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TO: Loren Larson
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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 1×10^8 cells $\cdot L^{-1}$ of green algae, Selenastrum capricornutum, and $5 \text{ mg} \cdot L^{-1}$ 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·L⁻¹ as CaCO₃; mean total alkalinity ranged from 28.3 to 100 mg·L⁻¹ as CaCO₃; 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 \pm standard deviation, range (in parentheses), and sample size.

| Compound ^a | Species ^b | Type of Test | Dissolved Oxygen (%) | Total (EDTA) Hardness (mg·L ⁻¹ as CaCO ₃) | Total Alkalinity (mg·L ⁻¹ as CaCO ₃) | pH | Temperature (°C) |
|------------------------------|----------------------|--------------|---|--|---|--|--|
| Acrylamide 79-06-1 | FHM & Amphipod | Flow-through | 86.5 \pm 3.5 (83.9-92.4) n=12 | 50.8 \pm 0.9 (50.0-52.0) n=4 | 43.3 \pm 1.3 (41.6-44.8) n=4 | 7.08 \pm 0.06 (7.01-7.18) n=12 | 24.1 \pm 0.6 (23.2-25.8) n=60 |
| Acrylamide 79-06-1 | FHM | Static | 70.1 \pm 6.1 (63.4-82.1) n=12 | 55.5 \pm 2.2 (52.6-58.0) n=4 | 50.6 \pm 3.2 (47.0-53.6) n=4 | 7.07 \pm 0.09 (6.91-7.17) n=12 | 22.1 \pm 0.1 (21.8-22.3) n=60 |
| Benzene 71-43-2 | FHM & Amphipod | Flow-through | 85.3 \pm 4.8 (6.8-8.2) n=12 | 50.7 \pm 0.0 (50.7-50.7) n=4 | 42.5 \pm 1.0 (42-44) n=4 | 7.04 \pm 0.01 (7.02-7.05) n=4 | 22.3 \pm 0.5 (21.7-22.9) n=50 |
| Benzene 71-43-2 | FHM | Static | 74.5 \pm 9.5 (47.2-83.4) n=12 | 51.2 \pm 0.5 (50.4-51.6) n=4 | 43.0 \pm 0.8 (42.2-43.6) n=4 | 6.96 \pm 0.12 (6.77-7.11) n=12 | 21.7 \pm 0.26 (21.3-22.1) n=60 |
| Carbon tetrachloride 56-23-5 | FHM & Amphipod | Flow-through | 81.1 \pm 4.9 (74.5-86.0) n=10 | 49.2 \pm 1.1 (48.2-50.6) n=4 | 39.6 \pm 0.4 (39.0-39.8) n=4 | 6.82 \pm 0.06 (6.73-6.93) n=10 | 21.7 \pm 0.1 (21.5-22.1) n=60 |
| Carbon tetrachloride 56-23-5 | FHM | Static | 87.2 \pm 10.9 (70.2-100.6) n=11 | 52.3 \pm 0.5 (52.0-53.0) n=4 | 44.0 \pm 0 (44.0-44.0) n=4 | 7.20 \pm 0.11 (7.07-7.34) n=4 | 22.5 \pm 0.56 (21.5-23.4) n=56 |
| 1,4-Dioxane 123-91-1 | FHM & Amphipod | Flow-through | 93.3 \pm 3.3 (86.4-98.3) n=12 | 50.2 \pm 1.0 (48.6-50.7) n=4 | 42.0 \pm 0.0 (42.0-42.0) n=4 | 7.32 \pm 0.10 (7.12-7.47) n=12 | 22.1 \pm 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 \pm standard deviation, range (in parentheses), and sample size.

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

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

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

| Compound ^a | Species ^b | Type of Test | Dissolved Oxygen (%) | Total Hardness (mg·L ⁻¹ as CaCO ₃) | Total Alkalinity (mg·L ⁻¹ as CaCO ₃) | pH | Temperature (°C) |
|-----------------------|----------------------|--------------|--|---|---|--|---|
| Styrene 100-42-5 | FHM | Flow-through | 92.5 \pm 4.35 (82.8-97.8) n=12 | 52.8 \pm 2.4 (49.2-54.6) n=4 | 40.6 \pm 1.1 (39.0-41.0) n=4 | 7.15 \pm 0.06 (7.05-7.25) n=12 | 21.3 \pm 0.4 (20.6-22.1) n=60 |
| Styrene 100-42-5 | FHM | Static | 90.3 \pm 5.8 (80.4-102.1) n=12 | 51.0 \pm 1.2 (50.0-52.0) n=4 | 42.8 \pm 1.0 (42.0-44.0) n=4 | 7.28 \pm 0.27 (6.86-7.64) n=12 | 23.54 \pm 0.96 (22.4-25.1) n=60 |
| p-Xylene 106-42-3 | FHM | Static | 73.4 \pm 13.9 (54.7-91.8) n=12 | 51.1 \pm 1.2 (49.4-52.3) n=4 | 50.2 \pm 2.4 (48.0-52.8) n=4 | 7.22 \pm 0.23 (7.03-7.59) n=12 | 21.7 \pm 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-Kärber 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 $1.13 \text{ ug}\cdot\text{L}^{-1}$.

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 $2.2 \text{ mg}\cdot\text{L}^{-1}$.

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 endpoint 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 $0.64 \text{ mg}\cdot\text{L}^{-1}$.

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 $100.8 \pm 1.2\%$ (N=6) and a $99.0 \pm 1.1\%$ agreement of duplicate analyses were found for this method. The detection limit was $4 \text{ ug}\cdot\text{L}^{-1}$.

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 $95.1 \pm 5.6\%$ (N=6) and $98.5 \pm 0.4\%$ (N=6) agreement of duplicate analyses were found. The detection limit was $0.036 \text{ mg}\cdot\text{L}^{-1}$.

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 cross-linked 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: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: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 $100.4 \pm 7.8\%$ (N=12) spike recovery and $96.1 \pm 3.7\%$ (N=14) agreement of duplicate analyses were found. The detection limit was $7.6 \text{ mg}\cdot\text{L}^{-1}$.

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 $104.6 \pm 5.7\%$ (N=9) spike recovery and $95.0 \pm 3.3\%$ (N=8) agreement of duplicate analyses were found. The detection limit was $1.6 \text{ mg}\cdot\text{L}^{-1}$.

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 $93.7 \pm 7.9\%$ (N=20) spike recovery and $97.0 \pm 2.1\%$ (N=16) agreement of duplicate analyses were found. The detection limit was $0.33 \text{ mg}\cdot\text{L}^{-1}$.

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 $102.2 \pm 4.8\%$ (N=11) recovery of spikes and $95.7 \pm 3.4\%$ (N=11) agreement of duplicate analyses were found. The detection limit was $0.42 \text{ mg}\cdot\text{L}^{-1}$.

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 ($55^{\circ}\text{C}/\text{min}$, $55\text{-}250^{\circ}\text{C}$, @ $10^{\circ}\text{C}/\text{min}$). A $99.0 \pm 7.2\%$ (N=4) recovery of spikes and $98.2 \pm 1.7\%$ (N=4) agreement of duplicate analyses were found. The detection limit was $1.4 \text{ ug}\cdot\text{L}^{-1}$.

Hexachlorocyclopentadiene (Aldrich, lot 00817JH, 98% pure). Exposure samples were extracted with hexane. The solvent layer was analyzed by GC-ECD using temperature programming ($55^{\circ}\text{C}/\text{min}$; $55\text{-}250^{\circ}$ @ $10^{\circ}/\text{min}$) 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 $3.0 \text{ ug}\cdot\text{L}^{-1}$.

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 $98.0 \pm 5.3\%$ (N=4) recovery of spikes and $96.2 \pm 3.9\%$ (N=3) agreement of duplicate analyses were found. The detection limit was $0.14 \text{ mg}\cdot\text{L}^{-1}$.

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 $96.8 \pm 3.6\%$ (N=9) recovery of spikes and $98.3 \pm 3.1\%$ (N=10) agreement of duplicate analyses were found. The detection limit was $0.04 \text{ mg}\cdot\text{L}^{-1}$.

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 $99.8 \pm 11.0\%$ (N=7) recovery of spikes and $95.8 \pm 5.7\%$ (N=5) agreement of duplicate analyses were found. The detection limit was $0.7 \text{ mg}\cdot\text{L}^{-1}$.

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 $94.3 \pm 2.9\%$ (N=5) spike recovery and $98.4 \pm 0.6\%$ (N=5) agreement of duplicate analyses were found. The detection limit was $0.05 \text{ mg}\cdot\text{L}^{-1}$.

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 ($55^{\circ}/\text{min}$, 55° - 250° @ $15^{\circ}/\text{min}$). A $99.9 \pm 9.1\%$ (N=16) spike recovery and $93.0 \pm 5.0\%$ (N=17) agreement of duplicate analyses was found. The detection limit was $0.172 \text{ ug}\cdot\text{L}^{-1}$.

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 $100.2 \pm 6.3\%$ (N=5) recovery of spikes and $96.9 \pm 2.4\%$ (N=5) agreement of duplicate analyses was found. The detection limit was $0.04 \text{ mg}\cdot\text{L}^{-1}$.

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 ($\mu\text{g}\cdot\text{L}^{-1}$) 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.

| Compound | Species ^a | Wet Weight + s.d. (g) | Flow-through ^b | | Static ^b | |
|---------------------------|----------------------|--------------------------|------------------------------------|---|---|---|
| | | | Nominal Analyticals | Complete Analyticals | Nominal 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 | | | | | | |
| 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 ($\mu\text{g}\cdot\text{L}^{-1}$) 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 | | Complete Analyticals |
|--------------------------------|------------|--------------------------|-------------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | | | Nominal Analyticals | 0-Hr Analyticals | Nominal Analyticals | 0-Hr Analyticals | |
| Ethylbenzene | FHM | 0.079±0.041 | 9,100 (7,500-11,000) | 25,700 | 77,300 (66,400-90,000) | 25,700 | 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) | 44,300 | 39,600 (36,100-43,400) | 44,300 | 26,300 (23,200-29,700) |
| | Furfural | 0.061±0.024 | 21,020 (16,790-26,350) | 29,900 | 41,600 (35,900-48,300) | 29,900 | 16,100 (13,400-19,300) |
| Hexachloro- butadiene | Amphipod | 0.041±0.013 | 11,890 (9,180-15,400) | | | | |
| | FHM | 0.093±0.044 | 90 ^d (90-100) | 507 (418-614) | 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.) |
| | Amphipod | 0.030±0.012 | 700 (580-840) | | | | |
| Hydrazine | FHM | 0.077±0.033 | 2,840 (2,070-3,890) | 2,730 | 2,460 (1,780-3,410) | 2,730 | 2,250 (1,810-2,790) |
| | Cladoceran | | 280 (226-348) | | | | |
| Pentachloro- ethane | FHM | 0.057±0.028 | 7,530 ^d (7,220-7,850) | 13,900 (12,720-15,230) | 66,000 (60,100-72,400) | 13,900 (12,720-15,230) | 5,750 (5,090-6,490) |

TABLE 2 Cont. LC50 or EC50 ($\mu\text{g}\cdot\text{L}^{-1}$) 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 | |
|--------------------------|----------|--------------------------|---|---------------------------|----------------------------|---------------------------|
| | | | Flow-through ^b | Nominal Analyticals | 0-Hr Analyticals | 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.) | 91,500 (85,500-97,900) | 94,800 (88,800-101,200) | 68,300 (63,400-73,600) |
| | | | 93,800 ^f (85,500-103,000) | | | |
| Tetrachloro- ethylene | FHM | 0.073±0.030 | 13,400 ^d (12,400-14,400) | 53,600 (45,000-63,800) | 25,600 (21,100-31,000) | 10,800 (8,600-13,500) |
| | | | 20,300 ^d (17,900-23,000) | | | |
| 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) |
| | | | 4,080 (3,290-5,050) | | | |
| Styrene | FHM | 0.083±0.035 | 2,990 (2,090-4,290) | 61,400 (50,000-75,400) | 21,100 (16,700-26,800) | 9,900 (6,750-14,500) |
| | Amphipod | 0.041±0.017 | | | | |
| 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·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 | 103.8 |
| 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·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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