

AD _____

AD-A225 908

REVIEW OF REACTIONS OF BIOTOXINS IN WATER
(CBIAC TASK 152)

Final Report

J. Scott Warner

August 24, 1990

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

MIPR No. 90MM0530

Battelle Memorial Institute
Columbus Division
505 King Avenue
Columbus, Ohio 43201-2693

DOD DISTRIBUTION STATEMENT

Approved for public release; distribution unlimited

The findings in this report are not to be construed as
an official Department of the Army position unless so
designated by other authorized documents.

90 08 28 139

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION <u>Unclassified</u>			1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE			5. MONITORING ORGANIZATION REPORT NUMBER(S)	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)			7a. NAME OF MONITORING ORGANIZATION	
6a. NAME OF PERFORMING ORGANIZATION Battelle Memorial Institute Columbus Division		6b. OFFICE SYMBOL (If applicable)	7b. ADDRESS (City, State, and ZIP Code)	
6c. ADDRESS (City, State, and ZIP Code) 505 King Avenue Columbus, Ohio 43201-2693			9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER MIPR 90MM0530	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command		8b. OFFICE SYMBOL (If applicable)	10. SOURCE OF FUNDING NUMBERS	
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21702-5012			PROGRAM ELEMENT NO. 61102A	PROJECT NO. 61102BS12
			TASK NO. AA	WORK UNIT ACCESSION NO. 176
11. TITLE (Include Security Classification) Review of Reactions of Biotoxins in Water (CBIAC Task 152)				
12. PERSONAL AUTHOR(S) J. Scott Warner				
13a. TYPE OF REPORT Final Report		13b. TIME COVERED FROM 12/1/89 TO 8/31/90	14. DATE OF REPORT (Year, Month, Day) 1990 August 31	15. PAGE COUNT 13
16. SUPPLEMENTARY NOTATION				
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP		
06	09		RA-1; Biotoxins; Potable Water; Hydrolysis;	
07	03		Disinfection; Photochemistry	
19. ABSTRACT (Continue on reverse if necessary and identify by block number) A review of the literature, both open and classified, was conducted on the hydrolytic and photolytic reactions and reactions with common disinfectants of U.S. and suspected threat biotoxins in dilute aqueous solution. References were reviewed covering Botulinum toxin A, Tetanus toxin, Diphtheria toxin, Ricin, Palytoxin, Tetrodotoxin, Saxitoxin, Conotoxin, Microcystin, and Anatoxin A. Information obtained includes estimates of stability under various pH and temperature conditions.				
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia M. Miller			22b. TELEPHONE (Include Area Code) (301) 663-7325	22c. OFFICE SYMBOL SGRD-RM1-S

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

hls Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

hls Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

Karen L. Schwatke 8/24/90
PI Signature Date

for Scott Warner

This report is a work prepared for the United States by Battelle.
 In no event shall either the United States or Battelle have any
 responsibility or liability for any consequences of any use, misuse,
 inability to use, or reliance upon the information contained herein,
 nor does either warrant or otherwise represent in any way the
 accuracy, adequacy, efficacy, or applicability of the contents hereof.

Availability Codes	
NTIS - C.A.B.	☒
DTIC - TAB	☐
Unannounced	☐
Justification	
By	
Distribution /	
Availability Codes	
Dist	Availability and/or Special
A-1	



INTRODUCTION

In a battlefield contaminated with biotoxins used in warfare, water supplies may be contaminated intentionally or incidentally. Part of the mission of the U.S. Army Biomedical Research and Development Laboratory (USABRDL) concerns evaluating the potential for adverse human health effects from consumption of contaminated water. It is essential that the fates of biotoxins in water subjected to Army field water treatment be learned.

This report describes a literature review conducted to obtain a data base of hydrolysis and photochemical reactions of biotoxins and reactions of biotoxins with common disinfectants.

SCOPE OF WORK

The review covered both open and classified literature. The work was directed specifically to obtaining information on the four protein neurotoxins and six nonprotein neurotoxins listed below.

Protein Neurotoxins

Botulinum toxin A

Tetanus toxin

Diphtheria toxin

Ricin

Nonprotein Neurotoxins

Palytoxin

Tetrodotoxin

Saxitoxin

Conotoxin

Microcystin

Anatoxin A

Data sought on hydrolysis reactions included solubilities, reaction rates, and reaction products of biotoxins in water, with particular emphasis on concentrations below 1 g/L. The effects of temperature, pH, ionic strength, and general or specific catalysis were considered

of importance, as well as, discussions of reaction mechanisms when such information might contribute to an understanding of the fates of biotoxins in natural waters.

Data sought on photochemical reactions included reaction rates, quantum yields, and photochemical degradation products of biotoxins in dilute aqueous solution, particularly when such data might be relevant to the fate of biotoxins in natural waters.

Data sought on reactions with disinfectants included reaction rates and reaction products, particularly data from reactions with dilute aqueous solutions of hypochlorite or iodine. Decontamination data was of interest if no other data were available.

DESCRIPTION OF INFORMATION SEARCH

Computer-assisted on-line searches of the following data bases were conducted.

- Chemical Abstract Service (CAS)
- National Technical Information Service (NTIS)
- Defense Technical Information Center (DTIC)
- Chemical/Biological Information Analysis Center (CBIAC)
- BIOSIS
- MEDLINE

The key limiters used for the searches were the names and CAS registry numbers of each of the ten toxins, and the following terms:

hydrolysis	photochemical	reaction rate	breakdown
water	solubility	chlorine	fate
destruction	aqueous	quantum yield	iodine
reaction	disinfect	aquatic	chemical
hypochlorite	degradation	destroy	reaction

In addition to the computer-assisted searches, a manual search of Chemical Abstracts for the years 1946 to 1967 was conducted.

RELEVANT INFORMATION RETRIEVED

The computerized searches yielded the following numbers of references.

- CAS - 356
- MEDLINE - 474
- BIOSIS - 513
- NTIS - 23
- DTIC/CBIAC - 271

Despite the large numbers of "hits" obtained, very little relevant information was found. Most of the references were devoted to isolation, synthesis, toxicology, or biological studies. The abstracts of all of the references were reviewed. Hard copies of the complete original references were obtained for about 40 of the more relevant citations and reviewed in detail.

The most useful reference was a review of chemical, biological, and toxicological properties of selected toxins and venoms conducted in 1983 by Thomas Facklam, of Battelle's Tactical Technology Center, for the U.S. Army Chemical Systems Laboratory.⁽⁶⁾ Facklam's review covered five of the ten toxins of interest to the current program. The information provided by references covered by Facklam's review and other references is presented in the following sections.

Botulinum Toxin A

Botulinum toxin can be isolated in crystalline or lyophilized form, can be destroyed or inactivated by alkali, chlorine developer, or iodine.⁽⁷⁾ Water infected with botulinum toxin was decontaminated by chlorination, boiling, sterilization, or charcoal treatment.⁽¹⁷⁾ Vigorous agitation of a dry blend of the toxin in air at 24°C causes a loss of activity.⁽²²⁾ Silverman et al. in studying the environmental persistence of botulinum neurotoxin A on surfaces, found that the

toxin is stable enough to require detoxification.⁽²⁴⁾ These workers also found that the effectiveness of the various detoxifying agents tested was dependent upon temperature, pH, and purity of the toxin.

Tetanus Toxin

Tetanus toxin was found by Lahiri and Dutta to be denatured rapidly under acidic conditions; purification was conducted in the cold to avoid degradation.⁽¹⁶⁾ In work conducted by Helting and Zwisler on the enzymatic degradation of tetanus toxin, the toxin was hydrolyzed with papain at pH 6.5 at 55°C but was stable below 40°C.⁽¹³⁾ Wright et al. in work on the development and evaluation of a purified tetanus toxin, studied the stability of the toxin.⁽²⁷⁾

Diphtheria Toxin

Blewitt et al. reported that diphtheria toxin switches from a hydrophilic conformation to a hydrophobic conformation at pH 4 to 5.⁽⁴⁾ Ramsay et al. reported that the toxin is most stable at pH 7 to 8 and unfolds at high and low pHs.⁽²¹⁾

Ricin

Ricin is a glycoprotein having a molecular weight of 62,000 and consisting of two different polypeptide chains joined by a disulfide bond; the disulfide bond can be broken by incubating the toxin at 37°C for 3 hours in pH 8.5 Tris buffer containing 2 percent 2-mercaptoethanol.⁽⁹⁾ Ricin was reported by Bushueva and Tonevitsky to be stable over the range of pH 3 through 8.⁽⁶⁾ Taira et al. reported that intact ricin D is stable at pH 1 through 11 for 24 hours at 25°C and stable at 50°C for 1 hour at pH 7.8.⁽²⁶⁾

Ricin is not hydrolyzed by trypsin or pepsin, but is partially hydrolyzed when incubated with an alkaline protease, nigrase, at 30° C and pH 8.0 for 16 hours; it is hydrolyzed by trypsin only in the presence of guanidine hydrochloride.^(10, 29) Yamasaki et al. reported that ricin is denatured and insolubilized by freeze-thawing.⁽²⁸⁾

Palytoxin

Palytoxin is a generic term for water-soluble toxins from a genus of soft corals. The toxins are moderately stable and have molecular weights of approximately 3,000. Moore and Scheuer have reported that the half-lives of palytoxin in 0.05 N HCl and 0.05 N NaOH are 85 and 55 minutes, respectively.⁽¹⁸⁾

Tetrodotoxin

Studies on tetrodotoxin have been reviewed by Goto et al. and Kao.^(12, 14) The toxin is extracted by boiling water to prevent autolysis and is soluble in slightly acidic water. Mosher et al. have reported that tetrodotoxin is rapidly converted to the nontoxic tetrodonic acid if the acid is very strong.⁽¹⁹⁾ The toxin is weakly basic, and, although it is insoluble in water under basic conditions, it is rapidly destroyed under such conditions.

Saxitoxin

The review by Kao indicates that saxitoxin is a basic substance that is very soluble in water as the dihydrochloride.⁽¹⁴⁾ Burke et al. have reported that saxitoxin is water soluble and acid stable, and survives boiling with 0.15 M trichloroacetic acid for 30 minutes.⁽⁵⁾ However, there is an appreciable loss in activity when the toxin is boiled in 0.03 N HCl for 30 minutes. The toxin decomposes rapidly in basic solution. Ghazarossian et al. have reported that saxitoxin is

hydrolyzed to decarbamoylsaxitoxin in 7.5 N HCl at 100°C for 3 to 8 hours without loss of activity.⁽¹¹⁾ Koehn et al. achieved the conversion by heating the toxin in 7.5 N HCl at 115°C under nitrogen for 90 minutes and reported a 40 percent loss in activity.⁽¹⁵⁾ Robinson has reported that the acid hydrolysis product is at least 100-fold less toxic than saxitoxin.⁽²³⁾

Conotoxin

No information was found on the stability of conotoxin.

Microcystin

Microcystin appears to be reasonably stable in water in that it leaches from cells into water over a 40-day period at 17°C.⁽²⁾ A review by Bishop et al. indicates that there have been conflicting reports on the stability of microcystin ranging from unstable to heat when wet to stable when autoclaved in neutral solution.⁽³⁾ Rabin and Darbre reported that there is no change in the toxicity of microcystin when it is heated for 1 hour at 100°C at pH 1 through 7.⁽²⁰⁾ Amann and Juettner have reported that microcystin is readily deactivated even when frozen.⁽¹⁾

Anatoxin A

Smith and Lewis have reported that Anatoxin A in water under ambient conditions is converted to the nontoxic anhydro derivative in several days.⁽²⁵⁾

REFERENCES

1. Amann, M. J., and F. Juettner. "A Rapid Procedure for the Isolation of an Unstable Microcystis Toxin with FDF-features," FEMS Letters 12: 191-193 (1981).
2. Berg, K., O. M. Skulberg, and R. Skulberg. "Effects of Decaying Toxic Blue-Green Algae on Water Quality, a Laboratory Study," Arch. Hydrobiol. 108: 549-564 (1987).
3. Bishop, C. T., E. F. L. J. Anet, and P. R. Gorham. "Isolation and Identification of the Fast-death Factor in Microcystis Aeruginosa NRC-1," Can. J. Biochem. Physiol. 37: 453-471 (1959).
4. Blewitt, M. G., J. Zhao, B. McKeever, R. Sarma, and E. London. "Fluorescence Characterization of the Low pH Induced Change in Diphtheria Toxin Conformation Effect of Salt," Biochem. Biophys. Res. Commun. 120: 286-290 (1984).
5. Burke, J. M., J. Marchisotto, J. J. A. McLaughlin, and L. Provasuli. "Analysis of the Toxin Produced by Gonyaulax Catenella in Axenic Culture," Ann. N.Y. Acad. Sci. 90: 837 (1960).
6. Bushueva, T. L., and A. G. Tonevitsky. "The Effect of pH on the Conformation and Stability of the Structure of a Plant Toxin - Ricin," FEBS Lett. 215: 155-159 (1987).
7. Dold, R. "Botulin -- The Weapon of Silent Warfare," Army Biological Labs Report Trans. 39. AD Number 841139 (February 1966).
8. Facklam, T. J. "Review of the Chemical, Biological, and Toxicological Properties of Selected Toxins and Venoms," Contractor Report ARCSL-CR-83041, U S Army ARRADCOM, Aberdeen Proving Ground MD 21010. AD Number B079421L (June 1983).
9. Foxwell, B. M. J., T. A. Donovan, P. E. Thorpe, and G. Wilson. "The Removal of Carbohydrates From Ricin with Endoglycosidases H, F and D and α -Mannosidase," Biochim. Biophys. Acta 840: 193-203 (1985).
10. Funatsu, G., and M. Funatsu. "Biochemical Studies on Ricin. Part XV. Limited Hydrolysis of Ricin D with Alkaline Protease From Bacillus Subtilis," Agric. Biol. Chem. 41: 1309-1310 (1977).
11. Ghazarossian, V. E., E. J. Schantz, H. K. Schnoes, and F. M. Strong. "A Biologically Active Acid Hydrolysis Product of Saxitoxin," Biochem. Biophys. Res. Commun. 68: 776-780 (1976).

12. Goto, T., Y. Kishi, S. Takahashi, and Y. Hirata. "Tetrodotoxin," Tetrahedron 21: 2059 (1965).
13. Helting, T., and O. Zwisler. "Enzymic Breakdown of Tetanus Toxin," Biochem. Biophys. Res. Commun. 57: 1263-1270 (1974).
14. Kao, C. Y. "Tetrodotoxin, Saxitoxin and Their Significance in the Study of Excitation Phenomena," Pharmacol. Rev. 18: 997 (1966).
15. Koehn, F. E., H. K. Schnoes, and C. Y. Kao. "On the Structure and Biological Properties of Decarbamoylsaxitoxin," Biochim. Biophys. Acta. 734: 129-132 (1983).
16. Lahiri, B., and S. K. Dutta. "A Simple Method for Purification of Tetanus Toxoid," Indian J. Exp. Biol. 22: 557-559 (1984).
17. Markarjan, M. K., N. V. Ryshow, and J. V. Stannikow. "Decontamination of Water Infected with Botulin Toxin," Army Medical Research Inst of Infectious Diseases Report No. USAMRIID-MUL-0760. AD Number B965762L (1960).
18. Moore, R. E., and P. J. Scheuer. "Palytoxin: A New Marine Toxin from a Coelenterate," Science 172: 495-498 (1971).
19. Mosher, H. S., F. A. Fuhrman, H. D. Buchwald, and H. G. Fisher. "Tarichatoxin -- Tetrodotoxin: A Potent Neurotoxin," Science 144: 1100 (1964).
20. Rabin, P., and A. Darbre. "An Improved Extraction Procedure for the Endotoxin from Microcystis aeruginosa NRC-1," Biochem. Soc. Trans. 3: 428-430 (1975).
21. Ramsay, G., D. Montgomery, D. Berger, and E. Freire. "Energetics of Diphtheria Toxin Membrane Insertion and Translocation Calorimetric Characterization of the Acid pH Induced Transition," Biochemistry 28: 529-533 (1989).
22. Riesen, W. H., and E. J. Hawrylewicz. "Physical and Biological Stability Studies of Botulinum Toxin", IIT Research Inst Report No L6003 15. AD Number 347295 (February 10, 1964).
23. Robinson, K. A. "HPLC Separation and Comparative Toxicity of Saxitoxin and Its Reaction Products," Biochim. Biophys. Acta. 687: 315-320 (1982).
24. Silverman, G. J., G. G. Roy, Jr., C. T. Woodbury, C. H. Lee, and J. J. Previte. "Environmental Stability and Detoxification of Staphylococcal Enterotoxin A and Botulinum Neurotoxin A," Proceedings of NRDEC Science Symposium held at US Army Natick RD&E Center, Natick, MA, June 2-4, 1986 pp 69-78. AD Number D750069L (June 4, 1986).
25. Smith, R. A., and D. Lewis. "A Rapid Analysis of Water for Anatoxin A, the Unstable Toxic Alkaloid From Anabaena Flos-Aquae, the Stable Nontoxic Alkaloids Left After

Bioreduction and a Related Amine Which May Be Nature's Precursor to Anatoxin A," Vet. Hum. Toxicol. 29: 153-154 (1987).

26. Taira, E., N. Yoshizuka, G. Funatsu, and M. Funatsu. "Effects of Physical and Chemical Treatments on the Biological Activity of Ricin D," Agri. Biol. Chem. 42: 1927-1932 (1978).
27. Wright, G. G., W. C. Latham, and L. Levine. "Development and Evaluation of a Purified Tetanus Toxin," Report from Tufts University School of Medicine. AD Number B016381L (July 31, 1975).
28. Yamasaki, N., T. Hatakeyama, and G. Funatsu. "Denaturation of Ricin D in Frozen Aqueous Solutions," Agric. Biol. Chem. 52: 2547-2553 (1988).
29. Yoshitake, S., K. Watanabe, and G. Funatsu. "Biochemical Studies on Ricin. Part XXV. Limited Hydrolysis of Ricin D with Trypsin in the Presence of Sodium Dodecyl Sulfate," Agric. Biol. Chem. 43: 2193-2195 (1979).