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Vapor Sampling Device for Interface with Microtox Assay for Screening Toxic Industrial Chemicals

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Foreword

The characterization of multi-component detection systems for chemical and biological agents is an integral part of the EPA National Homeland Security Research Center, Safe Buildings Program. The work described in this report was designed to meet the following guideline referred to in the EPA's Safe Buildings Program "conduct research to adapt existing technology for the purpose of homeland security." More specifically, each of the herein reported sampling and assay technologies has been previously described for an application other than homeland security.

The primary goal of this project is to adapt semipermeable membrane devices (SPMDs) sampling technology to chemical vapor monitoring, and interface this technique to commercially available bioassay methods. This EPA report is a product of this effort and describes the characteristics and potential use of a toxicity-based screening system for the cleanup and remediation of buildings that may have been contaminated with toxic industrial chemicals.

Abstract

A time-integrated sampling system interfaced with a toxicity-based assay is reported for monitoring volatile toxic industrial chemicals (TICs). Semipermeable membrane devices (SPMDs) using dimethyl sulfoxide (DMSO) as the fill solvent accumulated each of 17 TICs from the vapor phase. Uptake kinetics experiments for one of these compounds (acrolein) indicated that it was significantly sequestered (i.e., 10 percent of the 24 hr maximum) in as little as 10 min and was concentrated by a factor of over 200 in 24 hr as measured using both mass and toxicity assays. The effect of each of the TICs on the Microtox bacterial luminescence assay was determined both from a direct assay and a vapor accumulation assay using SPMDs. Microtox EC_{50} values (concentration yielding 50 percent inhibition) were determined for each of the TICs analyzed and ranged from 0.070 parts per million volume (ppmv) for diketene to 322 ppmv for 1,2-dibromoethane. The rank order of the Microtox EC_{50} values for each compound measured directly from liquid was similar but not identical to the Apparent (App) EC_{50} values determined from the vapor accumulation assay. The ratios of the EC_{50} and the $AppEC_{50}$ values were used to calculate toxicity-derived Concentration Factors (i.e., the toxicity equivalents of compound that concentrate from vapor into the SPMD). These Concentration Factors ranged from 17 to 5400 and primarily reflected differences in partitioning characteristics between air and DMSO for each compound. Acrolein was chosen as a representative compound for the vapor dilution experiment and it showed a toxicity-based detection limit of 19 mg/m^3 which was less than the LD-50 by a factor of 100, but greater than the National Institute for Occupational Safety and Health (NIOSH) 40 hr/week exposure limit also by a factor of 100. Consequently, for acrolein, this system in its current configuration, shows potential for development as an initial screening tool for mid to high acute vapor phase toxicity determinations.

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List of Acronyms

AppEC_{50}	Similar to the EC_{50} except that the assay was performed using a stock solution of DMSO that had accumulated the test compound through an SPMD.
CWA	chemical warfare agent
DMSO	dimethyl sulfoxide
DoD	Department of Defense
DOE	Department of Energy
EC_{50}	For the purpose of this report, the EC_{50} is the concentration of test compound yielding 50 percent inhibition in luminescence response (as compared to a control assay) and performed using a DMSO stock solution of this compound.
GC/MS	gas chromatography/mass spectrometry
LD-50	The concentration of a test compound that yields 50 percent mortality under specified conditions and for a specified species.
HSARPA	Homeland Security Advanced Research Project Agency
NHSRC	National Homeland Security Research Center
NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupational Safety and Health Agency
ppbv	parts per billion by volume
ppmv	parts per million by volume
SPMD	semi permeable membrane device
TIC	toxic industrial chemical
USGS-CERC	United States Geological Survey-Columbia Environmental Research Center

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Section 1
Background

Monitoring of toxic industrial chemicals in support of potential building remediation applications presents some unique analytical challenges. More specifically, remediation and re-occupation of a chemically contaminated building typically takes place over a period of months to years. Because structural building materials that have been contaminated with volatile or semi-volatile toxic industrial chemicals (TICs) may slowly release these compounds, there exists a potential for human exposure throughout the cleanup process. Consequently, continuous and time-integrated air monitoring for possible exposure to these acutely toxic and potentially genotoxic compounds is a task of considerable importance. Although several analytical methods are available to measure a limited number of specific compounds, air monitoring techniques able to capture and screen for a broad range of compounds and agent simulants that are toxic, but not expected to be detected by currently available chemical sensor technologies, may prove to be extremely useful in the cleanup of chemically contaminated buildings.

In contrast to the analytical needs of the DoD, DOE, and Department of Homeland Security (through the Homeland Security Advanced Research Project Agency, HSARPA) which appear to be primarily focused on prevention and first responder monitoring needs [2,3], the analytical needs of the EPA's Safe Buildings Program are focused on support of cleanup and remediation of contaminated buildings. In addition, the efforts of HSARPA, as evidenced by recent calls for proposals, have been focused on 20 target compounds, more than half of which are well known chemical warfare agents (CWAs) and focused little attention on the dozens, if not hundreds, of potentially toxic industrial chemicals.

The research strategy reported herein focuses on two areas. The first area involves the development of sampling technology that will provide a time-integrated concentration record for numerous locations throughout a potentially contaminated building. The next involves the use of screening assays to detect accumulated toxic chemicals on the basis of their potential biological / biochemical function. This report describes the adaptation and characterization of semipermeable membrane devices (SPMDs) as time integrated air monitors. SPMDs have been extensively used for water monitoring and are often interfaced with toxicological and genotoxicological screening assays for exposure assessment and remediation of environmental pollution, particularly in ecological assessment of sediments [4,5]. The adaptation of these techniques for a screening level assessment of indoor air is expected to provide new tools in the remediation of buildings that have been contaminated with an unknown mixture of hazardous compounds. These devices have been integrated with the Microtox acute toxicity screening assay. Although the Microtox assay has also been extensively used to screen for hazardous chemicals associated with water quality in waste water treatment systems [6,7], it has not been widely used for air monitoring, nor has it been evaluated for many of the herein reported TICs. Potential applications for this work with respect to building decontamination will involve providing (i) a time-integrated record of chemical toxin concentrations present in the air over a specified time period; (ii) a passive sample collection system to which acute toxicological screening assays can be interfaced; and (iii) a demonstration of the feasibility of toxicity-based assays such as the Microtox system for use in building decontamination applications.

Section 2
Methods

2.1 Reagents and Protocols

All reagents, other than the Microtox reagents, were obtained from Sigma/Aldrich (St. Louis, MO). A complete list of the TICs evaluated in this study is found in Appendix A.

The Microtox assay is based on observed changes in luminescence due to exposure of the test organism *Vibrio fisheri* to toxic chemicals. The organisms are supplied as lyophilized reagent that is reconstituted in 2 percent NaCl buffer prior to assay. The assay measures light output of the organism after exposure to the sample as compared to the control blank. Light output is measured after 5 and 15 min exposures. A serial dilution profile is used to determine the concentration of sample resulting in a 50 percent reduction in the luminescence as compared to the blank under the same conditions. This value is reported by the Microtox Omni software as the EC_{50}. For an example of the Microtox report see Appendix B.

The Microtox 500 analyzer and acute toxicity reagent (i.e., lyophilized *V. fisheri*) were obtained from SDI (Newark, DE). Assays were performed following the SDI basic protocol for pure compounds [8]. The EC_{50} values for both phenol and zinc sulphate, typically used as positive controls, were similar to previously reported values [8]. For direct measurement of the Microtox toxicity responses to the TICs, stock solutions were prepared in dimethyl sulfoxide (DMSO). From these stocks a dilution of 1 to 100 was made into the Microtox assay buffer. This solution was the maximum concentration in the Microtox serial dilution profile. The "Basic Protocol for Pure Compounds" was used with 4 or 9 dilutions. EC_{50} values for the 5 and 15 min assays were recorded along with 95 percent confidence limits.

The SPMDs were prepared using low density polyethylene tubing (25 mm x 88 µm wall thickness) as previously described [5] with the exception that DMSO rather than triolein was used as the trapping solvent. The tubing was cut into 75 mm lengths and heat sealed on one end, followed by addition of 100 µL DMSO and heat sealed on the other end. These SPMDs were then placed into 40 mL vials or 4 L glass bottles (suspended near the center of volume). For accumulation experiments, a specific volume of compound was spiked onto the side of the vial without contacting the SPMD and the container sealed. Each of the compounds analyzed completely vaporized in the vial head space in less than 2 min. After a specified time (typically 24 hr), the SPMD was removed from the vial, cut open and the DMSO analyzed for toxicity (Figure 1). Photographs of SPMDs and exposure chambers are shown in Appendix C. Apparent EC_{50} (App-EC_{50}) values were also calculated using the "Basic Protocol for Pure Compounds" from dilutions of the DMSO in the SPMDs after exposure to vapor phase TICs.

GC/MS analyses were conducted using a Finnigan Voyager system. Separations were performed using a 0.1 µL head space injection onto a capillary column maintained at 30° C until elution of the acrolein, then the temperature was ramped to 200° C for elution of the DMSO. The 55 + 56 mass ions were used to construct a calibration curve.

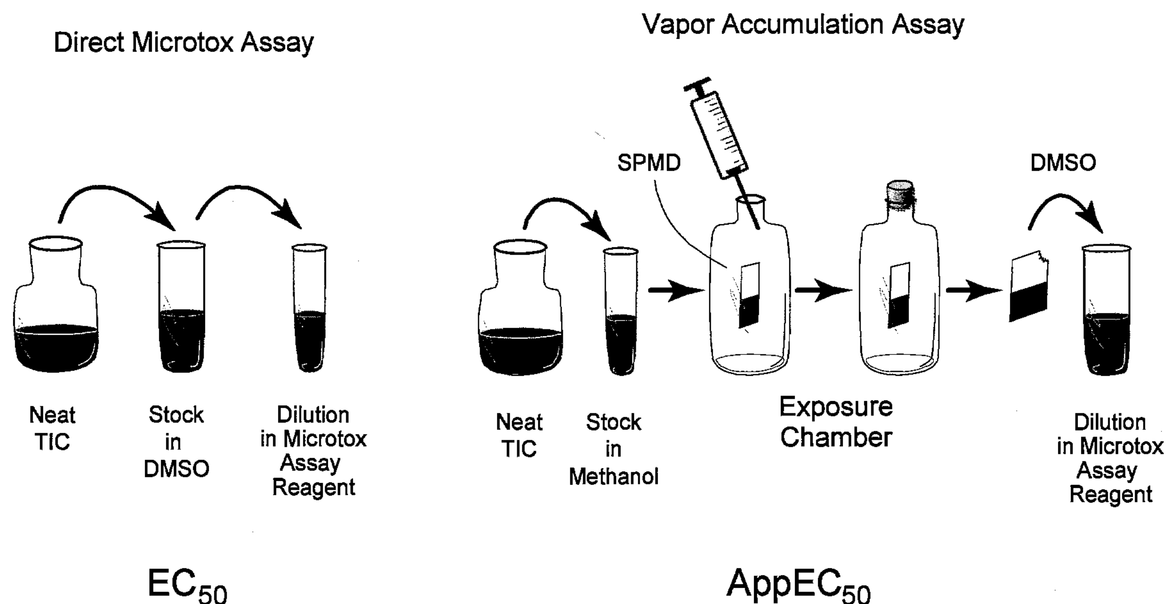

Figure 1. Experimental protocol diagram.

2.2 Calculations and Data Handling

The Microtox Omni software calculates the EC_{50} values for 5 and 15 min assays based on the comparison of luminescence between treated and control organisms at various times and at various dilutions [8]. For the pure compound protocols, the primary operator-entered variable is the initial concentration of the test compound. EC_{50} values for each test were determined using stock solutions of the compounds in DMSO. The value of the initial dilution (i.e., highest concentration) used in the Microtox assay allows the software to calculate the EC_{50} value for a series of serial dilutions.

In the case of the vapor accumulation experiments, the actual concentration of the test compound present in the DMSO was not known. Although the vapor concentration of the compound added to the exposure chamber was known, the amount of compound that actually accumulated into the DMSO in the SPMD was not directly measured (except for in the case of acrolein). The amount of test compound introduced to the Microtox assay from the DMSO and entered into the Omnitox software was based on an estimate of the lowest expected concentration of test compound (i.e., no concentration of the test compound vapor into the SPMD). Because $AppEC_{50}$ values were lower than EC_{50} values, this observation indicated the accumulation of test compounds from the vapor phase to solution phase in DMSO.

To determine the extent that a compound was concentrated in the DMSO in the SPMD, the following rationale was used: if the toxicity measured using the Microtox assay for a 100 µL solution (DMSO) spiked with a specific volume of pure test compound was the same as in the 100 µL of DMSO in the SPMD, then the Concentration Factor (calculated as the ratio of EC_{50} to $AppEC_{50}$) would be one.

The timescale for which the Microtox organisms are inhibited is not the same for all compounds. Consequently, 5 and 15 min incubation protocols were used. To compare assay results for these protocols among various test compounds, a relative change indicator was used. The percent relative change in EC_{50} between 5 and 15 min was calculated using the following relationship:

$$\% \text{ Relative Change} = \frac{EC_{50/5} - EC_{50/15}}{EC_{50/5}} \times 100$$

where $EC_{50/5}$ is the EC_{50} value determined for a 5 min assay time and $EC_{50/15}$ for a 15 min assay time.

Safety note: Due to the volatile and toxic nature of the compounds analyzed, all manipulations using these compounds with the SPMDs were performed in a fume hood with appropriate personal protection equipment.

Section 3
Results and Discussion

3.1 EC_{50} Values for Test Compounds in Solution

The toxic industrial chemicals included in this study are listed in a recent National Institute of Justice Report. For the herein reported study, these compounds were evaluated for acute toxicity using the Microtox assay [1]. Table 1 shows data from assays using stock solutions prepared directly in DMSO from neat compounds (listed as EC_{50} values) and those sampled from DMSO that had been placed in SPMDs and exposed to compound vapors for 24 hrs (listed as $AppEC_{50}$ values). The EC_{50} values were calculated for both 5 min and 15 min assays (i.e., corresponding to the incubation time of the sample with the Microtox reagent).

The EC_{50} values were determined for each of the TICs yielded values ranging from 322 parts per million by volume (ppmv) to 0.070 ppmv (Figure 2). These EC_{50} values form a continuum but appear to fall into 3 groupings consisting of low (e.g., diketene – sulfuryl chloride), medium (e.g., formaldehyde – methylhydrazine) and high (acetone cyanohydrin – 1,2-dibromoethane) values. It should be noted that low EC_{50} values indicate a more toxic response.

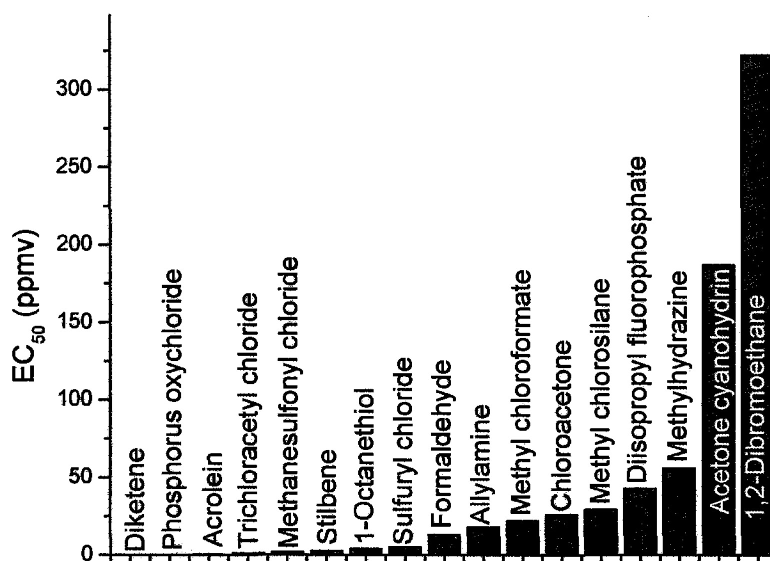

Figure 2. Rank order for EC_{50} values. EC_{50} values were determined using TICs assayed from solution.

3.2 $AppEC_{50}$ for Test Compounds Accumulated from Vapor

$AppEC_{50}$ values were determined for the accumulation of compounds in the vapor phase into DMSO present in the SPMD. These vapor phase experiments were conducted using smaller (40 mL) or larger (4 L) incubation chambers to measure the 24 hr accumulation of test compounds into SPMD/DMSO. For the vapor measurements, DMSO was recovered from SPMDs and diluted 100 fold prior to being assayed (see Figure 1). Although the initial vapor phase concentrations of compounds in the vials were known, the concentrations of the compounds in the DMSO (sampled by SPMDs) from the vapor phase (with the exception of acrolein) were not directly determined. The Microtox assay responses (i.e., toxic activities) of compounds sampled from the SPMD, however, were determined and reported as $AppEC_{50}$ values (Figure 3).

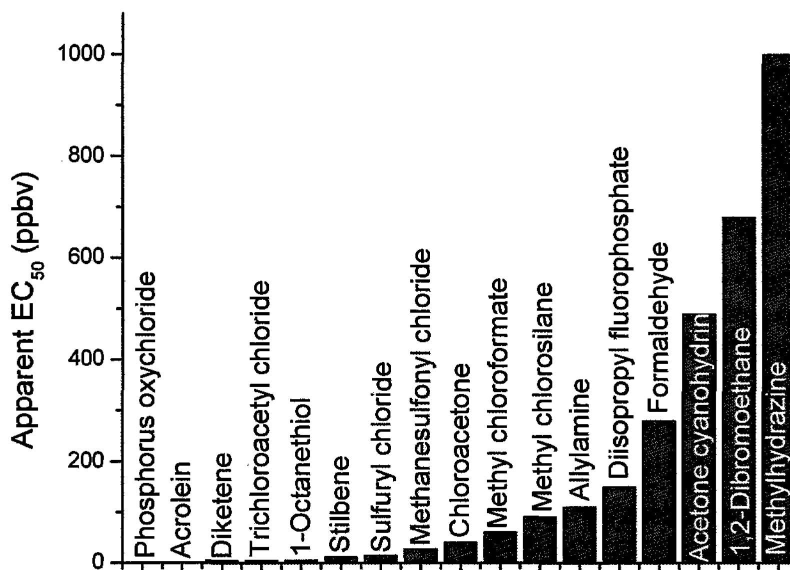

Figure 3. Rank order for $AppEC_{50}$ values. $AppEC_{50}$ values were determined from TICs accumulated into SPMDs from vapor.

If the molar concentration of a particular compound per unit volume was the same in the DMSO from the SPMD as in the vapor phase in the exposure vial (i.e., no concentration effect), then the $AppEC_{50}$ would be the same as the EC_{50}. This was not the case for any of the compounds tested, however, and the $AppEC_{50}$ values ranged from 1000 parts per billion by volume (ppbv) to 0.035 ppbv (Figure 3). These values were between 2 and 3 orders of magnitude smaller than the EC_{50} values which were determined from solution (Figure 2). This result indicated that the compounds were, on average, concentrated by between 200-400 times.

Although the rank order of EC_{50} values (Figure 2) and $AppEC_{50}$ values (Figure 3) were not identical, the compounds on the left (e.g., diketene through sulfuryl chloride) and compounds on the right (e.g., methylhydrazine through 1,2 dibromoethane) of both figures remained in their low / medium / high groupings. Small shifts in the rank order of these compounds between Figures 2 and 3 may be expected due to differences in partition coefficients between vapor and DMSO. Because of the highly reactive nature of many of these compounds, the relative shift in assay response (apparent toxicity) may not be entirely due to partition coefficients alone. More specifically, some of these compounds may react with the DMSO or water and form the breakdown products that may show higher or lower toxicity than the parent compounds.

3.3 Concentration of TICs into SPMD/DMSO

For all compounds measured, the vapor appeared to concentrate into the DMSO. This observed concentration effect can be measured as the ratio of EC_{50} to $AppEC_{50}$ and is reported as a Concentration Factor for each compound. The Concentration Factors reflected the accumulation of the TICs into the SPMD/DMSO as measured from the 5 min Microtox assay and ranged from 17 to 5400 (Figure 4). Again, these values formed a continuum but tended to segregate into 3 groups with most of the values falling between 200 and 400. Because it is likely that the Concentration Factors were highly influenced by partitioning between air/polyethylene/DMSO and kinetics of steady state accumulation, it was not expected that the Concentration Factors would be directly related to the EC_{50} values. It is interesting to note that diketene, which was particularly toxic to the Microtox organisms, as indicated by its low EC_{50} value, did not appear to readily concentrate into the SPMD/DMSO and yielded a Concentration Factor of only 17 (Table 1). Nevertheless, it remained at the left (i.e., low $AppEC_{50}$) side of the chart (Figure 3). This is contrasted by phosphorus oxychloride which was also particularly toxic but showed significant accumulation into the SPMD/DMSO with a Concentration Factor of 5400. These two factors contributed to this compound being the most toxic of the TICs measured using the SPMD-Microtox assay.

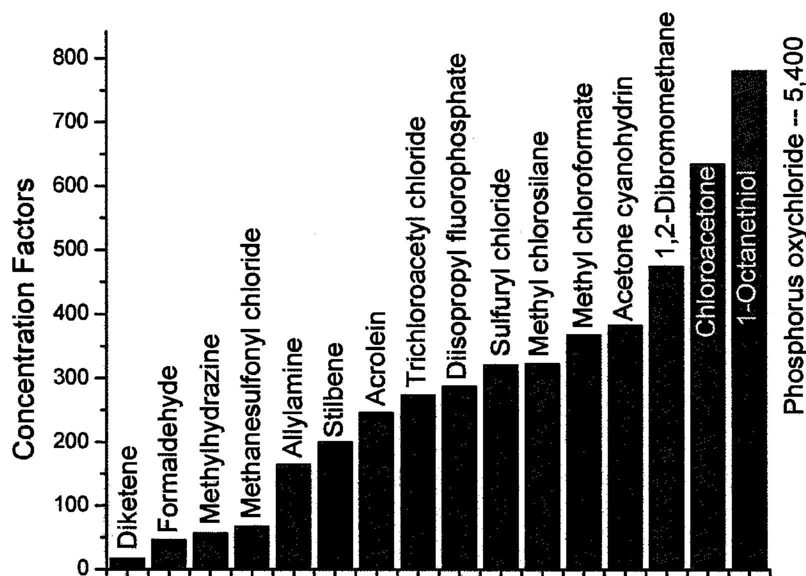

Figure 4. Rank order for Concentration Factors.

Although the Concentration Factor indicates the proportion of compound that concentrated into the DMSO from the vapor under particular conditions, it does not account for the amount of compound that concentrated into the polyethylene portion of the SPMD. These values were not determined for this study, however, previously reported SPMD studies suggest that for compounds of similar molecular weight, about 1/3 of the mass of these compounds concentrates into the polyethylene and 2/3 concentrates into the triolein which has been traditionally used in these devices [5].

3.4 Effect of Microtox Assay Time on EC_{50} Values

Because different compounds affect the light output of *V. fischeri* by different mechanisms and with different kinetics, the Microtox assay protocol suggests the use of 5 and 15 min exposure times for unknown compounds and samples. Results for the compounds measured using the 5 and 15 min protocols reflect these differences. Figure 5 shows the relative change in EC_{50} values between 5 and 15 min with bars extending to the left of center indicating compounds that were measured more effectively using the 5 min protocol; those near the center responding similarly for the 5 and 15 min protocols; and those with bars extending to the right indicating compounds more effectively measured after a 15 min assay. Responses for reference compounds such as phenol (i.e., more effective at 5 min) and zinc sulfate (more effective at 15 min) were typical of those previously reported [8]. It should be noted that the decrease in the apparent toxicity with extended incubation for compounds such as methane sulfonyl chloride, trichloroacetyl chloride, and phosphorus oxychloride (-1400% not included in Figure 5) may be due to the reaction/breakdown of these compounds upon introduction to an aqueous matrix. These compounds showed visible signs of reactivity with water and the breakdown products are less likely to be as toxic as the highly reactive parent compounds. Whatever the mechanism for differences between 5 and 15 min assay protocols, given the range of responses, it would appear prudent to include both protocols in any attempt to apply this assay to unknown compounds.

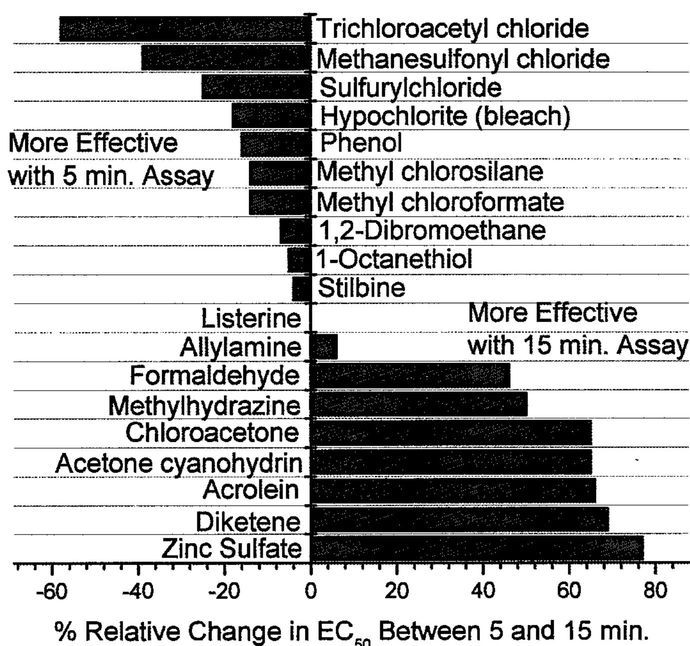

Figure 5. Time dependence of the Microtox assay.

3.5 The Effect of DMSO Concentration

The effect of DMSO on the Microtox assay was examined for final solvent concentrations between 1 and 10 percent (Table 2) for compounds (i.e., acrolein and 1-octanethiol). For this experiment, the SPMDs contained 500 μL DMSO rather than the 100 μL typically used. This allowed for various amounts of the DMSO containing toxicant from the same SPMD to be directly compared in the Microtox assay. In the case of the 1-octanethiol, the $AppEC_{50}$ values for both 5 and 15 min assays did not appear to be greatly affected by the DMSO over the range of 1 to 10 percent. For the acrolein, the EC_{50} values for DMSO concentrations between 2.5 and 10 percent were also similar to each other. For the 15 min assay, however, the EC_{50} value at 2.5% DMSO was somewhat lower than expected.

3.6 Vapor Accumulation Kinetics

Due to its intermediate observed toxicity, acrolein was chosen to conduct uptake kinetics and mass comparison studies. The kinetics for the accumulation of acrolein as measured by both mass and Microtox assay response (toxicity) was compared. The mass of acrolein recovered in the SPMD/DMSO was measured by GC/MS. A calibration curve for acrolein in DMSO was constructed by monitoring the 55 + 56 mass ions. A representative chromatogram for acrolein is shown in Figure 6. The acrolein eluted at 5.44 min, prior to the DMSO solvent peak which eluted at 10.69 min. A relatively low column temperature of 30°C followed by a temperature ramp to 200°C maintained acrolein peak integrity (i.e., prevented excess tailing) and allowed for the removal of the DMSO solvent peak, thus, regenerating the column for the next injection.

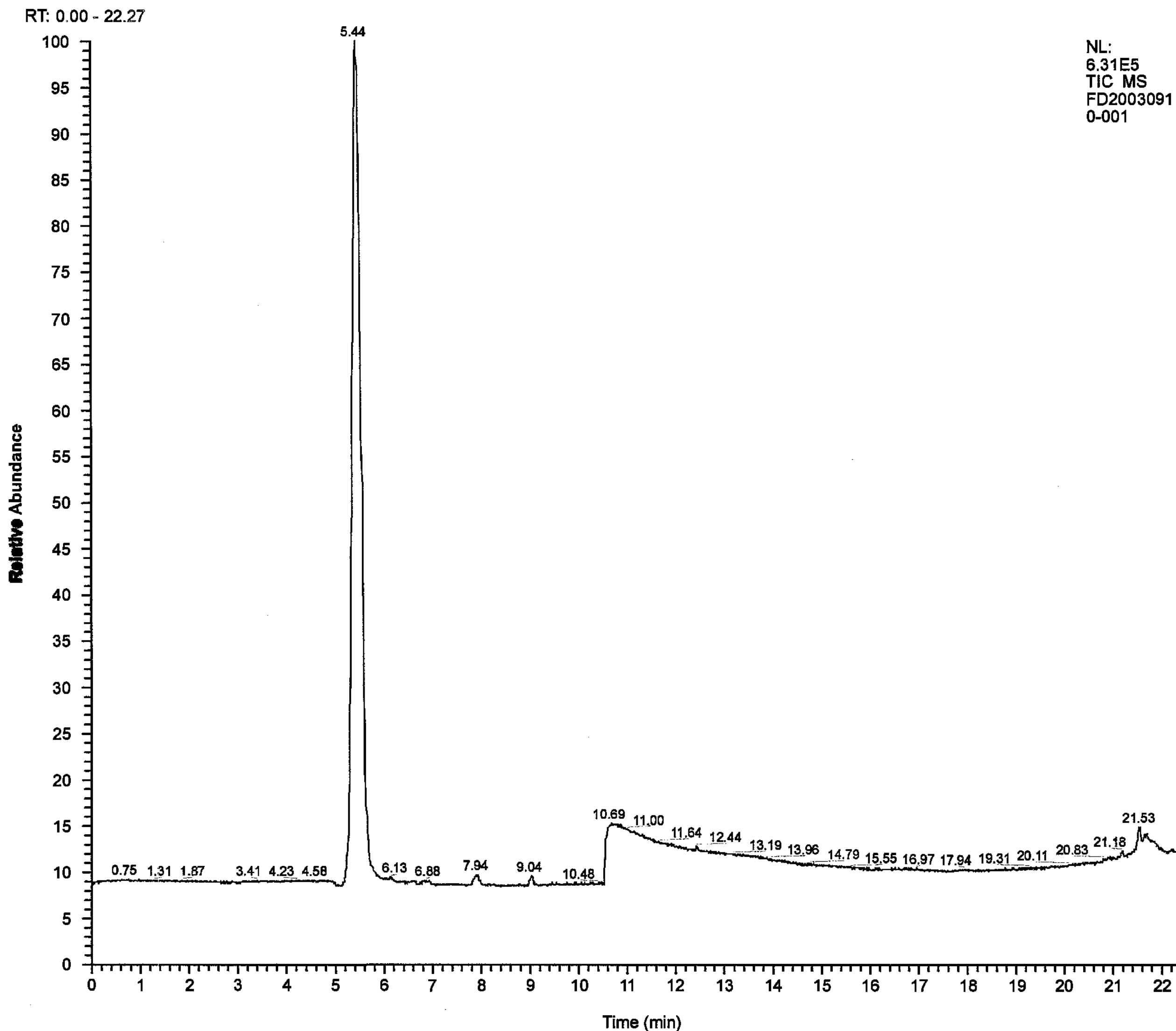

Figure 6. Representative chromatogram for acrolein.

The time course for acrolein uptake on the basis of mass and Microtox assay response is shown in Figure 7. To compare the two uptake profiles, the relative toxicity as measured by the Microtox assay and mass as measured by GC/MS were normalized to a 24 hr maximum value and fit to a sigmoidal curve. An accumulation time of 2 hrs yielded about a 50 percent uptake as compared with the 24 hr maximum.

Significant uptake of compound as measured by both mass and Microtox assay response (i.e., 10 percent) could be measured after as little as a 10 min exposure of the SPMD/DMSO sampler to the acrolein vapor. The determination of the mass of accumulated acrolein as a function of time also allowed a comparison of the Concentration Factor calculated on the basis of Microtox assay response (see Table 1) to a value calculated on the basis of mass. The Concentration Factor for acrolein based on Microtox assay response was 246 and the mass-derived value was 210. This difference most likely arises from the toxicity measurement protocol which required that the acrolein be diluted into an aqueous matrix and may have resulted in the slight degradation of this compound. The amount of acrolein accumulated into the DMSO in the SPMD accounted for 52 percent of that introduced into the vial as vapor. The amount sequestered into the polyethylene of the SPMD was not determined.

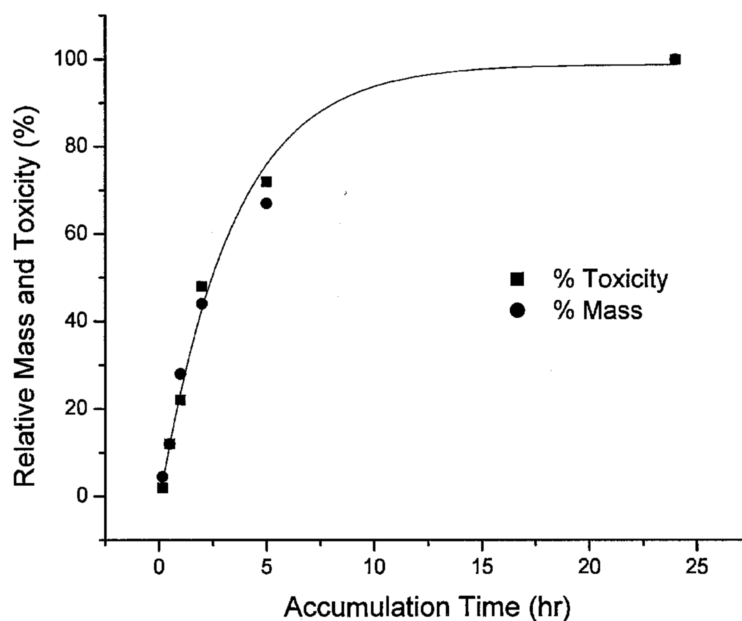

Figure 7. Accumulation of acrolein into SPMDs. Toxicity was determined as Microtox assay response and mass determined by GC/MS.

Because the exposure chamber had a finite volume and the SPMD (in the case of acrolein) accumulated a significant percentage of the total test compound added, it is expected that the observed accumulation rate was lower than would have been observed if the vapor concentration were not to have been depleted during the course of the experiment. It is also likely that the final concentration of test compounds that concentrated into the DMSO would have been greater if the compound vapor in the exposure chamber were not depleted.

The lowest vapor concentration of a particular compound yielding a response for the SPMD-Microtox assay would depend on several variables including: EC_{50} (5 or 15 min depending on the compound), Concentration Factor, exposure time (for the SPMD to the toxic environment), and volume of DMSO sampled in the Microtox assay as well as factors not examined in this study such as temperature. To determine the detection limit of this assay for acrolein, a serial dilution experiment was performed using a 4 L incubation chamber with the typical 75 mm SPMD containing 100 µL DMSO. Shown in Table 3, an acrolein vapor concentration as low as 0.1 µL/L could be detected using both the 5 min and 15 min protocols, whereas a 0.01µL/L concentration could be detected using the 15 min protocol. This, in fact, represents a conservative approach because in order for the Microtox software to calculate an $AppEC_{50}$ value, the highest concentration sample in the nine tube dilution series must inhibit the assay response by 30 to 40 percent.

Experience with this assay has indicated that inhibition values as low as 15 to 20 percent can be reproducibly observed.

The lowest concentration of acroline for which an $AppEC_{50}$ could be determined using the SPMD/DMSO-Microtox assay is shown in Table 4. The accumulation time for the SPMD/DMSO sampler was 24 hr and the Microtox assay time was 15 min. The limit of detection for acrolein using the SPMD/DMSO-Microtox system was 19 mg/m^3 which was about 100 times less than the LD-50 value listed in the NIOSH database and about 100 times greater than the NIOSH occupational exposure limit. Consequently, as evidenced for acrolein, this system in its current configuration, shows the potential for development as a screening tool for acute toxicity determinations. Areas that require further characterization include mass accumulation and Microtox response to chemical mixtures.

Table 1. Summary of Microtox Data

Name	Liquid EC50 (ppmv)[a]	95% confidence	Liquid EC50 (ppmv)[b]	95% confidence	% Change 5-15[c]	Vapor AppEC50 (ppmv)[d]	95% confidence	Vapor AppEC50 (ppmv)[e]	95% confidence	Conc Factor
Acetone cyanohydrin	187	112-310	66	41-103	65	0.49	0.33-0.70	0.21	0.10-0.42	382
Acrolein	0.32	0.26-0.38	0.11	0.10-0.13	66	0.0013	0.0010-0.0016	0.0004	0.0002-0.0008	246
Allylamine	18	2-146	17	2-147	6	0.11	0.05-0.19	0.09	0.02-0.36	164
Chloroacetone	26	21-33	9	7-11	65	0.041	0.037-0.044	0.012	0.009-0.015	634
1,2-Dibromoethane	322	33-3000	346	89-1300	-7	0.68	0.65-0.72	0.75	0.67-0.83	474
Diisopropyl fluorophosphate	43	33-58	44	35-56	-2.3	0.15	0.12-0.17	0.16	0.14-0.18	287
Diketene	0.070	0.053-0.090	0.022	0.005-0.080	69	0.0041	.0036-.0045	0.0013	.0010-.0016	17
Formaldehyde	13	12-14	7	5-8	46	0.28	0.20-0.37	0.19	0.16-0.21	46
Methanesulfonyl chloride	1.8	0.8-3.6	2.5	0.8-7.0	-39	0.027	0.021-0.035	0.03	0.02-0.04	67
Methyl chloroformate	22	9-53	25	NA	-14	0.06	0.01-0.23	0.06	0.01-0.36	367
Methyl chlorsilane	29	18-45	33	16-65	-14	0.09	0.06-0.12	0.12	NA	322
Methylhydrazine	56	52-59	28	23-31	50	1	0.2-4.6	0.4	0.1-1.4	56
1-Octanethiol	3.9	3.4-4.3	4.1	3.6-4.7	-5	0.005	0.004-0.006	0.007	0.004-0.013	780
Phosphorus oxychloride	0.19	0.17-0.19	3.0	0.7-11	-1400	0.000035	0.000027-0.000038	0.00008	0.00007-0.00011	5400
Stilbene	2.4	2.2-2.5	2.5	2.2-2.7	-4	0.012	0.010-0.014	0.016	0.011-0.022	200
Sulfuryl chloride	4.8	2.4-10	6	NA	-25	0.015	0.005-0.025	0.02	NA	320
Trichloroacetyl chloride	1.2	0.7-1.9	1.9	1.3-2.8	-58	0.0044	0.0038-0.0050	0.0060	0.0044-0.0085	273
Listerine	0.15%	0.12-0.18	0.15%	0.12-0.18	0	NA	NA	NA	NA	NA
Phenol	18	15-21	21	18-24	-16	NA	NA	NA	NA	NA
Zinc Sulpate	37	34-41	8.4	8.1-8.8	77	NA	NA	NA	NA	NA

a Microtox 9-point protocol for pure compounds, dilution into DMSO, 5 min incubation protocol. Each EC50 or AppEC50 value was representative of at least 3 assays.

b. Same as (a) with 15 min protocol

c Time-dependence of Microtox assay, data from Figure 4. Positive values indicate that the 15 min protocol is more effective and negative values indicate that the 15 min protocol is more effective.

d Microtox 9-point protocol for pure compounds, SPMD-DMSO was exposed for 24 hr to vapor then run using the 5 min incubation protocol.

e Same as (d) with 15 min protocol

f Concentration Factors were determined using the ratio of the EC_{50} to $AppEC_{50}$ for the 5 min incubation protocols.

Table 2. Effect of Solvent Volume on the $AppEC_{50}$ Values for Acrolein and Octanethiol [a]

Compound	% DMSO	App EC_{50} 5 min (ppbv)	App EC_{50} 15 min (ppbv)
Acrolein	2.5	1.6	0.26
	5	1.6	0.40
	10	1.2	0.44
Octanethiol	1	2.4	2.8
	2.5	1.8	2.0
	5	1.8	2.2
	10	2.2	2.4

a Experiments were performed in 40 mL exposure chambers using a 75-mm SPMD containing 100 µL DMSO.

Table 3. Effect of Acrolein Vapor Concentration on the $AppEC_{50}$ Measured Using the SPMD/DMSO Sampler

Dilution (µL/L)[a]	$AppEC_{50}$, 5 min (ppbv)[b]	$AppEC_{50}$, 15 min (ppbv)
10	0.60	0.17
1	0.65	0.18
0.1	0.60	0.21
0.01	NA[c]	0.20

a Experiment performed in a 4 L exposure chamber using a 75-mm SPMD containing 100 µL DMSO.

b Microtox 9-point calibration with 95% confidence limits.

c $AppEC_{50}$ value could not be established using the Microtox Omni software.

Table 4. Comparison of Detection Limits for Acrolein Toxicity Using the SPMD/DMSO-Microtox Assay

SPMD-Microtox (µL/L)	SPMD-Microtox (mg/L)[a]	Acrolein conversion (mg/m^3)[b]	LD-50 (cat) (mg/m^3/2 hr)[c]	NIOSH (mg/m^3/hr)[d]	OSHA (ppm/40 hr)[d]
0.01	0.0084	19	1570	0.25	0.10

a Density determined from Aldrich Materials Safety Data Sheet (MSDS)

b Conversion Factor from reference 9

c See reference 10

d See reference 11

Section 4
Conclusions

Several significant goals were accomplished by the development of methods to extend the SPMD-Microtox assay system to volatile TICs. These goals included the following: 1) The SPMD sampler was shown to accumulate (to varying degrees) each of the assessed TICs. The use of DMSO rather than triolein, typically used in SPMDs, allowed the Microtox assay to be run directly from the SPMD solvent without the usual processing and cleanup procedures. 2) Microtox EC_{50} values were determined for each of the 17 TICs analyzed and ranged from 0.070 ppmv for diketene to 322 ppmv for 1,2-dibromoethane. $AppEC_{50}$ values measured from vapor accumulation into the SPMD/DMSO were lower than the EC_{50} values measured from liquid and ranged from 0.035 ppbv for phosphorus oxychloride to 1000 ppbv for methylhydrazine. Although the 24 hr vapor accumulation values for the SPMDs include variability due to accumulation and possible reaction or breakdown, each of the compounds was concentrated and consequently increased in apparent toxicity as measured by the Microtox assay.

Listed in Appendix A is a summary table for $AppEC_{50}$ values for each of the TICs examined as well as LD-50, NIOSH and OSHA occupational limits (where available). Whereas acrolein appears to be somewhat average, it is not representative of all listed compounds. Because the values for $AppEC_{50}$, LD-50 and occupational exposure limits vary widely, and often without a clear relationship to each other, it is difficult to predict the safety margin afforded by a negative response to the SPMD/DMSO-Microtox assay. These results, however, suggest that the SPMD/DMSO-Microtox assay would respond to the TICs examined in this study at concentrations below their LD-50 values.

Section 5

Future Directions

The Microtox assay was shown to be stable in its response to specific compounds and robust in its application to air toxics. However, characterization of the Microtox assay for a structurally and mechanistically diverse group of TICs has shown a variable range of toxicity responses. It appears that the key to the application of this assay to screening of air toxics would be the pre-concentration of the compounds followed by elution or solvent exchange into a Microtox-compatible solvent such as DMSO or assay diluent (essentially an NaCl solution). Preliminary experiments have suggested that carbon/Empore membranes can accumulate several TICs to a significantly greater extent than DMSO alone. The contaminated membranes can then be extracted with DMSO with a relatively high yield. Continued research will optimize these protocols and correlate mass and toxicity measurements. In addition, GC/MS analyses will be used to indicate reaction or breakdown of these compounds prior to the Microtox assay.

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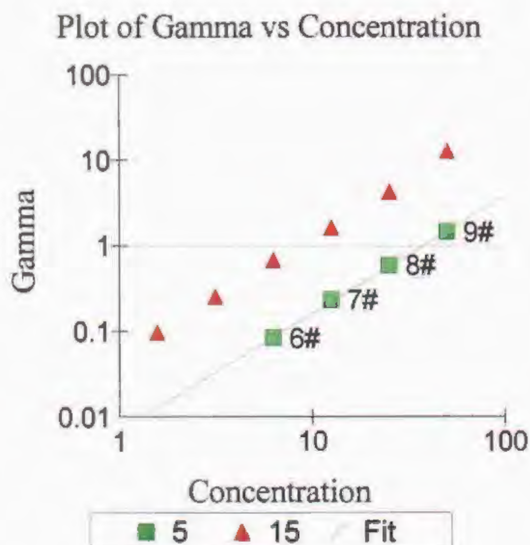
Appendix A

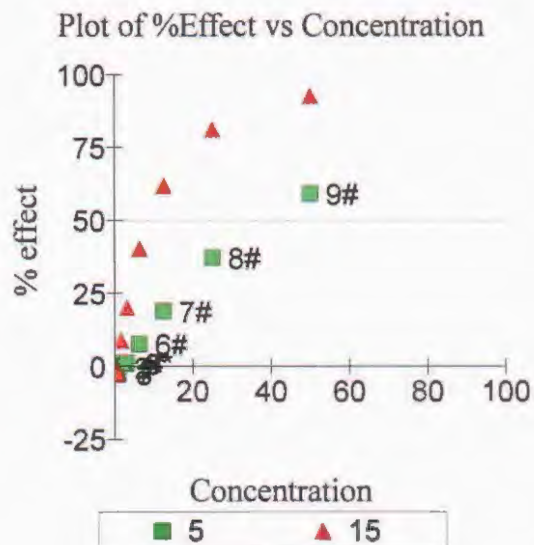
Selected Physical Parameters for TICs

Name	FW	BP	Density	Vapor EC-50-5 (ppbv)	Vapor EC-50-15 (ppbv)	LD-50 LC-50 (mg/m3/hrs)	OSHA ppm	NIOSH mg/m3/hr	Formula
Acetone cyanohydrin	85.11	82	0.93	490	210	70-2hr			$(CH_3)_2C(OH)CN$
Acrolein	56.06	53	0.84	1.3	4.0	1570-2hr	0.1	0.25	$H_2C=CHCHO$
Stilbene	180.25	82		12	16	NA	0.1	0.50	$C_6H_5CH=CHC_6H_5$
Methyl chlorsilane	Mixed	155	1.20	90	120	100	NA	NA	NA
Allylamine	57.10	53	0.76	110	90	320	NA	NA	$H_2C=CHCH_2NH_2$
Chloroacetone	92.53	119	1.16	41	12	262-1hr	NA	NA	$ClCH_2COCH_3$
Diketene	84.07	70	1.09	4.1	13	3000-1hr	0.50	0.90	
1,2-Dibromoethane	187.87	131	2.18	680	750	14,000-0.5hr	20	0.045	$BrCH_2CH_2Br$
Methyl chloroformate	94.50	71	1.22	60	60	180-2hr	NA	NA	$ClCO_2CH_3$
Methanesulfonyl chloride	114.55	60	1.48	27	30	620-2hr	NA	NA	CH_3SO_2Cl
Methylhydrazine	46.07	87	0.87	1000	400	270-4hr	0.2	0.35	CH_3NHNH_2
Phosphorus oxychloride	153.33	106	1.64	0.035	0.80	404-2hr	NA	NA	$POCl_3$
Sulfuryl chloride	134.97	69	1.68	15	20	10 ppm-6hr	NA	NA	SO_2Cl_2
1-Octanethiol	146.30	197	0.84	5	7	NA	NA	NA	$CH_3(CH_2)_7SH$
Trichloroacetyl chloride	181.83	115	1.63	4.4	6	400	NA	NA	CCl_3COCl
Formaldehyde	30.03		1.09	280	190	204-4hr	0.75	0.016	HCHO
Phenol	100.07	182	1.14	NA	NA	NA	NA	NA	C_6H_4OH
DFP	184.15	62	1.05	150	160	NA	NA	NA	$[(CH_3)_2CHO]_2P(O)F$

Appendix B
Microtox Omni Test Report

Date/Time: 9/18/2003 03:18 PM
Test Protocol: 90% Basic Test for Pure Compounds
Data File: Untitled Data File





			5 Mins Data			15 Mins Data		
Sample	Conc	Io	It Gamma		% effect	It Gamma		% effect
Control	0.000	95.05	67.47 - 0.7098	#		60.88 - 0.6405	#	
1	0.1953	104.67	75.44 - 0.0151	*	-1.536%	68.97 - 0.0279	*	-2.876%
2	0.3906	102.29	72.85 - 0.0033	*	-0.3316%	66.08 - 0.0085	*	-0.8589%
3	0.7813	100.61	73.25 - 0.0250	*	-2.567%	65.88 - 0.0218	*	-2.233%
4	1.563	102.83	72.17 - 0.0114	*	1.127%	60.00 - 0.0977	#	8.902%
5	3.125	100.04	70.25 - 0.0108	*	1.073%	51.14 - 0.2530	#	20.19%
6	6.250	102.26	66.98 - 0.0837	#	7.726%	39.09 - 0.6756	#	40.32%
7	12.50	100.32	57.75 - 0.2331	#	18.90%	24.48 - 1.625	#	61.90%
8	25.00	102.21	45.51 - 0.5942	#	37.27%	12.32 - 4.314	#	81.18%
9	50.00	99.18	28.70 - 1.453	#	59.23%	4.65 - 12.66	#	92.68%

= Used in calculation
* = Invalid data
D = Deleted from calcs

Calculations on 5 Mins data:
EC_{50} Concentration: 37.21 mg/L (95% confidence range: 33.63 to 41.17)
95% Confidence Factor: 1.106
Estimating Equation: LOG C = 0.7291 x LOG G + 1.571
Coeff. of Determination (R^2): 0.9990
Slope: 1.370
Correction Factor: 0.7098

Calculations on 15 Mins data:
EC_{50} Concentration: 8.432 mg/L (95% confidence range: 8.074 to 8.805)
95% Confidence Factor: 1.044
Estimating Equation: LOG C =0.7192 x LOG G + 0.9259
Coeff. of Determination (R^2): 0.9993
Slope: 1.389
Correction Factor: 0.6405

Appendix C
SPMD Samples

SPMD in 40 mL VOA vial
(colored dye added for contrast)

SPMD in 4 L bottle
(colored dye added for contrast)