CANADA

BACKGROUND STUDY TO THE HANDBOOK FOR THE INVESTIGATION OF ALLEGATIONS OF THE USE OF CHEMICAL OR BIOLOGICAL WEAPONS

SEPTEMBER 1987

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Many people have contributed to the work presented in this report, although not all have been involved in attempting to bring together under one cover the various elements which had already served the earlier purpose for which they were intended.

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1.0 INTRODUCTION

In December 1985, Canada submitted to the United Nations Secretary-General a Handbook for the Investigation of Allegations of the Use of Chemical or Biological Weapons. The Handbook drew upon Canadian experience acquired during the previous four years in relation to efforts to investigate certain disturbing refugee reports and, subsequently, allegations concerning the use of chemical/toxin weapons in Southeast Asia. Early Canadian efforts occurred in the context of broadening international concern as reflected, notably, in General Assembly Resolution 35/144C.

In fact, the Handbook was but one of a number of submissions made by Canada to the United Nations relating to allegations of the use of chemical/toxin weapons. Canadian investigations in Southeast Asia in 1981 and 1982 led to the submission of information that had been collected by Canadian officials and visiting scientists (who, however, did not have access to the actual countries and sites of the alleged use of the weapons).

In February 1984, a critical review panel of experts representing a variety of disciplines was convened in Ottawa to evaluate Canadian investigative experience for its possible relevance to efforts initiated by the United Nations and still underway at the time. The panel recommended that the methodologies and procedures, developed in part on an ad hoc basis under field conditions, be reviewed, refined and documented for the benefit of the United Nations and other

interested organizations. As a part of this overview, it suggested that a field trip to test and validate proposed procedures under operational conditions would be beneficial.

A "case study" scenario was developed and, in March-April 1984, a Canadian team (two epidemiologists and a laboratory technician) simulated the undertaking of a field investigation in Southeast Asia, using commercially available equipment and transportation. Medical doctors proceeded to Thailand in mid-March, and meetings took place there with Thai officials and medical officers over the next two weeks. Body-fluid samples were then collected over approximately a three-week period in April.

Development and validation of the analytical procedure took much longer than expected, due to problems involved with co-extractants and the need to develop new sample clean-up procedures. In view of the large number of samples, it was necessary to schedule instrument (GC/MS) time around other higher priority work of the laboratory. It had been predicted that the testing of specimens would proceed over the next 6-12 months. As it turned out, method development took approximately 8 months and analysis was performed during the following 2 months, with the laboratory effort being terminated after 10 months. All blood samples and certain key urine samples were tested. The scientist responsible for the analysis visited Thailand and met with Thai officials and

scientists in April 1985. The Handbook for the Investigation of Allegations of the Use of Chemical or Biological Weapons was written in the summer/fall of 1985 and submitted to the United Nations Secretary-General in December 1985.

The field trip phase of this study consisted essentially in the collection of body-fluid samples from citizens of Thailand and refugees from Laos and Kampuchea, and included some limited interviewing of the subjects and documentation of medical histories when available. The majority of samples was collected from people who claimed not to have been subjected to hostile use of chemical/toxin agents. Various remote areas and groups were chosen in keeping with the "case study" scenario. The samples were returned to Canada for analysis, and the investigators were especially attentive to matters related to the collection, labelling, documentation, packaging, storage and transportation of these fragile samples from a remote area over long distances to an analytical laboratory.

It should be recalled that, prior to the late 1970s and early 1980s, there was no previous historical experience involving an investigation of the possible use of trichothecene mycotoxins for hostile purposes. Toxins, in general, are toxic chemical substances produced in nature under specific conditions by biological systems, although some of these chemicals can also be synthesized in the laboratory. While some research had been conducted on

mycotoxins over the years, it was principally related to the contamination of food and feedstuff. There still remain many unknown factors concerned with the trichothecenes, both as to their natural occurrence and, more particularly in this case, as to their potential for use as weapons.

The very positive response to the Handbook has, in some instances, been accompanied by requests for further information on contributory material. This report was written while keeping in mind the nature of those requests. The report is organized to highlight the division of labour between field work and laboratory analysis, and the results from the analysis of the samples collected in Thailand in 1984 are presented in the text.

2.0 FIELD WORK

2.1 Terms of Reference and Preliminary Activities

2.1.1 Terms of Reference

The purpose of the overall study has been mentioned in the Introduction. The tasks set for the field team included:

- the collection of blood and urine samples from citizens of the host country living in areas remote from any allegations of the use of toxin/chemical weapons;
- the collection of blood and urine samples from refugees (in refugee camps) who made no claim to having been exposed to toxin/chemical weapons;
- the collection of blood and urine samples from refugees (in refugee camps) who claimed to have been exposed to toxin/chemical weapons;
- to obtain other collateral information from the subjects using a standard questionnaire (through interpreters), and any other medical information from authorities (e.g., in the event of a hospitalized patient);
- should the occasion arise, to investigate any current allegations of the use of toxin/chemical weapons; and
- to record experiences with a view to the development of a "standard operating procedure" for use in the investigation of allegations of the use of chemical or biological weapons.

2.1.2 Preliminary Activities

Approximately one month prior to the trip, it was possible to give some thought to each of the activities identified in this and the following chapter, although at varying levels of detail. Of particular concern were matters related to equipment, sample handling, routing for the return trip, preparation of fortified samples, development of the analytical method, sample clean-up, optimization of Mass Spectrometer (MS) parameters, and determination of the sensitivity of the procedure.

Since refrigeration of the samples was especially important, various coolers and ice packs were tested, not only to choose suitable equipment but also to determine the margin of safety that would apply in the event of travel delays. Dry ice was not considered suitable for chilling samples for extended periods, being more appropriate for freezing materials for short periods. Furthermore, there was some uncertainty as to various airline regulations on the subject (i.e., concerning escaping carbon dioxide gas in the luggage/freight compartment and elsewhere). Simplicity of equipment was also considered a most desirable feature.

Sample questionnaires (one for people claiming to have been exposed to toxin/chemical weapons, another for those not making such a claim) were prepared for reproduction in the host country.

Liaison with host officials and with commercial air carriers was particularly important, and provision was made for host country personnel and interpreters to accompany the team into the field. The liaison function was handled by the Canadian embassy in the host country, and certain other requirements (e.g., provision of a secure "base" facility -- in this case, in the embassy; and road transportation) were identified well in advance. A draft itinerary was prepared, bearing in mind the need for flexibility.

Details relating to the routing and refrigeration requirements for the return trip received considerable attention. Due attention was also paid to the issue of sample security and integrity of packaging.

2.2 The Team

The team from Canada consisted of two medical doctors from the Field Epidemiology Division, Laboratory Centre for Disease Control, Health and Welfare Canada; and a laboratory technician from the Toxicology Research Centre of the University of Saskatchewan.

In the field, these were accompanied on most occasions by an officer and medical technician (who also acted as an interpreter) from the Armed Forces Research Institute of Medical Sciences (AFRIMS) of the host country; and, as necessary, by Canadian embassy personnel.

The Canadian medical doctors and host medical staff were responsible for venepuncture, as well as for interviewing each person donating blood and urine specimens. The Canadian laboratory technician was responsible for sample safety, sample security/integrity, and for the transport of samples to the analytical laboratory in Canada.

2.3 Equipment

Equipment taken by the team included:

2.3.1 Sample Collection - Blood

- 500 Monoject Sterile Blood Collection Tubes (in 5 boxes), approximate draw 10 ml; no interior coating; stopper lubrication, silicone; dry additive, Sodium Heparin (U.S.P. Grade; source, hog intestinal mucosa, 143 U.S.P. units; Monoject, Sherwood Medical, St. Louis, MO, USA). The silicone lubrication of the stopper was intended to impede contact of any toxin/chemical in the sample with the often-used Tris-butoxyethylphosphate in the rubber stoppers which could produce unwelcome artifacts in the subsequent analysis.

- 1,000 Venoject Blood Collection Needles (in 10 boxes), sterile, 20 G X 1½" (Terumo Corporation, Tokyo, Japan).

2.3.2 Sample Collection - Urine

- 360 "Scintillation Vials", 18 ml, polypropylene (Beckman Instruments Inc., Toronto, Canada). These vials were chosen since GC-MS analysis showed that they were essentially inert.

  To each vial was added 250 ul. of Thymol in methyl alcohol (80 g/100ml) as a preservative.

2.3.3 Refrigeration

- 2 lightweight but rigid plastic coolers, each of 2.7 cubic feet (45 litre) capacity (outside dimensions approximately 14" X 24" X 14") were used (Coleman Model 5286L Cooler, Canadian Coleman Company Ltd., Toronto, Canada). These were of sufficient yet manageable size. The lid's flush-mounted latch system was considered unlikely to be broken during handling. The cooler could not be locked, and other security measures were devised using a number of luggage staps. Tape and labels would not adhere to the outer surface, and so indelible markers were used to label the containers as necessary.

- 16 polyethylene "Freeze Paks" (Reliance Products Ltd., Winnipeg, Canada) -- 5 lb. capacity, of the type that you fill with water, screw on cap and freeze -- were chosen over other possibilities after testing. In addition, the empty packs were found to be lightweight and collapsible, facilitating transport to the sampling area (as well as dropping off refills at points to be picked up on the return trip). Furthermore, when empty they made good packing material around fragile items.

- Note that during preliminary testing, it was determined that seven such ice packs in a cooler (standing at room temperature of 22°C) provided more than adequate chilling capability for a period of 72 hours plus (even though the cooler was opened for short periods after 24, 48 and 56 hours).

2.3.4 Miscellaneous Equipment

Additional items such as extra luggage straps, markers, labels, special note pads and log books, latex gloves and plastic sample bags were packed in the two coolers for the outward trip.

2.3.5 Clearances - Outward Bound

No special clearances were needed for the equipment and supplies, particularly since no toxic material (for example, as standards for base camp analysis for use in the calibration of instruments) nor electronic equipment was included.

Efforts were made beforehand to discuss transportation issues, including the matter of excess baggage costs, with airline representatives. This produced a false sense of security since, when the time came to travel, it was found that virtually everthing had to be discussed anew. Airline personnel as well as customs officials properly took an interest in the contents of the two coolers packed with a variety of equipment. A simple letter/manifest of contents helped to satisfy most inquiries.

2.4 Regional Activities

2.4.1 Chronology

Table 1 outlines the general flow of activity from the time of arrival in the host country until departure.

Table 1

Chronology in the Region

Date	Event
17 March 1984:	Arrival in Bangkok of two Canadian medical doctors.
18-28 March:	Preparations, briefings. Permission requested to travel to the areas of interest.
28 March:	Arrival of the Canadian technician with sampling and refrigeration equipment.
29-30 March:	Samples collected in Bangkok.
1-7 April:	1st excursion to collect samples near the Thailand-Burma border; then to the north-eastern Thailand-Laos border region; and return to Bangkok.
9-11 April:	2nd excursion, to collect samples near the Thailand-Kampuchea border; and return to Bangkok.
12-13 April:	Specimens prepared for shipment.
15 April:	1st and larger lot of samples dispatched to Canada accompanied by laboratory technician.
16-18 April:	3rd excursion, to collect samples near the north-western Thailand-Laos border; and return to Bangkok.
21 April:	One Canadian medical doctor departs for Canada.
23-25 April:	4th excursion, to collect samples near the Thailand-Kampuchea border (although no samples collected); and return to Bangkok.
27 April:	Final team member departs for Canada (with samples collected on the 3rd excursion).

2.4.2 Briefings and Clearances

Since this field trip was not directly related to any specific allegation of the use of toxin/chemical weapons, it was necessary to explain in greater detail the nature and purpose of the investigation to officials of the host country. Briefings were provided by the Canadian team to senior officials of the office of the Minister of the Interior, to senior officers of the office of the Supreme Commander, and to senior officers of the Armed Forces Research Institute of Medical Sciences (AFRIMS).

As a result of these briefings, a "study protocol" was developed by the Canadian team in conjunction with the AFRIMS staff and submitted for approval to the offices of the Minister of the Interior and the Supreme Commander. The protocol outlined methods to be used, the study groups and locations, and other procedural matters.

Clearances had to be obtained from military authorities to travel to certain border regions, particularly if there was some danger of military activity, and to gain access to refugee camps. On more than one occasion, an accompanying Thai senior officer succeeded in allaying the concerns of local authorities which might otherwise have prevented access in spite of possession of the appropriate clearances.

2.4.3 Base Camp

The Canadian embassy in Bangkok provided a secure facility for equipment and samples, and the four separate excursions radiated from Bangkok.

Although certain miscellaneous supplies were available in Bangkok as necessary, the team was pleased, in particular, that it had brought the necessary Monoject blood collection tubes and Venoject needles as well as suitably prepared urine collection vials.

As necessary, the embassy provided a station wagon, van and drivers to the team. On occasions when the team travelled internally by commercial aircraft, it was met at the interim destination by an embassy vehicle.

It was not considered necessary for the team to possess and operate its own communications equipment. Other means of communication were available to the team as necessary.

2.5 Actual Sample Collecting Activities

2.5.1 General Methodology

Venous blood specimens were collected in sterile 10 ml heparinized vacuum tubes (Monoject). Urine samples were collected in sterile 18 ml vials containing Thymol as a

preservative. Each blood specimen (or blood/urine pair, if appropriate) was coded with a unique random 3-digit number. Within a short period of time -- usually 10-30 minutes, but never more than two hours -- specimens were placed in a cooler and chilled (not frozen) using ice packs. Upon return to the "base camp" (i.e., the Canadian embassy in Bangkok), the specimens were locked in a refrigerator in a secure facility.

2.5.2 Site Selection and Sample Collection

The original plan envisaged the collection of blood and urine samples from a variety of sources:

- ill (hospitalized) people in Bangkok;
- healthy Thai civilians or soldiers residing near the Thailand-Burma border and, thus, far removed from any alleged "yellow rain" incidents;
- healthy Thai civilians or soldiers residing near the northern Thailand-Laos border;
- healthy Thai civilians or soldiers residing near the Thailand-Kampuchea border;
- residents of refugee camps in Thailand close to the Thailand-Laos border, but not claiming to have been exposed to "yellow rain";
- residents of refugee camps in Thailand close to the Thailand-Kampuchea border, but not claiming to have been exposed to "yellow rain"; and
- persons claiming to have been subjected to "yellow rain" attacks in the previous six months.

Thus, seven distinct groups were to be sampled, with males and females represented in each group if possible, and with fifty blood and fifty urine samples to be collected in each group.

The actual sample collection came reasonably close to the original plan, although the number of samples collected had to be adjusted to suit the circumstances. Sample collection may be summarized as follows:

Group I: Ill Civilians in Bangkok - Ramathibodi Hospital. Since this is a teaching hospital, permission was requested from and granted by the Dean of Medicine, Mahidol University, to collect samples from patients. Many such patients are referred to the teaching hospital from outside Bangkok. Samples (50) were collected over the two-day period 29-30 March.

Group II: Western Border Residents.
The sampling site was Mae Sot, a town located 6 km east of the Thailand-Burma border and about 400 km northwest of Bangkok. Samples were collected at the local hospital from ambulatory patients with a variety of health complaints. Samples (51) were collected over the two-day period 2-3 April.

Group III: Thai Military near the North-eastern Thai-Laos Border.

Loei training camp (Army) is located approximately 50 km south of the Thailand-Laos border. Samples (50) were obtained from male recruits on 5 April.

Group IV: Hmong Refugees recently arrived from Laos. Although Ban Vinai had been considered to be a convenient sampling site (37,000 Hmong residents), it was learned on arrival that the camp had been officially closed to new refugees for some time. New refugees were held for up to three months at border police stations along the Mekong River and then transferred to intermediate camps. On 6-7 April, the team collected blood samples (number obtained in parentheses) from Hmong refugees at border police stations: Sangkhum (3) and Sri Chiangmai (1). On 16-17 April, samples were obtained in the intermediate refugee camp Chiang Kham (6).

Group V: Thai Military near the Thailand-Kampuchea Border.

Taphraya military camp provided the soldiers from whom samples (50) were collected on 9 April.

Group VI: Refugees recently arrived from Kampuchea. Samples were collected from refugees at Khao-I-Dang (25), and from refugees at Nong Samet camp (25), over the two-day period 9-10 April. One of the fifty refugees claimed to have been exposed to "yellow rain" three years earlier.

Group VII: Persons claiming exposure to "yellow rain" within past six months.

On 6-7 April, samples were collected on the Thai-Laos border at Sangkhum (1), Sri Chiangmai (2) and at Nong Khai (1). On 16-17 April, samples were collected in Chiang Kham (5). (Note: A number of persons alleged exposure two or three years earlier but these are included in other groups.)

Table 2

Blood Samples Collected by Group and Site

	Group	Site	Subjects
I:	Civilian Thais	Bangkok hospital	50
II:	Civilian Western Thais	Mae Sot clinic	51
III:	Thai Military Thailand-Laos Border	Loei	50
IV:	Hmong (no recent exposure claimed to "yellow rain")	Sangkhum Sri Chiangmai Chiang Kham	3 1 6
V:	Thai Military Thailand-Kampuchea border	Taphraya	50
VI:	Khmer (no recent exposure claimed to "yellow rain")	Khao-I-Dang Nong Samet	25 25
VII.	Hmong claiming exposure to "yellow rain" within past six months	Sangkhum Sri Chiangmai Nong Khai Chiang Kham	1 2 1 5
	Total		270

2.6 Sample Handling

2.6.1 Packaging

The body-fluid samples had been collected in 10 ml tubes (blood) and 18 ml vials (urine), and these were placed in plastic bags when placed in the cooler (partially filled with locally-obtained loose ice); and, later, these plastic bags were put in cardboard containers for storage in the refrigerator. However, condensation caused some smearing of labels and the cardboard container to weaken and deteriorate somewhat. To counter this problem, the tubes/vials were transferred to dry racks. For the trip to Canada, the tubes/vials in the dry racks were simply placed in each cooler, covered with plastic bags, which were covered with the pre-frozen ice packs, and then the latch-cover of each cooler was secured in place with two luggage straps each. Materials were placed in the cooler in such a way as to minimize movement.

Each of the two loaded coolers weighed approximately 52 kg for the return trip. Unnecessary equipment (extra tubes, vials, needles etc.,) was simply left behind.

2.6.2 Labelling/Recording

Blood tubes and urine vials were numbered according to a three-digit random procedure. History/number sheets were retained by the Canadian medical doctors. (Team members also kept diaries of their daily activities.)

No problems were experienced with labelled tubes (blood); but, at the base camp, condensation was found to have affected the labelling on a number of the vials (urine). It was necessary to cross-check these urine samples and to re-label them. All but one could be positively identified.

For the return trip to Canada with the samples, a copy of the sample number sheets was carried by the escort. Similarly, a list of unused numbers was given to the escort who would, in turn, add appropriately-labelled spiked and blank samples to the coolers prior to delivery to the analytical laboratory.

2.6.3 Temporary Storage and Security

Samples remained in the custody of a team member in the field and during transportation to the Canadian embassy. Once returned to the "base camp", the samples were locked in a specifically-designated refrigerator in a secure area of the embassy.

2.6.4 Fortification of Samples

No fortification (or "spiking") in the form of the addition of fortified or blank samples, took place either in the field or in the base camp. This was done in Canada (see section 2.7.4).

2.7 Sample Transportation to the Laboratory

2.7.1 Two Batches of Samples and Chronology

Two batches of samples were dispatched to the analytical laboratory. The first and much larger batch departed the host country on 15 April. Fortified and blank samples were added in Canada on 16 April, and the lot was delivered to the analytical laboratory on 17 April. All samples were refrigerated during the stopover at the home base, after which they were re-packed in the coolers for onward transportation under escort to the analytical laboratory. The coolers were unpacked on 18 April in the presence of the escort and laboratory analyst, and all samples were found to be in order and undamaged. In this batch there were 269 blood samples (259 field samples plus 10 controls) and 267 urine samples (256 field samples plus 11 controls).

The second batch consisted of the 11 blood samples collected 16-17 April. They were handled in the host country in the same manner as indicated in section 2.6. The second

batch of samples was kept chilled throughout the return trip, including during a short stopover. This batch departed the host country on 27 April and was delivered to the laboratory on 2 May, at which time the escort and laboratory analyst confirmed that the samples had arrived undamaged. No control samples were added to this second batch.

The following table provides details concerning the two deliveries of samples to the analytical laboratory.

Table 3

Samples Delivered To The Laboratory

	Blood		Urine	
	Field	Control	Field	Control
1st batch	259	10 (8 fortified) (2 blanks)	256	11 (10 fortified (1 blank)
2nd batch	11	Ø	Ø	Ø
Total	270	10	256	11

2.7.2 Sample Clearances

Prior to departure of the team from Canada, arrangements were made to obtain the necessary permit from Agriculture Canada to allow the blood/urine samples to enter Canada. While in Bangkok, confirmation was received that an import permit had been issued. The team was cautioned that soil or plant material could not be guaranteed entry.

Return travel arrangements with the samples and equipment were more difficult to arrange with the airline than was the case on the outward bound trip. With security and the chain of custody being foremost on the team's mind, it was hoped the airline would allow the coolers inside the passenger compartment of the aircraft and, thus, remain with the escort throughout. For its part, the airline wanted the coolers sent as air freight with "special" handling instructions, at considerable cost. This was clearly not satisfactory. Through the fortuitous and very welcome intervention of an airline employee, special ground handling arrangements were made and the coolers were placed in the aircraft's diplomatic locker under lock and key. The airline made similar arrangements for handling at the transfer point, which was confirmed by the escort. No excess baggage charge was levied.

2.7.3 Safety and Security

The special handling arrangements for the coolers did not facilitate verification of the status of the cooler ice packs at the international transit point. However, the lapsed travel time was but a small fraction of the safety margin determined during pre-trip testing, and so this change in procedure was not a cause for concern. Upon entry into Canada, the ice packs were verified by the escort and, after

a lapsed time of approximately twenty-four hours, were still found to be "rock hard". As a result, no change of ice packs was required for the next phase of the trip. (Empty ice packs had been left on the outward bound trip at what would be the re-entry point, and the escort was met on the inward bound trip with substitute frozen ice packs which, as mentioned, were found not to be required.)

The security arrangements for the containers and the samples have already been described and need not be repeated. Every effort was made to have samples accompanied at all times by an escort or locked in a secure facility. One obvious deficiency is that the coolers themselves could not be locked, although extra luggage straps provided an additional measure of security.

2.7.4 Addition of Fortified Samples

In order to provide a reference point concerning the analytical methodology, a number of fortified and blank samples were included with the field samples. Prior to this, a number of decisions had to be made concerning:

- a "reasonable" level of fortification;

- when, before or after control sample collection, the toxins should be added to the blood/urine tube or vial;

- storage of the fortified and blank samples in conditions approximating those in the field;

- timing, to coincide approximately with collection of samples in the field; and

- the procedure for adding the fortified and blank samples to those collected in the field.

Based on a survey of the literature, it was concluded that 50 or 100 ppb of either T-2 toxin or HT-2 toxin in blood, and 25 or 50 ppb of either T-2 toxin or HT-2 in urine would be appropriate. The fortified samples (and level of fortification) are identified in sections 3.4.1 and 3.4.2.

The toxins were added to the blood collection tubes immediately after the blood samples were obtained. For the urine samples, thymol (as preservative) and the mycotoxins were introduced first into the vials, then filled with urine.

Fortification was carried out from 10 ug/ml stock solutions of T-2 toxin or HT-2 toxin in 0.2M Na_2PO_4 at PH 7.2. The blood and urine were obtained from a healthy Canadian citizen fifty-five years of age.

To ensure mixing of the fortified blood samples, the tubes were shaken thoroughly for 2-3 minutes. They were then kept for 2 hours in an upright position at room temperature (approximately 22°C), since it was assumed that the samples collected in the field would likely be kept at a non-refrigerated temperature for approximately 1-2 hours. The fortified samples were then transferred to a refrigerator until collected by the escort en route to the analytical laboratory. A similar procedure was followed for the fortified urine samples.

The sampling and fortification of control samples took place on 12 April 1984. Most sampling in the field took place within a short time prior to this date (i.e., 29-30 March, 2-3 April, 5 April, 6-7 April, 9-10 April); although some field sampling also took place after this date (i.e., 16-17 April).

It was decided that the fortified samples would be held at the home base and picked up by the escort en route to the laboratory. The other alternative of taking small quantities of toxins abroad to be added to control samples at the base camp was rejected, largely due to the legal/administrative problems associated with transporting toxic materials even in small quantities. Furthermore, it was considered desirable to collect blood and urine samples for control purposes from a subject removed from the region and who would be readily-available for any follow-on action if desired.

When the fortified and blank samples were retrieved by the escort, existing identification numbers were removed and re-numbered according to the 3-digit random numbering sequence. A key for the number change was prepared and left at the home base laboratory. The control samples (indistinguishable outwardly from those collected in the field) were distributed randomly throughout the racks of samples collected in the field. This represented a double blind situation for the analyst.

2.8 Interviews and Field Observations

In the host country, a revised standard questionnaire was prepared and used to collect demographic data on people donating samples, as well as to record details of alleged attacks. The English and Thai versions of the questionnaire appear in Appendix 1. This shortened form for recording responses does not reflect the less formal conversational manner in which the questions were asked. Interpreters sometimes accompanied the team on an excursion, and on other occasions were recommended by local authorities.

While efforts were made to avoid asking leading questions, it was soon realized that more work needed to be done to develop certain basic interviewing techniques and better questionnaires. In particular, problems were encountered in dealing with such notions as time, location, distance and colour, to name but a few.

A brief medical case history was compiled on each subject who was hospitalized or obviously ill. Healthy subjects were simply asked the general questions as indicated on the questionnaire.

In the case of the nine Hmong refugees claiming to have been attacked with "yellow rain", further details were sought, not only as regards the reported sickness and/or death of humans, but also as to any other visible effects, such as on plants and animals. Among the symptoms reported were nausea, vomiting, diarrhea, bloody diarrhea, headache and weakness. Epidemic diseases might normally explain illnesses affecting many villages over a short period, but they would not explain the accompanying death of crops or livestock.

It should be noted that many of the people sampled, including those not claiming to have been exposed to "yellow rain", had heard stories about the use of such substances. This was particularly true of the Khmer refugees sampled, only one of whom claimed "yellow rain" exposure. In view of frequent Khmer Rouge reporting of Vietnamese use of toxin/chemical weapons, the Khmer refugees were generally aware of such allegations.

3.0 LABORATORY ANALYSIS

3.1 Terms of Reference

The laboratory study included the following tasks:

- to develop an analytical procedure for the analysis of the trichothecenes T-2, HT-2 and DAS in blood and urine matrices;

- to validate the analytical procedure using fortified samples of blood and urine from Canadian sources; and

- to analyse blood and urine samples, which numbered 280 and 267 respectively.

3.2 Sample Handling

3.2.1 Receipt/Documentation

The containers transporting the samples were opened in the presence of the escort and the laboratory analyst to confirm the condition of the samples. All samples arrived in an undamaged condition. Copies of the sample random number sheets were left with the analyst, and reports were to correspond to that random number system.

3.2.2 Sample Storage

Upon receipt, the blood samples were refrigerated at 0°C until the plasma and red cells were separated (within two days); and the urine samples were refrigerated at -20°C.

Blood samples were received as heparinized whole blood in 10 ml tubes. The total volume of each sample was estimated by comparison with a calibrated tube, after which the tubes were centrifuged (5 minutes at 5,000 rpm) and the plasma removed by pipette. The plasma was centrifuged a second time and decanted into a new tube, care being taken to exclude all cellular material. The volume of plasma was estimated as before, and the presence noted of any undue hemolysis, icterus or elevated amounts of lipids. These observations were recorded and are presented in Appendix 2.

Both the plasma and the red blood cells were then stored at -20°C until analysed.

3.3 Analytical Procedure

3.3.1 Supplies and Equipment

All glassware used was new and rinsed with methanol prior to use. All solvents (Caledon Laboratories, Georgetown, Ontario) were distilled in glass. All glass

clean-up columns were pre-washed in acetone and then methanol and used for one sample only, after which they were discarded.

The Gas Chromatograph and Mass Spectrometer conditions were:

GC/MS: FINNIGAN MAT Model 4500, with INCOS Data system.

Column: DB-5 capillary column, 0.32 mm x 20 m, film thickness 0.25 u.

GC conditions: Initial column temperature: 140°C, hold for 0.1 minutes.
Programmed at 20°C/minute, 140°C-260°C, hold for 3 minutes.
Programmed at 5°C/minute, 260°C-285°C, then cycled for the next injection.
Carrier gas: Helium UHP.
Inlet Pressure: 8 psi.

MS conditions: Ion source temperature: 120°C.
Separator and transfer line: 280°C.
Townsend Discharge ionisation current: 45 u Amp.

Electron multiplier: -1100V.

Pre-amp sensitivity: 10^{-8} amps/volt.

CI gas: oxygen, UHP. Introduced through make-up valve to produce pressure in the source of 0.43 Torr.

3.3.2 Methodology

Analysis was performed using a procedure involving solid phase extraction followed by two clean-up steps for the plasma and urine samples (i.e., including controls as discussed in section 2.7.4). Analysis of the extracts was achieved by Gas Chromatography/Mass Spectrometry (GC/MS) in the CI mode. Negative ions produced in the presence of oxygen gas were monitored using Multiple Ion Detection (MID) for maximum sensitivity. The minimum detectable amount for both T-2 and HT-2 toxins was determined in preparatory work as approximately 20 pg, while sensitivity of the method was approximately 1 ppb for blood samples. In the case of urine samples, the sensitivity of the method was approximately 4 ppb for both T-2 and HT-2.

3.3.2.1 Sample Extraction

ClinElut disposable extraction columns (Fisher Scientific Ltd., Ottawa, Ontario) were used to perform solid phase extraction, which was simple, quick and more efficient than the traditional liquid-liquid methods. These

columns are pre-packed with an inert hydrophilic matrix having a large surface area, on to which the aqueous phase is first adsorbed. Subsequent additions of an appropriate organic solvent removed the extractable material from the adsorbed aqueous phase.

Each 1 ml ClinElut was first washed by allowing 8 ml ethyl acetate (EtAC) in the case of blood samples or EtAc/CH_2Cl_2 (20:80 v/v) in the case of urine samples, followed by 8 ml acetone, to drain through. In the case of urine, the less polar solvent was as efficient in extracting the toxins, but less likely to co-extract other contaminants. The columns were then allowed to dry overnight at room temperature. This removed extractable contaminants, which were found to vary according to the particular batch of ClinEluts.

1 ml of thawed, mixed plasma was pipetted on to a washed, dry 1 ml ClinElut. The same procedure was also followed for urine. After a few minutes, the ClinElut was extracted with 8 x 1 ml aliquots of EtAc, allowing a few minutes between the addition of each aliquot of EtAc. The eluate was collected in a 15 ml tube and was taken to dryness using a rotary evaporator.

3.3.2.2 Clean-Up of Extract

The first clean-up step was performed on a silica gel column. Approximately 400 mg of silica gel (Kieselgel 60, 70-230 mesh ASTM; Merck, Darmstadt, FRG) was loaded into a Pasteur pipette plugged with glass wool, which had been pre-washed with methanol. 3 ml of toluene were pipetted on to the silica gel and allowed to drain until the solvent level reached the surface of the gel.

The residue from the sample extraction step (section 3.3.2.1) was taken up in approximately 0.2 ml CH_2Cl_2 and loaded on to the silica gel column. The sample tube was rinsed with 0.2 ml CH_2Cl_2 and this was also added to the column. When the solvent level had reached that of the gel, 0.5 ml of toluene-acetone (95:5, v/v) was pipetted on to the gel, followed by a further 3 ml. The collection tube was changed and 4 ml of dichloromethane-methanol (95:5, v/v) was added. The column was allowed to drain completely and this second fraction was taken to dryness under a stream of nitrogen at 40°C.

The second clean-up step used a charcoal-alumina column. Equal weights of charcoal (non-activated, Darco G-60, J.T. Baker Chem. Co., Phillipsburg, New Jersey, USA) and

aluminium oxide 90 (70-230 m; Merck, Darmstadt, FRG) were mixed and columns of approximately 100 mg of the mixture were prepared in Pasteur pipettes. The columns were pre-washed by passing 0.5 ml CH_2Cl_2, followed by 0.5 ml MeOH. To speed up the process, a slight positive pressure was applied.

Each residue from the preceding clean-up step on the silica gel column was taken up in approximately 0.5 ml CH_2Cl_2 and passed through the column, which was then rinsed with 0.5 ml MeOH. The combined filtrates were stored at -5°C until required for analysis, at which time they were reduced to dryness under a stream of nitrogen at 40°C.

A second clean-up step was introduced for blood samples received from the field since, during the initial phase when the samples were centrifuged prior to storage (see section 3.2.2), conditions of hemolysis, icterus and elevated amounts of lipids were found to exist in some cases (see Appendix Z). For the execution of the analysis, it was necessary that these contaminants, as well as any other constituents present in abnormally large amounts, be removed as their presence were found to result in a significant loss of sensitivity and resolution when injected on the GC column. Although this second clean-up step undoubtedly reduced the recovery levels of the mycotoxins, it greatly alleviated the problem of GC

contamination. The second clean-up step was not found necessary in the case of the Canadian blood samples used initially to develop the analytical procedure.

3.3.2.3 GC/MS Analysis

The cleaned-up extracts were chromatographed (without being derivatized) on a DB-5 capillary column, the eluted analyte being ionized in an oxygen atmosphere using a Townsend Discharge Ionizer. Minor fragmentation of the ions occurred, with both HT-2 and T-2 producing one high intensity ion (M + 32) at 456 and 498 m/z respectively, and lower intensity ions (M) at 424 and 466 m/z respectively. A Multiple Ion Detection (MID) profile of Monoacetoxyscirpenol (MAS), Diacetoxyscirpenol (DAS), T-2 toxin and HT-2 toxin was devised to monitor these four characteristic ions. Instrumental conditions for the gas chromatograph and mass spectrometer have already been given in section 3.3.1.

Each residue after clean-up was taken up in 50 ul EtAc and 1 ul was taken into a gas chromatographic syringe which had already been loaded with 1 ul EtAc followed by a small plug of air. The sample was injected directly on- column using an on-column injector system (J & W Scientific Inc., Brockville, Ontario) and the column temperature was programmed. When data acquisition was completed, the GC column temperature was increased to remove contaminants, and 5 ul hexane was injected to aid this process. GC column

contamination was further reduced by replacing the first 2 metres of the column daily, or more often if required. To facilitate this operation, a zero dead volume fused silica union (J & W Scientific, Brockville, Ontario) was fitted to the front end of the column, to which a fresh 2 metre length of identical column could be joined to replace the contaminated piece, which was discarded.

Contamination of the mass spectrometer elements (lenses, ion volume, source and rods) was minimized by the use of oxygen as the CI reagent gas. These elements were cleaned only when deterioration of the peak shape or sensitivity indicated it to be essential.

Performance was checked using an external standard: a 200 pg/ul standard solution of HT-2 and T-2 toxins was run after every two or three samples. No carry-over of toxins from the standards to the next sample was found. Nevertheless, any sample giving a positive response was re-injected, with a blank run immediately preceding it.

3.3.3 Recovery of HT-2 and T-2 in Whole Blood

Whole blood from the Ottawa blood bank was used for this study. The plasma was extracted and analysed by the procedure described, which gave a fairly constant background intensity when monitored for 4 characteristic ions for the trichothecenes DAS, T-2 and HT-2 using a Multiple Ion

Detection (MID) profile, with no discernible increase in intensity at the appropriate retention times. Recovery studies were then carried out, in which known amounts of HT-2 and T-2 were added to measured volumes of plasma and whole blood. Gentle mixing (5 minutes at room temperature) was followed by extraction, clean-up and analysis by GC/MS/MID. Data manipulation removed the background intensity and gave an area count for each ion at the appropriate retention time. Quantitation was achieved by comparison of this value with the area produced by standard solutions treated in an identical manner.

It was found that at high concentrations (i.e., greater than 1 ppm), the recovery of both HT-2 and T-2 was 85% or greater. The recovery was less at lower concentrations, and was almost identical whether plasma or whole blood was fortified.

Table 4

Recovery Study - Canadian Blood Samples

Concentration	Toxin	Recovery %
1 ppm	HT-2	85
1 ppm	T-2	85
20 ppb	HT-2	50
20 ppb	T-2	75
10 ppb	HT-2	25
10 ppb	T-2	50

3.3.4 Metabolization of HT-2 and T-2 in Whole Blood and Plasma

The possible metabolism of HT-2 and T-2 was also studied using blood from a Canadian source. Whole human blood and plasma samples were fortified with T-2 and HT-2 toxins at a level of 100 ppb each, then the samples were incubated at 37°C (whole blood and plasma). Other similarly fortified whole blood samples were kept chilled at 5°C for 17 days.

A time study (Figure 1) shows that at 37°C in whole blood, HT-2 toxin was essentially ccmpletely metabolized after 2 hours; whereas, only 46% of the T-2 toxin had been metabolized during the same time period. The level of T-2 toxin had decreased to 10% after 7 days. In contrast, metabolism of the two mycotoxins at 37°C was less in plasma. In the case of HT-2 toxin, 50% remained after 2 hours, and slightly less after 6 hours.

When stored at 5°C, T-2 was not degraded to any extent in whole blood after 17 days, whereas HT-2 toxin had again undergone considerable degradation during that time and only 8% was recovered.

This experiment proved the importance of storing the blood samples at low temperature as soon as possible after they had been collected. It also pointed to the desirability of separation of the plasma from whole blood in addition to storing at low temperature.

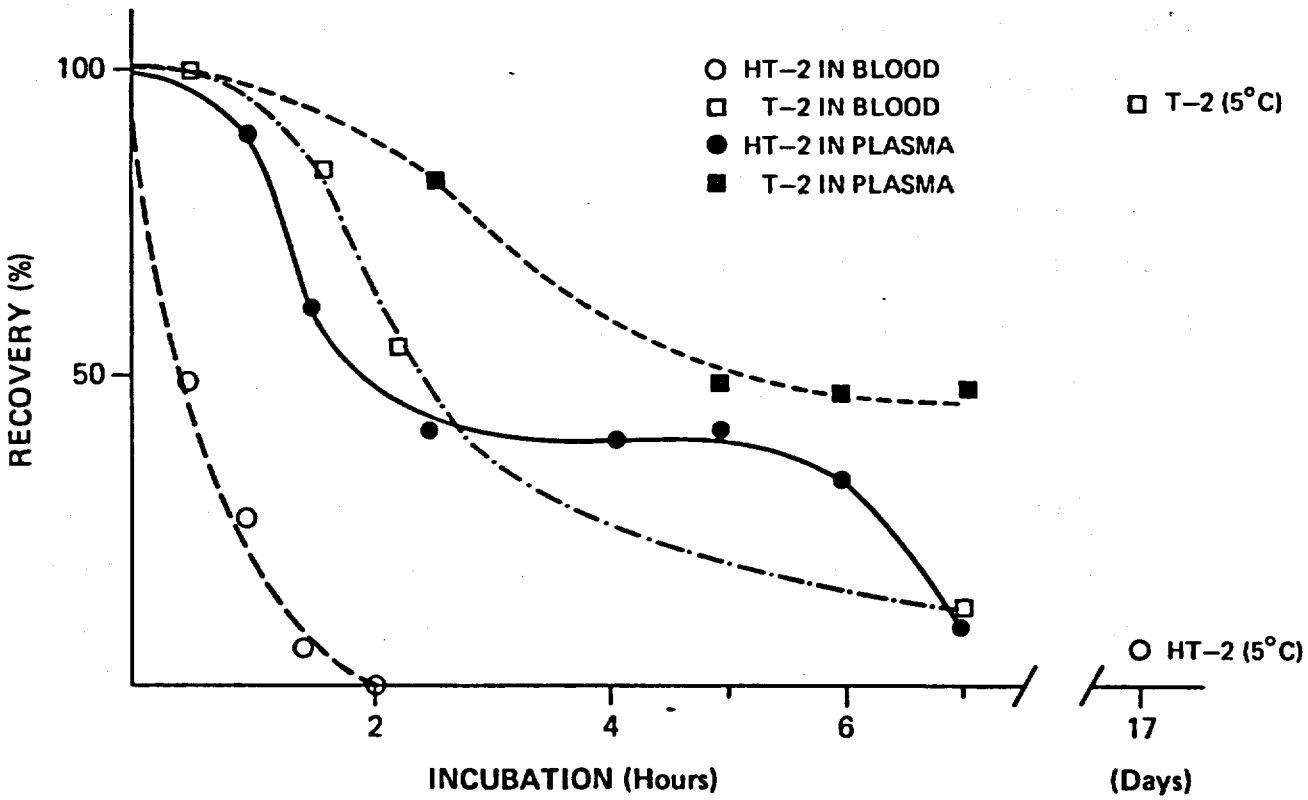

Figure 1. Recovery of HT-2 and T-2 from fortified (100 ppb) whole blood and plasma incubated with mixing at 37°C or stored at 5°C.

3.4 Results

A total of 280 human blood samples, including 8 fortified and 2 blank samples, were received by the analytical laboratory. 267 urine samples were also received, of which 10 were fortified and 1 was blank. Results relating to the control samples and to positive field samples are presented in this section.

It took approximately eight months to develop a method for analysis of HT-2 and T-2 in blood, sensitive to the desired level, which was a considerably longer time period than had been anticipated. This was due to the need for a second clean-up for the field samples. All of the 280 blood samples were then analysed over a period of approximately one month.

By that time, it was considered that the additional work and expense that would be involved in the analysis of all the urine samples were not warranted. Six months later, however, 16 urine samples were analysed corresponding to the 11 urine control samples and the 5 urine samples matching the 5 positive blood samples from Thailand. This was done in an attempt to obtain confirmation of the presence of the trichothecenes found in the blood. All urine samples gave a negative result.

3.4.1 Control Blood Samples

Seven of the eight fortified blood samples (of the total of 280 blood samples tested) were identified as being positive (Table 5). The eighth sample had been fortified with HT-2 toxin (which had a poor recovery rate as discussed in section 3.3.4). There were two blank control blood samples included in the total number of samples, and analysis found them to be negative, i.e., there was no false positive. The use of random numbers to document the samples ensured a double blind check.

Table 5

Control Blood Samples - Recovery

Random Number	Level of Fortification	Found
560	blank	negative
569	blank	negative
893	50 ppb T-2	61 ppb T-2
611	50 ppb T-2	54 ppb T-2
657	100 ppb T-2	75 ppb T-2
347	100 ppb T-2	69 ppb T-2
750	50 ppb HT-2	6.3 ppb HT-2
354	50 ppb HT-2	14 ppb HT-2
244	100 ppb HT-2	negative
825	100 ppb HT-2	14.4 ppb HT-2

3.4.2 Control Urine Samples

There were ten fortified and one blank control urine samples. Of the ten fortified samples, only six were identified as being positive. The single blank control sample was found to be negative.

Table 6

Control Urine Samples - Recovery

Random Number	Level of Fortification	Found
234	blank	negative
611	25 ppb T-2	6 ppb T-2
244	25 ppb T-2	negative
750	25 ppb T-2	7.2 ppb T-2
657	25 ppb HT-2	negative
893	25 ppb HT-2	negative
825	25 ppb HT-2	negative
560	50 ppb T-2	34.6 ppb T-2
354	50 ppb T-2	36.0 ppb T-2
347	50 ppb HT-2	4.2 ppb HT-2
569	50 ppb HT-2	5.2 ppb HT-2

The analytical results of the limited urine study have to be viewed with great caution, as insufficient time was available to study in detail the metabolic breakdown rate of trichothecenes in urine.

3.4.2 Results - Field Samples

Five blood samples from Thailand registered positive upon analysis. The urine samples corresponding to these positive blood samples were all found to be negative. It should be noted that the urine analysis took place some six months after completion of the analysis of blood samples.

Table 7

Positive Field Samples - Blood

Location	Random Number	Results	Clinical Observations at time of sampling[1]
Mae Sot[2] (Thailand-Burma border area)	072	51 ppb T-2	nil
	476	2.6 ppb T2	nil
	574	6.6 ppb HT-2 2.2 ppb T-2	abdominal pain
Loei Army Base[3] (near northern Thailand-Laos border)	183	76 ppb T-2	shortness of breath; asthmatic?
Khao-I-Dang[4] (near Thailand-Kampuchea border	874	2 ppb T-2	nil

[1] Clinical observations were recorded on individual history sheets.
[2] Corresponds to Group II, "Civilian Western Thais", in Table 2.
[3] Corresponds to Group III, "Thai Military", in Table 2.
[4] Corresponds to Group VI, "Khmer" (no recent exposure claimed), in Table 2.

4.0 LESSONS LEARNED AND OBSERVATIONS

4.1 Field Work

On the basis of this experience, it was concluded that it would be important that the analyst (who will later analyse the samples) be involved in the preparatory discussions relating to the matrices to be collected for analysis. While it was recognized that such a preparatory phase may be very short, and that the inspection team may have to improvise in view of unforeseen circumstances, it was clear that the inspection team should be aware of the limitations and constraints which would have to be faced by the analyst. For the same reason, it was concluded that a member of the inspection team should be experienced in at least some of the analytical techniques which will later be used in the laboratory.

It was concluded that it would be important for an investigating team to carry with it all the required specialized supplies (e.g., blood collection tubes, needles, other containers), thus avoiding problems related to availability or to compatibility with other equipment. It was found to be practical and convenient to pack the specialized and miscellaneous supplies in the coolers for the outward bound trip.

The team recognized the need not only to be briefed on the scientific and military considerations which must be borne in mind when investigating an allegation of chemical or biological warfare; but also to be briefed on certain political, geographical and cultural issues pertaining to the region in question. An accompanying political officer would be very useful, but all team members should be aware of particular political complexities and sensitivities.

The estimated time for travel (return) and field work, including briefings, was 2-3 weeks. In all, 5-6 weeks were actually required, in view of the nature of the study. In other words, this was not an investigation of a specific allegation of the use of toxin weapons, which would have focussed the briefings and field trips considerably. Instead it was more in the nature of a scientific field trip, and so there is some hesitation in drawing specific conclusions. Nevertheless, it is probably safe to say that a more focussed field investigation could have been completed in 1-2 weeks, bearing in mind that briefing requirements, the need to obtain clearances to travel to certain areas or facilities, actual travel to remote areas, sampling (including control samples) and interviewing will likely take more time than expected. Due to the sequential nature of many of the activities, a delay in one area would likely cause other activities to back up.

As regards the composition of the team, it was concluded that a multi-disciplinary team would be most effective. It should probably include: a chemist with experience in handling and analysing highly toxic substances; a medical doctor, preferably with experience in epidemiological methods; a toxicologist; a medical technician/nurse; a member (could be one of the above) with knowledge of chemical and/or biological weapons (based on initial reports from the area), their means of dissemination and characteristics, and of personal protective measures; a person with interviewing experience, preferably with a knowledge of the local culture(s); and a liaison officer to deal with government and local officials and, therefore, conversant with the political situation. The team should probably have its own interpreter, even if only to laise with other locally-provided interpreters. The minimum requirements for a sampling team would be: an analyst; a medical doctor/epidemiologist; medical technician/nurse; liaison officer; interpreter; driver; and an observer from the host government who would also liaise with local authorities. Any personnel assigned in situ to the team should be relieved of all other taskings.

The coolers and frozen ice packs were found to be perfectly adequate when specimens have to be chilled (not frozen). This proved to be the case for a period in excess

of 72 hours, bearing in mind that the coolers were not opened too frequently nor left open for extended periods (for example, at customs). Commercially-available, durable and lightweight coolers performed effectively. The issue of container security (i.e., locking) has to be addressed.

It was concluded that much attention has to be paid to arrangements for the transportation of equipment to, and of equipment and samples from, the host country if commercial transport is to be used. Contacts and key personnel at stopover and transfer points have to be identified and, if possible, correspondence (or telexes in more urgent cases) exchanged. Commercial carriers should be made fully aware of the size and nature of the cargo, and of the particular need for security and a clear chain of custody involving the escort. This may also involve liaison through the identified contacts with airport security officials. It would be preferable that the escort be (or include) a person carrying a diplomatic passport. To avoid surprises, any excess baggage costs should be discussed well in advance with the carrier(s). If, as is often the case with respect to excess baggage, such costs must be paid prior to take-off, provision should be made for the escort to carry surplus funds to deal with such requirements.

Official clearances to allow certain types of material (e.g., biological matter) and other substances (e.g., toxic "standards", should they be required) will need to be arranged well in advance. Surprises, in terms of including in the shipment any materials other than those for which clearances have been obtained, could occasion delays and considerable difficulties. It would be better to ship any such additional materials separately.

It was concluded that time spent in preparatory activities (obtaining equipment and supplies and testing, and considering all of the details which would have to be taken into account) could be substantially reduced through the availability of a check-list of items to take (or, at least, to consider taking) and of things to do. An illustrative questionnaire would also be useful.

Finally, it was considered that training programmes would greatly contribute to the effectiveness of, and reduce the risk to, personnel who might be tasked with carrying out such an investigation. These programmes could include personal protection, hazard identification, sample collection (environmental and biomedical) and handling, decontamination, analysis in the field (if base camp analysis is envisaged), and first aid, to name a few subjects of interest.

4.2 Laboratory Analysis

The laboratory (or, preferably, laboratories) selected to conduct the analysis should be advised as far in advance as possible and, ideally, the analysts should be involved in pre-travel briefings with the investigating team when matters related to sample collection and handling are discussed.

It was recognized that it would be necessary to have the laboratory (or laboratories) analyse the samples quickly and on a priority basis, hence the need for laboratories specializing in certain types of analysis. This would entail certain economic costs in the case of a commercial laboratory, or other opportunity costs in the case of a government laboratory.

That being said, in dealing with "novel" agents, a considerable amount of time will likely have to be devoted to refining analytical procedures. In addition to that, other more basic research (for example, work related to recovery rates or to the metabolic breakdown of substances in body-fluids) may well have to be done in order to be able to interpret the analytical results. All of this takes time.

Analysis of the blood samples was further complicated in that approximately one-third of the blood samples were found to be hemolyzed and/or icteric, or lipid-rich (see Appendix Z). While hemolysis may be associated with improper

handling of the samples, it can also be associated with malarial infection. In this case, the number of icteric samples supported the latter viewpoint. Such complications not only cause difficulties with respect to analysis, but also point to the need for strict enforcement of occupational health regulations when handling such specimens. Furthermore, in this case the analytical procedure had to be modified through the introduction of a third clean-up step for blood samples.

A high lipid content may or may not be avoidable. It is known that temporary hyperlipemia occurs after a fat-rich meal, and it may place undue restrictions on those who provide samples to ask them to refrain from eating twelve hours prior to sampling.

When the 10 ml whole blood samples were centrifuged, each yielded about 4 ml of plasma. Since 1 ml of plasma was needed for one analysis, this pointed to an obvious limitation to the analytical phase. This, combined with the observation concerning hemolysis mentioned above, led to two conclusions:

- 2 x 10 ml of blood should be collected from each subject, and multi-draw needles should be considered; and
- one of the 10 ml samples from each subject should be centrifuged as soon as possible after collection (possibly using a hand-operated spinner), while the other should be retained as whole blood for confirmation and/or whole blood studies.

It has already been mentioned that the method development and analysis of 280 blood samples and of 16 urine samples were carried out over a ten month period. Provided a suitable laboratory does not have to cope with method development and can proceed directly to work on the samples, the following appeared to be a realistic time-frame for two technicians analysing for trichothecenes:

- sample extraction and clean-up:
  20 samples/day; and

- actual GC/MS analysis (one station):
  15 samples/day.

In theory, the study reported here should have taken approximately 14 working days for extraction and clean-up; and approximately 20 working days for analysis. Allowing for weekends, almost assured temporary breakdown of equipment of a minor nature, and the necessary calibration of instruments every so often would suggest a turn-around time for 280 samples of slightly in excess of two calendar months. Provision of more technicians and several work stations could shorten this time significantly. The participation of more than one laboratory would not necessarily shorten the time-frame, since the purpose would likely be corroborative analysis.

Following from the last point, it was clear that at least a second laboratory would be required to analyse the same samples (which also accounts for the point made earlier about obtaining 2 x 10 ml blood samples from each subject). Similarly, some of each sample should be stored for future reference in the event of any problems. This pointed to the need to split the samples for distribution to more than one laboratory, which was not done in this study.

Finally, just as it was mentioned that training programmes would greatly contribute to the effectiveness of a field investigation team, the equivalent at the analytical level would also be very useful.

Appendix 1. Draft Questionnaire

BASELINE TOXICOLOGY STUDY - THAILAND 1984

GROUP [ ] SITE [ ] SAMPLE # [ ]

1. Name _____ 2. Sex M __ F ___ 3. Age ____ yrs

4. How long living here _____ mo/yr

5. If less than 6 months, previous residence _____

_____

6. Civilian _____ Military _____

7. Present state of health or current diagnosis if in hospital _____

_____

8. Any health problems in past 12 months? Indicate system involved:

skin/cardiovascular/respiratory/gastrointestinal/central nervous _____

_____

9. Hospitalization in past 12 months (diagnosis) _____

_____

_____

10. Did you ever encounter "yellow rain" or other poison attack?
Yes/No
If never, no more questions
If yes, answer questions 11-17.

11. Last time _____ 12. Where _____

13. Was attack by aircraft _____ artillery _____ Other _____

14. Colour of smoke or spray: Yellow ___ White ___ Blue ___ Other ___

15. Were you made sick by the attack? _____ If yes, what symptoms?
Headache/dizzy _____ Breathing/cough _____ Itch/Red spots _____
Vomiting _____ Bleeding _____ Other _____

16. Were other people ill after attack? # ___ Did any die? # _____

17. Were animals sick or dead after attack? _____

Date _____ Technician _____ Observer _____

แบบสอบถาม

กลุ่ม [ ] สถานที่เก็บตัวอย่าง [ ] ตัวอย่างเลขที่ [ ]

๑. ชื่อ _____ ๒. เพศ ชาย ____ หญิง ____ ๓. อายุ _____ ปี

๔. ได้ย้ายมาอยู่ที่อยู่ปัจจุบันนี้เป็นเวลา _____ เดือน/ปี

๕. ถ้าย้ายมาภายใน ๖ เดือน ที่อยู่ก่อนหน้านี้คือ _____

_____

๖. มีฐานะเป็น พลเรือน _____ หรือ ทหาร _____

๗. สุขภาพทั่วไปในปัจจุบัน หรือ กำลังป่วยด้วยอาการ หรือโรคอะไร ถ้าหากขณะนี้เป็นผู้ป่วยอยู่ในโรงพยาบาล _____

๘. มีปัญหาทางสุขภาพอย่างใดหรือไม่ในช่วง ๑๒ เดือนที่ผ่านมา ถ้ามี ขอให้บรรยายว่าเกี่ยวข้องกับระบบใด เช่น ผิวหนัง/ ระบบหลอดเลือดและหัวใจ/ ระบบทางเดินหายใจ/ ระบบทางเดินอาหาร/ ระบบประสาท _____

๙. ในช่วง ๑๒ เดือนที่ผ่านมา เคยเข้าอยู่ในโรงพยาบาลหรือไม่ ถ้าเคย ได้รับการวินิจฉัยโรคว่าอย่างไร _____

๑๐. ท่านเคยถูกยิงเฉี่ยวหรือได้รับการโจมตีด้วยสารพิษอย่างอื่น หรือไม่ เคย/ไม่เคย

ถ้าเคย โปรดตอบคำถามข้อ ๑๑ - ๑๙ ด้วย

๑๑. ครั้งสุดท้ายที่เกิดเหตุ _____

๑๒. สถานที่ที่เกิดเหตุ _____

๑๓. การโจมตีเกิดจาก เครื่องบิน _____ กระสุนปืนใหญ่ _____ อื่นๆ _____

๑๔. สีของควัน หรือสารที่ถูกฉีดพ่นออกมา สีเหลือง_____ สีขาว ____ สีฟ้า ____อื่นๆ ____

๑๕. ท่านมีอาการเจ็บป่วยจากการถูกสารพิษหรือไม่ _____ ถ้ามี ด้วยอาการอะไรบ้าง

ปวดศีรษะ/เวียนหัวตา _____ หายใจขัด/ไอ _____ คัน/มีผื่นแดงเกิดขึ้น _____

อาเจียร _____ มีเลือดออก _____ อื่นๆ _____

๑๖. มีผู้คนอื่นเจ็บป่วยหลังจากนั้นด้วยหรือไม่ จำนวน _____

มีผู้เสียชีวิตหรือไม่ จำนวน _____

๑๗. มีผู้คนอื่นเจ็บป่วยหลังจากนั้นด้วย หรือไม่ _____

วันที่ _____ เจ้าหน้าที่ สอบถาม _____ ผู้ให้สัมภาษณ์ _____

Appendix 2. Field and Control Samples, Random Numbers, Condition

SAMPLE NUMBER	VOLUME OF BLOOD (mL)	VOLUME OF PLASMA (mL)	HEMOLYSIS	ICTERUS	ELEVATED LIPID	OTHER
331	9.5	4.0				
526	9.5	3.8		+++		
909	9.5	4.0				
954	9.0	4.0				
343	9.5	4.0				
147	9.5	3.5				
325	9.5	4.0				
978	9.5	3.5				
647	9.0	3.5				
218	9.5	3.2	+			
841	9.5	6.0				
099	8.0	3.2	+++			Clotted
354	9.0	3.8				
201	8.0	2.5				
569	9.0	3.5				
489	8.0	3.5	+			
968	9.0	3.2	++			
390	9.0	3.8	+			
148	9.0	3.8				
870	9.5	3.5				
108	9.5	4.2				
045	9.5	4.8				
305	8.0	5.0				
926	9.0	5.0				
096	8.5	4.5		+		
362	9.5	3.5	+			
415	9.0	4.8				
228	9.5	4.2	+			
594	9.0	3.8	+			
445	6.0	2.5	+			
359	9.5	4.0	+++			Clotted
564	9.0	3.8	+++			
904	8.5	4.2	+++			
357	3.0	1.2	++			
007	3.5	1.8				
929	9.0	4.0				
932	9.0	4.5				
419	8.5	4.5				
190	9.0	5.0			+	
323	9.0	4.5				
255	9.0	4.0				
606	9.0	4.0				
013	9.0	4.2				
668	9.0	5.5				
399	9.5	2.5				

SAMPLE NUMBER	VOLUME OF BLOOD (mL)	VOLUME OF PLASMA (mL)	HEMOLYSIS	ICTERUS	ELEVATED LIPID	OTHER
521	4.0	1.8	+			
650	8.0	3.0				
807	9.5	4.0				
967	7.5	2.8				
054	4.5	2.0				
250	4.0	2.2	+			
262	8.5	3.5	+			
244	9.5	3.8				
039	9.0	3.5				
739	8.5	5.0	+			
487	9.5	4.0				
217	9.5	4.2				
683	9.0	4.2	++			
049	9.5	4.0				
393	9.0	3.5	+			
442	6.0	3.0				
755	9.5	6.0	++	++		
888	9.0	4.2			+	
478	9.0	3.5				
154	9.0	3.5			++	
289	9.0	4.5	+			
878	9.0	3.8				
747	9.5	3.8				
625	9.0	2.5	+			
180	9.5	3.2				
237	9.0	4.0	+++			
183	9.0	4.0				
592	9.5	3.8	++			
271	8.0	3.5	+			
200	4.5	2.0	+			
693	4.5	2.0	+			
198	9.5	3.5				
566	9.0	6.5				
927	9.0	5.0				
197	9.5	4.2	++			Clotted
146	9.0	4.0			++	
977	9.5	5.5				
748	9.5	4.8				
113	9.5	3.5			+	
44	9.5	4.5				
471	9.0	4.5				
815	9.0	4.2				
777	9.5	3.0				
933	8.5	3.0				
699	9.5	4.5				

SAMPLE NUMBER	VOLUME OF BLOOD (mL)	VOLUME OF PLASMA (mL)	HEMOLYSIS	ICTERUS	ELEVATED LIPID	OTHER
475	9.0	3.0				
022	9.5	4.0				
634	5.5	1.8	+			
658	8.5	4.0				
875	9.5	3.5				
684	9.5	4.2				
881	9.5	3.8				
611	9.5	3.8				
090	8.0	3.5	+			
094	8.0	3.0	+			
839	9.5	4.5				
037	7.5	2.5			+	
490	5.0	1.5				
139	8.0	3.8				
383	9.0	3.0				
5	8.0	3.8				
208	10.0	4.0	+			
867	9.5	4.2	+++			
563	9.5	4.0	++			
288	9.5	4.0				
610	5.5	2.8				
454	9.0	4.5	+			
373	8.5	3.0	++			
876	9.0	5.0				
984	9.0	3.8				
901	9.0	3.8				
725	9.0	3.0				
945	8.5	3.2				
142	8.0	3.0				
251	9.0	3.0			+	
729	6.5	2.5				
524	6.5	2.5				
243	9.0	3.5	+			
057	9.5	3.5	+			Clotted
509	6.0	2.8	++			
798	9.5	3.5	+			
896	9.5	3.8				
987	9.0	3.8				
981	7.0	2.5	+			
159	7.0	3.0	+			
31	9.0	3.8				
893	9.0	3.5				
384	7.5	3.5	+			Clotted
995	9.0	4.5	++			
849	10.0	3.5				

SAMPLE NUMBER	VOLUME OF BLOOD (mL)	VOLUME OF PLASMA (mL)	HEMOLYSIS	ICTERUS	ELEVATED LIPID	OTHER
946	8.0	3.5				
406	10.0	4.0				
560	9.0	3.8				
697	9.0	3.0	++			
738	9.0	3.0				
434	7.0	2.8	++		+	
114	9.5	4.5				
648	7.0	3.0				
426	9.0	3.0				
786	9.5	2.5				
899	8.5	3.5				
537	9.0	3.8				
410	9.0	3.8				
874	9.0	4.0				
820	8.0	3.8				
256	9.0	2.8				
814	9.0	3.8				
333	9.5	4.2				
597	9.0	3.8				
555	9.0	6.0		+		
576	9.0	4.2				
290	9.0	3.8				
157	9.0	3.5	++			
395	9.0	4.0				
591	9.0	3.8				
382	9.5	3.8			+	
330	9.5	4.8				
958	9.5	4.8				
622	9.5	3.5				
783	9.0	3.8				
433	9.0	3.8				
892	9.0	4.5		+		
879	9.0	4.0				
420	9.0	4.2				
82	7.0	2.5	+			
750	9.0	3.8				
174	9.5	4.2				
363	9.0	3.8				
740	9.5	4.2				
450	9.5	4.5		+		
505	9.0	3.8				
917	10.0	4.0		++++		
812	9.5	3.5				
709	9.5	4.0				
657	9.5	3.8				

SAMPLE NUMBER	VOLUME OF BLOOD (mL)	VOLUME OF PLASMA (mL)	HEMOLYSIS	ICTERUS	ELEVATED LIPID	OTHER
109	9.0	3.0				
808	9.5	4.5				
408	9.5	4.0				
671	7.0	3.5				
973	9.0	5.0				
698	5.0	2.5	++			
675	9.5	3.0			++	Clotted
476	9.5	4.5				
998	9.5	3.8				
994	9.5	4.2				
303	9.5	4.5				
350	9.5	3.8				
160	10.0	4.2				
617	9.5	5.0			+++	
574	9.5	4.5				
328	10.0	4.8				
969	9.5	4.0				
514	10.0	4.0				
821	9.5	4.0				
677	9.5	4.0				
886	9.5	4.5				
417	9.5	4.0			+	
920	9.0	4.2				
398	8.5	3.8	+			
394	9.5	4.2				
956	9.5	3.0	+			
347	9.5	3.8				
265	9.5	3.5				
666	9.0	3.0				
809	9.0	3.2				
660	10.0	5.0				
626	9.0	3.8				
134	10.0	4.2				
435	9.0	3.8				
898	9.0	4.5		+		
500	10.0	3.8				
636	9.5	4.5				
902	10.0	4.5		++		Clotted
985	8.5	3.0				
072	9.5	4.0				
707	9.0	3.5				
974	9.0	3.0				
628	9.0	3.5				
443	9.0	4.5		++		Clotted
188	9.5	4.5				

SAMPLE NUMBER	VOLUME OF BLOOD (mL)	VOLUME OF PLASMA (mL)	HEMOLYSIS	ICTERUS	ELEVATED LIPID	OTHER
609	9.5	4.0				
214	10.0	4.0				
543	10.0	4.0	+			
207	10.0	3.0				
915	8.5	3.5				
423	10.0	4.3				
126	10.0	4.0				
842	10.0	4.5			+	
461	5.5	2.0	++			
754	10.0	4.5				
260	10.0	3.8		++		
122	6.0	2.5				
877	10.0	5.0	+			Clotted
593	10.0	5.2		++		
567	10.0	4.0				
784	10.0	4.0				
424	10.0	4.0				
131	8.0	3.0	++			
535	10.0	5.0				
400	10.0	3.8				
249	10.0	4.0				
780	9.5	4.0		++		
438	9.5	4.0				
895	9.0	4.5				
635	10.0	3.8				
453	9.5	5.0				
023	10.0	4.0				
166	9.5	3.8			+	
941	10.0	4.2				
643	10.0	3.8				
845	10.0	4.0			+	
682	9.5	3.5				
336	9.5	4.0				
052	9.5	4.0				
474	8.5	3.5				
003	9.5	4.0				
727	10.0	4.0				
990	10.0	4.2				
687	9.5	4.0				
982	10.0	4.0				
259	10.0	5.0		++		
602	9.5	3.5	+			
965	9.5	3.5				
825	9.0	3.5				
928	8.0	4.0	+			

SAMPLE NUMBER	VOLUME OF BLOOD (mL)	VOLUME OF PLASMA (mL)	HEMOLYSIS	ICTERUS	ELEVATED LIPID	OTHER
612	7.5	3.0	+			
868	8.0	4.5	+			
796	8.0	4.2				
083	5.0	3.0				
633	8.0	3.0	+			
834	8.6	3.5	+			
619	8.5	4.5	+			
589	6.0	2.8	+			
632	8.5	3.0	+			
718	8.0	4.8