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Response Characteristics of an Aquatic Biomonitor Used for Rapid Toxicity Detection†

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INTRODUCTION

Recent concerns over water security have increased interest in technology that can rapidly detect the presence of toxic chemicals in water. Much effort has been directed toward developing on-line chemical monitors (e.g. fluorometers, spectrophotometers, gas or liquid chromatographs), but chemical monitors may not detect certain analytes or classes of analytes and, because they do not directly measure toxicity, chemical detectors cannot measure synergistic toxic effects that may be associated with chemical mixtures. By directly measuring aquatic toxicity, automated aquatic biological early warning systems (BEWS) can complement on-line chemical monitors by providing early warning of contamination.

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Automated BEWS detect toxicity by sensing changes in the behavior or physiological conditions in aquatic organisms. Many different types of organisms have been evaluated, including bacteria, algae, protozoans, bivalves, daphnids and fish (Kramer and Botterweg, 1991; Gerhardt, 1999). Among the first BEWS developed were those employing fish (e.g. Cairns *et al.*, 1970). Endpoints in fish BEWS include rheotaxis (Poels, 1975), activity levels (Cairns *et al.*, 1970; Morgan, 1977), movement patterns (Blubaum-Gronau *et al.*, 1994), electric organ discharges (Thomas *et al.*, 1996) and ventilatory patterns (Cairns *et al.*, 1970). Fish ventilatory patterns are conveniently monitored by remote electrodes that detect muscle-related electrical activity, and the patterns are known to change in response to a variety of environmental pollutants and toxicants (Cairns and van der Schalie, 1980; Diamond *et al.*, 1990; ASTM, 1995). Key data extracted from fish ventilatory signals include ventilatory (opercular movement) rate, depth or amplitude of ventilation and coughing or gill purge rate. A fourth parameter, whole body movement, is detected as a distortion of the ventilatory signal. Most fish BEWS monitor only one or two of these parameters, but a system developed at the US Army Center for Environmental Health Research (USACEHR) can monitor all four (van der Schalie *et al.*, 1988). The USACEHR aquatic biomonitor can be deployed in mobile trailers or fixed facilities and can be configured as a

compact self-contained unit (53 × 53 × 84 cm; length × width × height). Monitoring applications include surface waters (US EPA, 2001) and wastewater effluents (Shedd *et al.*, 2001; van der Schalie *et al.*, 2001).

Rapid detection of developing toxic conditions with minimal 'false alarms' are among the most important operational criteria proposed for BEWS (Evans *et al.*, 1986; Diamond *et al.*, 1988; Kramer and Botterweg, 1991; Baldwin and Kramer, 1994; Gruber *et al.*, 1994; ILSIRSI, 1999), but little information is available on the response of the USACEHR aquatic biomonitor to toxic chemicals. The EILATox-Oregon Workshop in Corvallis provided an opportunity to evaluate the USACEHR aquatic bio-monitor against a suite of 12 unknown samples, which included chemicals with varying modes of toxic action. Combined with in-house toxicity testing data, these test results help to define the toxicity response capabilities of the USACEHR aquatic biomonitor. This paper evaluates the toxicant concentrations causing rapid alarm responses in the aquatic biomonitor relative to corresponding 96-h LC_{50} levels and to the mode of toxic action of the tested chemicals. Historically, the LC_{50} has been the standard acute toxicity benchmark used in aquatic toxicity testing, and there are 96-h LC_{50} values for literally hundreds of chemicals for widely used test species such as the bluegill (*Lepomis macrochirus* Rafinesque). Understanding the relationship between the bluegill 96-h LC_{50} and aquatic biomonitor alarms would be helpful in predicting biomonitor responses to untested chemicals.

MATERIALS AND METHODS

Two types of toxicant response testing were conducted with the USACEHR aquatic biomonitor. In-house labora-tory tests performed at USACEHR followed procedures used in field deployments (van der Schalie *et al.*, 2001) and were conducted with continuous replacement of test solu-tions over exposure periods of up to 96 h. At the EILATox-Oregon Workshop, where the purpose was to screen a large number of unknown samples for toxicity, tests were conducted with recirculating water flows and a 1-h expo-sure period for each chemical. Other differences between in-house and workshop test procedures are noted below.

Test fish

Bluegills were used in all tests (length 4–8 cm; weight 1.6–14.7 g). Fish were acquired from local sources and acclimatized on site in control water with continuous light (wide-spectrum fluorescent bulbs) for a minimum of 2 weeks. Fish are held and tested under continuous light to eliminate diel changes in ventilatory patterns (Carlson, 1990). During acclimatization they were fed commercial trout chow and frozen brine shrimp, but once placed in the ventilatory chambers for testing the fish were not fed.

Test chemicals and analyses

In-house tests Reagent-grade chemicals tested include: cyanide (sodium cyanide, 99.98%; Aldrich Chemical Co., St. Louis, MO), malathion (99%; Chem Service Inc., West Chester, PA), pentachlorophenol (sodium pentachlor-ophenate, 99%; Aldrich Chemical Company, St. Louis,

MO), phenol (99%; Aldrich Chemical Company, St. Louis, MO), 1,1,2,2-tetrachloroethane (98%; Aldrich Chemical Company, St. Louis, MO), tricaine methanesulfonate (Aldrich Chemical Company, St. Louis, MO) and zinc (zinc sulfate heptahydrate, 99%; J. T. Baker, Mallinckrodt Baker, Inc., Phillipsburg NJ).

Test chemical solutions were analyzed as follows: zinc by inductively coupled argon plasma mass spectrometry (ICP-MS) using a Hewlett-Packard 4500 ICP-MS instrument equipped with a ChemStation data system; cyanide by ion selective electrode (Thermo Orion Model 9606 Combina-tion Cyanide Electrode, Thermo Orion, Beverly, MA); malathion by gas chromatography (Hewlett-Packard Model 6890 gas chromatograph with an electron capture detector and Hewlett-Packard 7673 autosampler; Hewlett-Packard, Avondale, PA); tricaine methanesulfonate, pentachloro-phenol and phenol by high-pressure liquid chromato-graphy (Hewlett-Packard 1050 series HPLC instrument equipped with variable-wavelength detector, autosampler and Hewlett-Packard 3396A integrator; Hewlett-Packard, Avondale, PA); and 1,1,2,2-tetrachloroethane by gas chromatography (Hewlett-Packard Model 6890 gas chro-matograph with an electron capture detector, Hewlett-Packard 7694 headspace sampler and ChemStation data system; Hewlett-Packard, Avondale, PA).

Workshop tests Blind samples were provided for testing at the workshop. Chemicals tested and sample prepara-tion are described elsewhere in this journal issue. Test solution concentrations are reported as nominal levels based on a sample dilution of 1:200 (as described in the next section).

Fish chambers and toxicant solution exposures

In-house ventilatory tests A customized solenoid toxicant diluter system supplied test solutions to four groups of eight fish (three toxicant concentrations plus a control). Dilution water was a mixture of 60% non-chlorinated well water obtained from a 150-m well adjoining the USACEHR facility at Fort Detrick, MD, and 40% dechlorinated, deionized tap water processed through a reverse osmosis system. Stock solutions of test chemicals in deionized water were delivered to the diluter using a peristaltic pump. Test solutions for each treatment were delivered either directly to individual test chambers (50 ml min^{-1}) or from a reservoir chamber with a 95% turnover time of ca. 2 h. During tests, the temperature, pH, dissolved oxygen and conductivity were measured at 30-min intervals in the controls by a Hydrolab® multiprobe monitor and were checked daily in all treatments. Water quality data were archived with the biomonitoring records. Ranges of dilution water quality parameters are shown in Table 1. Fish were held in individual cells (2.5 × 9.5 × 6 cm; 150-ml volume) in an eight-cell ventilatory chamber (23 × 15 × 12 cm). The cells allow source water to enter the back bottom of the chamber, pass through the chamber and exit at the front over a spillway. Chambers were constructed of Plexiglas® and walls of adjoining cells are translucent in order to minimize disturbance and interactions among fish.

All fish were held in test chambers receiving dilution water for a 3-day acclimatization period and a 4-day base-line data period. Fish then received either control water or a toxicant solution. The time to first alarm at each concen-tration tested was determined.

Table 1—Dilution water parameters

Parameter	In-house[a]	Workshop
Temperature (°C)	23.2–26.1	24.3–25.1
pH	7.3–8.5	6.7–7.4
Dissolved oxygen (mg l^{-1})	6.2–8.8	7.5–7.9
Conductivity (mS cm^{-1})	0.46–1.0	0.11–0.14
Alkalinity (mg l^{-1} as CaCO$_3$)	84–162	10[b]
Hardness (mg l^{-1} as CaCO$_3$)	156–272	9.0[c]

[a] Hydrolab® data unavailable for the malathion test; manual measurements were consistent with the parameter ranges reported.
[b] P. McFadden, personal communication.
[c] Water treatment plant value (Corvallis Public Works Department, 2003).

Concentration levels in the aquatic biomonitor toxicity studies were set to bracket the 96-h LC$_{50}$ level, and ventilatory tests continued for at least 96 h to permit calculation of a 96-h LC$_{50}$ when there was sufficient mortality of fish exposed in the ventilatory system. For chemicals where a 96-h LC$_{50}$ could not be estimated directly from the ventilatory studies, separate acute toxicity studies were conducted. Bluegills from the same stock as the ventilatory study fish were randomized into tanks containing 16 l of well water. Each tank received approximately five tank volumes per day of test solution, delivered by a toxicant diluter. Water quality parameters were consistent with values reported in Table 1. Each test included five treatments (four toxicant concentrations plus a control), with a dilution factor between treatments of 0.5; there were two replicates of 10 fish at each treatment level. The trimmed Spearman–Karber method (Hamilton et al., 1977) was used to compute 96-h LC$_{50}$ values.

Workshop tests Fish chambers similar to those used for the in-house tests were used. The test apparatus contained two eight-cell chambers, with each chamber linked to separate water reservoirs. Because flow-through dilution water was unavailable, fish in each group were exposed alternately to single unknown samples diluted into and recirculated from the reservoir. Fish initially placed in the test chambers were acclimatized to the test chambers for 16 h prior to the 1-h baseline period. Dilution water was dechlorinated tap water originally obtained from the Columbia River, which had much lower hardness, alkalinity, pH and conductivity than the water used for the in-house tests. As with the in-house tests, water measurements were taken during testing using a multiprobe water quality meter. Total water capacity for the reservoir plus test chamber was 5 l. Given that unknown samples were provided in 25-ml volumes, this provided a fixed 1:200 dilution. At the end of each exposure, test solution was drained from the reservoir and any remaining toxicant was flushed from the apparatus using several 5-l volumes of diluent water. If a fish group showed a response to unknown sample, that group was not exposed to a new sample until individual fish responses returned to near pre-exposure levels.
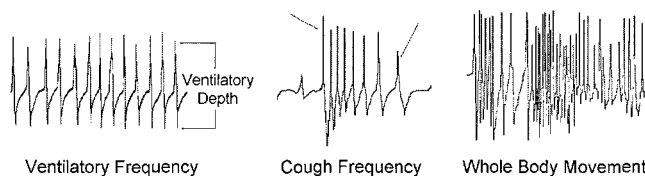
Ventilatory signal analysis

In both the in-house and workshop tests, ventilatory signals from individual fish were monitored by carbon block electrodes suspended above and below each fish in a chamber

Ventilatory Monitoring Chamber

Parameters Monitored

Ventilatory Frequency   Cough Frequency   Whole Body Movement
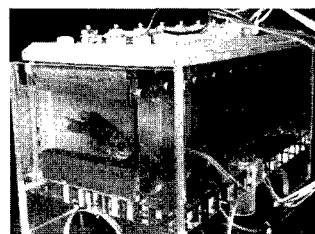
Figure 1. Fish ventilatory monitoring chamber and ventilatory patterns. Bluegills are placed in individual chambers with top and bottom carbon block electrodes. As the fish ventilate their gills, electrical signals generated by muscular contractions are received by the electrodes, allowing determination of the ventilatory frequency, depth of ventilation and cough frequency. If the fish actively swims in the chamber, the ventilatory signal is distorted and the overall body movement rate can be determined.

(Fig. 1). The electrical signals were amplified, filtered and passed onto a personal computer for analysis. Amplification was performed by PCI-20044T-1 and PCI-20045T-1 active analog signal conditioning termination panels (Intelligent Instrumentation, Tucson AZ). Each input channel was amplified independently by a high-gain true differential-input instrumentation amplifier; signal inputs of 0.05–1 mV were amplified by a factor of 1000. Signal interference by frequencies above 10 Hz was attenuated by low-pass filters.

Ventilatory parameters measured included ventilatory rate, ventilatory depth (mean signal height), gill purge (cough) frequency and whole-body movement (rapid irregular electrical signals) (Fig. 1). Specific algorithms are described elsewhere (Shedd et al., 2002). Each parameter was calculated at 15-s intervals, and any interval in which whole-body movement was detected was excluded from calculation of the other three parameters. The 15-s intervals are summed to create the selected monitoring record; for in-house tests, each record was 15 min, whereas for the workshop tests each record was 5 min. The accuracy of the system has been established by comparing the computer-generated values for ventilatory rate, average ventilatory depth and cough frequency with those obtained from strip chart recorder tracings. For a total of 128 records (2.5 min each) taken from six tests, the accuracy of ventilatory rate (and, by inference, average depth) was 99% with an R^2 value of 0.997 (slope 0.94), and the accuracy of the cough frequency was 118% with an R^2 value of 0.781 (slope 1.27; Shedd et al., 2001).

Data collected from each fish during a pre-exposure (baseline) period in dilution water is used to establish normal limits for each fish and the ventilatory parameter (Feder and Lordo, 1986). For in-house tests, a 4-day baseline of 15-min records was used; because of time constraints, workshop tests used a 1-h baseline. If, during the exposure period, an individual fish parameter becomes statistically different from its normal (baseline) response, the response is said to be 'out of control'. If >70% of fish

Table 2—Alarm responses to chemical exposures at or below the 96-h LC_{50} for in-house studies

Chemical[a]	Concentration tested[b] (mg l^-1)	Bluegill 96-h LC_{50} (mg l^-1)	Concentration (fraction of LC_{50})	First alarm time[c] (h)	Direction of response and time to alarm (h)[d]			
					Ventilatory rate	Ventilatory depth	Cough rate	Movement
1,1,2-Tetrachloroethane	14.6	21.0[e]	0.69	0.25	>>> 0.25	NR	NR	NR
Zinc	2.80	4.5[f]	0.62	0.25	NR	<<< 0.25	>> 1.0	NR
Cyanide	0.06	0.11[g]	0.55	**0.50**	>>> **1.75**	< 12.5	> **0.75**	NR
Phenol	11.66	12[f]	0.97	0.50	>>> 0.75	<< 17.75	>>> 51.75	> 0.5
Tricane methane sulfonate	60	64[f]	0.94	**1.25**	>>> **1.75**	> **3.25**	**NR**	> 3.75
Pentachlorophenol	0.25	0.40[g]	0.62	12.25	>>> 12.25	NR	NR	NR
Malathion	0.34	0.34[g]	1.0	88.5	NR	< 88.5	NR	NR

[a] Chemicals listed in order of rapidity of response.

[b] Concentration tested nearest to the 96-h LC_{50}.

[c] Response time is for 70% of fish in any of the four parameters during one 15-min recording interval. The minimum response time is 0.25 h. A response time in bold type indicates that more than one parameter contributed to the first biomonitor response.

[d] Arrows indicate direction and relative strength of response of each parameter. Times shown are the time to biomonitor alarm for each parameter individually. NR, no group response during the 96-h test period; however, as shown for tricaine methane sulfonate, NR in bold type indicates that individual fish responses for a parameter did contribute to a biomonitor alarm.

[e] Buccafusco *et al.* (1981).

[f] LC_{50} calculated from an acute toxicity test conducted prior to the ventilatory test.

[g] LC_{50} calculated from the ventilatory test.

in a treatment (i.e. six or more of eight fish) exhibit statistically different responses in any one of the four parameters monitored, the group response is said to be 'out of control' and an alarm response is generated. Ventilatory data were used to establish the time to first response at each exposure concentration tested.

RESULTS

Table 2 summarizes the results of in-house toxicant exposures under flow-through conditions with seven chemicals. For concentrations at or below the 96-h LC_{50}, five of the seven chemicals caused a biomonitor alarm response in close to an hour or less after the start of exposure. Pentachlorophenol and malathion required much longer than an hour to elicit a biomonitor alarm. No one response parameter was always the most sensitive. Ventilatory rate alone caused the first alarm for two chemicals, whereas cough rate, ventilatory depth and movement caused the first alarm for one chemical each. Multiple parameters caused the earliest alarms for cyanide and tricaine methane sulfonate. In contributing to an alarm, ventilatory rate, cough rate and movement always increased, whereas ventilatory depth either increased or decreased. The lowest concentrations causing alarms within the 96-h exposure period ranged from 20% to 100% of the 96-h LC_{50} except for phenol, which elicited an alarm after 41.5 h at ca. 8% of the LC_{50} (1.01 mg l^-1).

There were no alarms from control fish during the 96-h exposure period for any of the chemicals evaluated in the in-house tests, nor were there any alarm responses in the workshop tests to the three water blanks provided

as unknown samples (Table 3). The phosdrin sample caused complete mortality soon after a 40-min exposure, as might be expected for an exposure concentration >20-fold higher than the 96-h LC_{50}. Cyanide was the only chemical tested in both the in-house and workshop tests. The biomonitor response to cyanide at the workshop was rapid, consistent with the high exposure level. As with the in-house tests, there were no biomonitor alarms at the workshop at concentrations of <20% of the 96-h LC_{50}, whereas all chemicals tested at concentrations ≥39% of the 96-h LC_{50} caused an alarm within 1 h. Also consistent with the in-house tests, the ventilatory rate, cough rate and movement always increased, whereas ventilatory depth either increased or decreased when an alarm occurred.

Colchicine produced a weak sub-alarm response when first tested on group 1 at the workshop. To evaluate whether repeated toxicant exposure might alter fish sensitivity, the colchicine exposure was repeated as the next-to-last chemical tested with group 2. At the time of the second colchicine exposure, group 2 fish had been exposed to six other chemicals (chlordimeform, mercuric chloride, sodium arsenite, sodium cyanide, trimethylol propane phosphate and *p*-chlorophenol). No evidence of changed (enhanced or reduced) sensitivity was found in the second exposure.

DISCUSSION

Desirable operational characteristics for BEWS include reliability of alarm identification (no alarms in the absence of toxicants) and rapidity and sensitivity of response to developing toxic conditions. There were no alarms during the 96-h control exposures in the eight in-house tests (32

Table 3—Alarm responses to chemical exposures for workshop studies

Chemical[a]	Concentration tested[b] (mg l^{-1})	Bluegill 96-h LC$_{50}$	Concentration (fraction of LC$_{50}$)	Response time (h)[c]	Fish group[d]	Ventilatory rate	Ventilatory depth	Cough rate	Movement
						Parameters affected[e]			
Blank – 3 samples tested	NA	NA	NA	None	2,2,3	NR	NR	NR	NR
Trimethylolpropane phosphate	0.5	NA	NA	None	2	NR	NR	NR	NR
Chlordimeform	1.25	NA	NA	None	2	NR	NR	NR	NR
Colchicine – 2 samples tested	2.0	NA	NA	None	1,2	NR	NR	NR	NR
Arsenic (sodium arsenite)	0.5	30.0^f	0.02	None	2	NR	NR	NR	NR
Paraquat	0.5	13^f	0.04	None	3	NR	NR	NR	NR
p-chlorophenol	1.5	3.8^g	0.39	0.50	2	>>>	<<<	NR	NR
Mercury (mercuric chloride)	0.5	0.12^h	3.91	0.25	2	>	<	>	NR
Cyanide (sodium cyanide)	2.0	0.11^i	18.2	0.25	2	>>	>>>	>>	NR
Phosdrin	2.0	0.09^g	22.22	0.50^j	1	>	<	>	NR
Metham sodium	2.0	NA	NA	0.25	3	>	>	>	NR

[a] Chemicals listed by concentration (expressed as a fraction of the 96-h LC$_{50}$). Samples were furnished as unknowns and were tested in a different order than shown.
[b] Nominal concentration based on 1 : 200 dilution of sample provided; NA, not applicable or not available.
[c] Response time within a 60-min exposure period.
[d] Specifies which of three groups of eight fish were used for each exposure.
[e] Arrows indicate direction and relative strength of response of each parameter; NR, no response.
[f] Mayer and Ellersieck (1986).
[g] Buccafusco et al. (1981).
[h] Holcombe et al. (1983).
[i] This study.
[j] Toxicant was flushed from system at 40 min, but fish died 10 min later.

days' total duration) or during monitoring of the three blank samples provided at the workshop. However, variable water quality conditions in field testing can cause a greater frequency of non-toxicant related alarms, especially when patterns of variation in the exposure period differ from those in the baseline period, usually because of a storm event, passage of a cold front or diel or tidal fluctuations. In field deployments, the statistical response thresholds for the USACEHR biomonitor can be increased to offset greater water quality variations. Of course, lowering the sensitivity to water-quality-related alarms in this fashion may also lower sensitivity to toxicant-related events.

In deployments of the USACEHR aquatic biomonitor at a groundwater treatment plant (Shedd et al., 2001) and on an estuarine tributary (US EPA, 2001), most alarms were attributed to fish responses in ventilatory rate or depth related to variations in water quality (primarily temperature and dissolved oxygen), interruptions in water flow or changes in conductivity of the water. Diel or storm-related changes in pH or turbidity (water filtered to exclude particles >100 μm) did not seem to affect bluegill ventilatory patterns (US EPA, 2001), consistent with laboratory studies by Carlson (1984) that showed that bluegill ventilatory patterns varied little over a wide range of these parameters.

Establishing a general relationship between the response times of the aquatic biomonitor and bluegill 96-h LC$_{50}$ values would be useful in predicting system response for the very large number of chemicals for which there are bluegill LC$_{50}$ data. For example, the US EPA's ECOTOX database

(www.epa.gov/ecotox) has bluegill 96-h LC$_{50}$ data for several hundred chemicals. To characterize toxicant sensitivity, this study explored the relationship between the 96-h LC$_{50}$ and USACEHR aquatic biomonitor response times. A response time of ≤1 h was selected as a reasonable length of time for advanced warning of developing toxic conditions in water, considering applications such as surface water monitoring at drinking water intakes.

Table 4 groups bluegill response times to chemicals (from this study or the literature) by the acute toxicity mode of action. Overall, bluegill response ventilatory and movement responses were found to occur within 1 h for 12 of 15 chemicals at concentrations equal to or less than the 96-h LC$_{50}$. Furthermore, chemicals causing direct membrane irritation, effects on cellular respiration, and narcosis or polar narcosis are most likely to cause rapid aquatic biomonitor responses. Other modes of action may require longer exposure periods. For acetylcholinesterase inhibitors, the rapid response to carbaryl (compared to malathion) may be due to a secondary irritant effect present in carbaryl (Carlson, 1990). The classification of 2,4-pentanedione is somewhat uncertain. Carlson (1990) considered it to have an unknown mode of action, but observed that bluegill ventilatory responses were similar to responses of fish exposed to chemicals acting by narcosis. Russom et al. (1997) classified 2,4-pentanedione as an electrophile or proelectrophile, a class including classic membrane irritants such as acrolein or benzaldehyde but with a rather low level of certainty. However, the apparent slow response time of bluegill ventilatory patterns to 2,4-pentanedione

Table 4—Alarm response times to chemical exposures near the 96-h LC$_{50}$ for chemicals with varying modes of toxic action[b]

Response time[a] (h)	No. of chemicals	Mode of toxic action[b]						
		Direct gill effects	Cellular respiration inhibition	Narcosis	Polar narcosis	Acetylcholinesterase Inhibition	Oxidative phosphorylation inhibition	Electrophilic reactivity
0.25 – 1	11	Chlorine[c] Zinc[d]	Cyanide[d]	Acetone[e] Chloroform[f] Meparfynol[g] Tetrachloroethane[d] Tetrachlorethylene[f]	Phenol[d] p-Chlorophenol[d]	Carbaryl[g]		
>1 – 10	1				Tricaine methane sulfonate[d] (1.25 h)			
>10	3					Malathion[d]	Pentachlorophenol[d]	2,4-Pentanedione[g]

[a] Most rapid response time at or below the 96-h LC$_{50}$. Minimum biomonitor response time is 0.25 h.
[b] Sources for mode of toxic action classification: Carlson (1990) and Russom *et al.* (1997).
[c] Miller *et al.* (1980).
[d] In-house tests, this study.
[e] van der Schalie *et al.* (1979).
[f] Capute (1980).
[g] Carlson (1990).

exposure is inconsistent with other chemicals tested and classified as causing either direct gill effects or narcosis. Additional test data are required for chemicals with varying modes of toxic action to determine whether the relationships observed with the relatively small number of chemicals tested in this study can be confirmed.

Observed toxicant response times for bluegills in the biomonitor are as fast as or faster than responses reported for other fish ventilatory monitoring systems. Morgan and Kuhn (1984) found that changes in ventilatory patterns of largemouth bass could be detected within 2–6 h at 96-h LC_{50} concentrations. Sloof (1979) reported changes in rainbow trout respiration within 24 h of exposure at concentrations of 0.01–0.30 of the 48-h LC_{50}, whereas Baldwin et al. (1994) reported that, to achieve 40–80 min response times in rainbow trout ventilatory rates, toxicant concentrations of 0.1–2.5 of the 96-h LC_{50} were required.

The patterns of change in bluegill ventilatory and cough rates determined in this study were consistent with those measured by Carlson (1990) for tricane methane sulfonate, pentachlorophenol and malathion. Carlson found that a lack of increase in cough rate for chemicals causing narcosis (especially at sublethal levels) helped to distinguish them from chemicals with some modes of action (oxidative phosphorylation uncouplers, gill irritants) but not others (sublethal acetylcholinesterase inhibitors and the electron transport inhibitor rotenone). Of the four tested narcotic or polar narcotic chemicals in this study, only phenol caused an increase in cough rate. (Phenol was unique in another respect, in that a concentration as low as 8% of the LC_{50} eventually caused an alarm; no other chemical tested caused an alarm within 96 h at concentrations below 20% of the 96-h LC_{50}). Cough rate may have a use in distinguishing toxicant- and water-quality-related events in field situations, because ambient changes in pH, temperature, dissolved oxygen and turbidity are unlikely to cause changes in bluegill cough rate (Carlson, 1984; US EPA, 2001). However, given the variability in ventilatory

response patterns according to the pattern of exposure and the magnitude of exposure (e.g. lethal versus sublethal), it is difficult to formulate any definitive rules.

Monitoring multiple ventilatory and movement parameters can improve the biomonitor response time. In this study, no one parameter was always the quickest to respond, and in some cases two or more parameters combined gave the fastest alarm. In contrast, most BEWS that monitor fish ventilatory patterns have relied solely on changes in ventilation rate to detect developing toxic conditions (e.g. Sloof, 1979; Morgan and Kuhn, 1984; Baldwin et al., 1994; Gruber et al., 1989).

Biological early warning systems have been used or proposed for use in a variety of applications, such as surface water monitoring for watershed protection, wastewater treatment plant effluent monitoring or drinking water protection. For drinking water, BEWS can be used for source water protection (e.g. ILSIRSI, 1999; Grayman et al., 2001) or (with appropriate dechlorination) water distribution system monitoring. For these uses, and in view of recent terrorism concerns, it is especially important to understand how BEWS will respond to a wide range of toxic chemicals. This study has characterized the responses of an aquatic biomonitor relative to toxicant modes of action and a standard acute toxicity testing benchmark concentration. Such information can be useful in the design and implementation of BEWS that will be protective of water supply systems and surface waters.

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