W1 Torian

FOREIGN MEDICAL MATERIEL EXPLOITATION PROGRAM REPORT

A REPORT ON THE PROGRAM TO ASSESS THE MEANING OF MYCOTOXIN DETECTION IN BLOOD AND URINE SAMPLES FROM INDIVIDUALS IN SOUTHEAST ASIA

RICHARD TORIAN

APR 1987

SUMMARY

The detection in 1982 of mycotoxins in blood and urine samples from Southeast Asian chemical warfare(CW) victims led to a program of sample collection and analysis to verify and confirm the initial conclusion that mycotoxins were being used as a chemical warfare agent. By the end of 1986, when the last samples were analyzed, 1064 blood and urine samples had been collected from CW victims and controls and 479 of these had been analyzed by one laboratory for the presence of T-2 and HT-2 mycotoxins. This report provides details on this program. The author concludes that the program shows that the mycotoxins detected in blood and urine samples from CW victims in Southeast Asia cannot be used as evidence that mycotoxins were used as CW agents in Southeast Asia.

PURPOSE

The purpose of this report is to:

1. Summarize the important aspects of the program; namely sample collection, analysis, and the results obtained;

2. Condense a huge amount of data into an usable form;

3. Draw sensible meaning from the data;

4. Complete the program with a final review report;

5. Utilize the author's many hours of involvement in the program; and

6. Provide comments and conclusions concerning the program.

PART 1. INTRODUCTION

In 1982, blood and urine samples, taken from victims of chemical warfare attacks in Southeast Asia, were found to contain T-2 and HT-2 mycotoxins. The detection of the mycotoxins suggested a breakthrough in determining the agent that was being used in the chemical warfare attacks. To support or refute the conclusion that this detection showed that a CW agent being used was a mycotoxin or mycotoxins, blood and urine samples continued to be collected from CW victims and controls until the end of 1985, and analysis of the collected samples continued until the end of 1986. This effort of collection and analysis to fully understand the meaning of the initial detection of mycotoxins in victim samples constitutes the program described in this report. Part 2(page 4) of this report provides details on the sample collection aspects of the program; part 3(page 7) on the analysis aspects of the program; and part 4(page 10) on the results obtained. Part 5(page 14) provides interpretations and comments the author derives from the data presented in parts 2 to 4.

PART 2. SAMPLE COLLECTION

Table 1 shows the number of victim and control samples collected by the US Embassy in Bangkok and sent to the Armed Forces Medical Intelligence Center(AFMIC) from 1981 to 1985.

Table 1. Victim and Control Samples Collected, 1981 to 1985

Year Collected	Victim Samples	Control Samples
1981	28	8
1982	106	28
1983	139	8
1984	153	417
1985	33	142

Totals: 459 victim samples
603 control samples
1062 total samples

Details on sample collection:

1. The term sample as used in this report is a tube of blood or urine collected by the US Embassy, Bangkok from either a Southeast Asian CW attack victim or from a Southeast Asian non-CW attack control individual and sent to AFMIC. Frequently, multiple samples were collected from an individual, e.g. one blood sample and two urine samples from one individual.

2. Other biomedical samples, e.g. tissue samples other than blood, were collected by the Embassy. These other biomedical samples were small in number and have insufficient controls; results obtained from analysis of these samples have no bearing on the program described in this report.

3. Some of the samples that arrived at AFMIC were split into aliquots by the US Army Medical Research Institute of Infectious Diseases. Aliquots of a sample are considered in this report as one sample.

4. In 1984, a US military physician and a US Army Chemical Corps Officer were attached to the Embassy in Bangkok to collect samples. Prior to this, personnel in the Embassy's Defense Attache Office collected the samples. The attached personnel remained at the Embassy until the end of 1985.

5. The Embassy used a numbering system for the samples that shows: the country where the sample was collected; the year, month and day the sample was collected; a sequential number to distinguish samples collected on the same day; and a collector identification. AFMIC also gave each sample a number showing the year and month that the sample arrived at AFMIC and a sequential number to distinguish samples that arrived in the same month.

6. Samples were shipped frozen. Dry ice was used as the coolant. Shipments usually went on Japanese Airlines to New York and then to Dulles Airport taking approximately 48 hours. Personnel from the US Army's Technical Escort Team at Aberdeen Proving Ground picked up the shipments at Dulles and delivered them to AFMIC. All samples were kept frozen after arrival until analysis except for samples that were split into aliquots. These were thawed, split and refrozen. The Embassy messaged AFMIC with shipment information prior to each shipment. Sample tube breakage was a problem at first but better packing and use of plastic tubing eliminated this problem.

7. The Embassy provided background information to AFMIC for each sample. Information included: 1) the name of the individual from whom the sample was collected; 2) the type of sample, i.e. urine

or blood; 3) the individual's category, i.e. victim or control; 4) the details surrounding the collection, e.g. where the sample was collected and by whom; 5) the age of the individual; 6) the circumstances of exposure if the individual is a victim; 7) the symptoms of the individual following exposure; 8) the symptoms of the individual at the time of the collection of the sample; and 9) the individual's nationality.

8. The author does not attempt to qualify a victim. That is, no attempt is made to examine the victim's background to determine that the victim is a "hard" victim--e.g. one whose background is convincing in establishing the individual as a victim--or to determine that the victim is a "soft" victim--e.g. one whose background suggests suspicions about whether the individual could have been a victim. Likewise, no effort is made to separate "recent victims"--e.g. victims exposed within a recent time period prior to the collection of the sample--from "distant victims"--e.g. victims exposed at a distant time prior to collection of the sample. No doubt there are such "qualified" victims, i.e. not true victims. Also, no effort is made to define and to qualify controls, e.g. the control should be of the proper cultural background, age level, etc. to be a good control for a victim and controls meet this definition. If the results had been different, a qualification of victim and control samples would have to be considered. The category where the Embassy placed the sample (i.e. victim or control) has been accepted and used without qualification.

9. In 1985, 136 samples were collected from 68 individuals as part of the Hmong Control Project. This project selected two Hmong villages as control villages of individuals not exposed to CW attack.

PART 3. SAMPLE ANALYSIS

Table 2 shows the number of victim and control samples analyzed for T-2 and HT-2 mycotoxins by Dr Chester J. Mirocha of the Plant Pathology Department, University of Minnesota. Since in some cases 2 or 3 samples from the same individual were analyzed, the table shows how many victim and control individuals were tested for mycotoxins.

Table 2. Victim and Control Samples Analyzed

Year Collected	Victim Samples	(Individuals)	Control Samples	(Individuals)
1981	15	(12)	4	(4)
1982	89	(47)	23	(14)
1983	58	(45)	3	(3)
1984	31	(22)	90	(54)
1985	29	(16)	137	(71)
Totals:	222	(142)	257	(146)

479 total samples analyzed(from 288 individuals)

Details on sample analysis:

1. Only the results from Dr. Mirocha's laboratory are of sufficient numbers of victims and controls and unquestioned accuracy to be used in this report. Another laboratory, Dr. Hamilton's laboratory, analyzed 73 victim and 93 control samples, however these results are not being considered in this report because there are questions by other analytical chemists about the validity of the results. A few other laboratories also analyzed samples, but the number of samples analyzed were small with insufficient controls and their results have no bearing on the program described in this report.

2. As stated in Part 2, some samples were split into aliquots. More than one aliquot from a sample was sent for analysis in some cases. However, when more than one aliquot was sent it is counted as one sample analysis.

3. As seen by a comparison of tables 1 and 2, not all samples collected were analyzed. Selection criteria for sample analysis varied over the time span of the program. Since May 1985, when the author first became involved in the program, primary selection criteria were: 1) samples from individuals who tested positive on other of their samples or aliquots; 2) a balanced number between victim and control samples; and 3) the Hmong Village Control Project samples.

4. In early 1986, personnel assigned to the Embassy requested that all analyses be completed by August 1986. The last samples were sent for analysis in August 86 and the results were received in November 1986.

5. Until May 1985, AFMIC paid $800 per Mirocha sample analysis. At that time 219 samples had been analyzed and the cost of analysis was $175,200. In May 1985, AFMIC started paying $50 per sample analysis which in February 1986 increased to $75 per sample. 260 samples were analyzed using this new price scale for a total cost of $17,500. Therefore, for the 479 samples analyzed by Dr. Mirocha the total cost of analysis was $192,700. However, there were other analytical costs. First, tissue samples other than blood were analyzed. Second, blood and urine samples were analyzed by Dr. Hamilton's and other laboratories. Third, at least two quality control exercises in which mycotoxin-spiked samples were sent to various laboratories were conducted. And fourth, AFMIC had environmental samples (e.g. vegetation and grains) analyzed for mycotoxins. Altogether (the Mirocha blood and urine samples, the non-blood tissue and other laboratory-analyzed samples, the quality control samples, and the environmental samples), AFMIC paid $766,985 between FY81 and FY86

6. Until May 1985, the $800 per Mirocha sample analysis and other sample analysis costs were paid to Dr. A. Wallace Hayes (Health, Safety and Toxicology, Inc.). Hayes subcontracted the analysis of the samples to various laboratories. Most of the samples went to Dr. Mirocha's laboratory. In May 1985, AFMIC decided to stop using Dr. Hayes and contracted directly with Dr. Mirocha when the costs savings became obvious($50/sample versus $800/sample). AFMIC paid Dr. Hayes for Mirocha sample analyses and for analyses conducted by other laboratories $748,545 between FY81 and FY84.

PART 4. RESULTS OF ANALYSIS

Table 3 shows the number of victim and control samples sent to Dr. Mirocha for analysis that were positive for T-2 and/or HT-2. Table 3 also shows the numbers (in parentheses) of individuals represented by the samples. There is a difference since in some cases more than one sample from the same individual was analyzed. Table 4 shows the amounts of T-2 and HT-2 detected in the samples. A bracket in Table 4 is used to show those samples that came from the same individual.

Table 3. The Number of Positive Victim and Control Samples

Year Collected	Victim Samples Positive	(Individuals)	Control Samples Positive	(Individuals)
1981	3	(1)	0	
1982	29	(23)	4	(4)
1983	9	(9)	0	
1984	6	(6)	14	(13)
1985	1	(1)	7	(7)
Total:	48	(40)	25	(24)

Table 4. Amount of T-2 and HT-2 Detected in the Samples

Year Collected	Victim Samples		Control Samples	
1981	3 samples:			
	1.	9 ppb T-2	6 ppb HT-2	
	2.	10 ppb T-2	6 ppb HT-2	
	3.	9 ppb T-2	5 ppb HT-2	
1982	29 samples:			4 samples:
	1.	18 ppb T-2	22 ppb HT-2	1. 6 ppb T-2
	2.		18 ppb HT-2	2. 9 ppb T-2
	3.	11 ppb T-2	10 ppb HT-2	3. 59 ppb T-2
	4.	7 ppb T-2		4. 26 ppb T-2
	5.	3 ppb T-2		
	6.	3 ppb T-2	33 ppb HT-2	
	7.	5 ppb T-2	2 ppb HT-2	
	8.	7 ppb T-2		
	9.	4 ppb T-2	1 ppb HT-2	
	10.		8 ppb HT-2	
	11.	22 ppb T-2	7 ppb HT-2	
	12.	13 ppb T-2		
	13.	1 ppb T-2	32 ppb HT-2	
	14.	28 ppb T-2	16 ppb HT-2	
	15.	73 ppb T-2	22 ppb HT-2	
	16.	8 ppb T-2	97 ppb HT-2	
	17.		24 ppb HT-2	
	18.	16 ppb T-2	14 ppb HT-2	
	19.	48 ppb T-2	28 ppb HT-2	
	20.	19 ppb T-2		
	21.	3 ppb T-2	2 ppb HT-2	
	22.	14 ppb T-2	19 ppb HT-2	
	23.	110 ppb T-2	296 ppb HT-2	
	24.	41 ppb T-2		
	25.	101 ppb T-2	8 ppb HT-2	
	26.	33 ppb T-2	34 ppb HT-2	
	27.		65 ppb HT-2	
	28.		44 ppb HT-2	
	29.		13 ppb HT-2	

1983 9 samples:

1.		7 ppb HT-2
2.		10 ppb HT-2
3.		3 ppb HT-2
4.		13 ppb HT-2
5.		1 ppb HT-2
6.	3 ppb T-2	
7.		1 ppb HT-2
8.		7 ppb HT-2
9.	9 ppb T-2	32 ppb HT-2

1984 6 samples:

1.		5 ppb HT-2
2.	13 ppb T-2	55 ppb HT-2
3.	6 ppb T-2	3 ppb HT-2
4.	3 ppb T-2	
5.	6 ppb T-2	
6.		3 ppb HT-2

14 samples:

1.	57 ppb T-2	
2.	3 ppb T-2	2 ppb HT-2
3.		504 ppb HT-2
4.	6 ppb T-2	
5.		192 ppb HT-2
6.	29 ppb T-2	
7.	107 ppb T-2	
8.		13 ppb HT-2
9.		2 ppb HT-2
10.	10 ppb T-2	6 ppb HT-2
11.		5 ppb HT-2
12.		1 ppb HT-2
13.		5 ppb HT-2
14.		9 ppb HT-2

1985 1 sample:

1.	3 ppb HT-2

7 samples:

1.	119 ppb T-2	62 ppb HT-2
2.		12 ppb HT-2
3.		9 ppb HT-2
4.		10 ppb HT-2
5.		18 ppb HT-2
6.	10 ppb T-2	
7.	10 ppb T-2	

Details on results of analysis:

1. Only T-2 and HT-2 mycotoxins were consistently analyzed for in the samples, and therefore only results obtained for these mycotoxins are considered in this report. Other mycotoxins such as T-2 Tet, T-2,4OL, and NIV were analyzed for on a random basis and were detected.

2. As seen in Table 4, in 8 cases more than one sample from the same individual tested positive. However, for most of the positive results, other samples from the same individual tested negative. Moreover, for some of the positive samples aliquots were available. Not once when an aliquot tested positive did a second aliquot of the same sample also test positive. Neither of these findings seem logical, i.e. aliquots from the same sample do not both test positive. Regardless, all results from Dr. Mirocha are accepted as he has reported them to AFMIC. There is confidence in his results because of his scientific reputation and the results of the quality control exercises. Should AFMIC have a question about Dr. Mirocha's results, then his techniques and results should be judged not by AFMIC but submitted to the scientific community in a peer review process.

3. Six Hmong Control Project Villagers tested positive for mycotoxins.

4. The average T-2 amount in victim and control samples were 21 ppb and 35 ppb, respectively. The average HT-2 amount in victim and control samples were 25 ppb and 58 ppb, respectively.

PART 5. AUTHOR'S COMMENTS

Table 5 combines the data presented in Tables 1, 2, and 3. Table 5 shows that 25 control samples out of a total 257 tested (10%) tested positive for T-2 or HT-2 mycotoxin. This compares to 48 positives out of 222 (22%) for victim samples. The number of individuals represented by these samples is shown in Table 5 in parentheses. (These numbers are less since several individuals had more than one sample collected from them.) 40 victim individuals out of 142 (28%) and 24 control individuals out of 146 (16%), tested positive.

These findings mean that the positive results for victims shown in this report cannot be used as evidence that mycotoxins were used as chemical warfare agents in Southeast Asia. The detection of mycotoxins in victim samples shown in this report should not suggest a conclusion that the source of mycotoxins in victims is unique and different, e.g. the source is from exposure to a chemical warfare agent as the results might suggest if only victim samples were positive. Without further data, the source or sources of the mycotoxins for the victims and the controls cannot be differentiated, i.e. the source could be the same and if so not a chemical warfare agent. (We must assume that the controls have not been exposed to CW.) The results do not mean that mycotoxins were not used as chemical warfare agents, however they cannot be used as evidence that they were.

The question of where the mycotoxins detected are coming from cannot be answered by the program described in this report.

Table 5. Data From Tables 1, 2, and 3.

	# Victim Samples Collected	# Victim Samples Analyzed	# Individuals	# Victim Samples Positive	# Individuals Tested Positive
1981	28	15	12	3	1
1982	106	89	47	29	23
1983	139	58	45	9	9
1984	153	31	22	6	6
1985	33	29	16	1	1
Totals:	459	222	142	48	40

	# Control Samples Collected	# Control Samples Analyzed	# Individuals	# Control Samples Positive	# Individuals Tested Positive
1981	8	4	4	0	0
1982	28	23	14	4	4
1983	8	3	3	0	0
1984	417	90	54	14	13
1985	142	137	71	7	7
Totals:	603	257	146	25	24