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INDUSTRIAL ENVIRONMENTAL RESEARCH BRIEF

Use of Aquatic Oligochaete, *Lumbriculus variegatus,* for Effluent Biomonitoring

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Introduction

In a recent document for effluent bioassay methods, the U.S. Environmental Protection Agency (EPA) notes the importance of toxicity testing as it relates to the prevention of toxic discharges to the environment

"The Declaration of Goals and Policy of the Federal Water Pollution Control Act Amendments of 1972, Section 101 (a) (3), states that 'it is the national goal that the discharge of toxic pollutants in toxic amounts be prohibited.' Current Agency programs for the protection of aquatic life in receiving waters are based, in part, on effluent limitations for individual chemicals. However, toxicity data are available for only a limited number of compounds. The effluent limitations, therefore, may not provide adequate protection where the toxicity of the components in the effluent is not known, where there are synergistic effects between toxic substances in complex effluents, and/or where a complete chemical characterization of the effluent has not been carried out. Since it is not economically feasible to determine the toxicity of each of the thousands of potentially toxic substances in complex effluents or to conduct an exhaustive chemical analysis of the effluent, the most direct and cost-effective approach to the measurement of the toxicity of effluents is to conduct a bioassay with aquatic organisms representative of indigenous populations For this reason, the use of effluent bioassays to identify and control toxic discharges is rapidly increasing within the Agency and state NDPES programs "

A variety of biological approaches to the detection and assessment of effluent toxicity have been developed by various researchers. Monitoring objectives and practical limitations will determine the best available approach One application of biological techniques is the use of organisms as "early warning" mechanisms to detect changes in the toxicity of effluents and receiving waters While continuous on-site monitoring is ideal in situations where rapid detection of a potentially dangerous alteration in water quality is critical, approaches to the monitoring of behavior and physiology in test fish require complex equipment and techniques, and their widespread use is impractical

This report focuses on a simple, inexpensive short-term acute toxicity test which can be used in the detection of gross changes in effluent and receiving water toxicity. The approach is designed as an initial screening technique for detecting toxicity of cooling-water effluents. A "positive" toxicity test would

identify locations where more intensive biological and chemical analyses should be concentrated. Although this simple approach to biological water quality monitoring is not a substitute for more rigorous testing, its widespread use will improve the timely detection of toxic substances in the aquatic environment. This report describes the use of *Lumbriculus variegatus,* an aquatic earthworm, as a test organism for short-term acute toxicity tests. This organism's hardiness, sensitivity to fluctuating effluent quality, and response to a commonly used biocide (sodium pentachlorophenate) are described.

Conclusions and Recommendations

Oligochaetes may prove to be cost-effectively used to detect changes in gross toxicity of effluents and receiving waters because of their uncomplicated biology and life-cycle.

The bioassay tested requires only holding containers, temperature control, and the test organisms, *Lumbriculus variegatus* (Oligochaeta:Lumbriculidae). While not recommended as a substitute for more sophisticated biological techniques, bioassay testing promises to be a means of increased monitoring where the number of cooling-water biomonitoring stations are limited, particularly in the western United States, where recent emphasis on energy development is expected to result in the rapid increase of cooling towers and other sources of industrial pollution

Short-term static effluent tests for acute toxicity showed that *L. variegatus* responded differentially to industrial effluents collected at different dates from the same outlet, indicating their usefulness for detecting changes in the gross toxicity of complex effluents

L. variegatus also showed sensitivity to a specific pollutant, sodium pentachlorophenate (Na-PCP), which is commonly used as a fungicide in cooling towers. Definitive tests with Na-PCP resulted in a 96-hour LC_{50} of 0.57 ppm and a 48-hour EC_{50} for inactivity of 0 66 ppm.

Observations of effects of Na-PCP on *L. variegatus* at shorter and longer exposure times is needed to determine the minimum length of time required to detect high levels ($>$ 1 ppm) of a Na-PCP and whether a threshold of LC_{50} exists.

Additional work is recommended to determine response of *L. variegatus* to other important cooling-water toxicants and to a variety of cooling-water effluents, ideally with the chemical constituents of the effluents identified. Attempts can then be made to relate results from effluent tests to changes in toxicity predicted from chemical composition. It would also be useful to compare toxicant and effluent responses of this organism to those organisms indigenous to the receiving waters.

The potential of other oligochaetes or other hardy organisms for simple, initial-stage monitoring should be investigated.

Materials and Methods

Test Organisms

Most of the studies on the effects of pollutants on oligochaetes looked at sewage or other organic effluents. A number of investigations are described here.

Laboratory studies on the effects of specific heavy ion and biocide toxicants have been performed on a few species of oligochaetes with varied results, depending on the test species and toxicant. One study by Whitten and Goodnight found DDT nontoxic to worms at 100 mg/l, while marking fixed the LC_{50} of *Tubifex tubifex* to the pesticide, 2-(digeranylamino)-ethanol, at 0.054 ppm. Whitten established the sensitivity of tubificids to heavy metals from 24-hour LC_{50} of 49 0 ppm of lead and LC_{50} 46.0 ppm of zinc to a level well below the 1 ppm of cadmium, copper, and mercury reported by Brkovic and Popovic.

The sensitivity of many species of oligochaetes to industrial toxicants is indicated by field studies of the River Irwell in England and Kanawha River, West Virginia made by Eyers and Maciorawski, respectively. At some locations on the River Irwell, oligochaete diversity was lower than could be explained by organic pollution alone; the investigators speculated that toxins undetected by routine chemical analysis may have had adverse effects on the oligochaete community. Greatly reduced populations of all oligochaete species were found in the Kanawha River around areas of the highest industrial activity, with many of the species generally restricted to the cleaner water stations Lumbriculidae, although uncommon, was largely restricted to the less polluted reaches. These studies indicate aquatic oligochaetes are generally sensitive to a variety of toxic substances; it should be beneficial to investigate their applicability in simple bioassays for screening of cooling water toxicity

L. variegatus (Oligochaeta Lumbriculidae) was chosen for these investigations because it is readily available and in uniform supply. These organisms are raised under constant conditions and are highly sensitive to changes in their environment. For example, failure to change their holding water will

result in death of the organisms within two days (probably due to self-pollution caused by high organism density) Daily changing of their holding water (with dechlorinated tap water) insures their health and activity not only in the laboratory, but also in their function as test organisms

Test Media

Effluent samples were collected from a small outlet ditch at Basic Management, Inc., of Henderson, Nevada, an industrial complex. The effluents were left at 100% concentration for all effluent tests. Dechlorinated tap water was used as the control medium for the effluent tests.

The toxicant tested was sodium pentachlorophenate (Na-PCP). The Na-PCP was supplied by Dow Chemical Company of Midland, Michigan, as Dowicide™ G-St Beads (EPA Registration No. 464-380). The ingredients of this product were listed by the manufacturer as 79% sodium pentachlorophenate, 11% sodium salts of other chlorophenols, and 10% inert ingredients.

Na-PCP was chosen as the test toxicant because of its widespread and common usage. Na-PCP and pentachlorophenol (PCP) collectively are the second most heavily-used pesticides in the country. They are chiefly used for wood preservation and treatment, but also have many other uses and are registered by the U.S. Environmental Protection Agency as insecticides, fungicides, and algicides. Phenols and chlorinated or phenylated phenols represent a major class of cooling tower biocides used for slime control. Of this group, Na-PCP is probably the most frequently used. Because PCP and Na-PCP can become important and persistent environmental pollutants, they pose a potential health hazard. Also, they have been used extensively for toxicology testing.

The control and toxicant dilution water for the toxicant range-finding tests was dechlorinated tap water. The control and toxicant dilution water for the toxicant definitive tests was reconstituted soft water as recommended by EPA. This water was prepared by adding 48 mg/l $NaHCO_3$, 30 mg/l $CaSO_4 \cdot 2H_2O$, 30 mg/l $MgSO_4$, and 2 mg/l KCl to triple-distilled water (less than one micromho/cm). This water has the following properties: pH = 7.2-7.6, hardness = 40-48 mg/l $CaCO_3$, and alkalinity = 30-35 mg/l $CaCO_3$.

Equipment and Test Conditions

A Freas-815 low-temperature incubator kept at 17°C ± 2°C was used for all experiments. After the organisms were obtained, they were held in liter flasks filled with dechlorinated tap water which was changed daily. All effluent and toxicant tests were static tests The test containers consisted of 3-cm high, wide-mouth jars. For most tests these jars were filled with 50 ml of the appropriate medium, five organisms were placed in each filled jar. Maintaining a low number of test organisms per container eliminated self-contamination associated with overcrowding and facilitated counting. No aeration or other precautions were used to maintain the organisms during the experiments.

Experimental Design

Effluent Study

This test was designed to assess the ability of the organisms to respond to unknown and complex industrial effluents and to changes in the toxicity of those effluents. Effluent samples were collected on five different dates in February and March 1979. In the earliest test, ten organisms were placed in each of ten flasks filled with 100 ml of effluent. In all subsequent tests, five organisms and 50 ml of effluent were used with each experimental test set After 96 hours, the worms were removed from the incubator and inspected for mortality.

Toxicant Studies

Range Tests—Range tests were used to determine the Na-PCP concentration levels to be employed in the definitive toxicant tests. The first test utilized Na-PCP concentration levels to be employed in the definitive toxicant tests. The first test utilized Na-PCP concentrations of 0.5, 1, 2, and 4 ppm, and mortality was recorded at 24, 48, and 120 hours. The second test was conducted using Na-PCP concentrations of 0.125, 0.25, 0.5, and 1 ppm, with mortality recorded at 24, 48, 72, 96, and 168 hours. In each test, five organisms in five separate jars containing 50 ml of the appropriate medium were utilized for each experimental concentration and a control.

Definitive Tests—Definitive toxicant tests were carried out using nine concentrations of the test toxicant, ranging from 0.2 ppm to 1.0 ppm Na-PCP at intervals of 0.1 ppm. For each toxicant concentration and control, five organisms were placed in each of the five test jars filled with 50 ml of the appropriate medium. At 24, 48, 72, 96, 120, 144, and 168 hours the organisms were removed from the incubator and both mortality and inactivity were recorded.

Data Analysis

Graphical Determination of LC_{50} and LE_{50} Levels—ASTM graphical models were used for both toxicant

range-finding and definitive tests. The toxicant concentration was plotted on log scale against the probit scale for the percent of organisms affected at a given number of hours after the start of the experiment. A straight line was drawn to the plotted points, and the concentration corresponding to 50% mortality or inactivity was read from the graph as the LC_{50} or EC_{50}.

Probit Analysis of LC_{50} and EC_{50} Levels—Probit analysis was applied to results which met EPA criteria for definitive toxicant tests. These criteria are as follows (1) the concentration of toxicant in each treatment must be at least 60% of the next higher concentration, (2) one treatment other than the control must affect less than 65% of the exposed organism; and (3) graded responses must result for a minimum of five levels of the toxicant (a minimum of three partial kills or effects). Calculations were completed with the aid of a programmable handheld calculator

The probit analysis utilized was the maximum likelihood estimation described by Finney in 1971. The iteration was repeated until at no more than one concentration did the corresponding estimated probit value change by more than 0 1 and no concentration had a probit value shift more than 0.2. The chi-square value for precision of fit describes how well the data conform to the probit model; a smaller value indicates a better fit

Results from the probit analyses were used to plot percentage mortality against Na-PCP concentration for the exposure times of 48, 96, and 168 hours These regression lines show the rate of increase in mortality as a function of the increase in Na-PCP concentration The LC_{50}s were plotted against their exposure times (toxicity curve) to determine a possible threshhold LC_{50}. The threshold LC_{50} indicates the level of toxicity at which acute lethality has stopped

Results and Discussion

Suitability of Lumbriculus variegatus *as a Test Organism*

Collection and preparation of *L. variegatus* for bioassay testing can be cost effective for the following reasons (1) aquatic oligochaetes exhibit relatively few complex biological and life-cycle characteristics; (2) because they are hermaphroditic and do not molt, they do not have to be sorted acording to sex or molting stage; and, (3) since these organisms are commercially raised and shipped, they can be obtained from a uniform source. Variation factors that could occur because of sex, growth, and other complex biological characteristics are, thus, avoided by using this particular organism.

Handling, holding, and conditioning of test organisms can also require complex and expensive procedures to keep organisms healthy and to assure consistent results. A continuous flow of prepared water and proper feeding are required for many organisms. Survival rates exhibited by control organisms utilized in the tests described here (Tables 1-5) indicated that minimal preparation and maintenance procedures were adequate to assure that specimens remained healthy.

Sensitivity of Lumbriculus variegatus *to Effluent Toxicity*

The test organisms exhibited various responses to effluent samples collected on different dates (Table 1) The industrial outlet ditch from which the effluent samples were taken is shown to be subject to large variations in water quality, such fluctuations in water quality probably account for the observed variation in test organism response. While chemical analyses of the effluent are not available, the bioassay results suggest that *L. variegatus* is sensitive to fluctuations in water quality. The organism should be tested further to determine its usefulness as a biological screening agent for changes in effluent toxicity. For of 3 and 5500 ppm chlorides.

TABLE 1 MORTALITY OF *Lumbriculus variegatus* AT 96 HOURS TO 100% EFFLUENT COLLECTED AT VARIOUS DATES FROM THE OUTLET STREAM OF THE BMI INDUSTRIAL COMPLEX, HENDERSON, NEVADA

Date of Collection	No of Test Organisms	Test Water	Percent Mortality
2/28/79	100	Effluent	97
	100	Control	0
3/1/79	50	Effluent	2
	50	Control	2
3/5/79	50	Effluent	2
	50	Control	0
3/16/79	50	Effluent	0
	50	Control	0
3/21/79	50	Effluent	28
	50	Control	0

Effects of Na-PCP on Lumbriculus variegatus

One-hundred-percent mortality occurred within 24 hours at 2.0 ppm Na-PCP and within 48 hours at 1.0 ppm Na-PCP during the range-finding tests (Tables 2-3). The 24-hour LC_{50} was estimated to be slightly

TABLE 2 MORTALITY OF *Lumbriculus variegatus* TO SODIUM PENTACHLOROPHENATE (RANGE-FINDING TEST 0 5 - 4 0 ppm)

No of Test Organisms	Concentration NaPCP (ppm)	Percent Mortality 24 hr	at 48 hr	120 hr
25	4 0	100	100	100
25	2 0	100	100	100
25	1 0	12	92	100
25	0 5	8	28	100
25	0 0	0	0	0
LC_{50}, ppm, estimated from graph		1 16	0 60	

TABLE 3 MORTALITY OF *Lumbriculus variegatus* TO SODIUM PENTACHLOROPHENATE (RANGE-FINDING TEST 0 125 - 1 0 ppm)

No of Test Organisms	Concentration of NaPCP (ppm)	Percent Mortality at 24 hr	48 hr	72 hr	96 hr	168 hr
25	1 0	36	100	100	100	100
25	0 5	0	44	76	92	100
25	0 25	0	0	4	8	8
25	0 125	0	0	0	0	0
25	0 0	0	0	0	0	0
LC_{50}, ppm, estimated from graph		1 04	0 52	0 44	0 40	0 36

above 1 ppm, while the 48-hour LC_{50} was estimated to be slightly higher than 0.5 ppm. Only 2 of 25 worms had died at the 0.25 ppm concentration level after the full 7-day test period.

Results of the definitive test for mortality at the 1.0 and 0.3 ppm levels were similar to those of the range-finding test results (Table 4). However, there was a considerable difference between the results of the two tests for the 0.5 ppm concentration after 48 hours. This discrepancy may be due to differences in the dilution water used in the two tests.

The largest decrease in the estimated LC_{50} as generated from definitive test data occurred between 24 and 48 hours from initiation. The rate of decrease in estimated LC_{50} levels beyond 72 hours was low, and indicates that where Na-PCP concentration is sufficient to cause substantial acute mortality, the effects are largely apparent within 2 to 3 days. This finding may be important for the use of *L. variegatus* in biological screening, where the necessity for a longer test period would both increase cost and delay results.

None of the chi-square values for precision of fit to the probit line for the LC_{50} definitive test data showed statistical significance at the 0.05 probability level, that is, the probit model appears appropriate to these data. However, the confidence interval tends to narrow and the chi-square value decreases with increasing time of exposure, suggesting greater predictability of test organism response to Na-PCP after longer exposure periods.

Figure 1 shows that the slope of the probit regression line increases with increase in exposure time That is, as exposure time lengthens, increase in toxicant concentration will result in greater increases in mortality. A threshold LC_{50} is not apparent from the toxicity curve (Figure 2)

In 1968 Whitley, using dilution water with a pH of 7.5, found 100% mortality after 24 hours at 0.5 ppm concentration of Na-PCP for Tubifex and Limnodrilus as opposed to the 4% mortality for *Lumbriculus variegatus* reported here (with dilution of pH 7.2 - 7.6). Our results more closely approximate those obtained by Whitley with dilution water of pH 8.5 (24-hour mortality of 11%).

TABLE 4 MORTALITY OF *Lumbriculus variegatus* TO SODIUM PENTHAHLOROPHENATE

No. of Test Organisms	Concentration of NaPCP (ppm)	Exposure time (hours) 24	48	72	96	120	144	168
		Percent mortality						
25	1.0	36	88	96	100	100	100	100
25	0.9	32	44	76	100	100	100	100
25	0.8	16	52	84	96	96	100	100
25	0.7	16	28	56	76	84	96	100
25	0.6	12	32	52	52	68	88	96
25	0.5	4	8	20	40	40	44	68
25	0.4	0	0	0	4	8	20	28
25	0.3	0	0	0	0	0	0	0
25	0.2	0	0	0	0	0	0	0
25	0.0	0	0	4	8	8	8	8
		LC_{50} Results						
LC_{50}, ppm, estimated from graph		1.22	0.79	0.63	0.56	0.55	0.49	0.44
LC_{50}, estimated by probit analysis[1]		—	0.80	0.65	0.57	0.54	0.49	0.45
95% confidence limits of LC_{50}		—	0.74	0.60	0.53	0.51	0.46	0.42
		—	0.87	0.69	0.60	0.58	0.52	0.48
X^2 value for goodness of fit to probit line		—	9.66	6.62	4.55	1.17	2.18	0.96
degrees of freedom for X^2		—	5	5	5	5	4	3

[1]Probit analysis was applied only for those time intervals yielding definitive results. A definitive result must show graded responses at a minimum of five concentrations, including at least one response at greater than 65% and at least one at less than 35% (USEPA 1975)

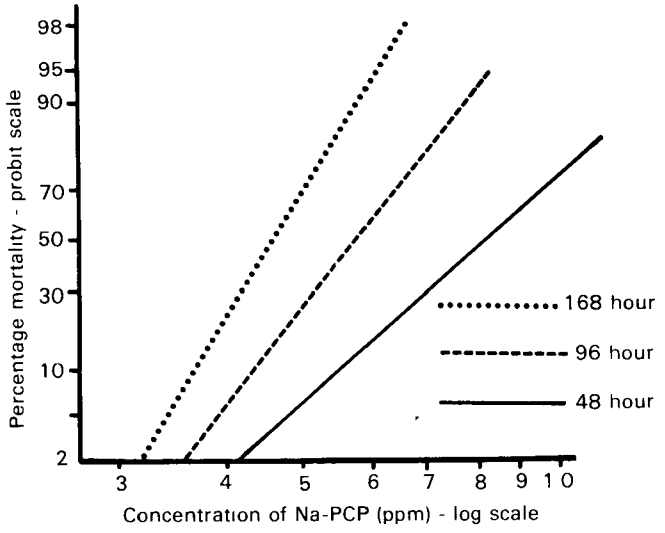

Figure 1. Probit regression lines showing relation of *Lumbriculus variegatus* mortality to concentration of Na-PCP (from Table 4).

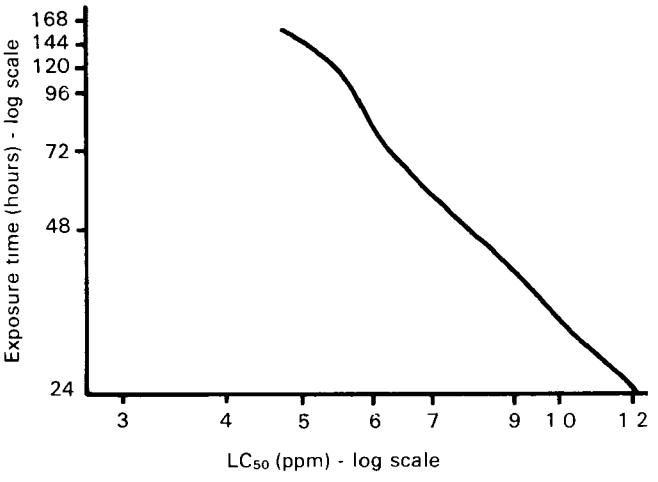

Figure 2. Toxicity curve showing change in LC_{50} as test proceeded (from Table 4)

EC_{50} levels for inactivity, estimated from the definitive test data, diverged most from the LC_{50} levels at shorter exposures and higher toxicant concentration levels (Tables 4-5). The lower EC_{50} levels under these conditions (in comparison to the LC_{50} levels) suggest that the use of sublethal criteria may be most effective in increasing test sensitivity where quick results are important and where relatively high concentrations are involved All chi-square values for precision of fit for the EC_{50} data fell within acceptable levels, except for the 24-hour exposure time (borderline at the 0 05 probability level).

In general, differences among investigators in determinations of mortality and inactivity were minimal, although there were some discrepancies at higher concentration levels for the shorter (48 hour) test period (Tables 6-7) A thorough familiarization with response criteria is recommended for improving the reproducibility of results.

TABLE 5 INACTIVITY RESPONSE OF *Lumbriculus variegatus* TO SODIUM PENTACHLOROPHENATE

No of Test Organisms	Concentration of NaPCP (ppm)	Exposure time (hours) 24	48	72	96	120	144	168
		Percent inactive						
25	1 0	80	100	100	100	100	100	100
25	0 9	44	92	88	100	100	100	100
25	0 8	16	88	88	96	96	100	100
25	0 7	24	48	88	76	92	100	100
25	0 6	20	36	60	56	76	88	100
25	0 5	12	12	28	40	40	64	68
25	0 4	8	0	8	12	12	20	32
25	0 3	0	0	0	0	0	0	0
25	0 2	0	0	0	0	0	0	0
25	0 0	0	0	4	8	8	8	8
		EC_{50} results						
EC_{50}, ppm, estimated from graph		0 88	0 66	0 59	0 55	0 52	0 47	0 45
EC_{50}, estimated by probit analysis[1]		0 91	0 66	0 57	0 55	0 52	0 47	—
95% confidence limits of EC_{50}		0 82 1 07	0 62 0 69	0 53 0 61	0 52 0 59	0 49 0 55	0 44 0 50	— —
X^2 value for goodness of fit to probit line		12 6	5 20	4 75	3 43	0 45	0 85	—
Degrees of freedom from X^2		6	5	6	5	5	3	—

[1]Probit analysis was applied only for those time intervals yielding definitive results A definitive result must show graded responses at a minimum of five concentrations, including at least one response greater than 65% and at least one less than 35% (USEPA 1975)

TABLE 6 MORTALITY OF *Lumbriculus variegatus* TO SODIUM PENTACHLOROPHENATE AS RECORDED FOR THE SAME SETS OF ORGANISMS BY TWO INVESTIGATORS

No. of Test Organisms	Concentration of NaPCP (ppm)	48 hr		144 hr		168 hr	
		Investigator I	Investigator II	Investigator I	Investigator II	Investigator I	Investigator II
		Percent Mortality					
25	1.0	88	48	100	100	100	100
25	0.9	44	40	100	100	100	100
25	0.8	52	44	100	100	100	100
25	0.7	28	28	96	96	100	96
25	0.6	32	32	88	88	96	92
25	0.5	8	8	44	44	68	68
25	0.4	0	0	20	20	28	20
25	0.3	0	0	0	0	0	0
25	0.2	0	0	0	0	0	0
25	0.0	0	0	8	8	8	8
				LC_{50} Results			
LC_{50}, ppm, estimated from graph		0.79	0.89	0.49	0.49	0.44	0.47
LC_{50}, estimated by probit analysis		—	—	0.49	0.49	0.45	0.47
95% confidence limits of LC_{50}		—	—	0.46	0.46	0.42	0.44
		—	—	0.52	0.52	0.48	0.50

TABLE 7 INACTIVITY RESPONSE OF *Lumbriculus variegatus* TO SODIUM PENTACHLOROPHENATE AS RECORDED FOR THE SAME SETS OF ORGANISMS BY TWO INVESTIGATORS

No. of Test Organisms	Concentration of NaPCP (ppm)	48 hr		144 hr		168 hr	
		Investigator I	Investigator II	Investigator I	Investigator II	Investigator I	Investigator II
		Percent Inactive					
25	1.0	100	76	100	100	100	100
25	0.9	92	56	100	100	100	100
25	0.8	88	68	100	100	100	100
25	0.7	48	40	100	96	100	100
25	0.6	36	36	88	88	100	100
25	0.5	12	16	64	64	68	68
25	0.4	0	4	20	20	32	20
25	0.3	0	4	0	0	0	0
25	0.2	0	0	0	0	0	0
25	0.0	0	0	8	8	8	8
		EC_{50} Results					
EC_{50}, ppm, estimated from graph		0.66	0.76	0.47	0.48	0.45	0.46
EC_{50}, estimated by probit analysis		0.66	0.75	0.47	0.47	—	—
95% confidence limits of EC_{50}		0.62 0.69	0.68 0.83	0.44 0.50	0.44 0.50	— —	— —

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