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EDGEWOOD ARSENAL
TECHNICAL REPORT

EATR 4441

TOXICITY OF VX AND GD IN AQUATIC ANIMALS
INDIGENOUS TO THE
CARROLL ISLAND TEST AREA WATER

by

- J. T. Weimer
- E. J. Owens
- J. B. Samuel
- J. S. Olson
- R. P. Merkey

September 1970



DEPARTMENT OF THE ARMY
 EDGEWOOD ARSENAL
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 Medical Research Laboratory
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TOXICITY OF VX AND GD IN
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Task 1B562602AD1102

DEPARTMENT OF THE ARMY
EDGEWOOD ARSENAL
Research Laboratories
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Edgewood Arsenal, Maryland 21010

FOREWORD

The work described in this report was authorized under Task 1B562602AD1102, Lethal Chemical Agent Investigations, Biomedical Evaluation of Lethal Agents (U). This work was started in September and completed in October 1969. The experimental data are recorded in notebook MN-2277.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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Acknowledgments

The authors wish to acknowledge the assistance of SP4 David A. Boyce and SP4 Robert L. Platt in collecting test species and observing the species during exposure to the test agents and the assistance of F. Prescott Ward, D.V.M., CPT Thomas F. Lemke, MSC, and 2LT George D. Edwards, MSC, in collecting test species.

DIGEST

This study was conducted to establish the sensitivity to VX and GD of aquatic species indigenous to the waters around Edgewood Arsenal. As an adjunct to this study, the bacterial and chemical characteristics of water taken from a number of areas around Edgewood Arsenal were determined.

The species tested with VX were the blue crab (*Callinectes sapidus*), the white perch (*Roccus americanus*), and the striped bass (*Roccus saxatilis*). Only the white perch was tested with GD. The water used was taken from Gunpowder River and allowed to come to room temperature (71°F). It was aerated for 24 hours before the fish and crabs were added and throughout the subsequent 24-hour period observation. No other adjustments were made.

It was found that the concentration of VX required to kill 50% of a group within 60 minutes is 215 ppm for blue crabs, 0.1 ppm for striped bass, and 0.085 ppm for white perch. The concentration of GD required to kill 50% of a group of white perch in 60 minutes is 0.0057 ppm. More than 300 grams of VX would have to remain in a 1-hectare by 10-foot volume of water for 24 hours to kill white perch and striped bass, and more than 30 kg would be required to kill blue crabs. These values apply only if there is no subsequent dilution of the waters and the fish and crabs remain in the waters for 24 hours. It was concluded that blue crabs, white perch, and striped bass are not sensitive enough to VX to be good indicators of nerve agent contamination in their natural habitat.

In degradation studies, VX was stable when added to samples taken from the waters around Edgewood Arsenal; GD was not stable. The waters that were tested had high total and coliform bacterial counts.

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TOXICITY OF VX AND GD IN AQUATIC ANIMALS INDIGENOUS TO THE CARROLL ISLAND TEST AREA WATER

I. INTRODUCTION.

In July 1969, the US Army agreed to suspend open-air testing of lethal chemical agents at Edgewood Arsenal until the safety procedures at Edgewood Arsenal could be reviewed by an external committee of experts. On 21 July 1969, the Secretary of the Army established an Ad Hoc Committee for Review of Testing Safety at Edgewood Arsenal, Maryland, and Fort McClellan, Alabama. One of the recommendations of this committee was to modify existing safety procedures and precautions to use more frequently mechanical and biological monitors beyond the test-grid periphery, including the downwind shoreline and above-water areas.¹

A prerequisite for the ecological studies initiated at Edgewood Arsenal in the fall of 1969 was to establish for certain indigenous aquatic animals the toxicity of the compounds that might be dispersed in the area. If these species were sensitive enough, the level of chemical agent contamination in a particular area could be estimated from their mortality.

The use of fish in bioassays of chemical agents is not new. As early as 1928 tadpoles were used for extensive toxicity screening studies.^{2,3} During World War II, the effects of lewisite, sulfur mustard, nitrogen mustards, cyanogen chloride, diisopropyl fluorophosphate, and methyl fluoroacetate were studied in aquatic animals.^{4,5} With the advent of the G agents following World War II, comprehensive studies were undertaken to establish the toxicity of this series of lethal agents in a freshwater crustacean⁶ and in freshwater fish.⁷ Sunfish have been used to verify the potability of decontaminated water.⁸ When the V agents were introduced in the 1950's, field bioassay techniques for small fish were developed to measure the concentrations of these agents in water.⁹⁻¹¹

In all but one of these studies,⁸ however, dechlorinated tapwater or distilled water was used, and pH usually was controlled. The purpose of the present study was to test the toxicity of VX and GD in indigenous species in otherwise untreated water taken from their natural habitat. To furnish additional basic information for the ecological study, the bacterial and chemical characteristics of water from a number of areas around Edgewood Arsenal were determined.

II. MATERIALS AND METHODS.

A. Materials.

The areas from which the aquatic animals and the waters were taken are shown in table A-1* and figure B-1**. The aquatic species used were as follows: (1) Blue crabs (*Callinectes sapidus*) weighing 3 to 7 oz and having a shell span of 5 to 6 inches, (2) white perch (*Roccus americanus*) weighing an average of 1.2 oz and measuring 5 to 6 inches in length, and (3) striped bass (*Roccus saxatilis*) weighing an average of 0.5 oz and measuring 2 to 3 inches in length. On each morning of testing, the crabs were collected with a trotline, and the fish were caught with a net.

* Tables A-1 through A-VII may be found in appendix A.

** Figures B-1 through B-9 may be found in appendix B.

The VX, designated Lot TCD 29959, had a purity of 90.2%; the GD, designated Run 8, Cut 2, had a purity of 94%.

B. Methods.

Twenty-four hours before the beginning of each toxicity test, six 25-gallon stainless-steel cylinders were filled with 100 liters of water freshly obtained from White Oak Point on Carroll Island. The water was brought to laboratory temperature $71^{\circ} \pm 1^{\circ}\text{F}$; 21.7°C). It was aerated for 24 hours before the fish and crabs were added and throughout the subsequent 24-hour observation.

The agents were added to the water samples from a stock solution of 1 mg/ml in water or as neat agents 30 minutes before the crabs and fish were put into the cylinders. Uniform distribution of the agents throughout the water was assured by the turbulence of aeration and by stirring. The water in one of the six containers was not contaminated, and the animals put into this water were the controls.

The level of contamination in the waters to which the crabs were exposed ranged from 10.0 to 300.0 ppm VX, and the striped bass were exposed to 0.02 to 5.0 ppm VX. The white perch were exposed to 0.025 to 10.0 ppm VX and 0.001 to 0.080 ppm GD.

Ten animals were put into each cylinder. The crabs were in compartmented stainless-steel mesh cages that were withdrawn periodically from the water momentarily to observe the animals' responses. The fish were in stainless-steel mesh containers and could be observed without withdrawing the cages from the water.

Any preexisting anticholinesterase (antiChE) activity was measured by analyzing samples of the bioassay water before it was contaminated. The water samples drawn from the other areas around Edgewood Arsenal also were analyzed for antiChE activity. To study agent degradation, some waters were contaminated with VX and GD, and samples were withdrawn for analysis over a period of 29 days. Chemical analyses for antiChE activity were done by an automated enzymatic method that is sensitive to ng/ml or 0.001 ppm of nerve agent (T. L. Hess, unpublished data).

Waters taken from various locations (table A-I) around Edgewood Arsenal were analyzed for pH, total alkalinity, and total hardness (as CaCO_3), Mg^{++} , Ca^{++} , Fe^{++} , Na^+ , K^+ , and Cl^- ; and total and coliform bacterial counts were made. The effect of VX on bacterial count was studied in one sample contaminated 24 hours before the count.

III. RESULTS.

None of the control crabs and fish died during the 24-hour observation period. No evidence of the control waters having antiChE activity was found.

VX at a concentration of 0.02 ppm (table A-II) killed 7 of 10 striped bass between 850 and 1225 minutes (14.2 and 20.4 hours) after the beginning of the exposure. A concentration of 0.05 ppm killed all 10 bass within 315 minutes (5.3 hours). Higher concentrations reduced time to death; at the highest concentration tested (5.0 ppm), all the bass were dead within 16 minutes. Results with the white perch (table A-II) were similar. The blue crabs (table A-II) were much more resistant, a concentration of 10.0 ppm killing only 1 of 10 and then only after 1260 minutes (21 hours) of exposure. When the concentration was 40.0 ppm, all 10 crabs

died within 1035 minutes (17.2 hours). Time to death was reduced to 85 minutes (1.4 hours) at the highest concentration tested (300 ppm).

Only white perch were exposed to GD (table A-II). A concentration of 0.001 ppm killed all 10 fish within 225 minutes (3.8 hours). Increasing the concentration to 0.04 ppm, reduced time to death to 6 minutes.

The harmonic means of these data are tabulated in table A-III and are plotted in figures B-2 through B-5. This statistical treatment shows that the concentration of VX required to kill 50% of a group (LC50) within 60 minutes is 215 ppm for blue crabs, 0.1 ppm for striped bass, and 0.085 ppm for white perch. The concentration of GD required to kill 50% of a group of white perch in 60 minutes is 0.0057 ppm.

The Bliss¹² dose-response regressions from which the harmonic means were derived are shown in figures B-6 to B-9.

The first sign of toxic response seen in the fish was a forward extension of the pectoral fins. This occurred 5 to 60 minutes before death, depending on dosage, and was more evident in the white perch than in the striped bass. Approximately 15 minutes before death, the fish swam first on their sides, then upside down, making convulsive thrusts through the water. Gill respiration rate increased, then became slow and deep just before death. Neither the fish nor the crabs displayed any color change at death.

Studies on the degradation of VX in water (table A-IV) showed that the antiChE activity of samples with 0.12 to 1.2 ppm VX did not change for at least 24 hours; it then gradually diminished to about 44% after 29 days. Within 6 hours, samples containing 0.009 ppm and 0.012 ppm GD (table A-V) had lost half their activity.

The pH of the water used in the bioassay ranged from 7.2 to 7.7. The pH of the samples taken from other areas ranged from 7.2 to 8.3. The results of the chemical analyses of these waters are shown in table A-VI.

Bacterial counts (table A-VII) were extremely high, and coliform counts indicated the presence of fecal contamination in most of the water samples tested. VX had no effect on the total or coliform bacterial counts when water was tested 24 hours after contamination.

IV. DISCUSSION.

Since World War I, the US Army has guarded vigilantly against pollution of natural waters by chemical agents. From the results of this study, it is concluded that to kill white perch and striped bass, more than 300 grams of VX must remain 24 hours in 1 hectare (approximately 2.5 acres) of water in which the average depth is 10 feet. More than 30 kg of VX must remain under the same conditions to kill blue crabs. These values apply only if there is no subsequent dilution of the waters and the fish and crabs remain in these waters for 24 hours. Striped bass, white perch, and blue crabs, therefore, are not sensitive enough to nerve agents to be good biological indicators of contamination.

A more effective biological monitoring technique would be to establish control values for ChE activity in aquatic species and to compare these values with those of animals collected after contamination is suspected to have occurred. A more economical approach would be to

collect water samples in areas suspected of contamination and to analyze them chemically for antiChE activity using the automated enzymatic method.

It should be pointed out that the studies reported here were based on acute exposure for 24 hours. The effects of chronic exposures to lower levels of VX have not been studied.

V. CONCLUSIONS.

Open-air testing of V agents would present little, if any, hazard to blue crabs, white perch, and striped bass under present test controls.

Blue crabs, white perch, and striped bass are not sensitive enough to VX to be good indicators of contamination in their natural habitat. A chemical analysis for antiChE activity offers a more sensitive method of assay.

VX is stable in the waters taken from around Edgewood Arsenal; GD is not stable.

The waters around Edgewood Arsenal have high total and coliform bacterial counts, which may affect the general health of all marine life.

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APPENDIXES

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APPENDIX A

TABLES

Table A-1 Water, Crab, and Fish Collection Areas

Location	Remarks
Carroll Island North Shore Bengies Point (Saltpeter Creek) East Shore White Oak Point Carroll Point South Shore Hawthorne Cove	Chemical analysis All toxicity studies were used with blue crabs, perch, and striped bass; chemical analysis and bacterial counts Chemical analysis and bacterial counts Chemical analysis
Gunpowder Neck West (Gunpowder River) Maxwell Point Days Point Rickett Point	Perch and striped bass netted; chemical analysis and bacterial counts Chemical analysis Chemical analysis and bacterial counts
Gunpowder Neck East (Bush River) Sandy Point Briery Point	Perch and striped bass netted; chemical analysis Perch and striped bass netted; chemical analysis
Gunpowder River (Wright Creek to Rickett Point)	Crabs caught by trot line; chemical analysis and bacterial counts

Table A-II. Toxicity of Nerve Agents to Aquatic Test Species

Agent	Species ^a	Concn	Lt50 ^b	Time to 1st death	Time to 10th death	Death time span
		<i>ppm</i>		<i>min</i>		
VX	Blue crab	10.0	1421 ^c	1260(1) ^d	-	-
		20.0	923	595	1310(8)	715(8)
		40.0	480	300	1035	735
		80.0	184	130	300	170
		100.0	124	70	270	200
		150.0	101	50	180	130
		200.0	86	35	140	105
		300.0	62	20	85	65
VX	Striped bass	0.02	1042	850	1225(7)	375
		0.05	231	145	315	170
		0.07	171	160	196	36
		0.10	153	130	189	59
		0.20	74	55	91	36
		0.50	57	50	67	17
		1.0	29	25	34	19
		5.0	13	10	16	6
VX	White perch	0.025	413	300	550	250
		0.05	389	277	540	263
		0.10	169	153	193	40
		0.25	117	100	140	40
		0.50	72	66	76	10
		1.0	59	55	61	6
		5.0	14	10	18	8
		10.0	8	5	10	5
GD	White perch	0.001	180	130	225	95
		0.004	69	54	292	238 ^c
		0.006	50	45	58	13
		0.008	37	33	43	10
		0.040	5	3	6	3
		0.060	4	2	6	4
		0.080	2	1	5	4

^aTen of each species per concentration of agent.

^bFrom Bliss, C. I. *The Statistics of Bioassay*. Academic Press, New York, New York. 1952.

^cEight of the fish died within 11 minutes after the first death.

^dNumber dead in 24 hours indicated in parentheses when less than 10.

Table A-III. Nerve Agent Concentrations* Necessary to Kill 50% of Aquatic Test Species at Selected Times

(Based on the Harmonic Mean Statistical Analysis)

Exposure time	VX, LC50			GD, LC50
	Blue crab	White perch	Striped bass	White perch
<i>hr</i>	<i>ppm</i>			
1	215	0.085	0.10	0.0057
2	77	0.062	0.058	0.0055
4	48	0.052	0.044	-
8	36	0.048	0.039	-
16	32	0.047	0.036	-
24	29	0.046	0.035	-

Table A-IV. Degradation of VX in Test Waters Based on Inhibition of Bovine Serum Cholinesterase (ChE)

Time after contamination ^a	ChE activity ^b		
	1.2 ppm VX	0.6 ppm VX	0.12 ppm VX
<i>days</i>	<i>% of control</i>		
0	100	100	100
1	101(100-102)	101(100-102)	101(100-102)
8	85(84-87)	88(86-89)	83(79-88)
14	71(70-72)	73(72-75)	83(79-85)
22	59(56-63)	59(56-63)	58(54-62)
29	44(41-48)	44(42-48)	44(41-46)

^aSamples stored at 70°F throughout testing.

^bAverage of three samples: one composite sample of Gunpowder River waters around Carroll Island and two composite samples of Gunpowder River waters around Edgewood Arsenal.

Table A-V. Degradation of GD in Test Waters Based on Inhibition of Bovine Serum Cholinesterase

Time after contamination ^a	ChE activity ^b	
	0.009 ppm GD	0.012 ppm GD
<i>hr</i>	<i>% of control</i>	
0	100	100
1.3	79(72-84)	74(68-79)
5.7	53(42-60)	54(43-60)

^aSamples were stored at 70°F throughout testing.

^bAverage of three samples: one from White Oak Point, one from Maxwell Point, and one from Hawthorne Cove.

Table A-VI. Chemical Analyses of Bush River and Gunpowder River Samples

Analysis	White Oak Point ^a Carroll Island		Gunpowder River ^b		Bush River ^c	
	Average	Range	Average	Range	Average	Range
pH	7.52	7.20-7.70	7.70	7.15-8.25	7.79	7.64-7.95
Cl ⁻ (ppm)	3218	2680-3560	1795	1135-3560	4145	4000-4300
Na ⁺ (ppm)	1795	1520-2000	1020	645-1355	2300	2100-2600
K ⁺ (ppm)	66	55-77	41	25-68	73	71-75
Mg ⁺⁺ (ppm)	240	220-255	139	90-185	278	245-330
Ca ⁺⁺ (ppm)	207	18.2-22.5	12.2	11.5-18.2	24.3	23.8-25.0
Total hardness (ppm) (expressed as CaCO ₃) ^d	1031	955-1110	602	410-710	1202	1070-1420
Total alkalinity (ppm) (expressed as CaCO ₃)	<0.5	--	<0.5	--	44.3	43.0-45.3
Fe ⁺⁺ (ppm)	<0.5	--	<0.5	--	<0.5	--

^a Gunpowder River waters used in toxicity testing.

^b Samples taken from Bay Cove, Bengies Point, Hawthorne Point, and Maxwell Point shores and 200, 500, and 1000 yards offshore.

^c Samples from Sandy Point and Briery Point.

^d Calculations based on Ca⁺⁺ as CaCO₃ and Mg⁺⁺ as its CaCO₃ equivalent.

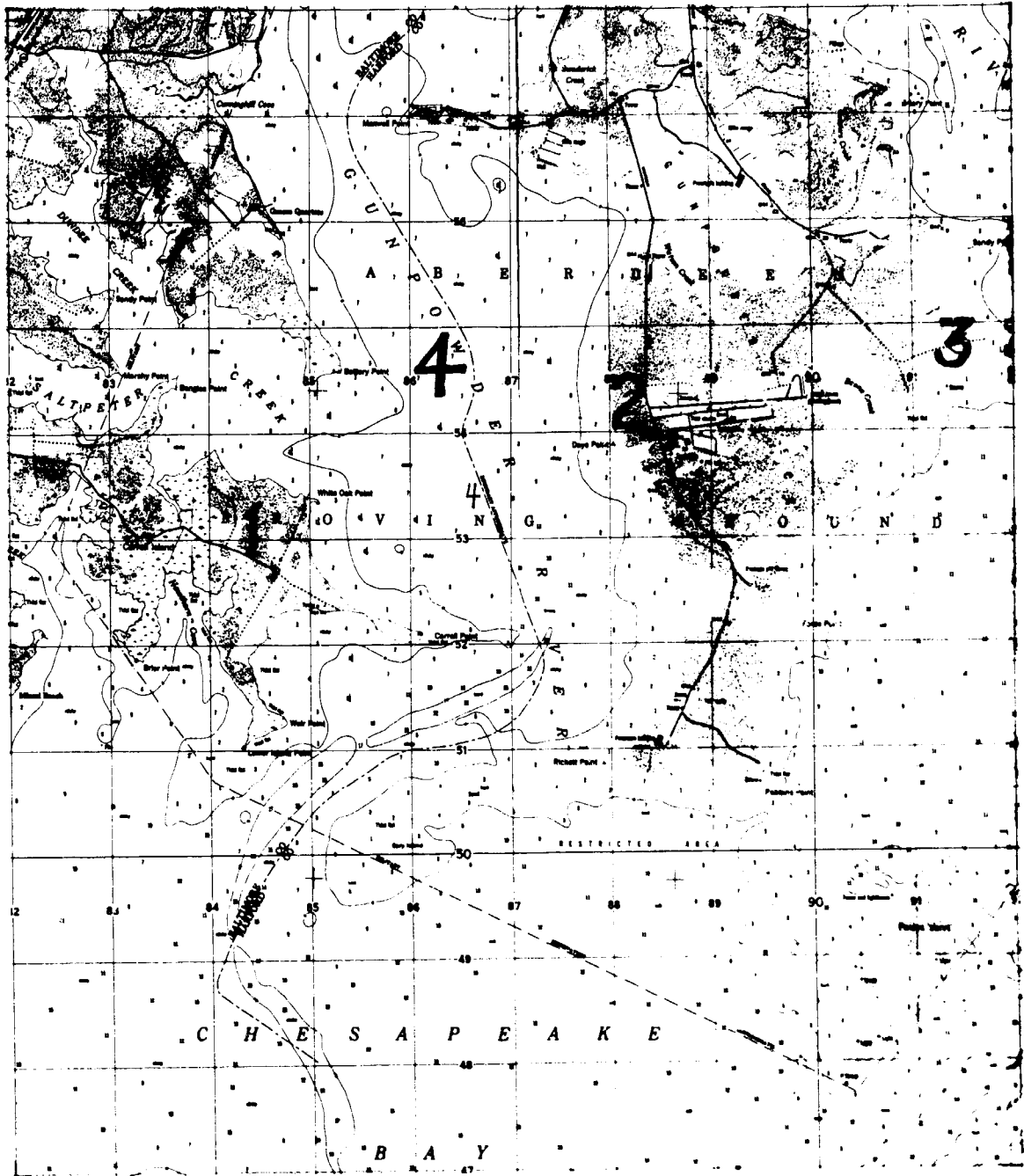
Table A-VII. Bacterial Analyses of Gunpowder River Samples

Location	Date collected	Counts	
		Plate	Coliform
		<i>No./cc</i>	
Gunpowder River at Wright Creek, Gunpowder Neck	11 Sept 69	980	>60*
Gunpowder River at Maxwell Point, Gunpowder Neck	16 Sept 69	97	39
Gunpowder River at Carroll Point, Carroll Island	16 Sept 69	40	10
Gunpowder River at White Oak Point, Carroll Island	16 Sept 69	39,500	>800*
Gunpowder River at White Oak Point, Carroll Island	25 Sept 69	≈ 600,000	Too many to count*
Gunpowder River at White Oak Point, Carroll Island	26 Sept 69	63,000	Too many to count*
Gunpowder River at White Oak Point, Carroll Island, with 1 ppm VX for 24 hr	25 Sept 69	≈ 600,000	Too many to count*

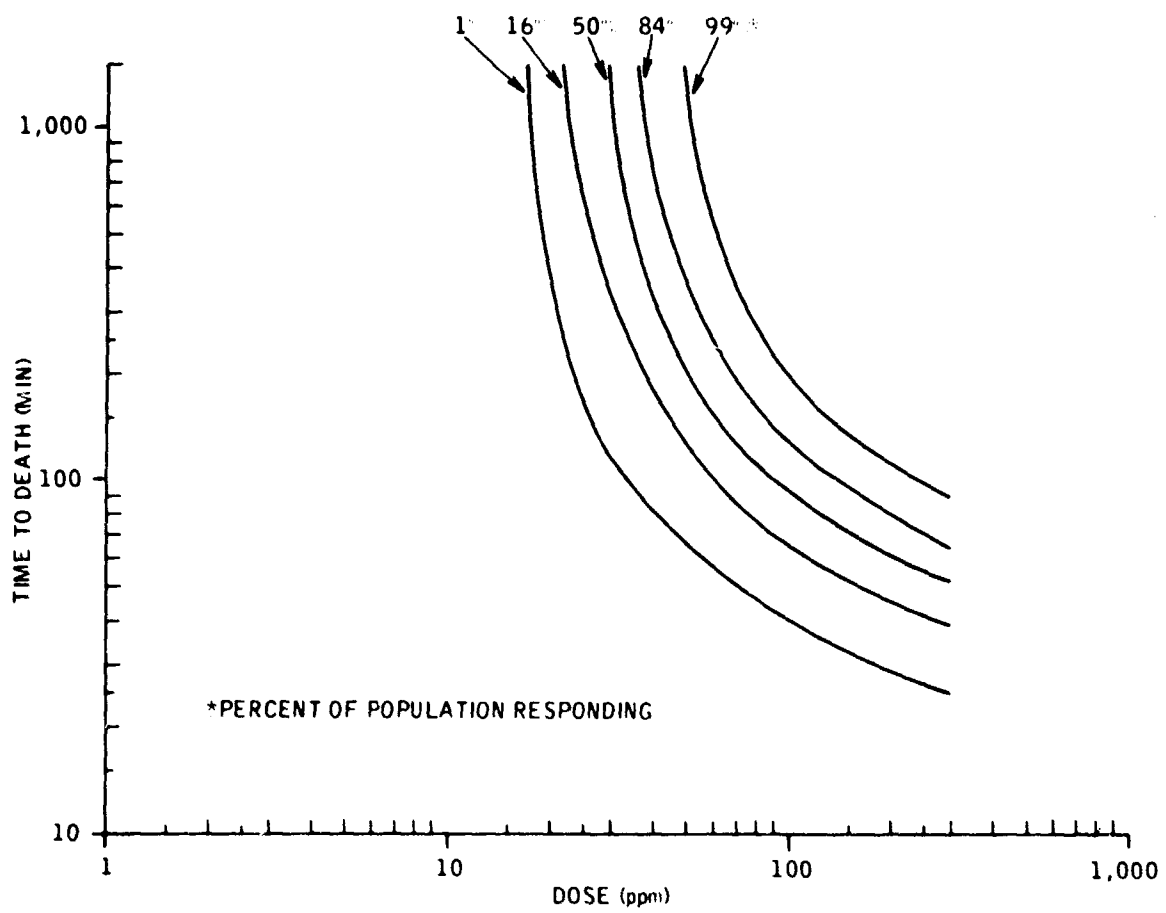
*Coliform count indicative of fecal pollution.

APPENDIX B

FIGURES

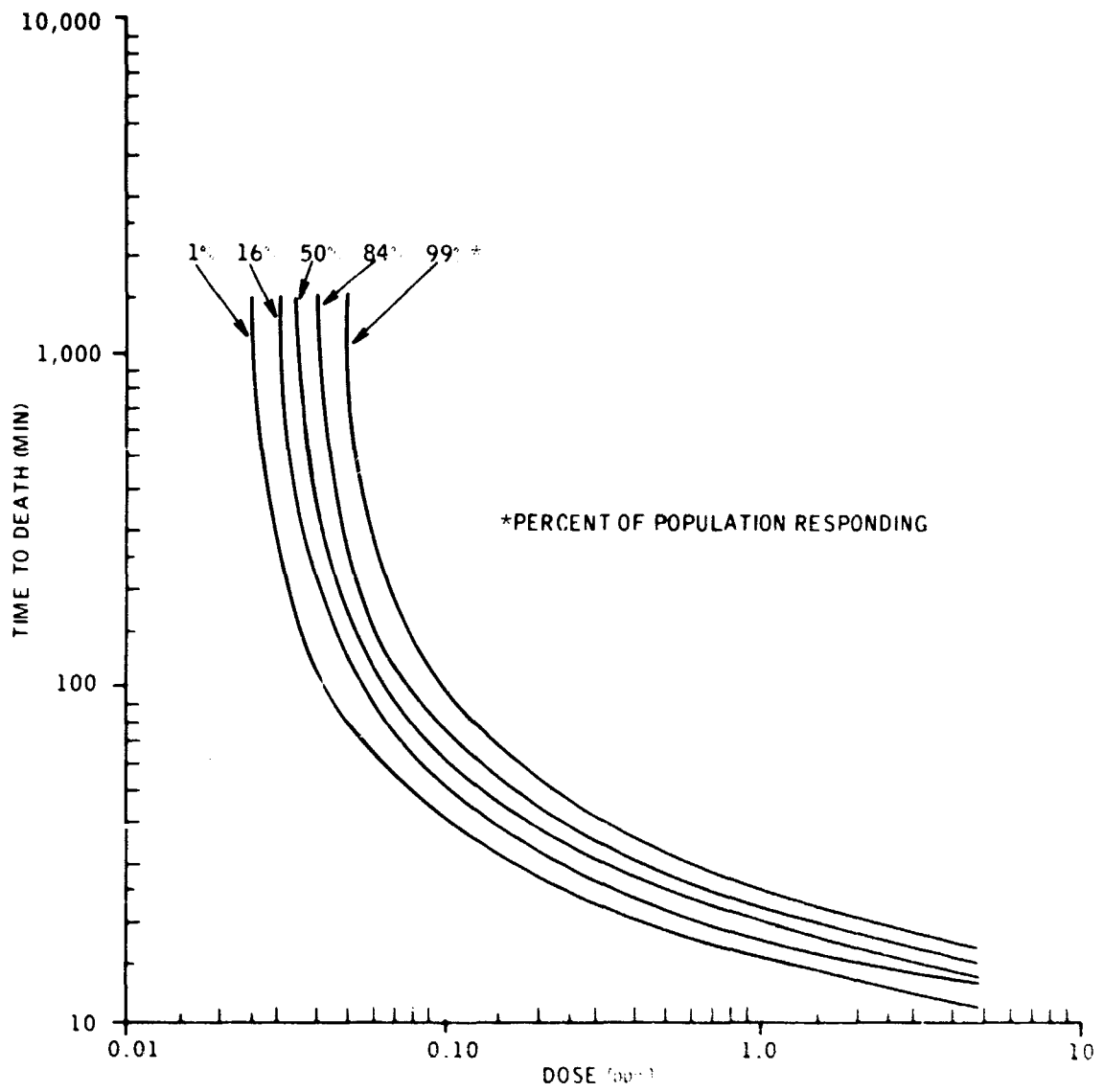


B-1. Waters Around Edgewood Arsenal

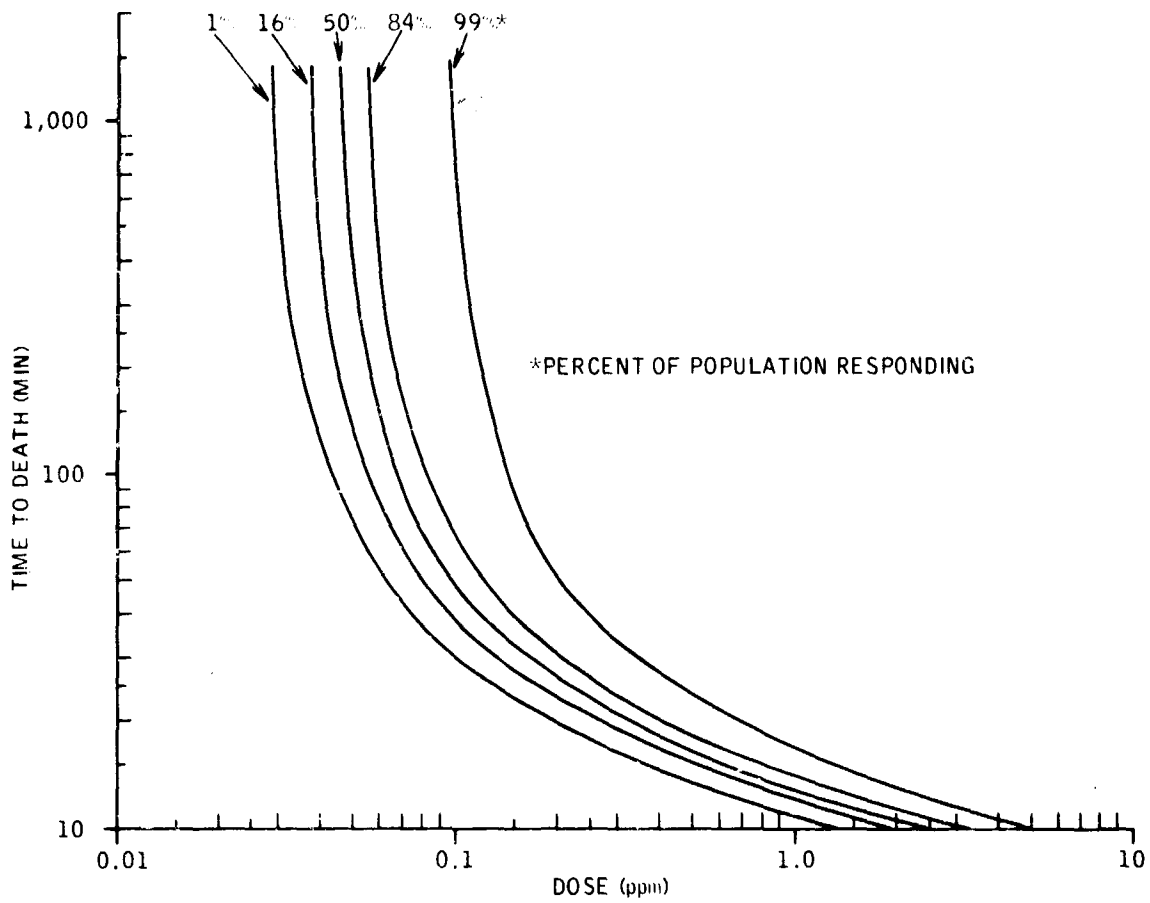


B-2. Time to Death in Blue Crabs Exposed to Various VX Concentrations in Water

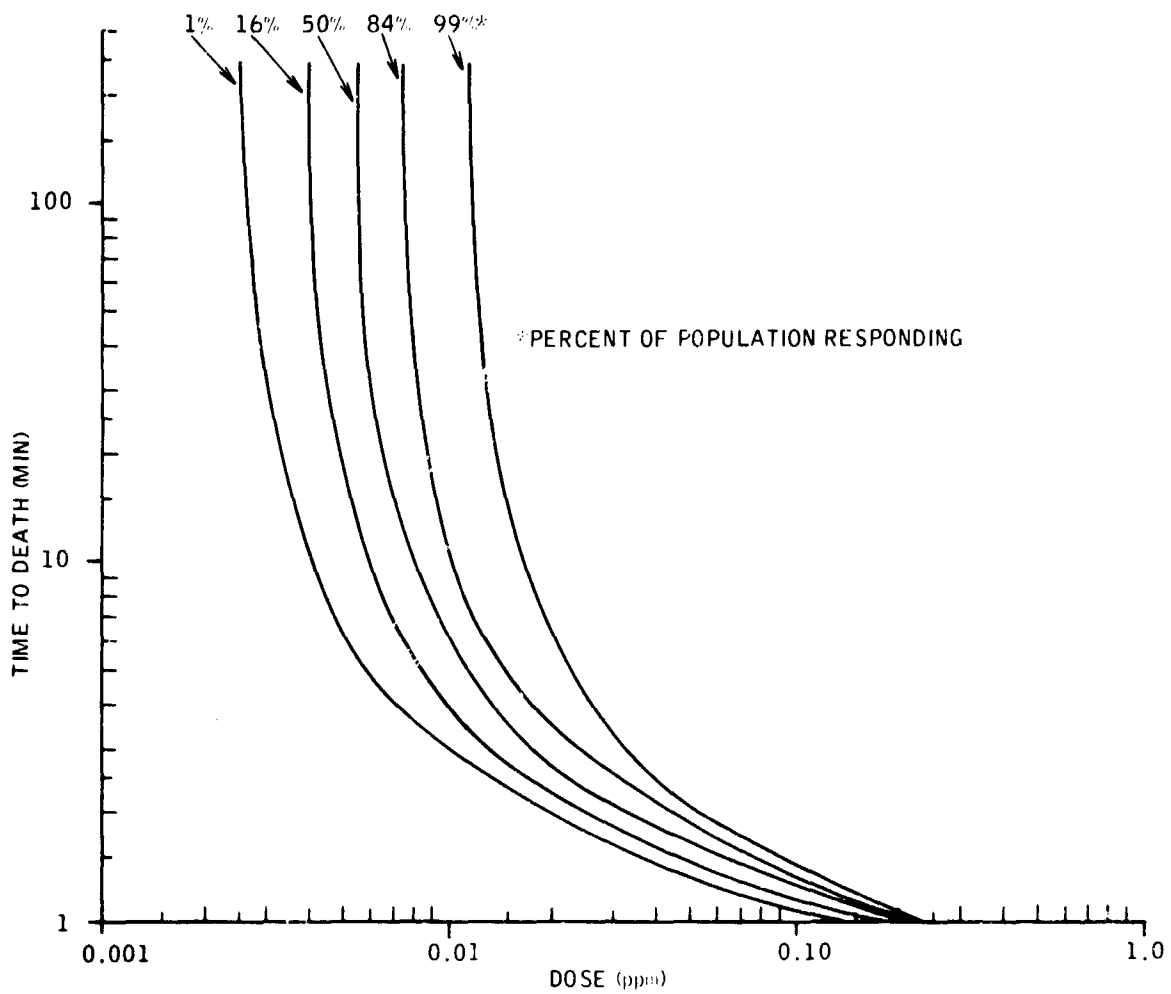
Figures 2 through 5 are based on a harmonic mean statistical analysis.



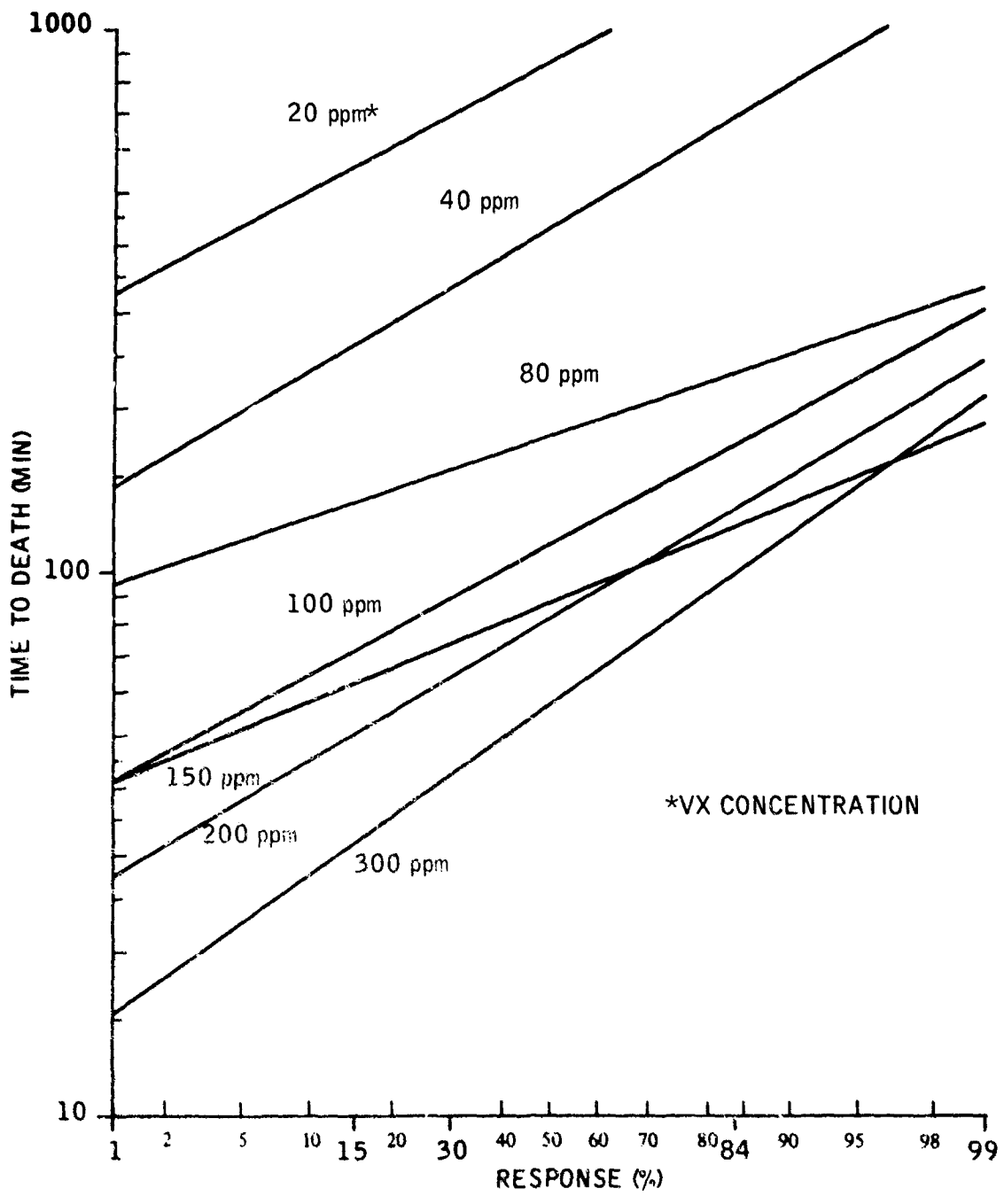
B-3. Time to Death in Striped Bass Exposed to Various VX Concentrations in Water



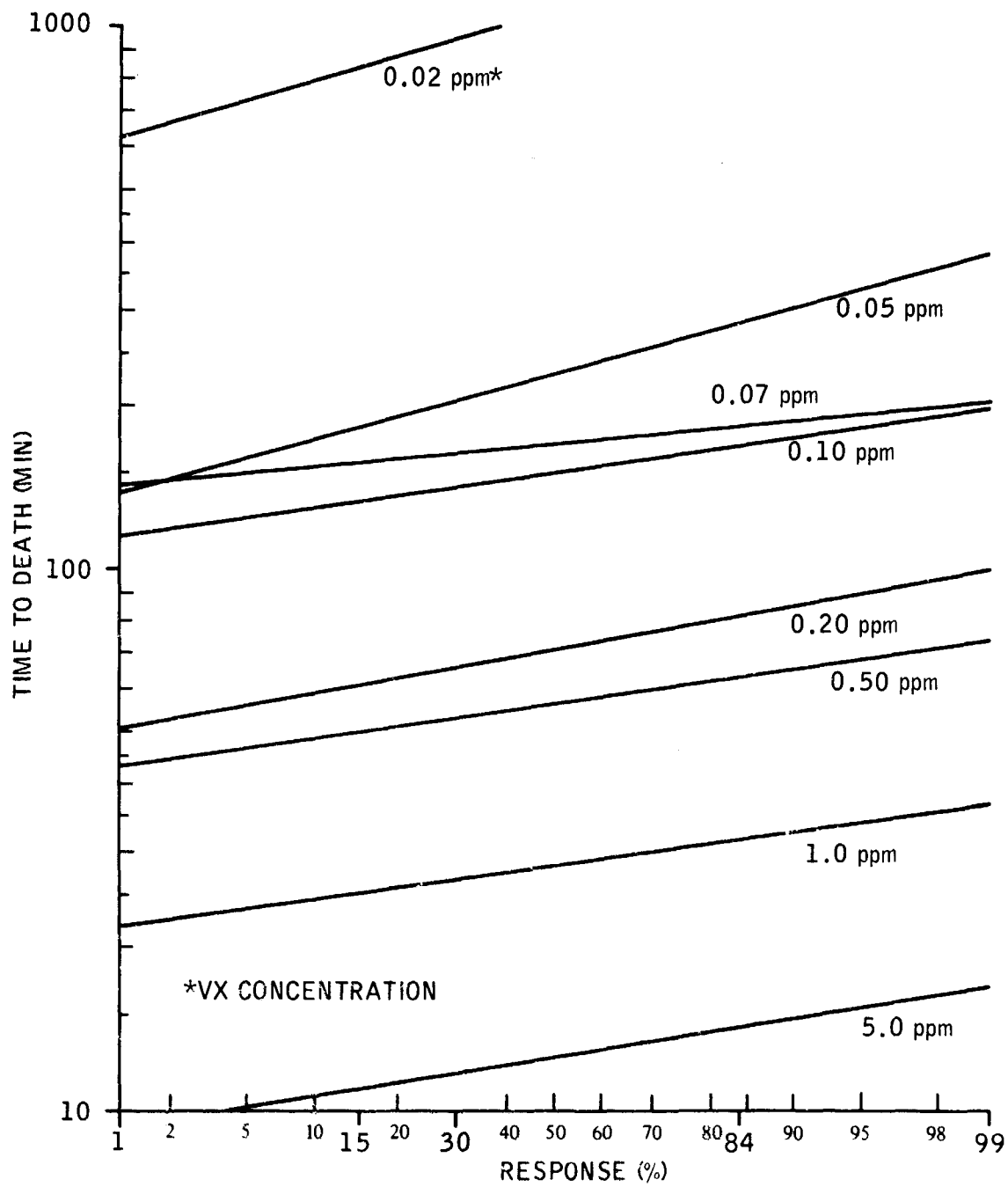
B-4. Time to Death in White Perch Exposed to Various VX Concentrations in Water



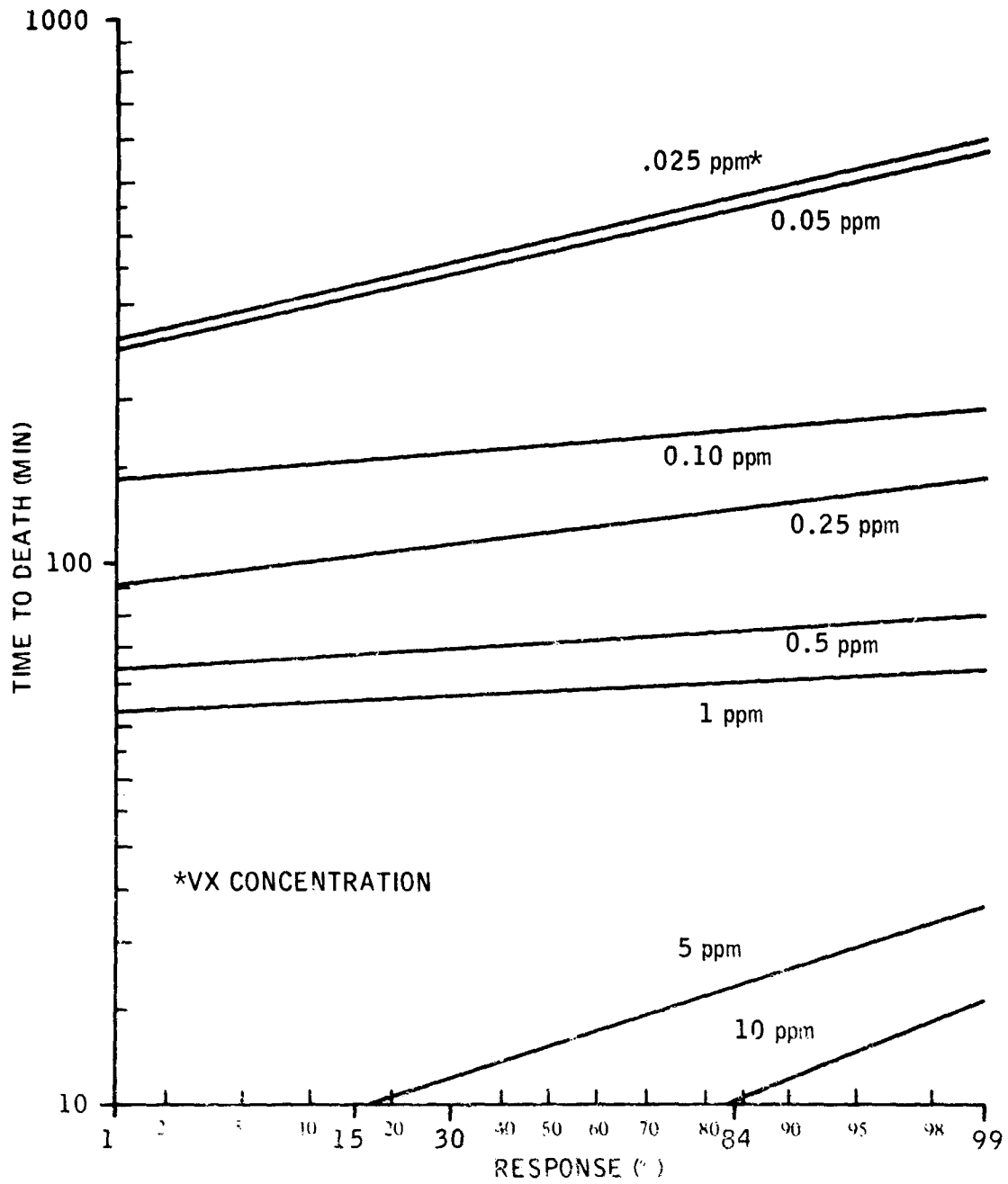
B-5. Time to Death in White Perch Exposed to Various GD Concentrations in Water



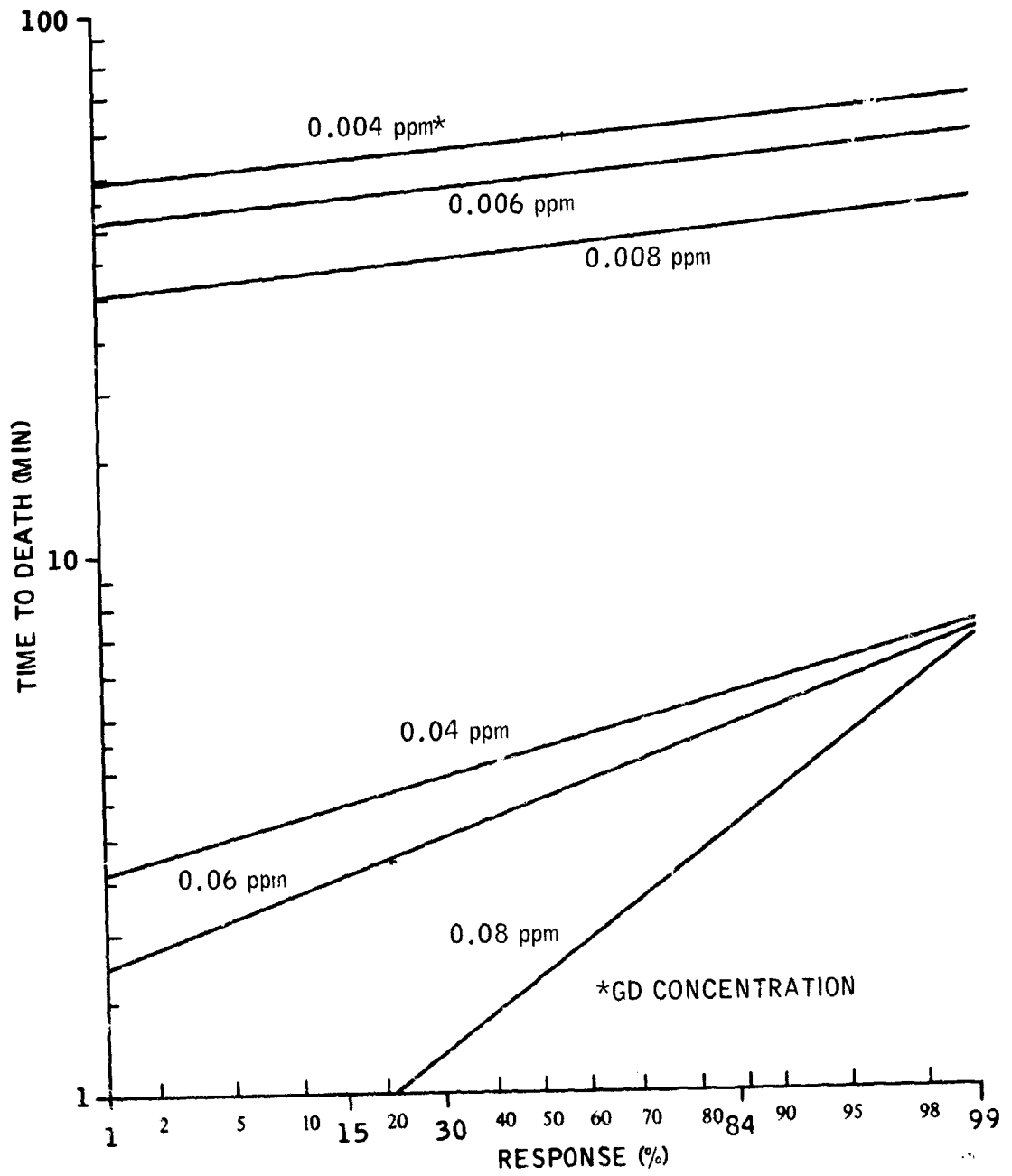
β-6. Time to Death in Blue Crabs Exposed to Various VX Concentrations in Water
 Figures 6 through 9 are the Bliss* dose-response regressions from which the harmonic means were derived.
 *Bliss, C. I. The Statistics of Bioassay. Academic Press, Inc., New York, New York. 1952.



B-7. Time to Death in Striped Bass Exposed to Various VX Concentrations in Water



B-8. Time to Death in White Perch Exposed to Various VX Concentrations in Water



B-9. Time to Death in White Perch Exposed to Various GD Concentrations in Water

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DOCUMENT CONTROL DATA - R & D		
<i>(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)</i>		
1. ORIGINATING ACTIVITY (Corporate author) CO, Edgewood Arsenal ATTN: SMUEA-RMT Edgewood Arsenal, Maryland 21010		2a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED
		2b. GROUP NA
3. REPORT TITLE TOXICITY OF VX AND GD IN AQUATIC ANIMALS INDIGENOUS TO THE CARROLL ISLAND TEST AREA WATER		
4. DESCRIPTIVE NOTES (Type of report & inclusive dates) This work was started in September and completed in October 1969.		
5. AUTHOR(S) (First name, middle initial, last name) J. T. Weimer, E. J. Owens, J. B. Samuel, J. S. Olson, and R. P. Merkey		
6. REPORT DATE September 1970	7a. TOTAL NO. OF PAGES 39	7b. NO. OF REFS 12
8a. CONTRACT OR GRANT NO.	8b. ORIGINATOR'S REPORT NUMBER(S) EATR 4441	
a. PROJECT NO.		
c. TASK NO. IB562602AD1102	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
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10. DISTRIBUTION STATEMENT Each transmittal of this document outside the agencies of the US Government must have prior approval of the Commanding Officer, Edgewood Arsenal, ATTN: SMUEA-TSTI-T, Edgewood Arsenal, Maryland 21010.		
11. SUPPLEMENTARY NOTES Lethal chemical agent investigations Biomedical evaluation of lethal agents	12. SPONSORING MILITARY ACTIVITY NA	
13. ABSTRACT This study was conducted to establish the sensitivity to VX and GD of aquatic species indigenous to the waters around Edgewood Arsenal. The bacterial and chemical characteristics of this water also were determined. The species tested with VX were the blue crab (<i>Callinectes sapidus</i>), the white perch (<i>Roccus americanus</i>), and the striped bass (<i>Roccus saxatilis</i>). Only the white perch was tested with GD. The water was from Gunpowder River and was used at room temperature (71°F). It was aerated for 24 hours before the fish and crabs were added and throughout the subsequent 24-hour observation period. No other adjustments were made. The concentration of VX required to kill 50% in 60 minutes is 215 ppm for blue crabs, 0.1 ppm for striped bass, and 0.085 ppm for white perch. The concentration of GD required to kill 50% of white perch in 60 minutes is 0.0057 ppm. It was concluded that blue crabs, white perch, and striped bass are not sensitive enough to VX to be good indicators of nerve agent contamination in their natural habitat. In degradation studies, VX was stable when added to samples of the waters around Edgewood Arsenal; GD was not stable. The waters tested had high total and coliform bacterial counts.		
14. KEYWORDS VX Carroll Island Test Area Ecology GD Gunpowder River Aquatic animals Water analysis, chemical and biological Blue crab LC50 White perch Agent stability Striped bass		

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