenes, nitrobenzenes, phenols and pyridines (Ribo 1984);

2. thioureas (Govers *et al* 1986);

3. biphenyls, azobenzenes, esters, ethers, N-substituted anilines, benzoyl and benzyl derivatives (Kaiser *et al* 1987);

4. 1,4 disubstituted benzenes (Kaiser 1987);

5. Mono nitrobenzenes (Deneer *et al* 1988).

These have been derived using measured or calculated parameters relating the:

1. hydrophobic (penetrative ability through lipid membranes and transport through an organism);

2. electronic (electronic arrangement); and

3. steric (spatial arrangement)

structural features of substances to toxicity. A commonly used index of hydrophobicity in QSARs is the octanol/water partition coefficient (K_{ow} or P), which reflects the level of toxicant partitioning into an organism and reaching the site of toxic action (Hermens 1986). For comparison of the toxicities of compounds on the basis of structural descriptors, values are transformed to molar units. Inverse values are then usually used in order that higher toxicities result in greater or more positive log values.

Chlorinated anilines, benzenes, nitrobenzenes, phenols and pyridines have shown similar slopes for QSAR based on K_{ow} (Ribo 1984), indicating that the transport of these chemicals into the cell through the membrane and the mode of action may be similar (Figure 3.1). Hermens *et al* (1985) have postulated that Microtox data generally reflect the minimum or aspecific baseline toxicity associated with a non-polar narcotic mode of action. In certain instances compounds with a specific mode of action in higher organisms will only exhibit minimum toxicity to Microtox.

Relative sensitivity should not be considered to be the only important factor when comparing the results of toxicity tests. A second very important consideration when evaluating the use of a toxicity test is its ranking

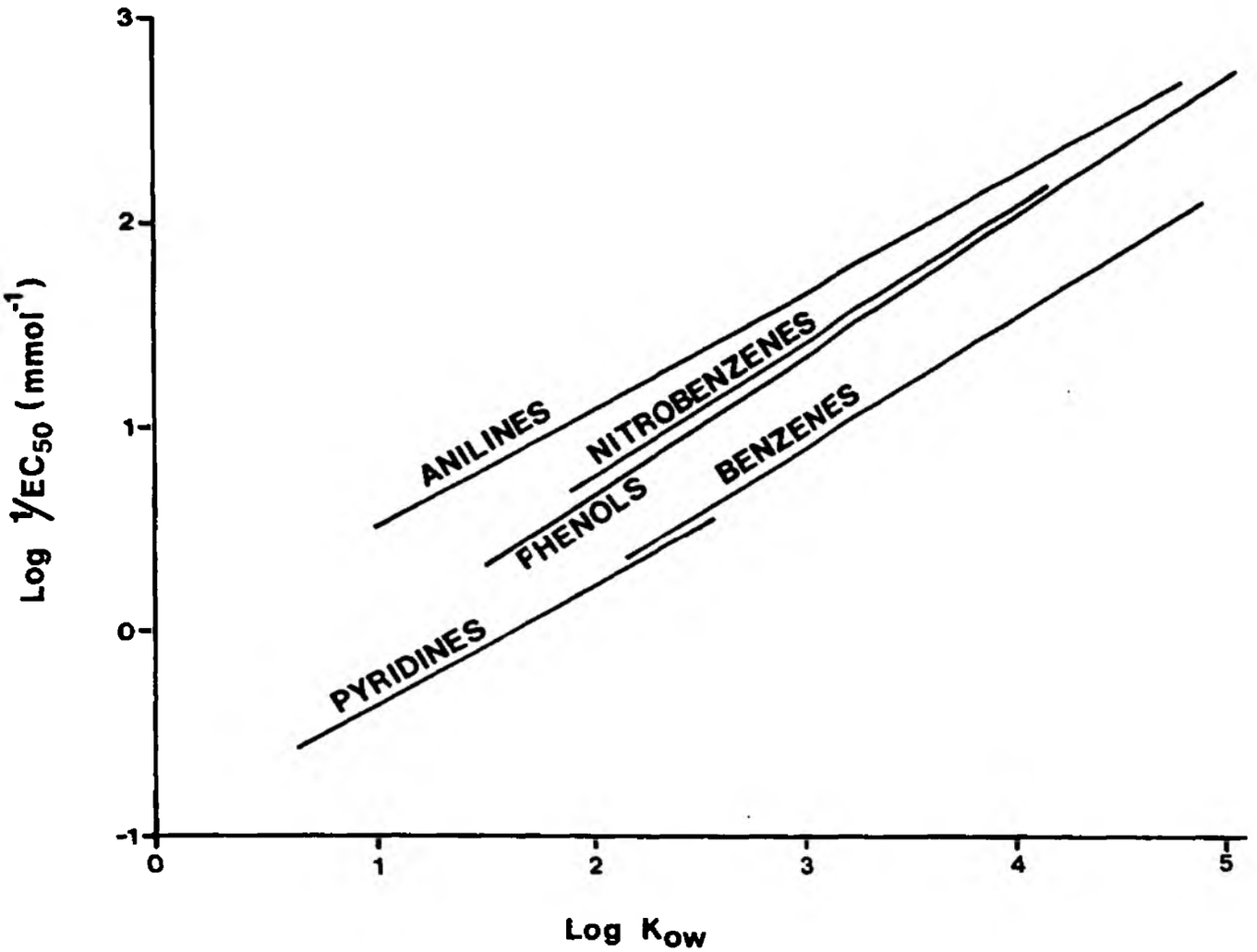

Figure 3.1 Microtox-log K_{ow} structure-activity relationships for chlorinated aromatic compounds

correlation with other test methods (Blum and Speece 1990). Although these values do not provide any information about relative sensitivity, they do indicate how similarly the two methods rank the toxicity of test substances. Indeed, it is often thought that a strong ranking correlation between species can be more important than finding similarities in sensitivities of the two organisms (Blum and Speece 1990).

Numerous comparative studies on the toxicity of organics have also been carried out and the available data for studies in which organics have been tested with Microtox and other commonly used acute toxicity test species are in the same laboratory given in Table A.2. Although the Microtox test is relatively sensitive to many organic compounds and correlates well with other toxicity tests, it has been shown to be relatively insensitive to certain low molecular weight organics such as urea (Table A.1).

Comparable sensitivities between the Microtox test and algal (*Chlorella pyrenoidosa*), invertebrate (*Daphnia magna*) and fish (rainbow trout and fathead minnow) tests were evident for a large proportion of the organics tested, which probably reflects the non-specific non-polar narcotic mode of action of these compounds.

The lower sensitivity of Microtox to certain organics may reflect their specific mode of action to higher organisms. Pesticides generally act on an organism's nervous system, with organophosporus compounds, for example, disrupting the activity of acetylcholinesterase. Since simple organisms such as bacteria do not possess a nervous system they are less affected and therefore less sensitive to the action of compounds, such as malathion and lindane, than higher organisms (Jones *et al* 1984, Hermens *et al* 1985).

McFeters *et al* (1983) described Microtox as being less sensitive than other assays to the herbicides diuron, monouron and simazine. However, a later study by Miller *et al* (1985) found Microtox to be more sensitive than *Daphnia* to the herbicides 2,4-D and Esteron 99.

The correlation coefficients (r^2) of Microtox EC_{50}s with other measures of toxicity in higher organisms for given chemical classes are given in Table A.3.

The r^2 values obtained were extremely high (>0.68) for organics grouped together on the basis of structural similarity, such as chlorobenzenes and chlorophenols, and also for groups of structurally different organics (Curtis *et al* 1982, Indorato *et al* 1984, Tarkpea *et al* 1986). Ribo and Kaiser (1983) found that the correlation coefficient for chlorobenzenes was lower than for chlorophenols and suggested this was due to their lower water solubility. Indeed, many other investigators have found that Microtox showed a decreased sensitivity to organics which have low water solubility (Bulich *et al* 1981; Ribo and Kaiser 1983; Hermans *et al* 1985; Vitkus *et al* 1985). Other studies have also suggested that the Microtox test is not sensitive to highly lipophillic organics such as PCBs (Vitkus *et al* 1985) and to chemicals, such as nitriloacetic acid, which do not easily penetrate cell walls (Surowitz *et al* 1987).

3.1.2 Inorganics/metals

The EC_{50} values obtained from studies of inorganic chemicals to Microtox and other toxicity tests are presented in Table A.4. In general, the Microtox test does not appear to be as sensitive as higher organisms to this group of chemicals. However, the test duration has been shown to be important in determining the toxciity of inorganics, as these substances require longer exposure times to exert their full toxic effect (Table A.4). Consequently to compare Microtox values for inorganics with higher organism data, a standardised test exposure has to be used.

On the basis of 30 min EC_{50} values the Microtox test has been shown to be less sensitive than the invertebrate test with *Daphnia* for the limited number of inorganic chemicals for which data are available. Microtox values, relative to 48 hr *Daphnia* LC_{50}s were 341, 3.8 and 1.3 times less sensitive for cadmium, copper and zinc respectively. For comparisons with 96 hr fish LC_{50} data, Microtox showed greater sensitivity to copper (39-156x) and comparable sensitivity to cadmium (1-7.8 x higher) and chromium (1.4-1.9 x lower).

3.1.3 Sewage effluents

There have been a few studies which have used the Microtox test to monitor the toxicity of sewage treatment wastes (Table A.5). Rowlen *et al* (1983) found that Microtox was more sensitive than the rainbow trout assay, although Qureshi *et al* (1982) did not find any major differences between these tests. Slattery (1988) studied the toxicity associated with dissolved and particulate fractions of sewage effluents, and showed Microtox was sensitive to the dissolved phase contaminants but relatively insensitive to contaminants associated with particulates.

3.1.4 Oil production and refinery effluents

The results of studies investigating the sensitivity of the Microtox test to oil production and refinery effluents are presented in Table A.5. In general Microtox exhibits comparable, though often greater, sensitivity than the *Daphnia*, rainbow trout or fathead minnow assays to this type of toxic effluent. Microtox has, therefore, proved very useful for monitoring relative changes in the toxicity associated with petroleum production effluents.

Burks *et al* (1981) confirmed that Microtox and *Daphnia* were more sensitive than the fathead minnow bioassay to oil production and refinery effluents. These authors reported that, when toxic/non-toxic designations were used, Microtox and *Daphnia* showed agreement in 9/11 instances. In contrast Mackinnon and Retallack (1982) and Mackinnon and Boerger (1986) found that for oil sand tailings water the rank order of sensitivity was rainbow trout > fathead minnow > *Daphnia* > Microtox. Qureshi *et al* (1982) had also found that for two oil refinery effluents, Microtox EC_{50}s were more sensitive indicators of toxicity than either *Daphnia* or rainbow trout. Strosher (1984) examined 48 waste drilling fluid samples and found a strong correlation between 96 hour rainbow trout lethality results and Microtox test data (r=0.89), with Microtox being more responsive in 13% of the tests.

3.1.5 Industrial effluents and complex wastes

Various studies have investigated the use of Microtox for toxicity screening and assessing the toxicity of industrial effluents and complex wastes (Table A.5). For the majority of these studies no chemical analyses of the samples were carried out and most comparisons between assays relied on the relative agreement between toxic/non-toxic designations.

Bulich (1982) compared the toxicity of a wide range of complex effluents to Microtox, *Daphnia* and fathead minnow using data ordered on a log-rank classification scheme shown in Table 3.1.

Table 3.1. Log-rank classification system

Class	% Effluent concentration causing LC_{50} or EC_{50}	Order value (log of effluent concentration)
1	<1.0	<0.0
2	>1.0-3.2	>0.0-0.5
3	>3.2-10	>0.5-1.0
4	>10-32	>1.0-1.5
5	>32-100	>1.5-2.0
6	>100 and nontoxic	>2.0

Comparisons between Microtox and fathead minnow data for 235 complex effluents showed the two tests were within the smallest rank interval of 0.5 log units in 68.5% of cases and within 1.0 log unit in 90.2% of cases (Table A.6). The high level of agreement between Microtox and fathead minnow toxicity data was comparable to that found between different fish bioassays for 320 complex effluents (US EPA 1978). The average log differences of 0.50 (Microtox vs fish) and 0.47 (fish vs fish) indicated that the Microtox data exhibited the same degree of similarity to fish results as was found between fish toxicity tests.

Bulich (1982) also showed that for 155 complex effluents Microtox results were within 1.0 log unit of *Daphnia* toxicity data in 80.2% of cases (Table A.7). The average log difference for Microtox-*Daphnia* comparisons was 0.34, whereas a comparison between *Daphnia* data for 169 effluents (US EPA 1978) showed only 55% of results were within 1.0 log unit and the average log difference was 1.06.

Vasseur *et al* (1983) investigated 162 complex industrial wastewaters and concluded that a strong correlation existed between Microtox and *Daphnia magna* based on toxic/non-toxic designations. All samples which were toxic to Microtox (i.e. for which an EC_{50} was derived) were also toxic to *Daphnia*, while 88% of samples which displayed no toxicity to the bacterium were non-toxic to daphnids.

In a later study Vasseur *et al* (1984) tested 39 industrial effluents and found a high level of agreement (86%) between Microtox and *Daphnia* (immobility) EC_{50}s, where the Microtox test has a coefficient of variation of 3% compared to 30% for *Daphnia*. Microtox indicated 19 of the 39 effluents studied were 'toxic', whilst *Daphnia* showed positive responses for 22 effluents including the 19 effluents detected by Microtox.

In general, as the complexity and toxicity of industrial effluent increases, the correlation and sensitivity also increases, whilst there is a corresponding decrease in the variability of EC_{50} values (Greene *et al* 1985; Munkittrick *et al* 1990).

Although the Microtox test generally exhibits good agreement with other toxicity tests for assessing the toxicity of complex effluents, it does not appear to be sensitive to those effluents containing high levels of cyanide, urea, ethanol or ammonia (Bulich *et al* 1981) or to those containing pharmaceuticals (Vasseur 1984). However, Microtox is more sensitive to phenol-based effluents and is of variable sensitivity to dyes (Qureshi *et al* 1982; Vasseur *et al* 1984).

Microtox appears to be particularly useful in monitoring relative changes in toxicity for pulp and paper effluents. Blaise *et al* (1987) investigated the rank agreement of toxicity of 39 pulp and paper effluents to rainbow trout and

Microtox and showed a >84% rank agreement between the tests. The correlation between Microtox 15 minute EC_{50}s, rainbow trout 96 hour LC_{50}s and *Ceriodaphnia* seven day chronic data for pulp and paper mill effluent has been investigated by Firth and Backman (1990). There was a high correlation between data for Microtox and rainbow trout (r=0.91, n=34), rainbow trout and *Ceriodaphnia* (r=0.92, n=8) and Microtox and *Ceriodaphnia* (r=0.94, n=8). The study showed that Microtox was more sensitive to these effluents than rainbow trout, but less sensitive than *Ceriodaphnia*.

3.1.6 Leachates and sediments

The results from studies investigating the Microtox response to leachates are given in Table A.5. Many comparative studies of leachates compared toxicity on the basis of positive versus negative response, or toxic/non-toxic designations. In general, it appears that the Microtox test is less sensitive to leachates than *Daphnia* and fathead minnow (Casseri *et al* 1983; Plotkin and Ram 1984; Calleja *et al* 1986). For example, Calleja *et al* (1986) reported Microtox was less sensitive than *Daphnia* to effluents containing pesticides (bendiocarb, propham, azinophos methyl, chloropropham, dimethoate, dichlorvos), leachates involving electroplating sludge and those containing DDT (see Section 3.1).

In the case of sediment-associated toxicity the issue is complicated by the fact that the sensitivity of Microtox, like other toxicity tests, is dependent on the sediment exposure route (e.g. testing whole sediment, elutriates or solvent extracts). Dutka and Kwan (1988) found Microtox to be much more sensitive to extracts using 1% dimethyl sulphoxide (DMSO), rather than aqueous extracts.

A study of the toxicity of 19 sediments resulted in only four positive responses with *Daphnia* tests on aqueous extracts whilst the Microtox test showed no positive responses. Microtox tests on organic extracts (1% DMSO) of the same sediment samples resulted in 18 positive responses. The authors suggested that more rigorous extraction procedures may measure toxicants which

would otherwise not be biologically available, whilst the solvent itself may potentiate the response of the test organism.

Greene and Peterson (1989) reported that in a large scale comparison of Superfund site toxicity, Microtox did not indicate toxicity in 65% of the samples shown as toxic by *Daphnia* or algal tests. In the comparison of the 326 samples tested, 185 showed toxicity to at least one test, with 48% toxic to algae, 41% to *Daphnia* and 21% to Microtox. The Microtox test did not show any response to samples of electroplating effluents and produced contradictory results for 64% of the samples considered toxic to algae and/or *Daphnia*. In a study of aqueous extracts of sediments, Microtox failed to show any positive responses, although *Daphnia* showed positive responses to four of 19 samples (Dutka and Kwan 1988). This lack of correlation was later confirmed in a study by Dutka and Gorrie (1989) where Microtox did not show any positive responses to aqueous-extracts of lake sediment, although *Daphnia* showed responses to 63 of 94 samples.

3.1.7 Mycotoxins

Two studies have investigated the use of Microtox for evaluating the toxicity of mycotoxins (Yates and Porter 1982; 1983). Although both studied the toxicity of a number of mycotoxins to Microtox, neither study reported any comparative sensitivities with other toxicity tests. However, Yates and Porter (1982) did report that the order of toxicity paralleled that found for mammalian cell cultures.

3.2 Correlation between Microtox and other toxicity tests

On the basis of the data presented in the previous sections the following general observations can be made concerning the correlation between Microtox and other commonly used toxicity tests.

3.2.1 Fathead minnow

Correlation studies which ranked the toxicity of samples have generally shown that a strong correlation exists between the Microtox test and the 96 hour fathead minnow lethality test. Correlation coefficients of 0.81, 0.99 and 0.96 have been reported for ketones, ethanes and alcohols respectively (Curtis *et al* 1982). For organics generally, correlations of 0.72 and 0.91 have been found by Curtis *et al* (1982) and Indorato *et al* (1984)

Sensitivity differences for pure compounds ranged from 25 times more sensitive for Microtox to 21 times less sensitive. On average the Microtox test was 2.9 less sensitive for pure substances than the fathead minnow test (Bulich 1991). In the case of complex samples the average of 8 separate studies showed Microtox to be 1.25 times more sensitive than fathead minnow tests (Bulich 1991).

3.2.2 Rainbow trout

A strong ranking correlation between Microtox and the 96 hour rainbow trout test has been found, with correlation coefficients of 0.93 for p-substituted phenols and 0.74 for chlorobenzenes having been reported (Ribo and Kaiser 1983).

The average sensitivity difference for pure compounds was four times greater for rainbow trout whereas for complex effluents the average of 11 separate studies showed Microtox to be 1.5 times more sensitive (Bulich 1991).

3.2.3 *Daphnia*

Correlation studies between Microtox and acute *Daphnia* toxicity tests have shown correlation coefficients of 0.87 for chlorophenols and 0.83 for chlorobenzenes (Ribo and Kaiser 1983).

In general *Daphnia* appears to be a more sensitive species than the Microtox bacterium with the average sensitivity difference being greater by a factor of 1.6 (Bulich 1991). For complex samples the average of 12 studies showed *Daphnia* to be more sensitive in eight studies with the test being two times more sensitive on average than Microtox (Bulich 1991).

3.2.4 *Chlorella pyrenoidosa*

The available data show that the algal test species *Chlorella pyrenoidosa* is generally more, or at least as sensitive, as the Microtox test to simple organic and inorganic compounds (De Zwart and Sloof 1983). Sensitivity differences ranged from 70 times more sensitive to 30 times less sensitive.

3.2.5 Oyster embryos

Few data are available to compare the oyster embryo development test with Microtox. However the oyster embryo test is apparently more sensitive, particularly for inorganic chemicals where the test was 2 to 32 times more sensitive than the Microtox test (Nacci *et al* 1986). Studies conducted at WRc by Butler and Horn (1990) have shown Microtox to be approximately one order of magnitude less sensitive to simple domestic sewage compared with the oyster embryo test. However toxic, complex effluents containing pharmaceutical wastes appear to result in comparable EC_{50} values.

3.2.6 Other micro-organisms

Dutka and Kwan (1981) compared Microtox with the *Spirillum volutans* motility test and growth inhibition of *Pseudomonas fluorescens* and *Aeromonas hydrophila*. The Microtox test was reported to be the most sensitive test for 8 of the 13 compounds tested. Dutka and Kwan (1983) reported similar results when comparing Microtox to the *Spirillum volutans* and *Pseudomonas fluorescens* assay as well as a 3-hour respiration inhibition assay using activated sludge. Microtox was also reported to be the least sensitive for 2 of the 13 chemicals.

Coleman and Qureshi (1985) compared Microtox and the *Spirillum volutans* motility test for assessing toxicity of environmental samples of unknown composition. In the study, 70.7% of the samples were negative for both tests, 12.2% were positive for both tests and 17.1% were positive for Microtox alone. On this basis Microtox was considered to be the more sensitive test.

Elnabarawy (1986) studied the sensitivity of Microtox compared to respiration activity, resazurin reduction and triphenyltetrazolium chloride activity in microbial cultures of activated sludge. The Microtox test showed greater sensitivity for the organic chemicals as well as for the majority of inorganic chemicals tested. Greene *et al* (1985) studied the effects of copper, acetone and methanol on Microtox, the resazurin reduction and dissolved oxygen depletion method, concluding that Microtox was the most sensitive and reliable of the tests.

Elnabarawy *et al* (1988) reported that the Microtox test was the most sensitive toxicity test when compared to the activated sludge respiration inhibition test (OECD 1984) and the Polytox Toxicity Procedure (Polybac Corporation 1986) for a number of organic and inorganic chemicals. Retuena *et al* (1986) compared the 5 minute Microtox test and the 3-hour activated sludge inhibition test for their sensitivity to copper and 3,5-dichlorophenol. Microtox was more sensitive, although the activated sludge respiration tests gave results which were probably more representative of surface micro-organisms. Schneider (1987) investigated the effects of mercury, nickel, dinitro-o-cresol (DNOC) and dichlorophenol on Microtox and the specific oxygen uptake, ATP reduction and chemical oxygen demand (COD) in batch tests. The Microtox test was the most sensitive test, except when nickel was tested. McGrath (1988) also concluded that the Microtox test was the most sensitive toxicity test when compared with the biological oxygen demand (BOD) inhibition test (APHA 1975) and the standard activated sludge respiration test.

McFeters *et al* (1983) investigated the toxicity of 35 chemicals using Microtox and the two-organism Tchan procedure which employs a luminescent bacterium and alga. The authors concluded that the Microtox assay was more sensitive than the Tchan bioassay in detecting most of the test chemicals. A notable

exception was the photosynthesis- inhibiting herbicides which were detected at lower concentrations with the Tchan procedure due to the presence of the algal species.

4. REGULATORY ROLES

The Microtox test has now been adopted as a standard test method by pollution regulation agencies in Europe, Canada and the United States. These are described in the following sections

4.1 Germany

After five years of evaluation and validation studies by a 15 member standards committee, the German Institute for Normalisation (DIN) has issued a standard document for the use of luminescent bacteria for toxicity testing. The DIN standard document does not refer to the proprietory name of Microtox, though the use of *Photobacterium phosphoreum* is specified. The document is referenced as DIN 38 412 L 34 and will be used in conjunction with other DIN standard methods for regulating effluent toxicity.

4.2 Canada

The final draft of the document "Toxicity Testing Using Luminescent Bacteria" has been issued by Environment Canada for final comment. The document is to be part of a series of approved Environment Canada Test Methods for the measurement and assessment of the biological effects of toxic substances on the aquatic environment. Its aim will be to provide guidance and to facilitate the use of consistent, appropriate and comprehensive procedures for obtaining aquatic toxicity data for samples of pure chemicals, effluents, leachates, lab-generated elutriates, ambient receiving waters and sediments.

4.3 USA

The Environmental Protection Agency (EPA) has recommended the Microtox test for toxicity testing of treated effluents, for predicting land treatability of organic wastes and for performing bioassessment of waste disposal sites (Elnabarawy 1986). In addition, the American Society for Testing Materials

(ASTM) has recommended Microtox for biological testing of wastewaters and leachates (Elnabarawy 1986).

4.4 Sweden

In 1990 the Swedish Environmental Protection Agency (SNV) issued a guidance document "Biological and Chemical Characterisation of Industrial Waste Water (CID)". The document describes the reasons for such CID tests as well as describing their principles and methods. Microtox is one of the acute toxicity test methods recommended by the document.

4.5 Netherlands

The Netherlands Institute for Normalisation has circulated a Microtox Protocol for comments, and a final version of the document is expected in 1991.

4.6 International Standards Organisation (ISO)

The Dutch "Standards Document" was used as a basis for submitting an ISO Standards Proposal in May 1990.

4.7 United Kingdom

WRc is currently evaluating a Direct Toxicity Assessment (DTA) protocol (Hunt 1989), for the National Rivers Authority, in which the Microtox test has been advocated for screening and prioritising effluents on the basis of their toxicity. Priority effluents, particularly those of a complex and variable nature, are then fully characterised using a range of appropriate sensitive test species alongside the Microtox test. A "calibrated" Microtox value would then be used in establishing Toxicity Based Consent (TBC) conditions for the discharge and the test is used to monitor compliance of the effluent with the derived consent.

A Microtox toxicity component has been added to a discharge consent by NRA Welsh Region and regulatory agencies are considering the wider use of the approach. The Microtox technique has also been used successfully by NRA Welsh Region in a recent prosecution of a pollution incident where only a small sample volume was available for testing.

5. THE ADVANTAGES AND LIMITATIONS OF THE TEST

5.1 Advantages

1. The guaranteed, all year round, availability of organisms for toxicity testing at short notice is crucial to monitoring programmes. This is difficult to achieve for any of the higher organism acute toxicity tests and large scale culture and maintenance facilities are expensive. However, *Photobacterium phosphoreum* (Microtox reagent) is available "off-the-shelf" in a freeze dried form which can, according to Microbics Corporation, be stored for up to a year without any change in sensitivity. Constant test capability can therefore be achieved with minimal laboratory space.

2. The relative ease of storage and transport makes the Microtox system superior to other toxicity tests for rapid toxicity testing at remote locations.

3. The controlled culture of the Microtox bacteria has enabled the production of test organisms of a consistent quality. This is not the case for other test organisms where factors such as age, sex, reproductive status, disease and nutritional history often affect the results obtained.

4. An important characteristic of any bioassay method is the precision or reproducibility of the test method. The use of a highly formalised and standardised reagent coupled with the ease of operation means that, in comparison to acute lethality tests Microtox has a considerably lower coefficient of variation and far greater reproducibility (McFeters *et al* 1983). Curtis *et al* (1982) reported a coefficient of variation of approximately 10% for duplicates of seven chemicals. Similarly, Reteuna *et al* (1986) found a coefficient of variation of <10% for triplicate samples of 3,5-dichlorophenol and copper sulphate. Casseri *et al* (1983) compared Microtox with fish and *Daphnia* in assessing wastewater treatment effectiveness. The authors reported the Microtox data to be

very reproducible with a coefficient of variation of 5-10% not only for duplicated tests, but also for tests conducted at different times on split samples. The variation is likely to be a result of pipetting inaccuracy which is the laboratory manipulation most likely to introduce error in the Microtox method. Variability has also been attributed to the duration of the assay, toxicant measurements and sample dilution (Qureshi *et al* 1983). Many of these factors, however, are also associated with other biological tests. In order to assess the reproducibility of the method in different laboratories Bulich (1981) analysed the results obtained from three different operators, instruments and production lots of reagents and found a coefficient of variation of 18% for all five minute EC_{50} values for sodium lauryl sulphate.

Strosher (1984) published the results of a large scale study which assessed the precision of Microtox. Three laboratories participated in an interlaboratory comparison of Microtox data for the testing of waste drilling fluids. The three laboratories generated 129 results on 29 shared samples. The coefficient of variation for Microtox five minute EC_{50} test data reached a maximum of 31%, but averaged 11%. For 15 minute tests, the average coefficient of variation was 13% and the maximum variability was 31%. In comparison, the results from the three laboratories performing the fish toxicity tests with the same samples showed a maximum coefficient of variation of 98%, with a mean value of 30%.

5. Bacterial toxicity tests utilise the response of a large "population" of organisms, compared to the relatively small populations of higher organisms. This contributes not only to improved precision but also to higher resolution, which means that small differences in toxicity can be detected.

6. An advantage of the Microtox system is associated with the type and quality of data obtained from the test system. The effect being measured, toxicant induced light loss, is a rate of biological activity rather than a count of organisms being affected (quantal data). Quantal

data can only be treated statistically when concentrations above and below a threshold effect are included in the experiment, and reasonable confidence levels can only be achieved for median results. For Microtox, the ability to extrapolate beyond these limits are far less restrictive than for other toxicity tests.

7. The measured value describing the slope of the dose response curve can also be used. The slope of the regression line relates to the mechanism of action of the toxicant as it reflects the number of toxicant molecules required to interact with the target molecule. Slopes of one and two are most common and slopes of <1.0 indicate either a mixture of toxicants or multiple targets or both. Slope values can be very useful when comparing toxicities of different samples. For example, two samples may have similar EC_{50}s but can display very different slopes indicating that the sample contents are qualitatively different.

8. The small sample volumes required for the Microtox test means the assay can be used in instances where obtaining a large test sample may be problematical, such as for interstitial pore waters, low yield leachate systems and chemical fractions produced at intermediate steps in Toxicity Identification Evaluation procedures.

9. The test has a rapid response time which is of utmost importance in many situations. For example, by monitoring toxicity in sewers, treatment plant operators may be alerted to high levels of incoming toxic pollutants in time for possible protective action to be taken. As a result of its rapidity it can also be used to evaluate the toxicity of unstable samples (Casseri *et al* 1983).

5.2 Limitations

There are a number of identified limitations to the Microtox test which will be discussed, though it should be remembered that these may also apply to other established toxicity tests.

1. *Photobacterium phosphoreum* is a marine bacterium considered to be of no economic and unknown ecological importance and environmental relevance. However, the use of indigenous, ecologically or economically relevant test organisms is only appropriate when the study objective requires such specific information (e.g. the use of an oyster embryo test to monitor effluents discharged near to oyster beds or a trout test to protect native or commercial fish stocks). A large proportion of toxicity tests are aimed at monitoring changes in relative toxicity, where a bacterial organism would be of equivalent utility to a fish species.

   In addition Ross (1991) has argued that since the bacterial trophic level is the most important in aquatic ecosystems, in terms of energy flow, metabolic activity and nutrient cycling, a bacterial test system should be routinely used to monitor pollutant impact.

2. The Microtox test is generally less sensitive to aquatic contaminants than acute toxicity tests with higher organisms, though typically by less than an order of magnitude. Low level toxicity, which is harmful to sensitive organisms, such as early life stages of bivalve molluscs, may not be detected by the Microtox test. However, the test can be considered as reliable over a broad band of test concentrations.

   In order to fully define the hazard of an aquatic contaminant, a suite of toxicity tests, with a variety of species should be performed. Neither the Microtox, nor any other single test result can be used in complete isolation to predict the environmental impact of contaminants except in the crudest terms.

3. The Microtox procedure requires the salinity of the sample to be adjusted to 2% (with sodium chloride), because of the marine origin of the luminescent bacterium. This modification may reduce sample integrity and have a large impact on metal speciation, particularly for freshwater samples. A resulting change in the availability of free metal ions could be translated into a toxicity difference.

4. Turbid effluent samples require filtration or centrifugation since suspended solids or colloids can attenuate the light signal and falsely indicate toxicity. In addition, toxicants, especially organics, may be bound to suspended material and be removed with filtration or centrifugation again resulting in lower apparent toxicities. These limitations, however, are applicable to most other tests, such as the oyster embryo larval test, algal growth inhibition tests, and the *Daphnia* test above certain low suspended solid loadings. Since *Daphnia* feed on suspended particulates they may in some instances be more sensitive to unfiltered effluents than other tests which cannot be used for effluents with low levels of suspended solids.

6. CONCLUSIONS

The Microtox test is a simple and rapid toxicity test method which displays sensitivity to a broad range of aquatic toxicants. Its ease of operation, rapid response, high precision, relatively low cost, independence from specialised laboratory facilities, guaranteed availability of test organisms and application at remote sites are some of the advantages of the technique over other aquatic toxicity tests involving higher organisms.

There are more than 250 publications containing information on the Microtox test. However, many of the comparative studies do not specify species of "fish" used or provide basic chemical analysis of "complex effluents". A thorough comparative review has therefore been difficult to compile.

However, with respect to acute lethality tests using fathead minnow, rainbow trout and *Daphnia magna* the following conclusions can be made:

1. Organics: Microtox appears to be marginally less sensitive to most pure organic compounds than acute tests. Microtox is more sensitive to complex compounds such as multi-chlorinated benzenes, phenols and ethanols, but was less sensitive to simple organics such as cyanide, ammonia, aniline, chloroform, ethylacetate, ethylpropionate or propanol. High ranking correlations have been reported between Microtox and these commonly used tests for a number of structurally similar compounds. These differences may reflect the mode of toxic action of these chemicals.

2. Inorganics: Microtox does not appear to be as sensitive as acute lethality tests to inorganic chemicals. However, since some of these substances require longer exposure times to exert their full toxic effect, the Microtox test can be extended to thirty or more minutes in order to reduce the difference in sensitivity between tests.

3. Sewage efluents: Microtox is apparently a sensitive technique for monitoring relative differences in toxicity.

4. Petroleum effluents: Microtox has considerable potential in monitoring relative differences in toxicity and showed strong ranking correlations with rainbow trout toxicity test data.

5. Industrial effluents: The available data showed that as the complexity and toxicity of industrial effluents increased, the correlations and sensitivity of Microtox increased, with a corresponding decrease in data variability. However, the test is unlikely to be sensitive to effluents with high levels of urea, cyanide or ammonia.

6. Other wastes: Microtox was less sensitive than acute lethality tests to insecticides, herbicides, textile effluents, highly lipophillic contaminants or to wastes with a high inorganic content.

7. Sediments: The sensitivity of the Microtox test varies depending on the sediment exposure route (i.e. extracts, elutriates or whole sediments). Microtox is therefore only likely to be useful in monitoring relative differences in toxicity, although its ability to use small sample volumes, such as interstitial pore waters, means it has advantages over other tests.

Although Microtox tends to be less sensitive than higher organism toxicity tests, EC_{50} and LC_{50} values do not normally differ by more than one order of magnitude. Compared to other microbial toxicity tests it appears to be more sensitive and reliable, suggesting that it may be the most appropriate screening or field monitoring method currently available (Dutka and Kwan 1981; Dutka and Kwan 1983; Schneider 1987; McGrath 1988).

Since the usefulness of the Microtox test as a preliminary cost effective toxicity test has been established, a "tiered approach" to assessing the potential impact of new or previously untested compounds, complex effluents and sediments is recommended.

In this approach Microtox is used as a primary screening test to rank samples on the basis of their toxicity (non toxic, toxic, highly toxic) and establish priorities for additional testing. This would involve the use of more

expensive acute toxicity tests, using algal, invertebrate and fish species, for priority samples and those to which Microtox is considered likely to be relatively insensitive. This approach would not replace but complement the traditional chemical-specific approach since chemical information is essential if sensitivity deficiencies are to be accommodated.

For complex effluents to be controlled by a direct toxicity approach the Microtox test can be 'calibrated' against the most sensitive species and used routinely to monitor the compliance of discharges with toxicity based consent conditions.

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APPENDIX A - TABLES OF RESULTS

Table A.1 - Relative sensitivity of the Microtox test to pure organic compounds (after Kaiser and Ribo 1988)

Compound	Formula	Microtox EC_{50} (mg/l)			Footnote
		5 min	15 min	30 min	
Acetaldehyde	C_2H_4O	392.7			e
"	"	342.0			
4-acetamidobenzoic acid	$C_9H_9NO_3$	103.0	98.5	96.2	98%
4-acetamidophenol	$C_8H_9NO_2$	1121	1046	999	t
Acetone	C_3H_6O	22086	29115		
"	"	22086			e
"	"	21576			
"	"	18365			
"	"	21577	21089		
"	"		14590		
Acetonitrile	C_2H_3N	24170		24170	
Aflatoxin B1	$C_{17}H_{12}O_6$	21.9	23.1		
"	"	21.9	19.4		b
Allylamine	C_3H_7N	19.8	16.4		
"	"	13387		12787	
2-allylphenol	$C_9H_{10}O$	9.9			
4-aminobenzophenone	$C_{13}H_{11}NO$	17.2	18.3	20.1	98%
4-aminophenylacetic acid	C_8H_9NO	169	126	126	96%
p-aminophenethyl	$C_8H_{11}NO$	4.3	4.3	3.8	98%
4-amino-2-nitro-phenol	$C_6H_6N_2O_3$	36.1			
1-amino-2-propanol	C_3H_9NO	27.3			
Ammonia (free)	NH_3	2.0			b
Aniline	C_6H_7N	426	489		
"	"	64.4	69.0	70.7	
Benzaldehyde	C_7H_6O	6.2	5.1	5.3	98%
Benzamide	C_7H_7NO	63.6	60.7	59.4	99%
Benzene	C_6H_6	201			
"	"	2.0			b
"	"		156		
"	"	215	236		
"	"	4.1			
"	"	83.7			g
"	"	74.6	78.1	74.6	
Benzene sulphonamide	$C_6H_7NO_2S$	217	249	244	98%
Benzenesulphonyl isocyanate	$C_7H_5NO_3S$	13.2	9.5	6.6	95%
Benzhydrazide	$C_7H_8N_2O$	82.1	65.1	76.5	98%
Benzil	$C_{14}H_{10}O_2$	0.53	0.58	0.63	98%
Benzonitrile	C_7H_5N	19.2			
"	"	10.1	10.5	11.6	99.9%
1,4-benzoquinone	$C_6H_4O_2$	0.009			
"	"	0.080			
"	"		0.020		98%

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l)			Footnote
		5 min	15 min	30 min	
Benzoyl chloride	C_7H_5ClO	10.4	11.7	12.2	99%
Benzoyl cyanide	C_8H_5NO	6.3	5.0	3.3	98%
Benzyl alcohol	C_7H_6O	63.7	63.7	71.4	99%
Benzylamine	C_7H_9N	21.4	17.0	17.0	99%
Benzylchloride	C_7H_7Cl	1.9	2.3	3.0	97%
Benzyl cyanide	C_8H_7N	1.5	1.2	1.3	99%
Benzylisothiocyanate	C_8H_7NS	0.014	0.011	0.010	97%
Benzyl mercaptan	C_7H_8S	0.96	1.1	1.4	99%
4-benzylphenol	$C_{13}H_{12}O$	0.26	0.26	0.25	96%
Benzylthiocyanate	C_8H_7NS	0.54	0.51	0.45	97%
4-biphenylmethanol	$C_{13}H_{12}O$	2.8	2.5	2.6	
Bromacil	$C_9H_{13}BrN_2O_2$	6.7			k
4-(bromomethyl) benzonitrile	C_8H_6BrN	1.0	0.57	0.33	98%
1-bromo-4-nitrobenzene	$C_6H_4BrNO_2$	14.0	15.3	16.8	
Bufencarb	$C_{13}H_{19}NO_2$	0.25			k
Butanal	C_4H_8O	16.5			
1-butanol	$C_4H_{10}O$	43657			
"	"	3311			b
"	"	2291			
"	"		2818		
2-butanone	C_4H_8O	5105			
2-butanone oxime	C_4H_9NO	955			
n-butylamine	$C_4H_{11}N$	18372		18372	
n-butyl ether	$C_8H_{18}O$	62.3			
Caffeine	$C_8H_{10}N_4O_2$	600			d
Catechol	$C_6H_6O_2$	31.8			
Cetyltrimethyl ammonium bromide	$C_{19}H_{42}BrN$	9.8			
Cetyltrimethyl ammonium chloride	$C_{19}H_{42}ClN$	0.80			d
2-chloroacetamide	C_2H_4ClNO	10.3	19.5	31.7	98%
Chloroacetone	C_3H_5Cl	27.3	9.9	5.8	90%
4-chloro-acetophenone	C_8H_7ClO	6.7	7.1	6.9	97%
4-chloro-N-acetyl-aniline	C_8H_8ClNO	38.0	44.6	48.9	
4-chloro-anisole	C_7H_7ClO	3.2	3.5	3.6	
2-chloro-aniline	C_6H_6ClN	16.1			d
"	"	14.3	15.0	15.7	
3-chloro-aniline	"	12.5	13.4	14.0	
4-chloro-aniline	"	3.2	3.8	5.1	
4-chloro-benzaldehyde	C_7H_5ClO	10.2	10.9	10.4	
Chloro-benzene	C_6H_5Cl	9.4	11.5	11.3	
"	"		14.8		
1-chloro-4-benzene-sulphonamide	$C_6H_6ClNO_2S$	69.6	83.6	100.6	

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l)			Footnote
		5 min	15 min	30 min	
4-chloro-benzoic acid	$C_7H_5ClO_2$	5.7	6.2	6.7	
4-chloro-benzoic acid amide	C_7H_6ClNO	42.9	42.9	43.8	
4-chloro-benzoic acid hydrazide	$C_7H_7ClN_2O$	59.2	64.9	60.5	
4-chlorobenzonitrile	C_7H_4ClN	4.1	3.9	4.5	
4-chlorobenzophenone	$C_{13}H_9ClO$	1.1	1.2	1.4	
4-chlorobenzotri-fluoride	$C_7H_4ClF_3$	2.7	3.5		f
4-chloro-benzoyl-chloride	$C_7H_4Cl_2O$	4.8	5.8	5.8	
4-chlorobenzylalcohol	C_7H_7ClO	10.3	11.6	10.6	
4-chloro-benzylamine	C_7H_8ClN	14.2	17.8	24.6	
3-chlorobenzylchloride	$C_7H_6C_{12}$	0.67	0.75	0.85	
4-chlorobenzylchloride	$C_7H_6C_{12}$	0.46	0.54	0.58	
3-chlorobenzyl cyanide	C_8H_6ClN	1.3	1.3	1.2	98%
4-chlorobenzyl cyanide	C_8H_6ClN	0.56	0.59	0.57	R
4-chlorobenzyl-mercapatan	C_7H_7ClS	0.57	0.46	0.49	98%
2-chloro-3,5-dinitro-pyridine	$C_5H_2ClN_3O_4$	6.0	0.43	0.34	99%
4-chloro-3,5-dinitro-benzotrifluoride	$C_7H_2ClF_3N_2O_4$	2.8	8.0	5.0	97%
2-chloroethanol	C_2H_5ClO	13361			
Chloroform	CHC_{13}	433			
1-chloro-4-fluoro-benzene	C_6H_4ClF	99.0	119	137	
Chlorohydroquinone	$C_6H_5ClO_2$	11.2	7.2	5.8	95%
1-chloro-4-iodobenzene	C_6H_4ClI	2.2	2.0	1.6	
4-chloro-N-methyl aniline	C_7H_8ClN	0.91	1.0	1.0	
2-chloro-5-nitro-aniline	$C_6H_5ClN_2O_2$	16.9	1.8	2.0	98%
4-chloro-3-nitroanisol	$C_7H_6ClNO_3$	3.9	4.3	4.5	98%
2-chloro-nitrobenzene	$C_6H_4ClNO_2$	4.1	4.2	4.3	
3-chloro-nitrobenzene	$C_6H_4ClNO_2$	15.0	17.3	19.8	
4-chloro-nitrobenzene	$C_6H_4ClNO_2$	20.8	21.3	23.8	
4-chloro-3-nitro-benzoic acid	$C_7H_4ClNO_4$	90.0	86.0	96.0	99%
2-chloro-phenol	C_6H_5ClO	22.3			
"	"	37.1	39.7	33.8	

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l)			Footnote
		5 min	15 min	30 min	
3-chloro-phenol	C_6H_5ClO	10.0	13.2	14.1	
4-chloro-phenol	C_6H_5ClO	8.5	9.1	8.3	
4-chlorophenoxy acetic acid	$C_8H_7ClO_3$	148	120	98.0	98%
4-chlorophenyl acetic acid	$C_8H_7ClO_2$	64.9	69.5	79.8	
4-chlorophenyl-acetone	C_9H_9ClO	5.3	5.8	5.6	
3-(4-chlorophenyl)-alanine,DL	$C_9H_{10}ClNO_2$	112	115	115	98%
"	"			934	
2-(4-chlorophenyl)-ethylamine	$C_8H_{10}ClN$	30.2	26.3	26.3	
4-chlorophenyl-isothiocyanate	C_7H_4ClNS	0.50	0.45	0.36	
4-chlorophenyl-isocyanate	C_7H_4ClNO	2.7	2.5	2.3	
4-chlorophenyl seleninic acid	$C_6H_5ClO_2Se$	75.9	57.6	38.0	
2-chloro-pyridine	C_5H_4ClN	70.0	71.6	70.0	
3-chloro-pyridine	C_5H_4ClN	66.9	54.3	68.4	
4-chloro-thiophenol	C_6H_5ClS	0.91	0.65	0.55	
2-chlorotoluene	C_7H_7Cl	5.9	6.1		f
4-chlorotoluene	C_7H_7Cl	4.9	5.8	6.5	
"	"	6.3	7.3		f
4-chloro-α, α, α-trichlorotoluene	$C_7H_4C_{14}$	6.3	7.3	10.8	
4-chloro-α, α, α-trifluorotoluene	$C_7H_4ClF_3$	11.1	13.4	14.3	
p-chloro-m-xylenol	C_8H_9ClO	1.6			d
"	"		8.0		99%
Citrinin	$C_{13}H_{14}O_5$	27.4	16.9		b
m-cresol	C_7H_8O	11.1			
"	"	8.2			
o-cresol	C_7H_8O	31.2			
"	"	20.6	15.3		
p-cresol	C_7H_8O	1.5			b
"	"	1.3			
4-cyanobenzaldehyde	C_8H_5NO	14.1	13.7	12.5	99%
1,4-cyclohexanediol	$C_6H_{12}O_2$	3846	3936	4028	99%
Cyclohexanol	$C_6H_{12}O$	115			
Cyclohexanone	$C_6H_{10}O$	18.7			
Cyclo-hexylamine	$C_6H_{13}N$	4232		119	

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l)			Footnote
		5 min	15 min	30 min	
Cyhexatin	$C_{18}H_{34}SnO$	9.9			
Cytrole (Amitrole)	$C_2H_4N_4$	180			l
2,4-D	$C_8H_6Cl_2O_3$	62.3			k
"	"	111	106	127	m
DL-phenylalanine	$C_9H_{11}NO$	445	434	405	99%
DL-α-phenylglycine	$C_8H_9NO_2$	6.0	5.5	5.5	98%
DDT	$C_{14}H_9Cl_5$	7.1			
3,4-diaminobenzoic acid	$C_7H_8N_2O_2$	121	115	105	97%
4,4'-diaminobibenzyl	$C_{14}H_{16}N_2$	10.9	11.4	10.4	97%
4,4'-diaminobibenzyl methane	$C_{13}H_{14}N_2$	6.0	6.6	6.6	99%
2,4-diaminotoluene	$C_7H_{10}N_2$	73.6		86.5	
2,7-diaminofluorine	$C_{13}H_{12}N_2$	31.1	30.4	29.0	97%
Diazinon	$C_{12}H_{21}N_2O3PS$	1.7			
"	"	9.8			
2,3-dibromophenol	C_3H_6Br2O	322			
Dichloran	$C_{21}H_{36}Cl_3N$	3.0			
2,3-dichloro-aniline	$C_6H_5Cl_2N$	2.5	2.8	2.8	
2,4-dichloro-aniline	$C_6H_5Cl_2N$	4.0	4.6	4.7	
2,5-dichloro-aniline	$C_6H_5Cl_2N$	3.4	3.6	3.8	
2,6-dichloro-aniline	$C_6H_5Cl_2N$	1.5	1.7	1.7	
3,4-dichloro-aniline	$C_6H_5Cl_2N$	0.45	0.56	0.65	
3,5-dichloro-aniline	$C_6H_5Cl_2N$	9.5	10.7	10.5	
1,2-dichloro-benzene	$C_6H_4Cl_2$	10.2			
"	"	2.7	3.1	4.0	
1,3-dichloro-benzene	$C_6H_4Cl_2$	3.1	4.1	5.1	
"	"		3.3		
1,4-dichloro-benzene	$C_6H_4Cl_2$	4.3	4.9	5.3	
3,4-dichlorobenzo-trifluoride	$C_7H_3Cl_2F_3$	1.6			f
5,5'-dichloro-2,2'-di-hydroxy diphenylmethane	$C_{13}H_{10}Cl_2O_2$		0.055		98%
1,2-dichloroethane	$C_2H_4Cl_2$	154			
"	"		1088		
Dichloromethane	CH_2Cl_2		2878		
"	"		2812		
"	"		998		pH 6.7
2,3-dichloro-nitro-benzene	$C_6H_3Cl_2NO$	1.3	1.4	1.5	
2,4-dichloro-nitro-benzene	$C_6H_3Cl_2NO$	2.9	3.0	3.2	
2,5-dichloro-nitro-	$C_6H_3Cl_2NO$	7.8	8.4	8.8	

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l)			Footnote
		5 min	15 min	30 min	
benzene					
3,5-dichloro-nitro-benzene	$C_6H_3Cl_2NO$	22.5	19.2	17.1	
3,4-dichloro-nitro-benzene	$C_6H_3Cl_2NO$	9.2	10.1	10.1	
2,3-dichloro-phenol	$C_6H_4Cl_2O$	4.3	4.8	4.9	
2,4-dichloro-phenol	$C_6H_4Cl_2O$	3.6			
"	"	4.7	5.0	5.5	
"	"	1.1	1.2	1.2	95%
2,5-dichloro-phenol	$C_6H_4Cl_2O$	8.4	9.6	9.4	
2,6-dichloro-phenol	$C_6H_4Cl_2O$	9.6	13.6	13.2	
3,4-dichloro-phenol	$C_6H_4Cl_2O$	1.3	1.7	1.6	
3,5-dichloro-phenol	$C_6H_4Cl_2O$	2.8			
"	"	4.5	4.2		
"	"		2.9		pH 6.7
"	"	3.9	3.2	2.8	
"	"	3.9	3.2	2.8	99%
3,4-dichlorophenyl-isocyanate	$C_7H_3Cl_2NO$	1.5	1.1	0.96	97%
2,4-dichlorophenyl-acetic acid	$C_8H_7Cl_2O_2$	93.7	89.5	85.5	99%
2,3-dichloro-pyridine	$C_5H_3Cl_2N$	35.5	33.9	33.9	
2,5-dichloro-pyridine	$C_5H_3Cl_2N$	81.3	83.2	81.3	
2,6-dichloro-pyridine	$C_5H_3Cl_2N$	81.3	83.2	83.2	
3,5-dichloro-pyridine	$C_5H_3Cl_2N$	74.2	72.5	70.8	
4,7-dichloroquinoline	C_9H_5ClN	3.3	3.2	3.1	99%
α, α-dichlorotoluene	$C_7H_6Cl_2$	2.1	3.4	5.8	99%
2,4-dichlorotoluene	$C_7H_6Cl_2$	2.3	2.5	2.7	99%
3,4-dichlorotoluene	$C_7H_6Cl_2$		1.4		
α, α-dichloro-p-xylene	$C_8H_8Cl_2$	0.044	0.052	0.054	
Diethanolamine	$C_4H_{11}NO_2$	72.8			n
Diethylamine	$C_4H_{11}N$	38384		31927	
Diethyleneglycol	$C_4H_{10}O_3$		29233		
Diethylether	$C_4H_{10}O$		5624		
2,4-difluoroaniline	$C_6H_5F_2N$	98.0	98.0	93.5	99%
4-(dimethylamino)-benzonitrile	$C_9H_{10}N_2$	0.18	0.18	0.17	98%
4-dimethylamino-3-methyl-2-butanone	$C_7H_{15}NO$	41.8			
4-dimethylamino-pyridine	$C_7H_{10}N_2$	26.7	24.9	26.7	99%
N-N-dimethylaniline	$C_8H_{11}N$	13.6	13.6	13.6	99%
N-N-dimethylformamide	C_3H_7NO	20133			
2,4-dimethylphenol	$C_8H_{10}O$	4.4			

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l) 5 min	15 min	30 min	Footnote
Dimethylsulphoxide	C_2H_6OS		63515		
"	"	103009			
Dimethyl yellow	$C_{14}H_{15}N_3$	0.023	0.019	0.019	pH 2.9-4.0
1,2-dinitrobenzene	$C_6H_4N_2O_4$	9.7	6.5	5.3	99%
1,4-dinitrobenzene	$C_6H_4N_2O_4$	0.38	0.11	0.095	o
4,6-dinitro-o-cresol	$C_7H_6N_2O_5$	6.6			
"	"	6.3		1.5	
2,4-dinitro-phenol	$C_6H_4N_2O_5$	6.1		6.1	
"	"	15.7			
3,5-dinitrosalicylic acid	$C_7H_4N_2O_7$	169	161	165	98%
2,4-dinitro-toluene	$C_7H_6N_2O_4$	33.1		20.9	
Diphenylmethane	$C_{13}H_{12}$	1.4	2.3	3.5	99%
"	"	1.4	2.3	3.5	99%
2,5-di-tert-butyl-hydroquinone	$C_{14}H_{22}O_2$	4.2	3.9	4.6	97%
Diuron	$C_9H_{10}Cl_2N_2O$	16.5			k
Esteron 99	$C_8H_6Cl_2O_3$	10.3	10.3	8.4	m,n
Ethanol	C_2H_6O		23090		
"	"	43997			
"	"	55389			
"	"	47143			
"	"	31147			b
p-ethoxybenzyl alcohol	$C_9H_{12}O_2$	32.5	31.8	29.7	98%
2-(2-ethoxyethoxy) ethanol	$C_6H_{14}O_2$	1142			
Ethylacetate	$C_4H_6O_2$	1189			e
"	"	5189	5822		
Ethylamine	C_2H_7N	31195		31195	
4-ethylaniline	$C_8H_{11}N$	0.20	0.20	0.21	99+%
4-ethylnitrobenzene	$C_8H_9NO_2$	1.3	1.4	1.4	99%
Ethylpropionate	$C_5H_{10}O_2$	616	811		
4-ethyltoluene	C_9H_{12}	2.0	2.2	2.6	98%
Fluorobenzene	C_6H_5F	192	163	183	99%
4-fluorobenzyl alcohol	C_7H_7FO	162	152	138	98%
4-fluorobenzylamine	C_7H_8FN	33.7	29.3	29.3	97+%
4-fluorobenzoyl-chloride	C_7H_4ClFO	10.7	13.2	15.5	98%
(4-fluorophenyl)-acetic acid	$C_8H_7FO_2$	48.7	51.0	56.0	98%
4-fluorophenyl isocyanate	C_7H_4FNO	32.1	26.1	20.3	99%

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l)			Footnote
		5 min	15 min	30 min	
Formaldehyde	CH_2O	907			
"	"	8.7			
"	"	3.0			b
Formanilide	C_7H_7NO	4.3	4.4	4.7	98%
Glyphosate	$C_3H_8NO_5P$	7.7			
Grotan	$C_9H_{21}N_3O_3$	42.8	28.9		78%
Heptachlorepoxide	$C_{10}H_5Cl_7O$	25.1			k
n-Heptanol	$C_7H_{16}O$		9.9		
"	"	14.6	20.2		
Hexachloroethane	C_2Cl_6	0.14			
1-hexanol	$C_6H_{14}O$	40.7			
"	"		67.5		
4-hydrazinobenzoic acid	$C_7H_8N_2O_2$	108	69.5	50.4	97%
Hydrogen cyanide	HCN	8.5			b
Hydroquinone	$C_6H_6O_2$	0.079			
"	"	0.042	0.038	0.038	
4-hydroxy-benzonitrile	C_7H_5NO	0.69	0.84	0.79	
4-hydroxy acetophenone	$C_8H_8O_2$	4.5	4.9	4.4	
4-hydroxy benzyl-alcohol	$C_7H_8O_2$	5.5	5.2	5.1	
4-hydroxy benzaldehyde	$C_7H_6O_2$	6.0	7.7	8.8	
4-hydroxy benzoic acid	$C_7H_6O_3$	12.3	11.8	10.5	
4-hydroxy benzophenone	$C_{13}H_{10}O_2$	8.7	7.9	7.9	
4-hydroxy benzotri-fluoride	$C_7H_5F_3O$	0.59	0.76	0.79	
3-hydroxy-4-methoxy-benzyl alcohol	$C_8H_{10}O_3$	294	268	256	98%
4-hydroxy-4-methyoxy-benzyl alcohol	$C_8H_{10}O_3$	154	144	134	98%
2-hydroxy-5-nitro-benzyl bromide	$C_7H_6BrNO_3$	22.2	19.7	19.7	98%
4-hydroxy propio-phenone	$C_9H_{10}O_2$	7.0	7.2	6.6	97%
4-iodoanisole	C_7H_7IO	1.2	1.3	1.3	98%
Isophthalaldehyde	$C_8H_6O_2$	21.3	16.5	125	97%
Isophthalonitrile	$C_8H_4N_2$	131	120	107	99+%
Kelthane	$C_{14}H_9Cl_5O$	0.45			
Lindane	$C_6H_6Cl_6$		11.1		
Maleic anhydride	$C_4H_2O_3$	38.1	41.8	43.8	99%
2-mercaptobenzoxazole	C_7H_5NOS	3.1	2.9	2.9	95%
2-mercapto-1-methyl-imidazole	$C_4H_6N_2S$	522	522	546	98%
2-mercapto-pyridine	$C_5H_5N_2S$	2.4	2.3	3.2	99%
Mercaptotriazole	$C_2H_3N_3S$	98.9			d

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l) 5 min	15 min	30 min	Footnote
Methanol	CH_4O	124650			
"	"	56976 *			
"	"		42237 *		
"	"		11368		
3-methoxyaceto-phenone	$C_9H_{10}O_2$	3.5	3.7	3.4	99%
4-methoxyaceto-phenone	$C_9H_{10}O_2$	8.4	8.3	7.5	98%
4-methoxyazobenzene	$C_{13}H_{12}N_2O$	0.10	0.11	0.11	99%
2-methoxy benzo-nitrile	C_8H_7NO	7.3	7.0	5.9	99%
4-methoxybenzyl alcohol	$C_8H_{10}O_2$	1.7	1.8	1.8	98%
"	"	1.1	1.1	1.0	98%
4-methoxybenzyl-amine	$C_8H_{11}NO$	34.7	34.7	35.5	98%
4-methoxy phenol	$C_7H_8O_2$	3.7	4.3	4.6	
(p-methoxyphenyl) acetic acid	$C_9H_{10}O_2$	57.6	56.3	56.3	99%
4-methoxyphenyl acetonitrile	C_9H_9NO	0.18	0.18	0.17	97%
4-methylacetophenone	$C_9H_{10}O$	3.3	3.3	3.1	90%
Methyl-p-amino benzoate	$C_8H_9NO_2$	12.6	12.3	11.0	98%
4-methylaminobenzoic acid	$C_8H_9NO_2$	85.0	85.0	79.3	97%
N-methyl-aniline	C_7H_9N	11.7	12.3	13.8	99%
Methyl benzoate	$C_8H_8O_2$	4.4	4.2	4.6	99%
p-methylbenzyl alcohol	$C_8H_{10}O$	14.0	14.4	12.8	98%
4-methylbenzylamine	$C_8H_{11}N$	20.6	22.1	25.9	98%
Methylenebis (thiocyanate)	$C_3H_2N_2S_2$	0.10			d
"	"		0.023		99%
6-methyl-5-hepten-2-one	$C_8H_{14}O$	17.4			
5-methyl-2-hexanone	$C_7H_{14}O$	972			
"	"	1438			
Methyl-4-hydroxy-benzoate	$C_8H_8O_3$	6.1	6.2	6.3	99%
Methyl isobutyl ketone	$C_6H_{12}O$	0.080			e
Methylisonicotinate	$C_7H_7NO_2$	198	208	217	98%

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l)			Footnote
		5 min	15 min	30 min	
2-methyl-2,4-pentanediol	$C_6H_{14}O_2$	3038			
4-methyl-2-pentanone	$C_6H_{14}O_2$	79.6			
4-methyl phenol (cresol)	C_7H_8O	2.1	2.3	2.4	
2-methyl-1-propanol	$C_4H_{10}O$	1659			
Monuron	$C_9H_{11}ClN_2O$	228			
Nitrilotriacetic acid	$C_6H_9NO_6$		1003		e
"	"		1003		pH 6.7
4-nitrobenzaldehyde	$C_7H_5NO_3$	11.7	8.3	6.8	99%
Nitrobenzene	$C_6H_5NO_2$	28.2	29.5	34.7	
2-nitrobenzene-sulfenylchloride	$C_6H_4ClNO_2S$	4.0	3.4	3.4	99%
4-nitrobenzyl acetate	$C_9H_9NO_4$	9.1	9.3	9.3	
4-nitrobenzyl alcohol alcohol	$C_7H_7NO_3$	32.0	33.5	35.9	97%
4-nitrobenzyl chloride	$C_7H_6NClO_2$	2.3	2.5	2.6	99%
4-nitrobenzyl-chloroformate	$C_8H_6NClO_4$	7.8	8.6	9.0	97%
4-nitrocatechol	$C_6H_5NO_4$	9.1	8.0	7.8	98%
4-nitrophenol	$C_6H_5NO_3$	13.0			
2-(4-nitrophenoxy) ethanol	$C_8H_9NO_4$	127	118	108	q
1-(4-nitrophenyl) acetone	$C_9H_9NO_3$	33.4	32.6	32.6	
4-nitrophenyl-acetonitrile	$C_8H_6N_2O_2$	6.2	6.5	7.1	p
4-nitro-DL-phenyl-alanine	$C_9H_{10}N_2O_4$	8.4	5.0	3.8	
4-nitro-o-phenylene-diamine	$C_6H_7N_3O_2$	27.9	22.1	21.6	97%
2-(4-nitrophenyl) ethanol	$C_8H_9NO_3$	51.7	48.2	45.0	98%
"	"	33.4	34.9	38.3	98%
4-nitrophenyl propionate	$C_9H_9NO_4$	2.2	2.1	2.0	98%
4-nitrophenyl sulphate	$C_6H_4KNO_6S$	46.8	38.9	37.2	98%
4-nitrophenyl-trifluoroacetate	$C_6H_4F_3NO_4$	13.2	13.2	13.8	98%
4-nitroso-N,N-di-methylaniline	$C_8H_{10}N_2O$	0.13	0.041	0.017	99%

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l) 5 min	15 min	30 min	Footnote
4-nitroso-α, α, α-triflurotoluene	$C_7H_4F_3NO_2$	16.3	14.8	15.2	96%
Ochratoxin A	$C_2OH_{18}ClNO_6$	18.5	16.1		b
1-octanol	$C_8H_{18}O$	6.2			
n-octanol	$C_8H_{18}O$		4.7		
2-octanone	$C_8H_{16}O$	17.7			
Patulin	$C_7H_6O_4$	7.5	2.7		b
"	"	3.5	1.3		x
Penicillic acid	$C_8H_{10}O_4$	15.9	8.7		b
"	"	4.7	1.5		w
Pentachloro-aniline	$C_6H_2C_{15}N$	13.0	12.1	11.9	j
Pentachloroethane	C_2HC_{15}	0.75			
Pentachloro-nitro-benzene	$C_6H_5NO_2$	3.8	3.8	3.8	
Pentachloro-phenol	$C_6HC_{15}O$	0.99			
"	"	0.70			d
"	"	0.92	0.61	0.52	
"	"	0.08			
2,4-pentanedione	$C_5H_8O_2$	1048			
Pentanol-3	$C_5H_{12}O$	1497			
Permethrin	$C_{21}H_{20}Cl_2O_3$	0.56			
Phenethyl alcohol	$C_8H_{10}O$	5.5	5.0	5.3	99%
Phenethylamine	$C_8H_{11}N$	14.9	13.0	12.4	99%
Phenol	C_6H_6O	25.9			
"	"	24.8			e
"	"	40.1			
"	"	30.5			
"	"	24.8			b
"	"	29.8	34.2	35.8	pH 6.7
"	"	29.8	34.2	35.8	
"	"	39.2			
"	"	42.0			
"	"	22.1			
"	"	24.8			
Phenoxyacetic acid	$C_8H_8O_3$	66.4	69.5	74.5	98%
N-phenylacetamide	C_8H_9NO	332	270	282	97%
Phenyl acetate	$C_8H_8O_2$	11.6	10.6	11.1	97%
Phenyl acetic acid	$C_8H_8O_2$	461	431	542	99%
Phenyl chloroformate	$C_7H_5ClO_2$	5.4	5.2	5.7	97%
Phenyl hydrazine	$C_6H_8N_2$	78.3	73.1	66.7	99%
Phenylisocyanate	C_7H_5NO	19.8	17.2	20.2	98%
1-phenyl-3-pyr-azolidinone	$C_9H_{10}N_2O$	3.0			d
Phenyl selenylchloride	C_6H_5ClSe	1.7	1.1	0.90	98%
1-phenylsemicarbazide	$C_7H_9N_3O$	8.9	9.3	10.7	99%

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l)			Footnote
		5 min	15 min	30 min	
4-phenylsemicarbazide	$C_7H_9N_3O$	85.0	69.1	57.5	97%
N-phenyl thiourea	$C_7H_8N_2S$	5.9	4.2	3.5	97%
4-phenyltoluene	$C_{13}H_{12}$	1.0	1.6	2.2	98%
Phthalonitrile	$C_8H_4N_2$	90.7	95.0	97.2	98%
Picric acid	$C_6H_3N_3O_7$	537		537	
Potassium cyanide	KCN	12.1			e
2-propanol	C_3H_8O	35390			
"	"	41579			b
n-propanol	C_3H_8O		8689		
"	"	17740	18576		
Propionitrile	C_3H_5N	5260		5260	
PR Toxin	$C_{17}H_{15}O_7$	7.8	2.1		b
Pyridine	C_5H_5N	2620	2129		
"	"			738	
Resorcinol	$C_6H_6O_2$	310			
Ridomil	$C_{15}H_{21}NO_4$	119			
Roundup	$C_6H_{17}N_2O_5P$	17.7			l
Rubratoxin B	$C_{26}H_{30}O_{11}$	32.0	35.1		b
Salicylaldehyde	$C_7H_6O_2$	16.5	14.3		
Simazine	$C_7H_{12}ClN_5$	237			l
Sodium cyanide	NaCN		2.8		pH 6.7
Sodium lauryl sulphate	$NaC_{12}H_{25}O_4S$	2.0			
"	"	1.2			e
"	"		1.5		99%
"	"	1.6			b
"	"		1.8		pH 6.7
"	"		1.2		
"	"		0.45		d
Sodium omadine 40%	NaC_5H_5NOS	0.12			40%
Sodium pentachlorophenate	NaC_6Cl_5O	0.93	0.76		
"	"	0.50			b
"	"		1.2		93%
Styrene	C_8H_8	5.5			
Sulphanilic acid	$C_6H_7O_3S$	43.5	60.1	114	99%
2,4,5-T	$C_8H_5Cl_3O_3$	79.0			l
"	"	157			k
Terephthalonitrile	$C_8H_4N_2$	82.7	73.7	70.4	r
2,3,4,5-tetrachloroaniline	$C_6H_3Cl_4N$	1.1	1.2	0.99	
2,3,5,6-tetrachloroaniline	$C_6H_3Cl_4N$	1.5	1.6	1.6	
1,2,3,4-tetrachlorobenzene	$C_6H_2Cl_4$	2.3	3.3	4.0	
"	"		1.9		a

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l)			Footnote
		5 min	15 min	30 min	
1,2,3,5-tetrachloro-benzene	$C_6H_2Cl_4$	3.3	3.5	2.5	
1,2,4,5-tetrachloro-benzene	$C_6H_2Cl_4$	10.1	6.5	4.5	
2,3,4,5-tetrachloro-nitrobenzene	$C_6HCl_6NO_2$	3.1	2.2	1.4	
2,3,4,5-tetrachloro-nitrobenzene	$C_6HCl_4NO_2$	7.2	7.0	8.3	f,g
2,3,4,5-tetrachloro-phenol	$C_6H_2Cl_4O$	0.34	0.20	0.18	
2,3,4,6-tetrachloro-phenol	$C_6H_2Cl_4O$	1.9	1.5	1.3	
2,3,5,6-tetrachloro-phenol	$C_6H_2Cl_4O$	2.8	2.5	2.2	
Tetrachloroethane	$C_2H_2Cl_4$	8.6			
Tetrachloroethylene	C_2Cl_4	17.0	19.5		f
Tetrahydroxyquinone	$C_6H_4O_6$	5.8	6.0	6.5	c,s
Tetrahydroxyquinone-bishydrate	$C_6H_8O_8$	5.9	6.1	6.7	
Thenoyltrifluoro-acetone	$C_8H_5F_3O_2S$	3.8			
Thiophenol	C_6H_6S	1.3	0.86	0.88	99%
Toluene	C_7H_8	49.5			
"	"	43.1			e
"	"		18.0		
"	"	33458			
"	"	48.4			
Toluene-4-sulphonyl-chloride	$C_7H_7ClSO_2$	2.0	2.3	2.4	99+%
Toluhydroquinone	$C_7H_8O_2$	0.40	0.39	0.43	94+%
p-toluoyl chloride	C_8H_7ClO	2.9	3.2	3.2	98%
p-tolylacetonitrile	C_9H_9N	0.11	0.12	0.12	98%
Trans-4-chloro-cinnamic acid	$C_9H_7ClO_2$	42.8	42.8	40.9	
Trans-p-nitro-cinnamic acid	$C_9H_7NO_4$	94.6	92.5	90.3	97%
Trans-4-nitro-cinnamic acid	$C_9H_7NO_4$	94.6	92.5	90.3	98%
2,4,6-tribromophenol	$C_6H_3Br_3O$	2.7			
2,3,4-trichloroaniline	$C_6H_4Cl_3N$	1.9	2.2	2.4	
2,4,5-trichloroaniline	$C_6H_4Cl_3N$	1.8	1.6	1.5	
2,4,6-trichloroaniline	$C_6H_4Cl_3N$	4.3	4.4	4.6	
3,4,5-trichloroaniline	$C_6H_4Cl_3N$	3.3	4.0	3.3	
1,2,3-trichlorobenzene	$C_6H_3Cl_3$	1.9	2.6	3.2	
"	"		2.5		

Table A.1 - continued

Compound	Formula	Microtox EC_{50} (mg/l)			Footnote
		5 min	15 min	30 min	
1,2,4-trichlorobenzene	$C_6H_3Cl_3$	2.3	3.7	4.0	
1,3,5-trichlorobenzene	$C_6H_3Cl_3$	12.8	14.1	14.1	
1,1,1-trichloroethane	$C_2H_3Cl_3$		8.0		
Trichloroethane	$C_2H_3Cl_3$	106			h
"	"	106			h
2,2,2-trichloroethanol	$C_2H_3Cl_3O$	1796			
Trichloroethylene	C_2HCl_3	162	117		
"	"	97.4	117		f
"	"		190		
2,3,4-trichloro-nitro-benzene	$C_6H_2Cl_3NO_2$	2.6	2.7	2.9	
2,4,5-trichloro-nitro-benzene	$C_6H_2Cl_3NO_2$	8.0	7.0	5.3	
2,4,6-trichloro-nitro-benzene	$C_6H_2Cl_3NO_2$	0.72	0.79	0.88	
2,3,4-trichloro-phenol	$C_6H_3Cl_3O$	1.8	1.6	1.2	
2,3,5-trichloro-phenol	$C_6H_3Cl_3O$	1.8	1.4	1.1	
2,3,6-trichloro-phenol	$C_6H_3Cl_3O$	14.0	13.3	12.7	
2,4,5-trichloro-phenol	$C_6H_3Cl_3O$	1.2	1.2	1.3	
2,4,6-trichloro-phenol	$C_6H_3Cl_3O$	7.2			
"	"	6.0	8.2	7.7	
3,4,5-trichloro-phenol	$C_6H_3Cl_3O$	0.44	0.38	0.36	
Triethylene glycol	$C_6H_{14}O_4$	32854			
Trifluoracetamide	$C_2H_2F_3NO$	15249	11567	11047	
4-trifluoromethyl-acetophenone	$C_9H_7F_3O$	6.5	7.2	7.5	95%
o ,o ,o -trifluoro-acetophenone	$C_8H_5F_3O$	138	120	95.7	99%
p-(trifluoromethyl-benzyl alcohol	$C_8H_7F_3O$	2.6	2.9	2.9	98%
3-trifluoromethyl-benzonitrile	$C_8H_4F_3N$	6.8	7.6	8.0	99%
o ,o ,o -trifluoro-toluene	$C_7H_5F_3$	19.7	23.2	32.0	99%
Trinitrotoluene	$C_7H_5N_3O_6$	19.8			b
Tyramine	$C_8H_{11}NO$	28.3	26.4	27.6	97%
Urea	CH_4N_2O	23914			b
Xanthone	$C_{13}H_8O_2$	7.8	7.0	7.5	99%
Xylene	C_8H_{10}	16.1			
o-xylene	C_8H_{10}		9.2		
Zearalenone	$C_{18}H_{22}O_5$	8.4	6.7		u
"	"	14.2	13.3		b

Notes for Table A.1

a - By extrapolation
b - Freshly reconstituted bacterial suspension
c - Mean of range given
d - Lowest concentration (highest toxicity) of range given
e - Measurements at room temperature
f - Measurements at 20 °C
g - Exposure time 10 mins
h - Isomer not stated in reference, value listed for all possible isomers
j - More than one compound with same name in reference
k - Analytical reference standard grade
l - Agrichemical grade
m - *Photobacterium fisherii*
n - Molecular weight assumed to be that of 2,4-D
o - Recrystallised from toluene/hexane
p - recrystallised from toluene
q - Recrystallised from hexane
r - Recrystallised from methanol
s - Values calculated from those for bishydrate
t - Recrystallised fron water/toluene
u - Measured at 10 °C; pH 6.0
v - Measured at 20 °C; pH 7.0
w - Measured at 25 °C; pH 6.5
v - Measured at 30 °C; pH 8.0
% values refer to the purity of the compound

Table A.2 - Comparison of the relative toxicity of pure organic chemicals to Microtox, *Daphnia*, rainbow trout, fathead minnow and *Chlorella pyrenoidosa* toxicity tests. Values in brackets represent the sensitivity of the acute lethality assay relative to Microtox (15 min Microtox EC_{50}/LC_{50} of other tests; values greater than one signify that Microtox was less sensitive while values less than one indicate that Microtox was more sensitive.)

Single Chemical	Microtox EC_{50} (mg/l) 5 min	15 min	30 min	*Daphnia magna* 48 hr LC_{50} (mg/l)	Rainbow trout 96 hr LC_{50} (mg/l)	Fathead minnow 96 hr LC_{50} (mg/l)	*Chlorella pyrenoidosa* 48 hr NOEC (mg/l)	Ref
acetone	22270	28940	-	15800 (1.8)	7400 [d] (3.9)	15000 [d] (1.9)	3400 (8.5)	1,2
allylamine	19.9	16.3	-	39 (0.4)	15 [d] (1.1)	2.1 [d] (7.8)	16 (1.0)	1,2
ammonia (total)	3607	-	-	129 (28)	62 (58)	-	-	4
ammonia (unionised)	1.5	-	-	0.8 (1.9)	1.4 (1.0)	-	-	4
aniline	425	488	-	0.64 (763)	43 [d] (11.3)	65 [d] (7.5)	11 (44.4)	1,2
benzene	214	238	-	400 (0.6)	56 [d] (4.3)	84 [d] (2.8)	-	1,2
benzonitrile	19	-	-	-	32 (0.6)	64 (0.3)	-	5
benzoquine	0.0085	-	-	-	0.13 (0.07)	0.045 (0.2)	-	5
catechol	32	-	-	-	8.9 (3.6)	3.5 (9.1)	-	5
Chlorobenzenes a								
mono	1.08	0.99	1.00	0.12 (8.25)	2.02 (0.49)	-	-	9
1,2	1.73	1.67	1.56	1.79 (0.93)	2.13 (0.78)	-	-	
1,3	1.68	1.55	1.46	0.72 (2.15)	2.00 (0.78)	-	-	
1,4	1.53	1.48	1.44	1.13 (1.31)	1.99 (0.74)	-	-	
1,2,4	1.89	1.69	1.66	0.56 (3.0)	2.01 (0.84)	-	-	
1,2,3,5	1.82	1.79	1.94	1.34 (1.33)	2.11 (0.84)	-	-	
1,2,4,5	1.33	1.52	1.68	-0.39	<1.66 (0.92)	-	-	
p-chlorobenzo-trifluoride	1.57	-	-	12.4 (0.13)	13.5 (0.12)	12.0 [b] (0.13)	-	8
2-chloroethanol	-	390.8	-	212 (1.8)	-	38.7 (10.1)	-	6
chloroform	435	-	-	758 (0.6)	32 (13.6)	-	-	4
Chlorophenols a								
2	0.54	0.51	0.58	<0.77 (0.66)	-	-	-	9
4	1.18	1.15	1.19	1.17 (0.98)	-	-	-	
2,4	1.54	1.51	1.47	<1.21 (1.25)	1.98 [c] (0.76)	-	-	
2,4,5	2.22	2.21	2.19	1.72 (1.28)	2.34 [c] (0.94)	-	-	
2,4,6	1.52	1.38	1.41	1.10 (1.25)	-	-	-	

Table A.2 - continued

Single Chemical	Microtox EC_{50} (mg/l) 5 min	15 min	30 min	*Daphnia magna* 48 hr LC_{50} (mg/l)
2,3,4,6	2.09	2.20	2.26	<2.37 (0.93)
2,3,5,6	1.92	1.96	2.02	1.97 (1.0)
2,3,4,5,6	2.46	2.64	2.71	2.25 (1.17)
o-chlorotoluene	6.14	6.19	-	-
o-cresol	20.7	15.4	-	9.5 (1.6)
	32	-	-	-
m-cresol	8.2	-	-	-
p-cresol	1.3	-	-	-
cyanide	13.3	-	-	6.1 (2.2)
	2.8-3.5	-	-	0.08-0.1 (28)
2,4-D	112	107	128	>240 (0.4)
3,4-dichlorobenzo-trifluoride	2.78	3.57	-	10.2 (0.35)
1,2-dichloroethane	-	158	-	1430 (0.11)
2,6-dimethyl-quinoline	5.7	6.3	-	-
6,7-dimethyl-quinoline	1.3	1.9	-	-
6,8-dimethyl-quinoline	2.2	2.4	-	-
2,3-dinitrotoluene	-	7.57 g	-	4.7 (1.6)
2,4-dinitrotoluene	-	8.26 g	-	35.0 (0.24)
2,5-dinitrotoluene	-	3.45 g	-	3.4 (1.0)
2,6-dinitrotoluene	-	20.45 g	-	21.7 (0.93)
3,4-dinitrotoluene	-	4.28 g	-	3.1 (1.4)
Esteron	10.3	10.3	8.4	13.1 (0.8)

Rainbow trout 96 hr LC_{50} (mg/l)	Fathead minnow 96 hr LC_{50} (mg/l)	*Chlorella pyrenoidosa* 48 hr NOEC (mg/l)	Ref
2.60 [c] (0.85)	-	-	
-	-	-	
3.13 [c] (0.84)	-	-	
2.3 (2.7)	7.5 (0.8)	-	8
13 [d] (1.2)	34 [d] (0.45)	34 (0.45)	1, 2
8.4 (3.8)	18 (1.8)	-	5
8.9 (0.9)	56 (0.15)	-	5
8.6 (0.2)	29 (0.04)	-	5
0.15 (89)	-	-	4
-	0.1-0.2 (14)	-	7
-	-	-	12
11.9 (0.3)	12.8 [b] (0.3)	-	8
198 (0.80)	-	-	4
6.2 [d] (1.0)	-	-	3
7.6 [d] (0.25)	-	-	3
2.6 [d] (0.92)	-	-	3
-	1.9 (4.0)	-	13
-	32.5 (0.25)	-	13
-	1.3 (2.7)	-	13
-	19.8 (1.02)	-	13
-	1.5 (2.9)	-	13
-	-	-	12

Table A.2 - continued

Single Chemical	Microtox EC_{50} (mg/l) 5 min	15 min	30 min	*Daphnia magna* 48 hr LC_{50} (mg/l)
2-(2-ethoxyethoxy)-ethanol	-	10954	-	4010 (2.73)
ethylacetate	5160	5870	-	590 (9.9)
ethylpropionate	612	811	-	250 (3.2)
n-heptanol	14.5	19.9	-	65 (0.3)
hexachloroethane	-	8.3	-	1.4 (5.9)
hydroquine	0.079	-	-	-
2-methyl-2,4-pentanedione	-	1447.5	-	7060 (0.21)
2-methyl-1-propanol	-	1224.6	-	1110 (1.1)
m-nitrobenzonitrile	-	3.96 [g]	-	48.1 (0.08)
p-nitrobenzonitrile	-	4.66 [g]	-	49.4 (0.09)
pentachlorophenol	0.94	0.76	-	0.48 (1.6)
	-	1.0	-	0.1 (10)
	0.08-0.15	-	-	0.14-0.28 (0.5)
2,4-pentanedione	-	373.0	-	47.6 (7.8)
phenol	22.0	-	-	32 (0.69)
	25	-	-	-
	21-41	-	-	10-23 (1.8)
	22.0-40.2	34.0	-	7.0-88.0
n-propanol	17700	18400	-	6300 (2.9)
pyridine	2590	2120	-	1080 (2.0)
resorcinol	310	-	-	-
salicylaldehyde	16.3	14.3	-	5.8 (2.5)

Rainbow trout 96 hr LC_{50} (mg/l)	Fathead minnow 96 hr LC_{50} (mg/l)	*Chlorella pyrenoidosa* 48 hr NOEC (mg/l)	Ref
-	26400 (0.41)	-	6
260 [d] (22.6)	270 [d] (21.7)	>1000 (<5.9)	1,2
56 [d] (14.5)	70 [d] (11.6)	320 (2.5)	1,2
43 [d] (0.5)	34 [d] (0.6)	18 (1.1)	1,2
-	1.3 (6.4)	-	6
0.097 (0.8)	0.044 (1.8)	-	5
-	8690 (0.17)	-	6
-	1510 (0.8)	-	6
-	60.2 (0.07)	-	13
-	24.4 (0.19)	-	13
0.2 [d] (3.8)	0.21 [d] (3.6)	-	1,2
-	0.3 (3.3)	-	6
-	0.2-0.5 (0.7)	-	7
-	142 (2.6)	-	6
9.9 (2.2)	-	-	4
8.9 (2.8)	68 (0.37)	-	5
-	-	-	7
5.0-11.6	24.0-67.5	-	10
3200 [d] (5.8)	5000 [d] (3.7)	1150 (16)	1,2
560 [d] (3.8)	115 [d] (18.4)	150 (14.1)	1,2
>100 (<3.1)	100 (3.1)	-	5
1.35 [d] (10.6)	4.2 [d] (3.4)	10 (1.43)	1,2

Table A.2 – continued

Single Chemical	Microtox EC_{50} (mg/l) 5 min	15 min	30 min	*Daphnia magna* 48 hr LC_{50} (mg/l)	Rainbow trout 96 hr LC_{50} (mg/l)	Fathead minnow 96 hr LC_{50} (mg/l)	*Chlorella pyrenoidosa* 48 hr NOEC (mg/l)	Ref
sodium lauryl sulphate	1.6-3.2	-	-	7.3-13	-	6.2-9.6	-	7
styrene	5.4	-	-	59 (0.09)	2.5 (2.2)	-	-	4
toluene	20-34	-	-	15-23	-	23-50	-	7
	30-135 [f]	-	-	-	8 (>3.8) [e]	-	-	11
2,2,2-trichloro-ethanol	43.9	-	-	148 (0.30)	-	173 (0.25)	-	6
trichloroethylene	156	115	-	94 (1.2)	42 [d] (2.7)	47 d (2.4)	-	1,2
2,3,6-trinitrotoluene	-	3.73 [g]	-	0.69 (5.4)	-	0.12 (31.1)	-	13
2,4,6-trinitrotoluene	-	6.54 [g]	-	11.9 (0.55)	-	2.40 (2.73)	-	13

Notes

[a] all values expressed as log (1/mmol)
[b] bluegill sunfish
[c] brown trout, *Salmo trutta*
[d] 48 hour LC50
[e] *Onchorhynchus gorbuscha*
[f] 10 min EC50
[g] exposure time not stated

References

1. De Zwart and Sloof (1983)
2. Sloof *et al* (1983)
3. Birkholz *et al* (1990)
4. Qureshi *et al* (1982)
5. Lebsack *et al* (1981)
6. Nacci *et al* (1986)
7. Elnabrawy (1986)
8. Casseri *et al* (1983)
9. Ribo and Kaiser (1983)
10. Walker (1988)
11. Vasseur *et al* (1984)
12. Miller *et al* (1985)
13. Shiotsuka *et al* (1980)

Table A.3 - Summary of correlations (r^2) between Microtox and specified toxicity tests

Response	Organism	chlorophenols (1)	chloro-anilines (5)	p-substituted phenols (1)	chlorobenzene (1)	substituted chlorobenzenes (2)	ketones (3)	ethanes (3)	alcohol (3)	organics
log P	chemical	0.91,n=18 [a]	0.80,n=15 [c]	-	0.76,n=11	0.77	-	-	-	0.68 (2)
	test	0.79,n=20	-	-	-	-	-	-	-	-
96 h LC_{50}	*Nitocra spinipes*	-	-	-	-	-	-	-	-	(0.75,n=16 [d] (6))
24 h LC_{50}	*Daphnia*	0.87,n=8	-	-	-	-	-	-	-	-
48 h LC_{50}	*Daphnia*	-	-	-	0.83,n=4	-	-	-	-	-
96 h LC_{50}	Shrimp	0.68,n=7	-	-	-	-	-	-	-	-
24 h LC_{50}	brown trout	0.92,n=6	-	-	-	-	-	-	-	-
24 h LC_{50}	bluegill	0.77,n=7	-	-	-	-	-	-	-	-
96 h LC_{50}	bluegill	-	-	-	0.71,n=6	-	-	-	-	-
95 h LC_{50}	sheepshead	-	-	-	0.80,n=5	-	-	-	-	-
96 h LC_{50}	fathead minnow	-	-	-	-	-	0.81,n=7	0.99,n=4	0.96,n=8	0.85,n=31 (2) 0.65,n=68 (3) 0.91 (4)
96 h LC_{50}	golden orfe	-	-	-	-	-	-	-	-	0.71 (4)
7-14 d LC_{50}	guppy (static)	0.89, n=11	-	-	0.86,n=9	-	-	-	-	-
ip LD_{50} [b]	rainbow trout	-	-	0.93,n=9	0.74,n=9	-	-	-	-	-

Notes

[a] without ortho-substituted phenols
[b] intraperitoneal injection
[c] chloroanilines excluding pentachloroaniline
[d] including some inorganic compounds

References

1. Ribo and Kaiser (1983)
2. Blum and Speece (1990)
3. Curtis *et al* (1982)
4. Indorato *et al* (1984)
5. Ribo and Kaiser (1984)
6. Tarkpea *et al* (1986)

Table A.4 - Comparison of the relative toxicity of pure organic chemicals to Microtox, *Daphnia*, rainbow trout, fathead minnow, oyster embryo and *Chlorella pyrenoidosa*. (Values in brackets represent the sensitivity of the acute lethality assay relative to Microtox; values greater than one signify that Microtox was less sensitive while values less than one indicate that Microtox was more sensitive.)

Single chemical	Microtox EC_{50} (mg/l) 5 min	15 min	30 min	*Daphnia magna* 48 hour LC_{50} (mg/l)	Rainbow trout 96 hr LC_{50} (mg/l)	Fathead minnow 96 hr LC_{50} (mg/l)	*Crassostrea gigas* 48 hr LC_{50} (mg/l)	*Chlorella pyrenoidosa* 48 hr NOEC (mg/l)	Ref
arsenate (As 5+)	35.0	-	-	5.4 (6.5)	43 (0.81)	-	-	-	2
cadmium	-	11.6	-	-	-	-	1.1 (10.5)	-	1
(Cd 2+)	70-90	-	-	0.02-0.16	-	0.01-0.14	-	-	3
	1070	218	-	0.046 (>4500)	0.15 (>1400) [a]	2.2 (100) [a]	-	3.1 (70)	4,5
	106	25	14	0.041 (610)	-	-	-	-	6
	-	-	7-60	-	-	55 (1-8) [b]	-	-	7
chromium	70-90	13	-	0.10-0.13 (100)	-	12-53 (1.0)	-	-	3
(Cr 6+)	-	-	42-58	-	-	31 (1.4-1.9) [c]	-	-	7
cobalt	135-177	16	-	4.7-13 (1.2)	-	50-70 (0.32)	-	-	3
copper	-	0.076	-	-	-	-	0.012 (6.3)	-	1
	7.4	-	-	0.02 (370)	0.25 (30)	-	-	-	2
	1.2	0.42	0.24	0.064 (6.6)	-	-	-	-	6
	-	-	0.5-2.0	-	-	78 (0.006) [b]	-	-	7
	4-20	-	-	0.01-0.06	-	-	-	-	3
lead	-	1.7	-	-	-	-	0.68 (2.5)	-	1
mercury	0.08	-	-	0.03 (2.7)	0.21 (0.38)	-	-	-	2
	0.03-0.07	-	-	0.01-0.06 (1.0)	-	0.16-0.66 (0.44)	-	-	3
	0.044-0.032	-	-	0.005 (6.4)	0.65 (0.5) [a]	0.05 (0.64) [a]	-	1.3 (0.03)	4,5
silver	-	0.595	-	-	-	-	0.019 (31.3)	-	1
zinc	-	0.44	-	-	-	-	0.207 (2.1)	-	1
	49	-	-	5.1 (9.6)	2.2 (22)	-	-	-	2
	2-14	-	-	1.0-1.2 (2.0)	-	0.5-1.7 (>1.2)	-	-	3
	12	1.6	0.7	0.54 (3.0)	-	-	-	-	6
	-	1.4-8	-	-	-	66 (<0.1)	-	-	7
zirconium	>4.3	-	-	-	>20 (0.22)	-	-	2.6 [d] (>1.65)	8

Table A.4 - continued

Notes

[a] 48 hour LC_{50}
[b] *Fundulus heteroclitus*
[c] *Aldrichetta fosteri*
[d] *Selenestrum capricornutum*

References

1. Nacci *et al* (1986)
2. Qureshi *et al* (1982)
3. Elnabarawy (1986)
4. De Zwart and Sloof (1983)
5. Sloof *et al* (1983)
6. Miller *et al* (1985)
7. Vasseur *et al* (1984)
8. Couture *et al* (1989)

Table A.5 - A comparison of Microtox with *Daphnia*, rainbow trout and fathead minnow toxicity tests for the assessment of toxicity of industrial effluents

Effluent type	Effluent concentration (%) causing a 50 % effect				Rank order of toxicity (sensitivity)	Reference
	Microtox 15 min EC_{50}	Rainbow trout 96 hour LC_{50}	*Daphnia* 48 hour EC_{50}	Fathead Minnow 96 hour LC_{50}		
Waste water influent	< 6.3		15.9	23.6	M > D > FM	1
Waste water influent	4.3		16.4	26.8	M > D > FM	1
Waste water influent	11.1		31.6	23.6	M > FM > D	1
Waste water influent	19.5		> 50.0	38.7	M > FM > D	1
Waste water influent	13.6		66.3	50.8	M > FM > D	1
Waste water influent	7.2		22.3	29.1	M > D > FM	1
Waste water primary effluent	< 6.3			28.7	M > FM	1
Waste water primary effluent	< 6.3		24.0	23.6	M > FM > D	1
Waste water primary effluent	< 6.3		34.7	26.8	M > FM > D	1
Waste water primary effluent	5.2		45.0	> 50.0	M > FM > D	1
Waste water primary effluent	23.9		72.6	53.5	M > FM > D	1
Waste water primary effluent	6.3		22.9	35.4	M > D > FM	1
Waste water secondary effluent	> 50.0			> 100.0	M = FM	1
Waste water secondary effluent	> 50.0			> 100.0	M = FM	1
Waste water secondary effluent	> 50.0		> 100.0	> 100.0	M = D = FM	1
Waste water secondary effluent	>100.0		> 100.0	> 100.0	M = D = FM	1
Waste water secondary effluent	>100.0		> 100.0	> 100.0	M = D = FM	1
Waste water secondary effluent	>100.0			> 100.0	M = FM	1
Electrofinishing discharge	6.8		5.7 (a)	100.0	D > M > FM	2
Electrofinishing discharge	40.0		7.5 (a)	NT	D > M > FM	2
Electrofinishing discharge	NT		NT (a)	NT	D = M = FM	2
Electrofinishing discharge	27.0		9.7	NT	D > M > FM	2
Pulp mill effluent	2.5 (b)	17.0	34.0		M > RT > D	3
Pulp mill effluent	8.4	37.0			M > RT	3
Chemical plant effluent	50<EC50<100	51.0			RT > M	3
Chemical plant effluent	15.0	71.0	23.0		M > D > RT	3
Chemical plant effluent	40.0	7.1			RT > M	3
Chemical plant effluent	34.0	NT	39.0		M > D > RT	3
Oil refinery waste	6.5	71.0	78.0		M > RT = D	3
Oil refinery waste	50<EC50<100	NT			M > RT	3
Packaging plant dye waste	1.5	0.9	0.3		D > RT > M	3

Table A.5 - continued

Effluent type	Effluent concentration (%) Microtox 15 min EC_{50}	Rainbow trout 96 hour LC_{50}
STP effluent	SS	NT
STP effluent	SS	NT
STP effluent	30	43
STP primary treated effluent	25.21 (+/-10.7)	88.12 (+/-11.5)
STP primary treated effluent	24.77 (+/-8.2)	88.12 (+/-11.5)
STP primary treated effluent	13.10 (+/-8.45)	74.0 (+/-5.26)
STP secondary treated effluent	98.1 (+/-3.4)	122.3 (+/-30.3)
STP tertiary treated effluent	>100	111.0
Pesticide Industry Waste Leachate	0.22	
Pesticide Industry Waste Leachate	0.20	
Pesticide Industry Waste Leachate	0.175	
Pesticide Industry Waste Leachate	0.28	
Pesticide Industry Waste Leachate	2.15	
Pesticide Industry Waste Leachate	6.7	
Pesticide Industry Waste Leachate	0.258	
Pesticide Industry Waste Leachate	0.225	
Pesticide Industry Waste Leachate	0.238	
Pesticide Industry Waste Leachate	0.325	
Pesticide Industry Waste Leachate	0.295	
Electroplating sludge leachate	36.0	
Electroplating sludge leachate	67.8	
Electroplating sludge leachate	58.0	
Electroplating sludge leachate	75.3	
Electroplating sludge leachate	0.243	
Electroplating sludge leachate	0.215	
Electroplating sludge leachate	0.220	
Electroplating sludge leachate	0.400	
Electroplating sludge leachate	0.210	
Electroplating sludge leachate	51.7	
Electroplating sludge leachate	0.448	

causing a 50 % effect *Daphnia* 48 hour EC_{50}	Fathead Minnow 96 hour LC_{50}	Rank order of toxicity (sensitivity)	Reference
NT		M = RT = D	3
NT		M = RT = D	3
16		D > M > RT	3
		M > RT	4
		M > RT	4
		M > RT	4
		M > RT	4
			4
0.0000625		D > M	5
0.00025		D > M	5
0.00007		D > M	5
0.00265		D > M	5
0.0038		D > M	5
0.0064		D > M	5
0.0018		D > M	5
0.0046		D > M	5
0.0011		D > M	5
0.000385		D > M	5
0.007		D > M	5
1.45		D > M	5
2.74		D > M	5
3.35		D > M	5
42.30		D > M	5
0.051		D > M	5
0.041		D > M	5
0.090		D > M	5
0.11		D > M	5
0.142		D > M	5
9.3		D > M	5
0.135		D > M	5

Table A.5 - continued

Effluent type	Effluent concentration (%) causing a 50 % effect				Rank order of toxicity (sensitivity)	Reference
	Microtox 15 min EC_{50}	Rainbow trout 96 hour LC_{50}	*Daphnia* 48 hour EC_{50}	Fathead Minnow 96 hour LC_{50}		
Fossil-fuel process water	1.3	0.42 (c)		0.57 (c)	RT > FM > M	6
Fossil-fuel process water	0.37	0.52 (c)		0.64 (c)	M > RT > FM	6
Fossil-fuel process water	1.27	0.068 (c)		0.071 (c)	RT > FM > M	6
Fossil-fuel process water	1.6	1.5 (c)		3.0 (c)	RT > M > FM	6
Fossil-fuel process water	0.27	0.18 (c)		0.19 (c)	RT > FM > M	6
Fossil-fuel process water	0.38	0.35 (c)		0.65 (c)	RT > M > FM	6
Fossil-fuel process water	0.11	0.09 (c)		0.17 (c)	RT > M > FM	6
Fossil-fuel process water	0.12			0.10	FM > M	6
Fossil-fuel process water	0.21			0.20	FM > M	6
Fossil-fuel process water	0.20			0.17	FM > M	6
Fossil-fuel process water	0.65			0.48	FM > M	6
Fossil-fuel process water	0.18			0.071	FM > M	6
Fossil-fuel process water	5.3			6.7	M > FM	6
Fossil-fuel process water	2.2			3.0	M > FM	6
Fossil-fuel process water	0.37			0.90	M > FM	6
Fossil-fuel process water	1.6			0.6	FM > M	6

Note: NT = Non toxic
STP = Sewage treatment plant
SS = slight stimulation at 100 % concentration
M = Microtox, RT = Rainbow Trout, D = *Daphnia*, FM = Fathead Minnow

(a) = *Daphnia pulex* (all other Daphnia results refer to D magna)
(b) = All Microtox EC50 values quoted for reference 3 are for 5 min exposure. All others are for 15 min exposure.
(c) = Flow-through toxicity tests

All concentrations are calculated on a volume : volume basis

References

1. Neiheisel *et al* (1983)
2. Szal (1985)
3. Qureshi *et al* (1982)
4. Rowlen *et al* (1983)
5. Calleja *et al* (1986)
6. Lesback *et al* (1981)

NRA

Awdurdod Afonydd Cenedlaethol
Rhanbarth Cymru

National Rivers Authority
Welsh Region

12th November 1991

Dear Dr Sweetmey

Please find enclosed a copy of the interim report **Review of the MICROTOX Toxicity Test** for your consideration - any comments you have could be made at our ECOTOX meeting on 21st November. I also hope to be able to send you a draft copy of the **TOXICITY INFORMATION SYSTEM** proposal by the end of the week (pending certain alterations which I have asked for). Sorry to load you up with reading at short notice.

Yours sincerely

R.A.Milne
Tox. Scientist