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FROM: Larry Brooke
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SUBJECT: Report of the flow-through and static acute test comparisons with fathead minnows and acute tests with an amphipod and a cladoceran

The Center for Lake Superior Environmental Studies of the University of Wisconsin-Superior, Superior, Wisconsin 54880 has completed acute toxicity tests with eighteen organic compounds. Fathead minnows, Pimephales promelas, (30±5 days old) were exposed to all compounds in flow-through and static exposures with measured chemical concentrations. An amphipod, Gammarus pseudolimnaeus, (adults) were simultaneously exposed with fathead minnows in flow-through exposures to eight of the chemicals. The cladoceran, Daphnia magna, (<24 hr old) were exposed to two chemicals in 48-hr exposures.

Test Organisms

Fathead minnows (30±5 day old), Pimephales promelas, were obtained from the University of Wisconsin-Superior and U.S. EPA Environmental Research laboratory, Duluth, MN culture units. Adult amphipods, Gammarus pseudolimnaeus, were collected from the Eau Claire River, Douglas County, WI. Recommended procedures for care, handling and acclimation of test organisms were followed (ASTM 1980). Amphipods were acclimated to test water and exposure temperature for a minimum of two weeks prior to test initiation. Cladocerans, Daphnia magna, were

cultured at the University of Wisconsin-Superior facilities.

Adult cladocerans were cultured in 2.0 L containers with 1600 mL of water with twenty individuals per container. Adults were transferred to new water every Monday, Wednesday and Friday. Each culture container was fed 1 x 10^8 cells·L^{-1} of green algae, Selenastrum capricornutum, and 5 mg·L^{-1} trout chow suspension on Mondays, Wednesdays and Fridays. Prior to test initiation, adults were separated into 100 mL glass beakers with 80 mL culture water containing the same food concentrations as the stock culture. Less than 24-hr old neonates taken from the individual adults were pooled for the initiation of the test. Transfer of young and adults was accomplished with a wide-mouth, fire polished pipette.

Water

The water supply for test organism acclimation and testing came from two sources. One source was directly from Lake Superior with sand filtration and the other source was municipal water from the City of Superior, WI. The municipal water was drawn from shallow wells beneath Lake Superior and was dechlorinated by charcoal filtration and sodium sulfite addition. The flow-through and static tests for each chemical were conducted in water from the same source. The desired water temperature (22°C) was maintained using a temperature controlled water supply for flow-through tests or a stable temperature water bath or room for static tests.

Toxicity Tests

All acute toxicity tests were conducted with duplicate controls and exposure treatments except for the cladoceran tests which had quadruplicate replication. All the tests consisted of five toxicant treatments with a dilution factor of 0.5. Flow-through tests were conducted on a modified Benoit-type mini-diluter (Benoit et al. 1982).

Flow-through tests were conducted in 23 x 15 x 10 cm chambers containing 1.5 L of test solution. Static tests with fathead minnows were conducted in 15 x 20 x 20 cm chambers containing 4 L of solution. Cladoceran tests were conducted in 100 mL beakers containing 80 mL of solution. Amphipod tests were conducted with some of the flow-through tests. Amphipods were placed into chambers containing the fathead minnows in stainless steel mesh wire cylinders (8-10 cm length x 3 cm diameter) and closed at the end with a neoprene stopper. Each cylinder also contained two tree leaf disks (Betula sp. and Populus sp.) 20 mm in diameter. Aeration was not added to any tests and all test chambers were open to the atmosphere. The number of organisms in each test chamber was ten for fathead minnow tests, five or ten for amphipods and five for cladocera tests.

Dissolved oxygen was measured in control, low, middle and high exposures at the initiation of each test and every 48 hr thereafter (Table 1). Total hardness (EDTA), total alkalinity, specific conductance and pH were measured at least once during each test on control, low, middle and high exposure (Table 1) in accordance with accepted procedures (APHA 1980). Temperatures were measured daily with mean temperatures calculated (Table 1). Dissolved oxygen (mean percent saturation) ranged from 63.6 to 97.4 for all tests; mean total (EDTA) hardness ranged from 46.6 to 55.5 $mg \cdot L^{-1}$ as $CaCO_3$; mean total alkalinity ranged from 28.3 to 100 $mg \cdot L^{-1}$ as $CaCO_3$; and pH ranged from 4.06 to 7.48.

Biomass loading requirements were met as stated by ASTM (1980). Tests were begun by placing the test organisms in the exposure chambers when concentrations had reached equilibrium in the flow-through tests and within 0.5 hr after the toxicant was added in the static tests.

The criteria for death was lack of reaction to gentle prodding. Exposures were checked every 24 hr for death and behavioral effects.

TABLE 1. Measured test water characteristics for acute toxicity tests with fathead minnow (Pimephales promelas), an amphipod (Gammarus pseudolimnaeus) and a cladoceran (Daphnia magna) exposed to several organic chemicals. Values are mean ± standard deviation, range (in parentheses), and sample size.

Compound[a]	Species[b]	Type of Test	Dissolved Oxygen (%)	Total (EDTA) Hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	Total Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	pH	Temperature (°C)
Acrylamide 79-06-1	FHM & Amphipod	Flow-through	86.5±3.5 (83.9-92.4) n=12	50.8±0.9 (50.0-52.0) n=4	43.3±1.3 (41.6-44.8) n=4	7.08±0.06 (7.01-7.18) n=12	24.1±0.6 (23.2-25.8) n=60
Acrylamide 79-06-1	FHM	Static	70.1±6.1 (63.4-82.1) n=12	55.5±2.2 (52.6-58.0) n=4	50.6±3.2 (47.0-53.6) n=4	7.07±0.09 (6.91-7.17) n=12	22.1±0.1 (21.8-22.3) n=60
Benzene 71-43-2	FHM & Amphipod	Flow-through	85.3±4.8 (6.8-8.2) n=12	50.7±0.0 (50.7-50.7) n=4	42.5±1.0 (42-44) n=4	7.04±0.01 (7.02-7.05) n=4	22.3±0.5 (21.7-22.9) n=50
Benzene 71-43-2	FHM	Static	74.5±9.5 (47.2-83.4) n=12	51.2±0.5 (50.4-51.6) n=4	43.0±0.8 (42.2-43.6) n=4	6.96±0.12 (6.77-7.11) n=12	21.7±0.26 (21.3-22.1) n=60
Carbon tetrachloride 56-23-5	FHM & Amphipod	Flow-through	81.1±4.9 (74.5-86.0) n=10	49.2±1.1 (48.2-50.6) n=4	39.6±0.4 (39.0-39.8) n=4	6.82±0.06 (6.73-6.93) n=10	21.7±0.1 (21.5-22.1) n=60
Carbon tetrachloride 56-23-5	FHM	Static	87.2±10.9 (70.2-100.6) n=11	52.3±0.5 (52.0-53.0) n=4	44.0±0 (44.0-44.0) n=4	7.20±0.11 (7.07-7.34) n=4	22.5±0.56 (21.5-23.4) n=56
1,4-Dioxane 123-91-1	FHM & Amphipod	Flow-through	93.3±3.3 (86.4-98.3) n=12	50.2±1.0 (48.6-50.7) n=4	42.0±0.0 (42.0-42.0) n=4	7.32±0.10 (7.12-7.47) n=12	22.1±0.4 (21.6-23.2) n=60

TABLE 1 Cont. Measured test water characteristics for acute toxicity tests with fathead minnow (*Pimephales promelas*), an amphipod (*Gammarus pseudolimnaeus*) and a cladoceran (*Daphnia magna*) exposed to several organic chemicals. Values are mean ± standard deviation, range (in parentheses), and sample size.

Compound[a]	Species[b]	Type of Test	Dissolved Oxygen (%)	Total (EDTA) Hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	Total Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	pH	Temperature (^{o}C)
1,4-Dioxane 123-91-1	FHM	Static	82.0±28.5 (58.2-106.6) n=12	51.2±1.0 (50.7-52.7) n=1	43±0.82 (42-44) n=4	6.52±1.98 (6.69-7.24) n=12	21.7±4.5 (24.1-24.6) n=60
1,4-Dioxane 123-91-1	Cladoceran	Static	86.4±2.9 (83.5-91.4) n=8	47.0±1.6 (45.6-48.6) n=3	41.3±1.7 (39.4-42.6) n=3	7.46±0.28 (7.00-7.73) n=8	23.3±0.3 (22.4-23.6) n=18
Ethanal (acetaldehyde) 75-07-0	FHM & Amphipod	Flow-through	86.0±6.4 (93.7-75.5) n=12	46.6±0.8 (45.6-47.6) n=4	40.50±1.29 (39.00-42.00) n=4	7.05±0.06 (6.95-7.17) n=12	21.6±0.19 (21.1-22.1) n=60
Ethanal (acetaldehyde) 75-07-0	FHM	Static	76.4±18.7 (50.3-97.1) n=12	48.0±0.6 (47.2-48.6) n=4	58.0±5.5 (50.4-63.0) n=4	6.97±0.17 (6.78-7.29) n=12	21.4±0.3 (20.7-21.8) n=60
Ethylbenzene 100-41-4	FHM & Amphipod	Flow-through	82.5±5.5 (76.2-97.8) n=12	50.0±0.0 (50.0-50.0) n=4	41.5±0.6 (41.0-42.0) n=4	7.18±0.08 (7.10-7.28) n=4	22.2±0.4 (21.3-22.8) n=52
Ethylbenzene 100-41-4	FHM	Static	80.5±8.3 (72.6-93.6) n=10	52.0±1.0 (51.0-53.0) n=3	43.0±1.0 (42.0-44.0) n=3	7.48±0.07 (7.41-7.54) n=3	22.2±0.6 (21.2-23.1) n=60
Formaldehyde 50-00-0	FHM	Flow-through	87.8±8.3 (73.0-98.6) n=11	50.8±2.1 (49.2-53.8) n=4	37.0±3.4 (32.0-39.0) n=4	6.83±0.09 (6.71-7.01) n=11	21.7±0.3 (21.2-22.4) n=60

TABLE 1 Cont. Measured test water characteristics for acute toxicity tests with fathead minnow (Pimephales promelas), an amphipod (Gammarus pseudolimnaeus) and a cladoceran (Daphnia magna) exposed to several organic chemicals. Values are mean ± standard deviation, range (in parentheses), and sample size.

Compound[a]	Species[b]	Type of Test	Dissolved Oxygen (%)	Total (EDTA) Hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	Total Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	pH	Temperature (^{o}C)
Formaldehyde 50-00-0	FHM	Static	68.3±8.5 (45.4-73.8) n=10	49.8±0.0 (49.8-49.8) n=3	42.9±0.8 (42.2-43.8) n=3	6.81±0.25 (6.53-7.13) n=10	21.3±0.3 (20.9-21.8) n=60
Furfural 98-01-1	FHM & Amphipod	Flow-through	93±2.8 (87.0-96.0) n=12	51.2±0.5 (50.7-51.8) n=4	40.5±0.5 (40.2-40.4) n=4	7.14±0.09 (7.07-7.24) n=8	21.9±0.30 (21.1-22.6) n=60
Furfural 98-01-1	FHM	Static	90.6±13.8 (71.9-104.3) n=8	52.6±1.73 (50.9-54.9) n=4	28.3±17.3 (8.4-50.6) n=4	6.40±1.06 (4.06-7.33) n=8	22.2±0.10 (21.9-22.3) n=60
Hexachloro-1,3-butadiene 87-68-3	FHM	Static	81.8±6.8 (71.5-92.8) n=12	49.2±0.69 (48.6-49.8) n=4	54.0±1.3 (42.0-45.0) n=4	7.21±0.25 (6.97-7.58) n=12	21.8±0.8 (21.3-22.3) n=60
Hexachlorocyclo-pentadiene 77-47-4	FHM	Static	89.7±16.5 (51.6-109.4) n=12	47.6±0.8 (46.6-48.6) n=4	43.8±0.5 (43.0-44.0) n=4	7.28±0.24 (6.85-7.41) n=12	21.5±2.0 (21.0-21.9) n=60
Hydrazine 302-01-2	FHM & Amphipod	Flow-through	95.0±2.0 (92.0-98.5) n=12	51.0±3.2 (48.0-54.3) n=4	43.0±6.0 (40.0-52.0) n=4	7.34±0.11 (7.17-7.56) n=12	22.8±2.6 (22.2-23.7) n=60
Hydrazine 302-01-2	FHM	Static	83.3±3.8 (80.5-88.9) n=4	52.9±1.9 (50.3-54.9) n=4	43.0±3.2 (38.2-45.0) n=4	7.02±0.17 (6.88-7.21) n=4	20.2±5.4 (21.3-22.0) n=14

TABLE 1 Cont. Measured test water characteristics for acute toxicity tests with fathead minnow (Pimephales promelas), an amphipod (Gammarus pseudolimnaeus) and a cladoceran (Daphnia magna) exposed to several organic chemicals. Values are mean ± standard deviation, range (in parentheses), and sample size.

Compound[a]	Species[b]	Type of Test	Dissolved Oxygen (%)	Total (EDTA) Hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	Total Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	pH	Temperature (^{o}C)
Hydrazine 302-01-2	Cladoceran	Static	97.4±2.3 (94.7-100.7) n=8	50.3±1.2 (49.6-51.7) n=3	39.3±6.6 (32.6-45.8) n=3	7.39±0.38 (7.01-7.77) n=8	22.3±0.76 (21.6-23.5) n=18
Pentachloro-ethane 76-01-7	FHM	Static	89.4±9.6 (73.6-102.5) n=12	47.8±0.5 (47.0-48.0) n=4	42.0±1.6 (40.0-44.0) n=4	7.33±0.10 (7.19-7.44) n=12	21.9±0.2 (21.7-22.1) n=60
Pentachloro-benzene 608-93-5	FHM	Flow-through	87.1±5.2 (77.2-93.4) n=12	51.2±4.5 (44.8-55.0) n=4	43.5±1.1 (42.2-44.8) n=4	6.82±0.34 (6.40-7.22) n=8	22.5±0.8 (21-23.8) n=60
Pentachloro-benzene 608-93-5	FHM	Static	63.6±5.0 (55.6-74.2) n=10	53.4±0.8 (52.4-54.0) n=3	45.4±0.9 (44.4-46.2) n=3	7.14±0.11 (6.93-7.23) n=10	22.3±0.5 (21.8-23.2) n=60
Pyridine 110-86-1	FHM	Static	79.4±6.2 (66.9-86.5) n=11	46.6±1.3 (45.0-49.0) n=7	100.0±67.4 (44-222) n=7	7.29±0.26 (7.03-7.68) n=4	21.9±0.2 (21.4-22.1) n=54
Tetrachloro-ethylene 127-18-4	FHM	Static	86.3±8.4 (68.4-96.4) n=12	50.4±0.51 (49.6-50.7) n=4	43.5±1.0 (42.0-44.0) n=4	7.31±0.06 (7.20-7.40) n=12	23.2±0.5 (22.4-24.3) n=60
Toluene 108-88-3	FHM	Static	75.2±17.6 (3.9-8.6) n=12	47.2±1.7 (45.0-49.0) n=4	42.8±1.0 (42.0-44.0) n=4	7.20±0.28 (7.02-7.50) n=8	22.5±1.0 (19.2-23.6) n=58

TABLE 1 Cont. Measured test water characteristics for acute toxicity tests with fathead minnow (Pimephales promelas), an amphipod (Gammarus pseudolimnaeus) and a cladoceran (Daphnia magna) exposed to several organic chemicals. Values are mean ± standard deviation, range (in parentheses), and sample size.

Compound[a]	Species[b]	Type of Test	Dissolved Oxygen (%)	Total (EDTA) Hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	Total Alkalinity ($mg \cdot L^{-1}$ as $CaCO_3$)	pH	Temperature (^{o}C)
Styrene 100-42-5	FHM	Flow-through	92.5±4.35 (82.8-97.8) n=12	52.8±2.4 (49.2-54.6) n=4	40.6±1.1 (39.0-41.0) n=4	7.15±0.06 (7.05-7.25) n=12	21.3±0.4 (20.6-22.1) n=60
Styrene 100-42-5	FHM	Static	90.3±5.8 (80.4-102.1) n=12	51.0±1.2 (50.0-52.0) n=4	42.8±1.0 (42.0-44.0) n=4	7.28±0.27 (6.86-7.64) n=12	23.54±0.96 (22.4-25.1) n=60
p-Xylene 106-42-3	FHM	Static	73.4±13.9 (54.7-91.8) n=12	51.1±1.2 (49.4-52.3) n=4	50.2±2.4 (48.0-52.8) n=4	7.22±0.23 (7.03-7.59) n=12	21.7±0.5 (21.3-22.7) n=44

a The number beneath the common name is the Chemical Abstract Service registry number.

b FHM = Fathead minnow (Pimephales promelas); amphipod = Gammarus pseudolimnaeus; cladoceran = Daphnia magna.

Statistical Treatment of Data

LC50's and EC50's with their respective 95% confidence limits were calculated by the trimmed Spearman-Karber method (Hamilton et al. 1977). None of the tests had more than 10% of the control organisms that died or appeared stressed during the test. No test had less than 60% dead or affected organisms in the highest exposure.

Water Sampling

In the static tests, samples for analysis were collected within 0.5 hr of test initiation. All tanks were sampled at 0, 48 and 96 hr or until the compound was no longer detectable. Additional samples were collected in the middle and highest exposures at 24 and 72 hr to determine the decay rate of the chemical.

The flow-through tests were sampled at 0, 48, and 96 hr in all tanks, and odd-or even-numbered tanks alternately at 24 and 72 hr. Analysis of samples proceeded without delay.

Duplicate analyses and spike recoveries were performed on approximately 10% of all samples. All test concentrations have been corrected for the mean spike recovery for the specific test.

Chemical Analysis

All compounds tested were of reagent-grade quality or better. Sources of the compounds were Aldrich Chemical Company (Milwaukee, WI). J.T. Baker Chemical Company (Phillipsburg, NJ), Eastman Kodak Chemical Company (Rochester, NY) or EM Science (Cherry Hill, NJ).

Two compounds, pyridine and acrylamide, were analyzed using high pressure liquid chromatography (HPLC). The HPLC system consisted of two Waters M-45 pumps, a Waters M-490 Programmable Multiwavelength Detector, a Waters Automated Gradient Controller, Spectra-Physics SP 4270 Integrator, and a Micromeritics

Model 725 Autoinjector with a 10 uL loop or a Valco Model C6W injector with a 20 uL loop. One of two columns were used in conjunction with a Hamilton PRP-1 pre-column; either a Waters uBondapak C18 column (3.9x150 mm, 10 um particle size), or, a Hamilton PRP-1 analytical column (4.1x150 mm, 10 um particle size).

Pyridine (Kodak, lot A9A, 95-96% pure). Pyridine was analyzed by HPLC at a wavelength of 256 nm using a Waters uBondapak C18 column, a mobile phase of 75% phosphate buffer (pH=7) -25% acetonitrile pumped at 1.0 mL/min, and a 10 uL sample size. A 101 $\pm$ 3.0% (N=5) spike recovery and 99.4 $\pm$ 0.4% (N=5) agreement of duplicate analyses were found. The detection limit was 1.13 $ug \cdot L^{-1}$.

Acrylamide (Aldrich, lot 02530LM, 97% pure). Acrylamide was analyzed by HPLC at a wavelength of 254 nm using a Hamilton PRP-1 analytical column, a mobile phase of 10% methanol-90% deionized water, and a 20 uL sample size. A 100.0 $\pm$ 3.5% (N=10) spike recovery, and a 98.8 $\pm$ 0.9% (N=10) agreement of duplicate analyses were found. The detection limit was 2.2 $mg \cdot L^{-1}$.

Hydrazine (Kodak, lot A15A, 97.3% pure). Hydrazine was analyzed by two methods, both of which yielded similar results. The first method, used for the fathead minnow flow-through and static tests, was a titrimetric method (Koupparis and Hadjiioannou, 1978). Samples were pipetted into a vessel and a known amount of chloramine-T solution, potassium iodide and acetic acid added. After the reaction was complete, the liberated iodine was titrated to a starch end-point using standardized thiosulfate solution. For this method, a spike recovery of 86.0 $\pm$ 6.6% (N=14) and 95.1 $\pm$ 3.8% (N=8) agreement of duplicate analyses were found. The detection limit was 0.64 $mg \cdot L^{-1}$.

The second method, used for the amphipod flow-through and cladoceran static acute hydrazine tests, was a spectrophotometric method ASTM 1976). This method was used because of the need for greater analytical sensitivity. Aqueous samples, diluted if necessary, were acidified with hydrochloric acid. Following

the addition of a p-dimethylaminobenzadehyde color reagent, the absorbances were read at a wavelength of 458 nm on a Perkin Elmer Hitachi 200 spectrophotometer using 1 cm cells. A spike recovery of 100.8 ± 1.2% (N=6) and a 99.0 ± 1.1% agreement of duplicate analyses were found for this method. The detection limit was 4 $ug \cdot L^{-1}$.

Formaldehyde (biological solutions: Paraformaldehyde, Kodak, lot B16D, 90% min. purity; analytical solutions: Formalin, Aldrich, lot 03707AT, 36.54% (w/w) formaldehyde). Due to the presence of methanol as a stabilizing agent in formalin solutions, and the oxygen demand of methanol in test exposures, paraformaldehyde was used to generate a stock of formaldehyde in water that was free of methanol. Paraformaldehyde was placed in a distillation flask and heated. Formaldehyde gas was driven off and bubbled directly into a flask of exposure water. The solution was filtered through a paper filter, analyzed, and then adjusted to the desired concentration. Stock solutions prepared using this method appear to be relatively stable for at least 96 hr.

Formaldehyde was analyzed using a spectrophotometric method (Hauser and Cummings 1964). The method is sensitive to numerous aldehydes, but because the stock had been shown to contain only formaldehyde (gas chromatographic analysis), the method was acceptable. A sample was added to a known volume of a 0.05% 3-methyl-2-benzothiazolone hydrozone (MBTH) solution. Following the addition of the oxidizing reagent (1.6% sulfamic acid and 1% ferric chloride solution) and color development, the absorbance was read at 628 nm. Concentrations were determined from a calibration curve of formaldehyde standards prepared from formalin. A spike recovery of 95.1 ± 5.6% (N=6) and 98.5 ± 0.4% (N=6) agreement of duplicate analyses were found. The detection limit was 0.036 $mg \cdot L^{-1}$.

The remaining compounds were analyzed by gas-liquid chromatography utilizing either a Hewlett Packard (HP) Model 5794A gas chromatograph with a HP Model 3390

Integrator, HP Model 7671A Autosampler and flame ionization detection (GC-FID); or a HP Model 5880A gas chromatograph with a HP Level 4 terminal, HP Model 7672 Autosampler and electron capture detector (GC-ECD). Hydrogen or helium were used as carrier gases and nitrogen (GC-FID) and argon-methane (GC-ECD) were used as make-up gases. The injector and detector temperatures were 250°C and 300°C, respectively. Injection volumes were approximately 1 uL. All GC-FID analyses were made in the split mode and all GC-ECD analyses were made in the splitless mode.

One of three J & W Scientific, Inc. (Folsom, CA) Durabond capillary columns were used in the analyses: (1) DB-1 (30M x .32 mm) with a 1.0 um film of crosslinked and bonded 100% dimethylpolysiloxane, (2) 45M x 0.258 mm) with a 1.0 um film of crosslinked and bonded 95% dimethyl - 5% diphenylpolysiloxane, and (3) DB wax (30M x .255 mm) with a 0.5 um film of crosslinked and bonded polyethylene glycol.

Benzene (Baker, lot 902176, 99.9% pure). Exposure samples were extracted with pentane. The solvent layer was analyzed by GC-FID on a DB-5 column using temperature programming (40° for 3 min; 40-135° C @ 20°/min). Toluene was used as an internal standard. A 98.2 $\pm$ 7.3% (N=10) spike recovery and 96.4 $\pm$ 4.1% (N=10) agreement of duplicate analyses were found. The detection limit was 0.54 $mg \cdot L^{-1}$.

Carbon tetrachloride (Kodak, lot 1143056, 99% pure). Exposure samples were extracted with pentane. The solvent layer was analyzed by GC-FID on a DB-5 column isothermally at 70°C. Chloroform was used as an internal standard. A 93.9 $\pm$ 12.3% (N=4) spike recovery and 95.1 $\pm$ 3.7% (N=5) agreement of duplicate analyses were found. The detection limit was 1.6 $mg \cdot L^{-1}$.

1,4-Dioxane (Kodak, lot C16A, 99% pure). Direct aqueous injections were analyzed by GC-FID isothermally (80°C) on a DB Wax column. Pyridine was used as

an internal standard. A 100.4 ± 7.8% (N=12) spike recovery and 96.1 ± 3.7% (N=14) agreement of duplicate analyses were found. The detection limit was 7.6 $mg \cdot L^{-1}$.

Ethanal (Acetaldehyde) (Kodak, lot A16A, 99% pure). Direct aqueous injections of samples were analyzed by GC-FID isothermally (50°C) on a DB Wax column. Methanol was used as an internal standard. An overall 104.6 ± 5.7% (N=9) spike recovery and 95.0 ± 3.3% (N=8) agreement of duplicate analyses were found. The detection limit was 1.6 $mg \cdot L^{-1}$.

Ethylbenzene (Aldrich, lot 4520 LK, 99% pure). Exposure samples were extracted with hexane. The solvent layer was analyzed by GC-FID isothermally (125° C) on a DB-5 column. The internal standard was 2-octanone. An overall 93.7 ± 7.9% (N=20) spike recovery and 97.0 ± 2.1% (N=16) agreement of duplicate analyses were found. The detection limit was 0.33 $mg \cdot L^{-1}$.

Furfural (Kodak, lot D16A, 98% pure). Aqueous injections of samples were analyzed by GC-FID isothermally (150° C) on a DB-Wax column. Pyridine was used as an internal standard. A 102.2 ± 4.8% (N=11) recovery of spikes and 95.7 ± 3.4% (N=11) agreement of duplicate analyses were found. The detection limit was 0.42 $mg \cdot L^{-1}$.

Hexachloro-1,3-butadiene (Aldrich, lot 01821LL, 98% pure). Exposure samples were extracted with hexane. The solvent layer was analyzed by GC-ECD on a DB-1 column using temperature programming (55° C/min, 55-250° C, @ 10° C/min). A 99.0 ± 7.2% (N=4) recovery of spikes and 98.2 ± 1.7% (N=4) agreement of duplicate analyses were found. The detection limit was 1.4 $ug \cdot L^{-1}$.

Hexachlorocyclopentadiene (Aldrich, lot 00817JH, 98% pure). Exposure samples were extracted with hexane. The solvent layer was analyzed by GC-ECD using temperature programming (55°C/min; 55-250° @ 10°/min) on a DB-1 column. A 101% (N=2) recovery of spikes and 92.7% (N=2) agreement of duplicate analyses

were found. The detection limit was 3.0 $ug \cdot L^{-1}$.

Pentachloroethane (Aldrich, lot 0092CJ, 96% pure). Exposure samples were extracted with hexane. The solvent layer was analyzed by GC-FID isothermally (130° C) on a DB-5 column. Ethylbenzene was used as an internal standard. A 98.0 ± 5.3% (N=4) recovery of spikes and 96.2 ± 3.9% (N=3) agreement of duplicate analyses were found. The detection limit was 0.14 $mg \cdot L^{-1}$.

Styrene (Kodak, lot B15A, 98% pure). Exposure samples were extracted with hexane. The solvent layer was analyzed by GC-FID isothermally (170° C) on a DB-5 column. The internal standard used was 2-octanone. A 96.8 ± 3.6% (N=9) recovery of spikes and 98.3 ± 3.1% (N=10) agreement of duplicate analyses were found. The detection limit was 0.04 $mg \cdot L^{-1}$.

Toluene (Omnisolv, lot 4331, 99.9% pure). Exposure samples were extracted with hexane. The solvent layer was analyzed by GC-FID isothermally (90° C) on a DB-5 column. The internal standard was pyridine. A 99.8 ± 11.0% (N=7) recovery of spikes and 95.8 ± 5.7% (N=5) agreement of duplicate analyses were found. The detection limit was 0.7 $mg \cdot L^{-1}$.

p-Xylene (Aldrich, lot PE0511PE, 99% pure). Exposure samples were extracted with hexane. The solvent layer was analyzed by GC-FId isothermally (125° C) on a DB-5 column. The internal standard used was 2-octanone. A 94.3 ± 2.9% (N=5) spike recovery and 98.4 ± 0.6% (N=5) agreement of duplicate analyses were found. The detection limit was 0.05 $mg \cdot L^{-1}$.

Pentachlorobenzene (Aldrich, lot AB060627, 98% pure). Exposure samples were extracted with hexane. The solvent layer was analyzed by GC-ECD on a DB-1 column using temperature programming (55°/min, 55°-250° @ 15°/min). A 99.9 ± 9.1% (N=16) spike recovery and 93.0 ± 5.0% (N=17) agreement of duplicate analyses was found. The detection limit was 0.172 $ug \cdot L^{-1}$.

Tetrachloroethylene (Aldrich, lot 04520PM, 98% pure). Exposure samples were

extracted with hexane. The solvent layer was analyzed isothermally (100° C) on a DB-wax column. Pyridine was used as an internal standard. A 100.2 ± 6.3% (N=5) recovery of spikes and 96.9 ± 2.4% (N=5) agreement of duplicate analyses was found. The detection limit was 0.04 $mg \cdot L^{-1}$.

RESULTS

Toxicity Tests - Fathead minnows were exposed to eighteen compounds in flow-through and static tests with measured concentrations of the compounds. All flow-through exposures, with the exception of two (hydrazine and pyridine), resulted in lower LC50 values than the static tests using nominal or unmeasured compound concentrations (Table 2). Agreement between nominal and 0-hour analyticals in static exposures was poor for approximately half the compounds and within a factor of two for the others. Comparison of the flow-through test results with static test results, generally, showed improvement when using 0-hour analyticals over nominals and further improvement when the complete analytical set for the static tests was used.

Amphipods were exposed to ten of the compounds with flow-through tests (Table 2). No attempt was made to contrast these results with static exposures. Cladocerans were exposed to two compounds; one a flow-through test and the other a static exposure (Table 2).

Chemical Loss from Solution

An attempt was made to estimate the rate of loss of each test compound from the static exposures. Two exposures differing by a factor of approximately 2 to 5 were measured daily through the 96 hr test to measure concentration decline. Loss of concentration could have been due to volatilization, sorption, biodegradation, organism uptake, photodecomposition or a combination of these factors.

TABLE 2. LC50 or EC50 ($ug \cdot L^{-1}$) estimates (95% confidence limits in parentheses) for exposure of fathead minnow (Pimephales promelas), an amphipod (Gammarus pseudolimnaeus) and a cladoceran (Daphnia magna) to organic compounds.

			Flow-through[b]	Static[b]		
Compound	Species[a]	Wet Weight ± s.d. (g)		Nominal Analyticals	0-Hr Analyticals	Complete Analyticals
Acrylamide	FHM	0.090±0.035	109,000 (103,000-115,300)	139,800 (118,100-165,600)	143,600 (120,900-170,600)	151,000 (127,100-179,300)
	Amphipod	0.008±0.004	55,400 (40,300-76,000)			
Benzene	FHM	0.063±0.020	12,500 (10,700-14,700)	107,200 (90,400-127,000)	83,200 (70,100-98,700)	35,700 (28,900-44,200)
	Amphipod	0.021±0.012	12,100 (6,700-22,000)			
Carbontetra-chloride	FHM	0.092±0.037	41,400 (36,300-47,300)	141,000 (129,000-154,000)	29,200 (26,600-32,000)	10,400 (9,680-11,300)
	Amphipod	0.008±0.003	11,100 (9,730-12,600)			
1,4-Dioxane	FHM	0.106±0.044	9,872,000 (N.R.)[c]	13,199,000 (11,178,000-15,586,000)	13,792,000 (11,654,000-16,322,000)	12,326,000 (10,306,000-14,742,000)
	Amphipod	0.039±0.014	2,274,000 (1,800,000-2,872,000)			
	Cladoceran					4,269,000 (3,494,000-5,215,000)
Ethanal [Acetaldehyde]	FHM	0.076±0.029	36,800 (30,800-43,900)	148,000 (135,000-161,000)	110,000 (102,000-118,000)	43,100 (39,800-46,800)
	Amphipod	0.034±0.017	19,300 (15,000-24,900)			

TABLE 2 Cont. LC50 or EC50 ($ug \cdot L^{-1}$) estimates (95% confidence limits in parentheses) for exposure of fathead minnow (Pimephales promelas), an amphipod (Gammarus pseudolimnaeus) and a cladoceran (Daphnia magna) to several organic compounds.

Compound	Species	Wet Weight ± s.d. (g)	Flow-through[b]	Static[b]		
				Nominal Analyticals	0-Hr Analyticals	Complete Analyticals
Ethylbenzene	FHM	0.079±0.041	9,100 (7,500-11,000)	77,300 (66,400-90,000)	25,700 (22,100-30,000)	11,900 (9,100-15,600)
	Amphipod	0.038±0.008	1,940 (1,260-2,990)			
Formaldehyde	FHM	0.068±0.038	24,500 (22,900-26,200)	39,600 (36,100-43,400)	44,300 (40,200-48,800)	26,300 (23,200-29,700)
Furfural	FHM	0.061±0.024	21,020 (16,790-26,350)	41,600 (35,900-48,300)	29,900 (25,800-34,700)	16,100 (13,400-19,300)
	Amphipod	0.041±0.013	11,890 (9,180-15,400)			
Hexachloro-butadiene	FHM	0.093±0.044	90[d] (90-100)	1,140 (940-1,390)	507 (418-614)	205 (166-253)
Hexachloro-cyclopentadiene	FHM	0.058±0.022	7.0[e] (6.4-7.6)	565.7 (N.R.)	29.6 (N.R.)	13.9 (N.R.)
Hydrazine	FHM	0.077±0.033	2,840 (2,070-3,890)	2,460 (1,780-3,410)	2,730 (2,080-3,580)	2,250 (1,810-2,790)
	Amphipod	0.030±0.012	700 (580-840)			
	Cladoceran		280 (226-348)			
Pentachloro-ethane	FHM	0.057±0.028	7,530[d] (7,220-7,850)	66,000 (60,100-72,400)	13,900 (12,720-15,230)	5,750 (5,090-6,490)

TABLE 2 Cont. LC50 or EC50 ($ug \cdot L^{-1}$) estimates (95% confidence limits in parentheses) for exposure of fathead minnow (Pimephales promelas), an amphipod (Gammarus pseudolimnaeus) and a cladoceran (Daphnia magna) to several organic compounds.

Compound	Species	Wet Weight + s.d. (g)	Flow-through[b]	Static[b] Nominal Analyticals	Static[b] 0-Hr Analyticals	Static[b] Complete Analyticals
Pentachloro-benzene	FHM	0.128±0.022	247 (210-291)	1,060 (N.R.)	672 (N.R.)	378 (N.R.)
	Amphipod	0.007±0.003	51.1 (39.2-66.6)			
Pyridine	FHM	0.059±0.030	106,000[f] (N.R.) 93,800[f] (85,500-103,000)	91,500 (85,500-97,900)	94,800 (88,800-101,200)	68,300 (63,400-73,600)
Tetrachloro-ethylene	FHM	0.073±0.030	13,400[d] (12,400-14,400) 20,300[d] (17,900-23,000)	53,600 (45,000-63,800)	25,600 (21,100-31,000)	10,800 (8,600-13,500)
Toluene	FHM	0.067±0.022	36,200[f] (29,400-44,600)	51,200 (47,900-54,800)	50,800 (48,200-53,500)	22,100 (20,500-23,800)
Styrene	FHM	0.083±0.035	4,080 (3,290-5,050)	61,400 (50,000-75,400)	21,100 (16,700-26,800)	9,900 (6,750-14,500)
	Amphipod	0.041±0.017	2,990 (2,090-4,290)			
p-Xylene	FHM	0.093±0.047	8,870[f] (8,010-9,810)	21,200 (18,400-24,500)	22,400 (19,700-25,400)	8,400 (7,200-9,900)

TABLE 2 Cont.

[a] FHM = Fathead minnow (Pimephales promelas) 96-hr exposure; amphipod = Gammarus pseudolimnaeus 96-hr exposure; cladoceran = Daphnia magna 48-hr EC50.

[b] Flow-through test LC50 or EC50 estimates calculated from complete set of analytical determinations. Static test LC50 or EC50 estimates calculated using nominal, 0-hr or complete test analytical determinations.

[c] N.R. = Not Reliable. The 95% confidence interval could not be reliably estimated due to death pattern of the organisms.

[d] Data from Geiger et al. 1985.

[e] Data from Spehar et al. 1979. Fry 1-day old were exposed for 96 hr by Spehar et al. in contrast to 30-day old fish in the static exposure reported in this memorandum.

[f] Data from Geiger et al. 1986.

Time to 50% of initial measured concentration was calculated by linear regression (Table 3) using only the data approaching linearity. A better estimate for compound half-life will be achieved when all the concentration data for each compound are fitted to a quadratic equation. The quadratic equation estimate may lower the half-life estimates from those predicted using linear equations.

Generally, all the compounds tested showed concentration dependent half-lives with the lower concentrations decreasing to 50% concentrations more quickly than higher concentrations. One compound, acrylamide, did not decrease in concentration during the 96-hr test. Others decreased to 50% of initial concentrations at various rates. p-Zylene, styrene, pentachloroethane and benzene declined most rapidly of the compounds tested. 1,4-Dioxane, hydrazine and pyridine declined the slowest. The compounds with the slower concentration decline rate had generally good agreement between flow-through and static test results.

TABLE 3. Linear regression equations describing the rate of chemical compound decline for two initial concentrations and the calculated half-life of the compound in exposure systems at 22°C.

Compound	Initial Concentration ($ug \cdot L^{-1}$)	Regression Equation for Chemical Concentration Decline ($Y = a-bx$)[a]	$T_{1/2}$ (hr)
Acrylamide	308,000	b	b
	75,900	b	b
Benzene	162,000	Y=100-2.86x	17.5
	39,000	=100-3.196x	15.6
Carbon tetrachloride	96,900	=100-2.506x	19.9
	23,700	=100-2.823x	17.7
1,4-Dioxane	25,988,500	=98.5-0.229x	212.0
	6,447,000	=98.5-0.290x	167.6
Ethanal (acetaldehyde)	152,200	=95.6-1.678x	27.1
	41,200	=93.9-1.819x	24.2
Ethylbenzene	87,040	=100-2.244x	22.3
	21,290	=100-2.775x	18.0
Formaldehyde	67,200	=102.0-0.738x	70.5
	15,600	=115.2-1.057x	61.7
Furfural	234,100	=106.0-1.105x	50.7
	53,900	=104.1-1.099x	49.2
Hexachloro-1,3-butadiene	1,790	=92.3-1.735x	24.4
	468	=88.3-1.883x	20.3
Hexachlorocyclopentadiene	95.7	=102.3-2.042x	25.6
	18.1	= 91.7-2.083x	20.0
Hydrazine	23,200	= 97.3-0.278x	170.4
	5,630	=100.4-0.463x	108.8
Pentachloroethane	20,250	=100-3.067x	16.3
	5,350	=100-3.317x	15.1
Pentachlorobenzene	320	=100-2.992x	16.7
	160	=100-3.012x	16.6

TABLE 3 Cont. Linear regression equations describing the rate of chemical compound decline for two initial concentrations and the calculated half-life of the compound in exposure systems at 22°C.

Compound	Initial Concentration ($ug \cdot L^{-1}$)	Regression Equation for Chemical Concentration Decline ($Y = a-bx$)[a]	$T_{1/2}$ (hr)
Pyridine	486,000	Y=99.4-0.479x	103.1
	129,000	=97.9-0.545x	87.9
Tetrachloroethylene	51,700	=100-2.312x	21.6
	11,300	=100-2.750x	18.2
Toluene	76,000	=100-2.315x	21.6
	16,700	=100-2.746x	18.2
Styrene	65,000	=101.5-3.043x	16.9
	18,700	=100-3.477x	14.4
p-Zylene	59,900	=100-3.012	16.6
	15,700	=100-3.529	14.2

a Y = Percent of original compound concentration; a = intercept on y axis at time 0; b = slope or rate of compound decline; x = time (hr).

b Compound concentration did not decline during the 96-hr test.

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July 14, 1987

TO: Advisory Guidelines Workgroup

FROM: Mary D. Balcer

SUBJECT: Adjustment factor for static test results

According to the Guidelines for Deriving Ambient Aquatic Life Advisory Concentrations dated May 1987, the LC50 values obtained from static tests must be adjusted if the concentration of the chemical in the test medium decreases by more than 50% from the beginning to the end of the test. The adjustment factor is to be obtained by dividing the time-weighted LC50 from a flow-through exposure by the LC50 from a comparable static exposure in which the chemical concentrations were measured at the beginning of the test.

In order to obtain data to derive these adjustment factors, the UW-Superior scientists have conducted a series of comparison tests with the fathead minnow. Preliminary results from several of these tests are shown in Table 1, along with ratios of the test results. The LC50 s derived from static tests measured at 0-hrs (SM_o) are often quite different from the flow-through (F M) LC50 s (i.e. Benzene and HCBD). A closer examination of the data also reveals that ratios obtained by dividing the flow-through measured results by results from static tests measured at 0-hr (FM/SM_o) are often quite different from ratios obtained by dividing flow-through measured results by results from static tests measured several times during the exposure (FM/SM). In some cases (i.e. Xylene), the data suggest that LC50 s from SM tests should not be adjusted while results from SM_o tests may differ significantly from the flow-through conditions. Correction factors also appear to differ for SM_o tests and static unmeasured tests (SU).

Because of these differences, I am concerned about the current guidelines which require that we divide all LC50 s obtained from static tests (regardless of whether they were based on nominal concentrations, measured at 0-hr, or measured several times) by a single factor $\left(\frac{SM_o}{FM}\right)$. I would suggest that we develop a separate correction factor for each set of test conditions and make adjustments accordingly. In this way the original authors' data will be adjusted to correspond to his test methodology.

I would appreciate it if the guideline committee would consider this issue at its earliest convenience. The choice of an appropriate correction factor will cause differences in the calculation of Genus Mean Aucte Values and therefore in the final Advisory Values. Until we hear from you, we will continue to adjust all static LC50 s by multiplying by the ratio of FM/SM_o.

jb

TABLE 1. Relationships Between Flow-through and Static Test Results for Fathead Minnows

Compound	96 Hr LC50 (mg/L)				Ratio and (% Difference)[b/]		
	FM[a/]	SM	SM_o	SU	FM/SM	FM/SM_o	FM/SU
Carbon tetrachloride	19.2	10.4	29.2	141[c/]	1.85 (+46%)	.657 (-52%)	.136 (-634%)
Furfural	21.0	14.0	29.9	41.6	1.50 (+33%)	.702 (-42%)	.505 (-98%)
Toluene	36.2	22.1	50.8	51.2	1.64 (+38%)	.713 (-40%)	.707 (-41%)
Pentachloroethane	7.53	5.75	13.9	66.0	1.31 (+24%)	.542 (-85%)	.114 (-776%)
Xylene	8.87	8.44	22.4	21.2	1.05 (+5%)	.396 (-152%)	.418 (-139%)
Acetaldehyde	36.8	43.1	110	148	.85 (-17%)	.384 (-199%)	.249 (-302%)
Tetrachloroethylene	16.5	16.9	28.6	70.7	.976 (-2%)	.577 (-73%)	.233 (-328%)
Ethylbenzene	9.1	11.9	25.7	77.3[c/]	.843 (-7%)	.358 (-276%)	.117 (-749%)
Styrene	4.08	7.36	21.1	61.4	.554 (-80%)	.193 (-417%)	.066 (-1405%)
HCBD	0.09	0.205	0.507	1.1	.439 (-128%)	.178 (-463%)	.082 (-1122%)
HCCPD	0.007	0.012	0.030	1.13	.583 (-71%)	.233 (328%)	.006 (16043%)
Benzene	12.5	35.7	83.2	107[c/]	.35 (-186%)	.150 (-566%)	.117 (-756%)

a/ FM = weighted flow-through measured results
SM = static test, measured at 0, 48 and 96 hr
SM_o = static test, measured at 0 hr only
SU = static test, based on nominal concentration

b/ % difference calculated as $\frac{FM-SM}{FM} \times 100$, $\frac{FM-SM_o}{FM} \times 100$ or $\frac{FM-SU}{FM} \times 100$

c/ These values are not based on strict nominal concentrations, but on the measured concentration of the stock solution