FIRST WORLD CONGRESS 1984, GHENT - BELGIUM

Editor : Prof. A. HEYNDRICKX
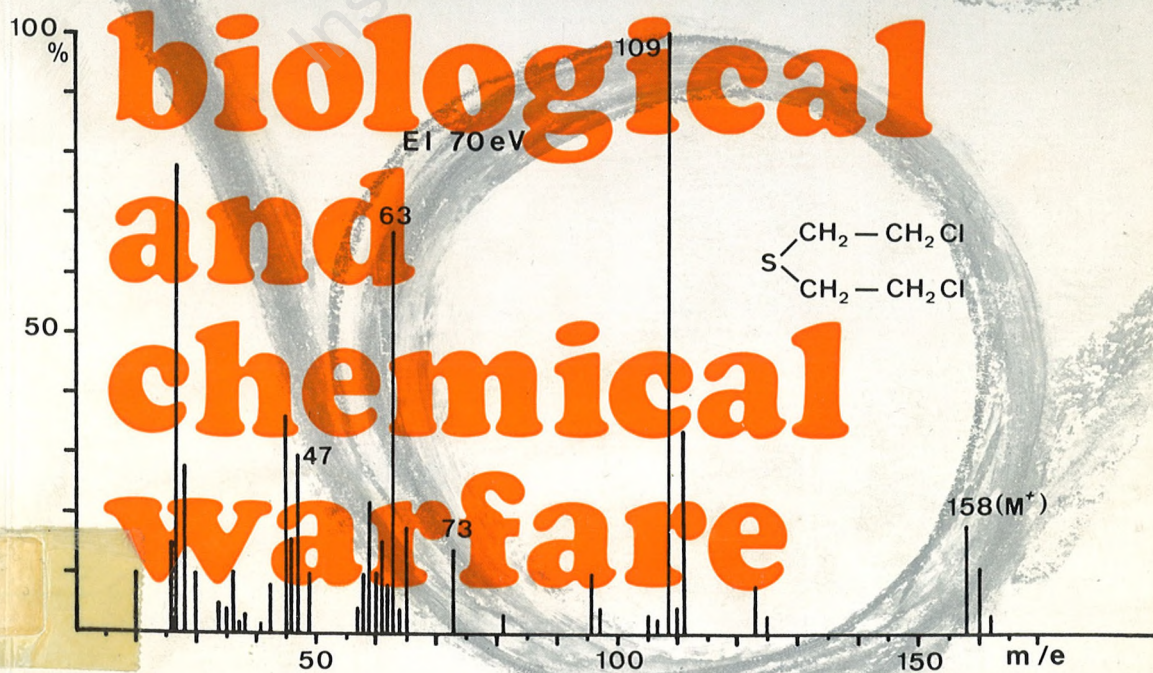
100 %

109

EI 70 eV

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biological
and
chemical
warfare

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FIRST WORLD CONGRESS

New Compounds in Biological and Chemical Warfare : Toxicological Evaluation

PROCEEDINGS

Ghent, May 21st - 23rd, 1984

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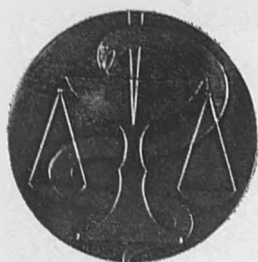Prof. P. GHYSBRECHT, State University of Ghent, Belgium.

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State University of Ghent, Belgium

EDITORIAL

by A. HEYNDRICKX

Chairman of the Congress
Department of Toxicology, State University of Ghent,
Hospitaalstraat 13, 9000 Ghent, Belgium

His Excellency, Mr. W. De Clercq, Vice-Prime Minister and Minister of Finance and Foreign Trade,

His Excellency Prince Norodom Ranariddh of Cambodia,

The Honourable Rector of the State University of Ghent,

Excellencies,

Ladies and Gentlemen,

Dear Colleagues,

On April 27th 1915 at 4 o'clock in the afternoon a green gas cloud was coming towards the Belgian defensive troups stationed near Ieper (Ypres) ; the first gas attack, made by the German Army with chlorine had taken place. Through that attack, five thousand Belgian troups and Allied Forces died.

The Belgian Army and the Allied Forces did not think about Gas Warfare. We had no gas masks, and no protective clothing.

Six thousand metal cylinders filled with chlorine were used in this battle.

In December 1915 the German forces first attacked with Phosgene and later on July 12th 1917 they first used mustard gas. Mustard gas got its name from the City of Ieper (Ypres) where the gas was first used, since then it was called also Yperite.

The first gas mask that was made by the British was introduced on May 1st and was a very simple system, called the Black Veil Respirator.

Since then also a kind of mask was developed for the horses which were of such great use in this warfare.

It was Prof. Dr. Fritz Haber, Head of the Berlin Kaiser Wilhelm Institute, Physikalische Chemie und Elektrochemie, who invented the technique.

Later on, after the War, he received the Nobel Price, for other inventions.

The war gas history started by the British in 1894 at Sebastopol in the Crimean War, where noxious sulfur fumes were used, which were quite ineffective. In the Boer War of 1899-1902 the artillery was using shells, filled with picric acid which had a vomiting reaction on the soldiers.

The War of 1914-1918 was numbering about 100,000 dead and over 1,200,000 of casualties, including civilians, due to chemical warfare.

I still remember, in the pharmacy of my father before World War II, that veterans of the First World War were coming in every week to receive from the Ministry of Defence and the Ministry of Health, the bromoform syrup for their daily use.

In 1930 Mussolini was using mustard gas in Ethiopia. And in 1965 Egypt was using also war gases, Yperite, in Yemen. Since 1975-1976 we have reports that chemical and microbiological warfare is used in Laos, later in Cambodia and Afghanistan. At this moment Irak is using it in Iran.

It means that today's First World Congress « New Compounds in Biological and Chemical Warfare : Toxicological Evaluation », is up to date.

I want to thank especially the Belgian Government and also His Excellency the Vice-Prime Minister present here, for the great help that I had in my research, and the support to organize this First World Congress.

At no time there was any influence or pressure from the Belgian Government or any Minister so that I could organize completely and independently this meeting.

During my research and also sampling in foreign countries, at no time I was influenced so that I could work completely independently. I want to thank also the Universities of Vienna, Munich, Hamburg, Essen, Lausanne and Ghent for their close collaboration and co-operation the last weeks, treating the patients, analysing the samples which were sent over every day to the University, and their close co-operation and reporting the results. I want to thank also especially Austrian Airlines, Lufthansa and

Swissair for the close collaboration sending over the samples and taking back the reports.

I also want to thank the Rector of the State University of Ghent, Prof. A. Cottenie for the possibilities given to my Department of Toxicology to do the analysis, and fundamental research which was needed in this field.

Unfortunately all our physicians, pharmacists and nurses in Western Flanders who had to treat our soldiers and civilians attacked by those terrible weapons passed away. We only have a few veterans left who still remember those hard days. One of them told me that by one of these attacks the soldier who was blowing the horn and announcing the gases, died, due to the fact that he had to breath many times, warning the others that they had to protect themselves. In those attacks, our soldiers only had a handkerchief that they had to wet with their own urine, bring on their mouth and nose so that they would have some protection.

It means that today, what the treatment is concerned, we do not know much, it is only by these results at those University Clinics, where all those physicians and nurses were doing such an outstanding job, that we gain some experience using modern pharmaceuticals and also new techniques ; we can see how we can cure and at the same time relieve.

The techniques of detection have been changing since 1914-1918, by using modern possibilities of analytical toxicological investigation.

It is surprising to find that many of those war gases do not hydrolyze or do not metabolize so fast, as we originally thought. Of course today our methods are much more reliable and sensitive than they were 69 years ago.

This Congress has one main purpose in humanitarian sight : see how we can help people who suffer. This conference has no political goal, everybody can express his own ideas, the way he was conducting his investigations, at the same time everyone can report what he has heard and seen.

In this way, Belgium, which was suffering so much during the First World War, has the honour to organize this First World Congress.

We hope, bringing these results known internationally, Governments and Countries involved will draw the necessary conclusions,

and bring them towards the United Nations, as it has already been done by some of them.

That all those weapons will be banned and that at the same time the basic compounds, the bulk materials, that one needs to manufacture them, will be under direct international control.

All other solutions will be ineffective.

We hope that industrialized countries will limit the transfer of technology, and limit technical experts in helping those countries to build this new manufacturing military industry.

In this way we will help to have more peace in some parts of the World and try to conserve it in those parts where we still have peace.

I want to thank the National Science Foundation of Belgium and the Faculty of Pharmaceutical Sciences of the State University of Ghent for the help given to organize this Conference.

I extend a warm welcome to the 324 scientists and delegates from the 31 countries : Afghanistan, Austria, Australia, Belgium, Bulgaria, Cambodia, Canada, China, Denmark, Ecuador, Finland, France, Germany, Greece, Iran, Israel, Italy, Japan, Norway, Philippines, Singapore, South Africa, Spain, Sweden, Switzerland, Thailand, The Netherlands, United Kingdom, USA, Western Germany, Yugoslavia.

Fifty-two communications will be discussed.

May I ask now His Excellency the Vice-Prime Minister of Belgium, Mr. W. De Clercq to open this First World Congress.

Allocution by His Excellency Mr. W. DE CLERCQ

Vice-Prime Minister, Minister of Finance and Foreign Trade

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His Excellency, Prince Ranariddh Norodom,

Excellencies Ambassadors,

Authorities,

It is with my warmest congratulations to the State University of Ghent that I extend my appreciation to organize the First World Congress « New Compounds in Biological and Chemical Warfare : Toxicological Evaluation », which takes place these coming days.

Belgium has suffered a lot in the First World War of Chemical attacks on its soldiers and thousands died in the fight for their freedom with the Allied Forces.

This Conference on high scientific level and humanitarian grounds, away from all international political involvement is a light for humanity in its search to live free from any war.

The program that is submitted for the detection of those compounds, the diagnosis and the treatment of the patients is of such a great importance for humanity and for our future.

Belgium, on humanitarian grounds has always supported, in the Western World and internationally, all efforts to attain those goals.

By comparing the scientific results of all great specialists of the world here united today, it is my sincere hope that more and more the truth will be known to the world and that all of us will do our best to reach peace for all men.

I hope that in those three days, the international exchange between scientists will improve and that the press of all Nations here united, will make it also known to the World.

Let us hope that in this way you and Belgium will make a step forward to reach our final destination in human rights and peace.

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Allocution by Prof. H. BRANDENBERGER

Gerichtlich-Medizinisches Institut der Universität Zürich,
Zürichbergstrasse 8, Postfach 8028, Zürich, Switzerland

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Dear Professor Heyndrickx,

Ladies and Gentlemen,

As the current President of THE INTERNATIONAL ASSOCIATION OF FORENSIC TOXICOLOGISTS (TIAFT), I have the pleasure and the honour to convey the greetings of our Society to our distinguished member Professor Heyndrickx and to the organizers and participants of this congress, our greetings and wishes for a successful event.

During the last 30 years, most of us toxicologists have neglected the study of war poisons. We have been busy looking after pharmaceuticals, drugs, metals, solvents and pesticides, but not after war poisons, since they have been banned.

However, the ban of a compound never excludes its application, as we know so well from the drug scene. It was therefore a very wise move of Professor Heyndrickx and his Department of Toxicology in Ghent to organize this meeting devoted to a neglected field. His action has already been fully justified by the very recent events in the Near East.

Let me therefore — in the name of TIAFT and in my personal name — congratulate Professor Heyndrickx for his foresight and his initiative. Once more he has been doing pioneering work in the field of toxicology.

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CHEMICAL WARFARE IN BELGIUM

First World War 1914-1918

MUSEUM BELGIAN ARMY - BRUSSELS

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Tranchées du canal.   Loopgraven aan 't kanaal.

Quelques Extraits de Livres

" Le Drame de l'Yser "

par le Général MORDACQ,
qui commandait, en avril 1915, la 90 brigade des
troupes d'Afrique, dans le secteur de Langemarck.

Dans ces tranchées, la journée du 22 avril s'était passée dans un calme presque absolu: quelques salves de 77 seulement avaient été tirées vers 16 heures sur les tranchées du 1ᵉ tirailleurs. Tout à coup, à 17 heures, sans qu'aucun bruit préalable ait pu donner l'éveil, une immense fumée jaunâtre s'abattait sur le front français, dans les tranchées, depuis Steenstraat jusqu'aux lignes anglaises, c'est-à-dire sur un front de 6 kilomètres environ.

Les guetteurs donnent l'alerte. La fusillade se déclenche immédiatement.

Les Allemands, sachant qu'à la gauche des territoriaux, vers Steenstraat, la surveillance était très relâchée (depuis plusieurs jours poilus et feldgrauen communiquaient, échangeant du tabac, des cigarettes, du vin, du pain, etc.), manœuvrèrent surtout de ce côté, si bien que les deux régiments territoriaux furent immédiatement tournés : presque tous leurs hommes furent tués, asphyxiés, blessés ou pris. les quelques isolés qui purent s'échapper, s'enfuirent par les ponts de Boesinghe et de Steenstraat; ce soir-là, on ne les revit plus.

Dans le secteur d'Elverdinghe, le 1ᵉ bataillon d'Afrique et les deux bataillons de tirailleurs essayent de tenir tête avec quelques unités qui ont pu sortir des tranchées ou qui étaient en réserve, mais suffoquant presque immédiatement sous les fumées asphyxiantes et accablés par un tir très violent d'artillerie lourde, tous les poilus qui ont essayé de rester sont tués ou faits prisonniers par les Allemands, les autres se retirent dans la direction d'Ypres et de Boesinghe.

Les Allemands les serrent de près et les ont même dépassés vers le nord, le long de la voie ferrée Langemarck-Boesinghe, que les territoriaux ont complètement abandonnée, sans tenter la moindre résistance. Ce drame s'était déroulé en une demi-heure environ; la surprise avait été complète. Les Allemands purent, en moins de trois quarts d'heure, atteindre la région de Het Sas et arriver en face des ponts de Boesinghe (200 à 300 mètres environ), mais jusqu'alors, ils avaient vainement essayé de les enlever.

Telle était la situation à ce moment, 18 h. 45 environ.

Dès les abords du village de Boesinghe, le spectacle était plus que lamentable, il était tragique. Partout des fuyards : territoriaux, « joyeux », tirailleurs, zouaves, artilleurs, sans armes, hagards, la capote enlevée ou largement ouverte, la cra-

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LES GRENADIERS A STEENSTRAAT

rente un caractère tragique. Les murs de sacs apparaissent en clair comme des récifs. Entre eux les boyaux enfoncent leurs méandres noirs. Les shrapnells qui se déchirent dans les arbres en dessinent pendant un instant le squelette sur le ciel.

Dans cet enfer, les hommes silencieux se glissent lentement, courbés car le danger vient de partout. Ils longent les parapets, entrent dans les couloirs qui mènent à la tranchée de combat. Dans celle-ci retentit le bruit sourd et continuel des coups de fusil. L'air est chargé de poudre et de cette odeur étrange, parfumée, qui serre les tempes comme un casque. Les fusées descendent en nappes de lumière émeraude, qui font songer à quelque pays sidéral.

Tandis qu'à genoux nous pansons un homme dans le chemin de ronde, une balle vient s'enfoncer dans la paroi même de celui-ci. Cette balle sûrement vient de derrière nous. Sans mot dire, nous continuons notre besogne.

A ce moment, il semble que le fracas reprenne avec plus de force. Nos pièces tirent par salves. Dans l'abri, au sortir de l'atmosphère du dehors, nous retrouvons la réalité laide, aux teintes d'ombre et de terre brune, la clarté jaune des bougies, le sang, les os, les faces contractées ou impassibles, les conversations entrecoupées et les plaintes, la besogne hâtive, les gestes précis. Pourtant une gêne pèse sur tout le monde.

Avant qu'une mauvaise nouvelle n'arrive, il semble que chacun la devine. Mille indices la font pressentir. Les Allemands sont à Lizerne. Ils sont même arrivés jusqu'au moulin, où nous avons passé il y a une heure à peine.

Nous sommes tournés. Tout semble plus tragique, la nuit, les lumières étranges et cette odeur qui passe par la porte. Et voici qu'un officier entre à tâtons, comme aveuglé. Il vient s'affaler au milieu de nous. Il respire avec peine. Ses yeux sont rouges et cette odeur le suit. Il semble que ses vêtements en soient imprégnés, car bientôt l'atmosphère devient pénible. Nos yeux commencent à brûler et les larmes en coulent comme des ruisseaux. En même temps, l'étau se resserre sur le front. Nous ne savons que faire pour le soulager ni pour nous soulager nous-mêmes. Dans le silence, c'est un moment pénible qui se prolonge. Mais voilà des blessés, il faut tout de même les aider. Il faut pour cela lutter contre cet engourdissement qui paralyse la volonté, et qui se fait plus lourd au moment des grands dangers. L'officier lui-même se relève. Ce n'est pas le moment de se laisser aller. Il retourne en titubant à son poste.

Sans que nous nous en apercevions, le jour vient doucement, le jour pâle qui ressemble au clair de lune.

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La tranchée est bourrée de monde. Nos grena-

diers sont le long du canal, les zouaves le long de l'Yperlée. Nos hommes sont accroupis contre les parapets, le fusil baïonnette au canon. Les zouaves sont dans les berges où ils ont creusé des niches ovales, dont une partie s'enfonce sous la terre et sert à protéger la tête. Les Allemands qui sont de ce côté-ci de l'eau, ont une tranchée à trente mètres de la nôtre, et l'on s'attend à un assaut. A tout moment vient l'ordre d'appuyer.

Un par un, des hommes reviennent, seuls ou soutenus par un camarade, pâles, l'uniforme défait, le regard fixe, la bouche entr'ouverte. L'un boite, l'autre a la tête bandée, un autre soutient son bras. Ils vont debout, là où les autres se penchent, comme si une première blessure assurait l'immunité. Quant aux morts, ils sont rejetés sur le parapet, le long des boyaux.

De l'autre côté de l'eau, il y a des cadavres allemands. Près de nous s'en trouvent trois. Ils portaient des passerelles. Ils sont tombés sur le talus. Les poutres écrasent leur capote grise.

Depuis ce matin, les communications avec l'arrière sont interrompues, aussi sommes-nous obligés d'entasser les blessés dans les abris. Bientôt tous les refuges qui avoisinent le poste se remplissent. Il faut tirer les hommes par les épaules et les pousser pour les faire entrer par les petites portes.

Il y a là des mourants. Ceux qui sont atteints plus légèrement se recroquevillent dans leur coin, pour éviter les gestes désordonnés de ceux qui n'ont plus connaissance et se débattent contre la mort. Un grand grenadier, le crâne fracassé, a le cerveau qui fait une énorme hernie dans son pansement. Il est couché sur le dos, inerte, les mains crispées, les yeux et la bouche ouverte. Un autre, qui a le bras presque détaché du tronc, à moitié nu sous sa capote rejetée, dans une continuelle agitation, se roule de droite à gauche et écrase son bras ensanglanté.

— Appuyez.

Il y a encore de la casse là-bas. Il faut d'autres hommes pour rester stoïques sous les feux convergents, pour demeurer derrière les remblais hâtifs, à guetter, à attendre. Il faut aller à pas lents, sous les balles. Les obus s'écroulent. Leurs éclats ronronnent dans l'air. Parfois ils éclatent parmi les sacs et creusent des brèches déchiquetées et sanglantes.

Une salve de 75 tirée trop court, vient nous tuer six hommes. Ceux qui étaient près d'eux les relèvent.

— Appuyez, appuyez encore.

Au dessus de tout ce saillant qui s'avance dans les lignes ennemies plane un nuage de poussière. Et quand les bombes éclatent dans le canal, il s'élève une trombe d'eau toute blanche et scintillante qui retombe en pluie. Le sol tremble et des coups sourds se propagent dans la terre. Les murs de sacs paraissent prêts à s'effondrer.

Dans les abris, les blessés continuent à s'entas-

DE GRENADIERS TE STEENSTRAAT

ser. Il faut courir de l'un à l'autre. Celui dont le bras était presque arraché est mort, le grand grenadier aussi. Leur dépouille orne les parapets et le corps demi-nu de l'un semble encore tourmenté par la souffrance. Un zouave, plus loin, est caché sous une couverture. Ses souliers ferrés la dépassent. Les pieds des morts semblent toujours énormes.

D'autres suivront. Dans l'abri de nos infirmiers, un caporal du 4e zouaves souffre avec courage. Une balle lui a traversé l'estomac. Il est sur la paille. Nos infirmiers lui parlent et le consolent.

À chaque visite, c'est partout la même demande anxieuse :

— Les brancardiers vont-ils venir? Vous n'allez pas nous laisser là?

Dans la tranchée, monotonément, à intervalles réguliers, l'ordre passe de bouche en bouche :

— Appuyez. Appuyez.

Encore un pas vers la mort.

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L'attaque est enrayée, l'ennemi ne progresse plus. La nuit notre régiment est relevé, mais nous restons avec les blessés qui n'ont pu être emportés et quelques hommes que l'ordre de relève n'a pu atteindre. Des obus tombent en pluie au milieu des terriers qui nous servent d'hôpital. Ce sont les derniers soubresauts, les derniers cris de la bête furieuse. Vers le soir, nous partons avec les deux derniers de nos grenadiers, deux épaves qui sûrement vont mourir en route.

Toute notre petite troupe s'en va en longeant la tranchée, par derrière le redan. Le sol est troué comme une écumoire. Pourtant, au fur et à mesure que nous avançons, nous arrivons dans des régions plus épargnées. Voici que finit le secteur du régiment, les uniformes des carabiniers apparaissent.

Le jour est clair encore, il faut attendre avant de nous engager dans la campagne. Nous nous asseyons un instant entre les tas de sacs, las, les membres détendus, loin du bruit enfin, mais heureux. Les pertes ont été fortes, mais le régiment n'a abandonné aucune de ses positions. La vague furieuse est venue mourir et se briser contre lui. Grâce à sa résistance, la ligne, qui était percée à notre droite, a pu être reconstituée. L'attaque allemande, malgré les moyens employés, malgré les succès du début, a abouti à un échec sanglant.

Ici, le calme règne. En attendant l'heure de partir, nous regardons l'endroit d'où nous venons, l'enfer dont nous sommes sortis. Les hommes, deux par deux, s'en vont, emportant leurs camarades mourants, victimes de la gloire. Là-bas, la tranchée disparaît dans un nuage de poussière épaisse, que les déflagrations dispersent dans l'air du soir.
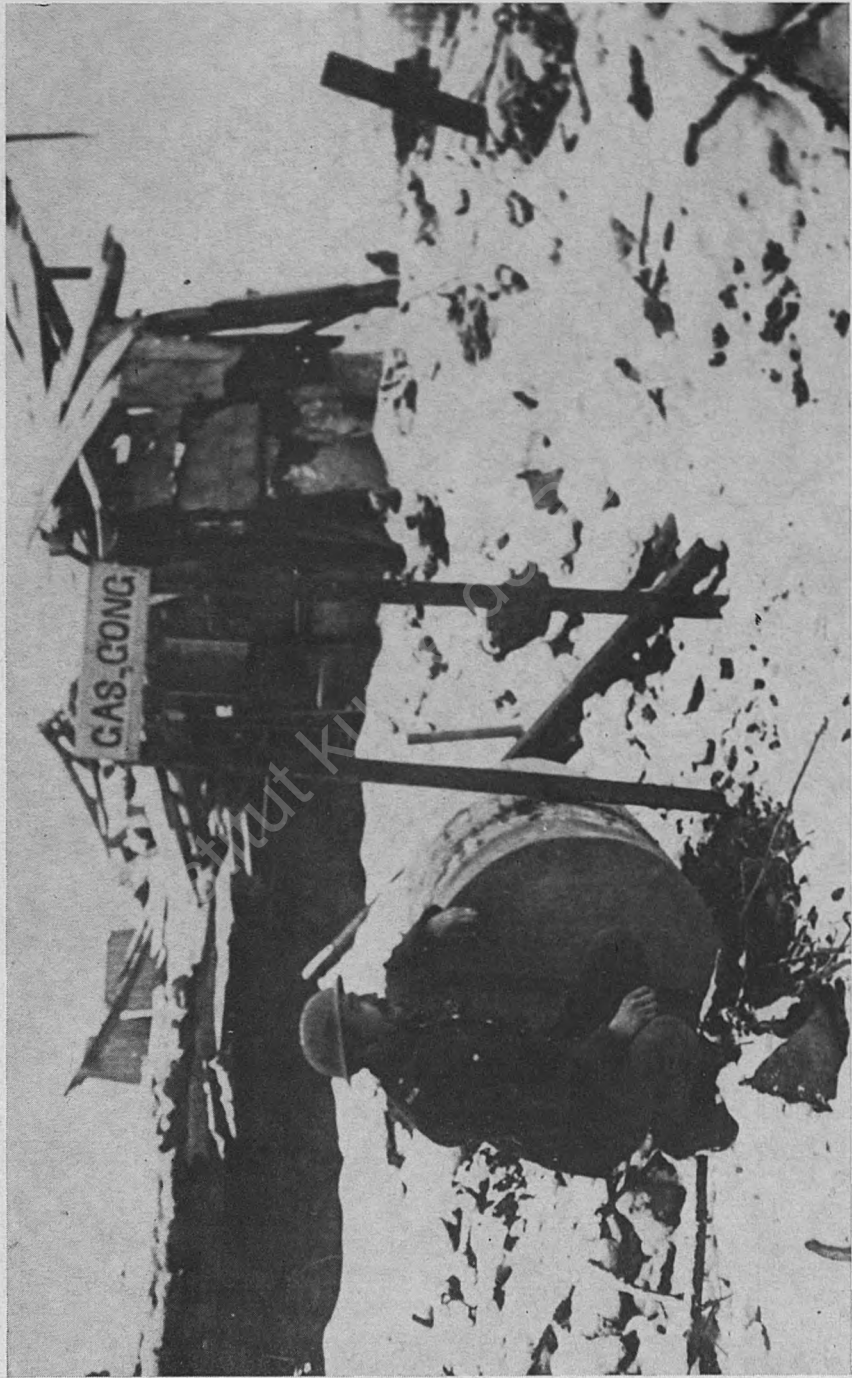
SECT. ALLEMAGNE
GUERRE 14-18
PROTECTION CONTRE GAZ
MUSEE DE L'ARMEE
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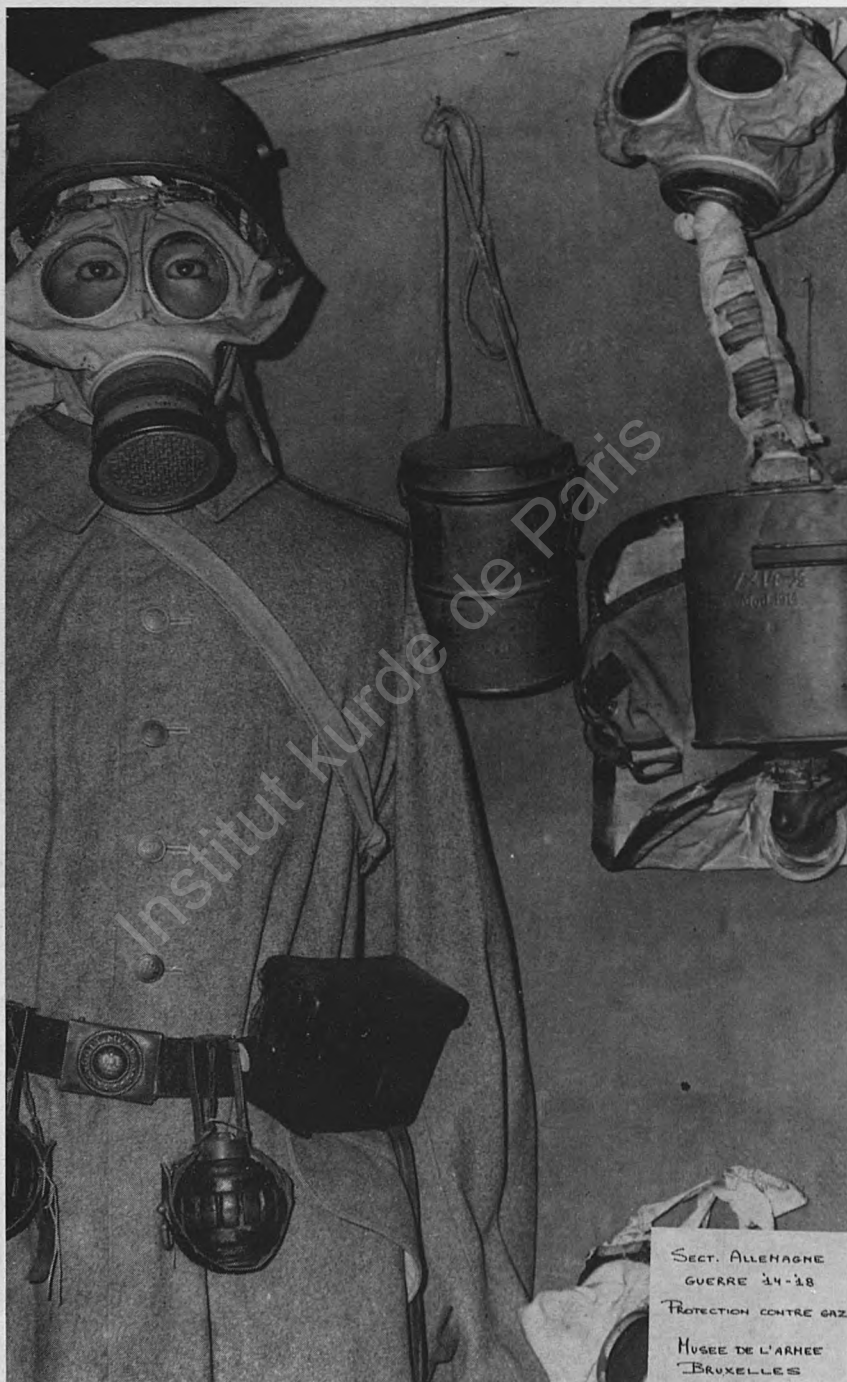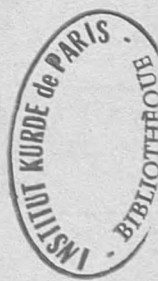
Human and Medical Factors

Analysis of snow samples
contaminated with chemical warfare agents

by B.A. JOHNSEN and J.H. BLANCH

Norwegian Defence Research Establishment, Division for Environmental Toxicology,
N-2007 Kjeller, Norway

SUMMARY.

Snow samples contaminated with chemical warfare agents such as nerve agents (sarin, soman, VX, tabun), mustard gas, irritating agents (CS, CN, DM), T-2 toxin and a mixture (1:1) of nerve agent precursors (methylphosphonyl dichloride and methylphosphonyl difluoride, didi precursor) have been analysed after outdoor exposure for 2 and 4 weeks under normal Norwegian winter conditions. The agents do not penetrate deeply into the snow so the main portion of the agents are recovered in the upper layer of the snow. After 2 and 4 weeks all agents except mustard gas and tabun were still present in concentrations sufficiently high for positive verification. Mustard gas and tabun could be verified after 2 weeks but after 4 weeks the concentration was below the detection limit of the method. It has further been demonstrated that snowfall covering the samples has a preserving effect due to less evaporation. This was demonstrated by the higher recoveries of all agents and especially by the finding of mustard gas even after 4 weeks. The possibility of positive identification of the nerve agents sarin and soman was increased by verification of the hydrolyzed products, isopropyl methylphosphonic acid and pinacolyl methylphosphonic acid respectively. These compounds are relatively stable, and were present in large amounts even after 4 weeks. In addition, sarin and soman usually contain impurities of diisopropyl methylphosphonate and dipinacolyl methylphosphonate. The evaporation and decomposition rates of these compounds are low, and they were recovered in large amounts even after 4 weeks.

Increased droplet size increases the possibility of positive identification of mustard gas, but this was found to have little effect for sarin.

For added realism, a CS grenade was discharged and snow samples were collected and analysed. CS was verified in all samples as far as 70 m downwind and as long as 4 weeks after dissemination.

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INTRODUCTION.

The use of chemical weapons in warfare has been prohibited by international law since the protocol prohibiting the use in war of poison gas and bacteriological weapons was signed at Geneva in 1925. Since then, however, several countries have been accused of violating the treaty. The difficulty of chemical agent identification is the critical problem in substantiation of these charges. Unless these weapons are used on a massive scale, compelling analytical evidence is generally difficult to obtain due to the large number of different compounds that may be used as chemical weapons. Chemical weapons are effectively used primarily against unprotected personnel, and all reports of their use are against unprotected or poorly protected personnel. Military objectives may be achieved with relatively small amounts of agents, thus demanding sensitive analytical methods for positive identification. Further, many of the agents are highly volatile, and on a battlefied, the amount of residual agents to be found and sampled is often very small. The agents will be spread all over the ground, and will therefore be present in low concentrations. In most cases deterioration occurs rapidly due to instability and decomposition. To verify if collected samples contain chemical warfare agents, to identify the agent, and to determine the quantity requires highly selective and sensitive analytical methods. Therefore the analysis itself must generally be performed in a well equipped laboratory by trained personnel.

EXPERIMENTAL.

The experimental work was done partly outdoors, and partly in the laboratory. The work performed outdoors was preparation and collection of samples. The investigations were based on a scenario in which the chemical agents, nerve-, mustard- and irritating

agents, mycotoxins and precursors, have been used at a low concentration (0.25 gm/m^2). This is the amount expected to be used against unprotected troops or civilians. After outdoor exposure the samples were brought to the laboratory for analysis.

Field experiments.

The field experiments were designed to include what might be expected to happen in a real chemical attack situation. Small samples (1 mg) of chemical warfare agents were placed on top of the snow surface, and samples were taken for analysis after 2 and 4 weeks. To examine the penetration of the agents into the snow the samples were divided into horizontal layers which were analysed separately. The effect of snowfall immediately after an attack was simulated by covering the samples with a snow layer of 5 cm immediately after application. A large number of chemical compounds are potential chemical warfare agents. For practical reasons the number of agents to be tested had to be restricted, and the agents chosen were the following :

1. Ethyl N,N-dimethylphosphoramidocyanidate (GA or tabun).
2. Isopropyl methylphosphonofluoridate (GB or sarin).
3. 1,2,2-Trimethylpropyl methylphosphonofluoridate (GD or soman).
4. Ethyl S-2-diisopropylaminoethyl methylphosphonothiolate (VX).
5. Bis(2-chloroethyl) sulphide (HD or mustard gas).
6. 2-Chlorobenzalmalononitrile (CS).
7. α-Chloroacetophenone (CN).
8. 10-chloro-5,10-dihydrophenarsazine (DM or adamsite).
9. Mixture (1:1) of methylphosphonyl dichloride and methylphosphonyl difluoride [didi, precursor-analysed as dimethyl methylphosphonate (DMMP)].
10. 4β,15-diacetoxy, 8α-(3-methylbutyryloxy)-3α-hydroxy-12, 13-epoxytrichothec-9-ene (T-2 toxin).

To increase the possibility for verification of sarin and soman the analyses of samples containing these two agents are extended to include the hydrolysis products isopropyl methylphosphonic acid and 1,2,2-trimethylpropyl methylphosphonic acid. In addition it is known that production of sarin and soman give up to 20 % of the byproducts diisopropyl methylphosphonate and di(1,2,2-trimethylpropyl) methylphosphonate respectively.

Therefore, samples containing a mixture of the agent and its impurity were analysed after outdoor exposure with respect to the agent itself, the impurity and also to the hydrolysis product.

Experiments were also carried out for some of the agents to study the effect of increased droplet size on the persistency of the chemical warfare agents.

To make the experiments as realistic as possible, a pyrotechnic tear gas grenade containing 250 g CS was disseminated. The experiment was carried out in an enclosed military forest area. At the time of dissemination, the ground was covered with a snow layer having a thickness of approximately 10 cm. Snow samples were taken at increasing downwind distances (1, 2, 3, 5, 7 and 10 m) 30 minutes after setting off the grenade. The samples consisted of snow taken from the upper 2-3 cm of a 10 × 10 cm area using plastic spoons. Additional samples were also taken after 7, 14, 20 and 29 days. The results of the analysis of the first samples showed higher amounts of CS than originally expected, and additional samples were therefore collected as far as 70 m downwind. During the whole experimental period, precipitation was marginal (about 2 cm snow), and the snow disappeared gradually by evaporation and melting. After 29 days, snow remained only in scattered patches, and it was therefore possible to take only a few samples.

There have also been carried out experiments in order to gain practical experience in the subject of sample handling. The experiments took place at Banak in the far north of Norway about 1,400 km north of the laboratory at Kjeller. Four samples of sarin, mustard gas and 2-chlorobenzal malononitrile (CS) were prepared and left overnight to outdoor exposure. On the following day the samples were collected and each agent was treated according to four different procedures.

One sample of each agent in a glass sampling bottle was put in a polystyrene box together with dry ice (solid carbondioxide) and thus kept at a temperature below —20°C. Another sample of each agent was transferred to a sampling bottle and put in a separate polystyrene box, without any artificial cooling. The third sample of each agent was kept in a sampling bottle without any special precautions. The last sample of each agent was melted in a sampling bottle by immersing it in hot water made on a simple commercial butane stove. When melted, the agents were extracted with 5 ml chloroform. The chloroform was transferred to sampling tubes and sodium sulphate was added. The

samples were transported to Oslo by an armyaircraft and **to Kjeller** by car.

Sample preparation.

The snow samples to be analysed were immediately melted in the laboratory at room temperature. After melting, the volume of the samples varied from 100 to 150 ml. The total volume was transferred to a separatory funnel and extracted with 5 ml chloroform (all compounds except methylphosphonic acids) or with 5 ml 0.02 M trioctylamine in chloroform (methylphosphonic acids). If necessary the extracted compound was derivatized before analysed by gas-chromatography, mass-spectrometry or high performance liquid chromatography. It is important that the agents can be extracted as soon as possible when melted, since most of the agents are easily hydrolysed.

RESULTS.

The agents ability to penetrate the snow layers differed from one agent to another and seems to be connected both to the volatility and solubility of the different agents. Hydrolytic stability is also an important factor.

The experiments, however, showed that the agents did not particularly tend to migrate into the snow. This means when collecting snow samples it is not necessary to collect more than the upper 3 cm.

The results show further that snowfall has a preserving effect on the agents and in all cases made it easier to verify the presence of chemical warfare agents (table I).

If diisopropyl methylphosphonate (DIPMP) and di(1,2,2-trimethyl-propyl) methylphosphonate (DTMP) are present as impurities in sarin and soman, respectively, they are useful in verification since they are relatively stable and easily analysed after 4 weeks.

When sarin and soman are hydrolysed they form isopropyl methylphosphonic acid and 1,2,2-trimethylpropyl methylphosphonic acid and these compounds have also shown to be stable and are easily verified as isopropylmethyl methylphosphonate (IPMMP) and methyl-1,2,2-trimethylpropyl methylphosphonate (MTMP), respectively, even after 4 weeks. Thus both the impurities and the

TABLE I

Total amount of agent in per cent of the applied amount (1 mg)
found 14 and 28 days after application to different snow samples.
Half of the samples were covered with snow immediately after application

Time (days)	Total amount of agent found in sample (%)			
	Uncovered	Snowcovered	Uncovered	Snowcovered
Agent:	VX		GA	
14	••	—	•	—
28	••	—	0	—
Agent:	GB		GD	
14	•	••	••	••
28	•	•	•	••
Agent:	HD		T-2 TMS	
14	•	•	•••	•••
28	0	•	•••	•••
Agent:	CS		CN	
14	•••	•••	•••	•••
28	•••	•••	•••	•••
Agent:	DM		DMMP	
14	•••	•••	•••	•••
28	•••	•••	•••	•••

••• 10-100 %.
•• 0.1-10 %.
• < 0.1 %.
0 not detectable.
— not analysed.

hydrolysis products can be valuable in the verification of sarin and soman.

The analytical results of the experiments with a mixture of sarin and diisopropyl methylphosphonate (4:1) and with a mixture of soman and di(1,2,2-trimethylpropyl) methylphosphonate (4:1) are shown in table II.

For mustard gas, increasing droplet size was postulated to increase both stability and recovery. The results showed that this was true and that larger droplets both evaporate and dissolve slowly in water.

The nerve agents are more easily dissolved in water and the recovery is not influenced by increased droplet size (table III).

The analytical results of the experiment using a CS grenade is further shown in table IV. Analyses verified the presence of CS in all samples even 29 days after dissemination and as far as 70 m downwind.

TABLE II

**Total amount of agent, impurity and hydrolysis product in per cent
of the applied amount (1 mg) found 14 and 28 days after application
of a mixture (4 : 1) of agent and impurity to different snow samples**

Time (days)	*Total amount of compounds found in sample (%)*		
Compound	*GB*	*DIPMP*	*IPMMP*
14	•	•••	•••
28	•	•••	•••
Compound	*GD*	*DTMP*	*MTMP*
14	••	•••	•••
28	•	•••	•••

••• 10-100 %.
•• 0.1-10 %.
• < 0.1 %.

TABLE III

**Total amount of agent in μg found 14 days after application
of different amounts of agents**

Droplet size	*GB (μg)*		*HD (μg)*	
	Uncovered	*Snowcovered*	*Uncovered*	*Snowcovered*
2	0.001	0.006	3	34
4	0.013	0.165	80	672
6	0.008	0.014	1587	3151
8	—	—	960	2688

TABLE IV

**Total amount of CS found in snow samples taken at increasing distances downwind
and at different time intervals after the discharge of a CS grenade.
Entries marked (—) signifies that no samples were taken**

Distance (m)	*Total amount of CS found in sample (μg)*				
	1	*7*	*14*	*20*	*29 (days)*
1	230	470	300	44	—
2	100	110	160	19	—
3	30	13	33	4	4
5	6	9	7	3	—
7	4	2	3	2	—
10	18	2	2	1	—
13	—	0.7	1	0.15	0.4
20	—	0.8	0.6	3	0.15
35	—	2	2	0.4	0.2
50	—	—	0.3	—	—
70	—	—	—	—	0.3

In table V the analytical results of the different procedures in sample handling are shown.

For CS the handling procedure is not important, because CS is relatively stable in a water solution.

<div align="center">TABLE V</div>

<div align="center">Comparable recoveries after different procedures of sample handling</div>

	GB (%)	HD (%)	CS (%)
Chloroform	100 (300 μg)	100 (413 μg)	100 (826 μg)
Polystyrene/CO$_2$	100	35	100
Polystyrene	86	13	99
No precautions	52	9	98
Water 20°	7	0.006	79

GB was recovered in high quantity even when no precaution was taken but when kept in water at $+20°C$ the recovery was below 10 %. For mustard gas the recovery is much lower. It is 9 % when transported with no precautions but very low when kept in water at $+20°C$.

CONCLUSIONS AND RECOMMENDATIONS.

The experiments carried out during the last two winters have clearly shown that under winter conditions there is a large variation in the stability of different chemical warfare agents. This will markedly influence the possibility of verification of use of chemical warfare agents by means of chemical analysis of snow samples taken some time after the alleged attack. Of the agents investigated the following are relatively stable :

— The physical incapacitating agents CS, CN and DM.
— The immediate decomposition product of the didi precursor.
— The mycotoxin T-2.
— The nerve agent VX.

For these compounds, except for VX, one expected that at least 25 % of the original agent is still available for analysis in samples taken as long as 4 weeks after the attack. VX is slightly less stable, with a recovery between 1 and 10 %. Very selective and sensitive analytical methods are available for all compounds and there would be no difficulties in verifying the presence of

these agents after a chemical attack under winter conditions even after 4 weeks.

The nerve agents GA, GB, GD and the blister agent HD were found to be markedly more unstable. After 2 weeks, generally less than 0.1 % of the applied agents were still present in the samples. The analytical methods used are, however, very selective and sensitive, and verification of use by chemical analysis of snow samples would be most likely. After 1 month, it was still possible to analyse sarin and soman, but the content of mustard gas and tabun was below the sensitivity limit of the method. The amount of nerve agents still left in the samples were in the order of 1/100000 of the original amount. The verification of use of sarin and to an even larger extent mustard gas and tabun is uncertain and highly dependent upon the weather condition. High temperature and strong wind is unfavourable to positive verification. As expected, a snowfall covering the samples reduces evaporation, and increases the possibility for verification. This was confirmed by the experiments and was specially important for the agents GB, GD and HD. Under this condition it was also possible to detect and analyse HD after 4 weeks.

Verification of sarin and soman was improved by analysis of their hydrolysis products and also by analysing the impurities formed in the production of sarin and soman. In the case of mustard gas it was found that the size of the droplet had large influence in verification of this agent.

In sample handling the use of immediate extraction or transportation in polystyrene/CO_2 are the methods recommended in the handling of G-agents and mustard gas. A general advice in sample handling is to keep the samples at as low temperature as possible.

*
* *

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˙ This paper is based upon results from the following reports :

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Herbicides, miracle agents or?
Fatal Danish cases

by A. STEENTOFT and B. KAEMPE

Institute of Forensic Chemistry, University of Copenhagen,
Copenhagen, DK Denmark

SUMMARY.

Herbicides, weed killers, have improved the production of corn in the agricultural countries and thus probably decreased the famine in the developing countries. The question is if the compounds have been worth the price. Can the advantage compensate for the human tragedies seen in the wake of the compounds. Here is referred to the occurrence of side products under the syntheses (dioxin in Seveso) removing of the remaining stocks of herbicides (Blast of the BT-kemi in Sweden) with the possibilities of formation of dioxins or simply an accumulation of poison in the nature or not to forget the misuse under warfare (Vietnam).

The Danish cases of suicide, accident and a case of attempted homicide with herbicides since 1960 are described. The cases have been investigated in detail for signs of poisoning, autopsy findings, chemical analyses. The results of the forensic chemical examination are evaluated according to values from the literature. A possible prevention is discussed.

Key words : Herbicides, 2.4-D, 2M4K, paraquat, allyl alcohol, atrazine, dinoseb. Fatal cases.

INTRODUCTION.

Herbicides are chemicals for destruction of noxious weeds. They can be classified according to their chemical configuration into following groups (8) :

I. INORGANIC (arsenites, chlorate, sulphuric acid).

II. ORGANIC Compounds.

A. Chlorinated phenoxy-acids (2.4-D).

B. Chlorinated aliphatic acids.

C. Carbamates and allyl alcohol.

D. Substituted ureas.

E. Triazines (atrazine).

F. Substituted phenols (dinoseb).

G. Bipyridyls (paraquat).

H. Miscellaneous.

Another classification is in relation to their selectivity and distribution in the plant.

A. I. *Selective :*

Growth hormones (2.4-D).

Phytotoxic (Chlorinated aliphatic acids).

Selective absorptions from soil (Dinoseb).

II. *Non selective :* (Kill all vegetation).

Free radical react. (Paraquat).

Allyl alcohol.

Inorganic compound.

B. *Distribution :*

Contact herb. : active only at the site of application.

Translocated herb. : distributed throughout the whole plant.

Residual herb. : are spread on or in the soil and are effective mainly against germinating seeds.

Because plants differ markedly from man in their morphology and physiology it might be expected that herbicides would present little hazard of chemical toxicity to man. However fatal poisonings in man have occurred.

In the following 7 danish fatal human cases and 1 case of attempted murder will be described in relation to autopsy findings, forensic chemical analyses and the toxicological evaluation.

Prevention of deaths (suicide and accident), pollution of the environment is discussed.

CASE REPORTS.

Case 1.

A 23 years old man was found dead (1961) with signs of convulsions before death.

Autopsy shows organs with acute congestions and a peculiar odour from the stomach content.

Microscopy shows brain with degenerative changes of the ganglion cells.

Forensic chemistry : extraction at acid reaction with chloroform. The chloroform extract was washed with phosphate pH 7.3 and then extracted with borate pH 10.6 for UV-spectrophotometry, m.p. and colour reaction.

Toxicology (19) : suicide with 2.4-D (Agent white) (7, 11, 20, 25, 26).

Case 2.

A 47 years old man was found dead (1970).

Autopsy shows liver, brain and lungs with acute congestions.

Microscopy shows trombangitis obliterans Buerger.

Forensic chemistry : as for case 1 supp. with thinlayer chromatography.

Toxicology : suicide with 2M4K (11).

Case 3 (14).

A 39 years old man was hospitalized (1984) but died 4 days later.

Autopsy ?

Forensic chemistry : aspirate is analysed as described for case 2 supp. with gas chromatography.

Toxicology : suicide ? with 2.4-D.

Case 4 (13).

A 40 years old man suddenly began to vomit (1974).

Physician diagnosticated a jaundice ? but the patient declared to have taken 8 prednisone tablets. Presumably 48 hours after drinking 25 ml allyl alcohol, he died.

Autopsy shows stomach content with a pungent odour.

The stomach was swollen with hyperaemic mucosa.

Liver and kidneys were swollen, pale and soft.

Lungs and brain showed oedema.

Microscopy : the liver showed typical peripheral zonal necrosis.

Forensic chemistry : blood : direct gas chromatography.
Toxicology : suicide with allyl alcohol (war gas).

Case 5.

A 45 years old man was found dead (1983).
A can of Agro Atrazine was found at the finding place.
Autopsy : in the respiratory organs and oesophagus a white viscous fluidum was found (looked similar to the content in the can).
Forensic chemistry : extraction at own pH-reaction with dichloromethane. Gas chromatography and thinlayer chromatography.
Toxicology : suicide ? with atrazine.

Case 6 (22).

A 57 years old woman was hospitalized (1980) with acute dysphnoea, cyanosis, confusion, restlessness, sign of stasis pulmonis. Eight days before she had swallowed a drop of « gammel dansk ». Afterwards she vomited and got diarrhoea.
Died 8 days later.
Autopsy : lungs were heavy, grey with honeycombphenomenon. Liver and kidneys were pale and swollen.
Microscopy : lungs with fibroblastproliferation and collagen in alveolae.
Forensic chemistry : urine (15 days after the intake).
Column chromatography and colour reaction with dithionite (4).
Toxicology : accident with paraquat filled in a wrong bottle (5, 23, 24).

Case 7.

A 47 years old woman was hospitalized (1983) suspected of swallowing a 3 times lethal dose of paraquat.
Treated with haemoperfusion but died 3 weeks later.
Autopsy ?
Forensic chemistry : as for case 6 supplemental with high pressure liquid chromatography on urine and plasma (12).
Toxicology : suicide ? with paraquat.

Case 8.

A dog ate some roasted hen. 90 min. later :
Vomiting, strong perspiration, convulsions and death.
The hen was intended for a married couple, but the man found the taste bitter and the colour peculiar yellow.
Autopsy of dog : no characteristics.

Forensic chemistry of the hen : extraction at acid reaction **with**
ether, evaporation and resolution in water. The water was made
alkaline and washed with chloroform, then made acid and extract-
ed with chloroform. Paperchromatography and UV-spectrophoto-
metry showed 2 sec-butyl-4,6-dinitrophenol (dinoseb).
Dog : liver and intestinal content showed no signs of dinoseb.
Results : attempted murder with dinoseb added to roasted hen
of son-in-law (28).
In table I is the concentration of the herbicides in organs given
for 7 of the above described 8 cases.

TABLE I

Concentration of herbicides in organs and manner of death
in 7 cases of fatal poisoning

Case No.	Age	Sex	Compound	μg/g or ml				Manner of death
				Stomach	Blood	Liver	Urine	
1	23	m	2.4-D	7770	669	183	264	Suicide
2	47	m	2M4K	2450	126	288		Suicide
3	39	m	2.4-D	(300)				Suicide ?
4	40	m	Allyl alcohol		< 10			Suicide
5	45	m	Atrazine	54650	20	79	2	Suicide ?
6	57	f	Paraquat				3	Accident
7	47	f	Paraquat		(< 0.04)		2.6	Suicide ?

DISCUSSION.

The discovery of *death owing to herbicides* is often difficult.

By possible performance of an autopsy the presence of herbicides
with peculiar odour such as allyl alcohol, amines of 2.4-D may be
disclosed by a simple nasal analysis.

Far more difficult is it in cases concerning intake of odourless
compounds such as paraquat and inorganic herbicides.

Therefore, a tracer should be added to preparations of these
compounds.

Paraquat, one of the most frequently used herbicides, causes
typical changes of the lungs but these changes will first appear
after some days latent period (6, 21) : the hit and run effect.

The initiate symptoms as vomiting are too unspecific. Findings of
high body temperature or rapidly produced rigor mortis (18) could
suggest an intake of nitrophenols. Securing of bottles on the find-
ing place is also important in clearing up the case. A simple

analytical chemical screening for the herbicidegroup is not possible, because the group of herbicides according to acid/basic properties and solubility is so heterogeneous that an isolation (10) of all herbicides would be very difficult and time consuming. Additionally should be mentioned interference from putrefactive compounds by the colorimetric analysis (27), UV-spectrophotometry (16) and gaschromatography (15). Spot test can initialy trace some compounds as paraquat/diquat (17).

Another serious problem concerning herbicides is their pollution of the environment using difficult subverted compounds and preparations containing very toxic by-products (dioxin) in the production of the herbicides (9). In addition removing of surplus stocks or closing of factories for herbicides production can result in pollution from new poisons formed by burning [2.4.5.-T, Agent Orange (2, 26) forms dioxins by heating (3)].

Misuse of herbicides as defoliants in war is another source of pollution. Pollution of the ground water by percolation of herbicides from buried drums, dumping grounds or overdose of herbicides in agriculture forms likewise a danger for a *chronic poisoning of the population* in a most injurious way leading to irreparable injuries on the central nerve system (2.4-D) (1).

Prevention of accident with herbicides can be done by :

Enforcing the prohibition of removing the original labels.

Enforcing the prohibition of decanting the poison to another bottle. Adding tracers to odourless herbicides.

Prevention of suicide by i.e. paraquat can be done by common informations of its hit and run effect. Unfortunately this information may increase the misuse of paraquat to remove undesirable persons. Selective permissions for using the herbicides exclusively in agriculture and by special trained people only and only allowing sale of herbicides in great portions.

Prevention of pollution of nature by prohibition of the use of difficult decomposing herbicides and misuse under war.

Removing of surplus stock and obsolete factories should be done in accordance with special trained experts only.

Prevention of pollution of the food chain by proper use of the herbicides. More control by authorities of the compounds taking part in the syntheses or formed as by-products.

Support to Green peace for their admirable work in the field of combating pollution.

CONCLUSION.

Poisonings by herbicides are often difficult to trace by autopsy or common forensic chemical screening. The number of suicides and accidents discovered using herbicides are relatively limited but may be further reduced by information about the development of the poisoning (hit and run) and by emphasizing the right handling of the herbicides. The pollution of the environment can be reduced by diminishing the use of herbicides and prohibition of the misuse in warfare.

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Plasma technology
in war hight production of toxic gas

by G. PROUVEUR

Industrial Research Laboratories Liège, Belgium

SUMMARY.

With the advent of plasma technology, an adequate heat source is now available for the attainment of high temperatures. The plasma rotary furnace and particularly the plasma rotary furnace with superposed electric current are examples of efficient devices using plasma as a heat source, $10.000/15.000°K$. This type of furnace is made of two main parts : a static part and a cylindrical part rotating inside the static part and containing particulate material to be fused : dioxine, combat gas, $C-Cl$, $COCl_2$ (...).

After 15 min. max. the plasma arc breaks the high toxicity of industrial waste. But, a great danger exists with the plasma technology : production of combat gas.

It is very simple for such a Company to receive industrial waste from chemical industry, oil products... and if the process work with 99,99974 % of success to break a toxicological molecule, it is possible for the Company to inverse the identical process and sale high toxic gas, for a weak price.

I. THE COMPANY.

IRELAB is a technical department since 1976, March 05, of SBID sprl. Liège, BELGIUM.

In 1984, the department started as a new Company : IRELAB SA EUROPE (capital 10.800.000 FB) at MOUSCRON (offices and plant) ; NAMUR (offices and laboratories) ; LIEGE (offices) and will this year too start at TAMPA — Florida — USA the IRELAB CORPORATION.

Technology and Departments.

Plasma rotary furnace, gun and spray.

1. *Testing of materials :*

— Metallurgy.
— Steel.
— Refractory and Composites.

2. *Military :*

— Aviation.
— Combat gas.
— Blindage.

3. *Oil :*

— prospection and extraction.
— Refinery.

4. *Industrial waste :* destruction.

5. *Biotechnologies :*

— Pharmacological products.
— Biometallurgy.
— Toxicology.
— Biology.

II. **THE PLASMA ROTARY FURNACE.**

With the advent of plasma technology, an adequate heat source is now available for the attainment of high temperatures. The plasma rotary furnace and particularly the plasma rotary furnace with superposed electric current are examples of efficient devices using plasma as a heat source.

This type of furnace is made of two main parts : a static part and a cylindrical part rotating inside the static part and containing particulate material to be fused.

Heating is achieved by means of an electric current carried by a dc plasma beam generated by two plasma jets fixed at the ends of the cylindrical rotary part.

The plasma acts as a gaseous resistor. Water sprays fixed to the static part cool the external wall of the rotary part.

The inside lining of the furnace is made of either a suitable refractory material or the same material as the melt. The distribution of the melt on the inside lining of the furnace depends on the rotating speed of the furnace. Temperatures : 3.000°C (see fig. 1).
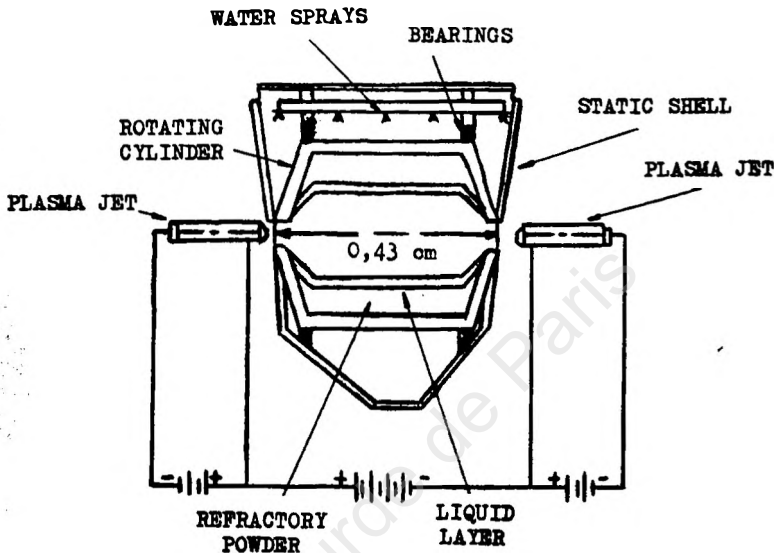
FIGURE. — Plasma rotary furnace.

III. **INDUSTRIAL WASTE.**

Form :

— liquid,
— solid,
— gas.

Industries :

— Textile.
— Chemical.
— Nuclear.
— Metallurgy.
— Alimentary.
— Oil-industry.
— Research center's.
— Military industries.

IV. **ANNUAL PRODUCTION IN EUROPE.**

For West Europe alone :

— 1 billion (toxic and non toxic).
— 10 million ton (toxic).
— 5 or 7 million ton (toxic).

This last point, 5 or 7 million ton, is the industrial annual production. What production : — dioxines — pesticides — combat gas as yperite and others — phosgène ($COCl_2$) — hydrocarbures and all the toxic products in the repertory of the Law. The plasma technology is the best system today in the World for a complete destruction with great success : 99,99974 %.

For some of the experiments reported herein, the rotating speed of the furnace was reduced so that the melt remained at the bottom of the rotor and the inside lining. In previous works on plasma furnaces of this type, the rotating speed was high and the melt was spread as a fairly uniform layer on the lining.

V. **RESULTS.**

Two types of materials were melted in the plasma furnace : dioxine and a mixture containing several toxic elements. Dioxine was used for preliminary experiments to evaluate the operational characteristics of the furnace. The mixture was melted after modifications were made on the furnace following experience with dioxine.

For the experiments with dioxine, the plasma jets were disposed at 3 cm from the circular end openings of the rotor. It should be noted that with this arrangement there are considerable heat losses through the end openings. The furnace using argon, nitrogen, hydrogen or other gas (5 at 30 l/min.) during 15 minutes, and 10,000 at 15,000°K temperatures. 60 kg toxic material is treated by plasma arc in 1 hr.

The average energy consumption was 0.53 kW/kg to 2.4 kW/kg ; identical consumption if the furnace attained 1 MW.

Following the experiments with dioxine, modifications were made on the furnace to reduce heat losses through the end openings and to allow melting on a continuous basis, in addition to batch operation. To achieve continuous melting, a gravimetric system that can feed 60 kg/hr of particulate material Into the furnace

was added. The speed of the rotor was also reduced from 375 to 2.3 rpm. With this low speed of rotation, the fused material remains at the bottom of the furnace and sweeps the internal surface of the refractory lining instead of forming a cylindrical layer around the central cavity of the rotor, as with high speeds of rotation. With the former level of rotation, the thermal efficiency of the furnace is better because of the absence of a thick cylindrical liquid layer, which prevents heat transfer from the plasma resistor to the solid material to be melted. The plasma jets were also fixed closer to the end openings of the furnace and installed so as to reduce heat losses through these openings.

For melting the mixture, the cavity of the rotor was not filled before the furnace was started, as for dioxine, but the material was introduced into the cavity by means of the gravimetric feeding system after the arc was stabilized and after a preheating period of 6-8 min. to increase the temperature of the inner surface of the lining to 2,300°C. The initial diameter of the internal cavity of the refractory lining was 15 cm. Feedings rates of 200 and 300 g/min. were used.

The difference between the fed and the collected quantity was only several percent. The furnace was tilted at 15° during the entire process. The liquid was finally cast into an insulated graphite mold. The average energy consumption was 9.1 kW/hr/kg. The consumption decrease with increasing size of the batch or ingot and appears to also decrease with increasing feeding rate because of a reduction of the total melting time.

Continuous melting, and then the destruction of the toxic element, was also achieved. For these experiments the furnace was fed at various rates and was kept tilted at 20° so that the liquid flowed continuously out of the furnace. The preheating period to 2,300°C lasted only 4 min. since the larger diameter of the cavity was only 11 cm. The lowest energy consumption for melting, destruction and casting the toxic mixture on a continuous basis was 3.3 kW-hr/kg.

These results were obtained at a total power of 100 kW, with cathode and anode power levels of 15 kW each, a cathode gas flow rate of 15 l/min. of argon, and anode gas flow rate of 15 l/min. of nitrogen, a feeding rate of 500 g/min., a —28+35 mesh material, and a tube feeding the material at the top of the arc column at 45° with respect to both the vertical and

horizontal axes. The total duration of the experiments was limited to 15 min. since larger samples were not required.

The best furnace efficiencies were obtained with finer particle size materials, but plugging of the feeding tube occurs with these materials.

Despite the small scale of operation, it appears that the plasma-arc rotary furnace with the feeding system described above is an efficient tool for reactions requiring temperatures above 2,000°C. It offers great advantages over existing devices, with its capability for continuous or batch operation. Great efficiency would also be achieved with a larger furnace. The particulate material injected into the furnace rotor is projected onto the inner wall of the furnace, which allows intimate and prolonged contact between reactants.

In other types of plasma reactors, reactions between solid particles are practically impossible by reason of residence times of only a few milliseconds in the reactor.

All the advantages claimed for the plasma arc rotary furnace are retained and considerably larger powers can be obtained with this new device because the electrical resistance of the plasma beam is increased by injecting particles in it through a suitable feeding system.

Indeed, since electrode life fixes a limit to the maximum electric current that can be used in the arc (300 A) an increase in the electrical resistance of the plasma beam makes it possible to obtain larger arc powers by operating at higher voltages. In the present case, arc powers above 100 kW could be used with the modified furnace, compared to a maximum of 60 kW for the furnace without the feeding system.

VI. **CONCLUSION.**

Your attention please. What we are above described, with several precautionary measures (the secret of the know-how) can help a State in War. How ?

It is very simple for a Company to receive an industrial toxic waste, and the engineers can inverse the process. The plasma furnace stops the destruction, changes its program and starts

to produce high toxic gas : purity : 99,99974 %. What I say is a secret but it is the reality. Our Company can produce yperite, phosgene $CoCl_2$, dioxines and more other dangerous materials since industrial waste is not controlled. No problems exist for a State today : purchase a little plasma rotary furnace, 60 or 250 kg of capacity.

This technology can be installed by a refinery, for example, for the cracking ; nothing for the destruction of oil-waste or oil production, but only for toxic gas tro produce. For a weak price.

The question is : what is the Company doing ? furnaces sale or not ? And the answer is : we sell.

The immediate decontamination of the skin

by F. LAMBRECHT

Department NBC, N.V. Lippens, Ghent, Belgium

SUMMARY.

We, Belgians have a continuous experience in vivo with war gases. The first gas attacks in WW I took place no more than 40 km from where we are. Even now, seventy years later, more un-exploded ammunition is found every day than our bomb disposal units can destroy. A large part of this unwanted heritage is gas ammunition.

In handling these gas shells, of which a large part are corroded and are leaking, accidents inevitably occur. But thanks to the good instruction of the personnel and the decontamination material used, practically all of these contamination accidents are benign.

The most important point in skin decontamination is speed. If Sarin, for example, is removed from the skin within two min-utes, there is a 80 % chance of survival. After five minutes only 5 % survive, and after 10 minutes nobody. Yperite and VX pene-trate light clothing in three minutes. Droplets of persistent war gases can be removed from the skin by chemical or physical means.

The chemical means decompose the combat gas, but practically all used chemicals attack the eyes and the mucous fibers. The violent exothermic reaction can give severe burns. In order to avoid these burns the decontaminating chemical can be diluted, but then the danger is a too long reaction time. There exists no universal chemical decontamination product. The chem-icals used : hypochlorite, sodium hydroxide, phenol, chloramine B, and zincchloride are themselves not without danger, and cause allergies. Washing with soap and water increases the danger by distributing the droplets all over the skin and some gases are water soluble.

The physical methods do not necessarily detoxify the chemical agents, but remove them. In Belgium decontamination gauntlets

are used. Their main content is Fullers Earth which adsorbs the toxic droplets. The adsorbens is powered on the contaminated skin through stamin, and removed after two minutes with the backside of the glove. The decontamination of Yperite or Sarin is complete in two minutes. Addition of certain chemicals improve the decontamination. For example small amounts of magnesiumoxyde, calciumoxyde, titandioxyde improve the decontamination of sulfuryperite. Addition of silverions gives promising results.

For the civilians : the best protection is to get under cover during a gas attack. A protective sheet impregnated with polyurethane stops any existing persistent gas. If there are parts of the body contaminated with droplets : dust with flour and wipe it off after two minutes.

We, Belgians, have a continuous experience *in vivo* with war gases. The first gas attacks in WW I took place no more than 40 km from where we are. Even now, seventy years later, more unexploded ammunition is found every day than our bomb disposal units can destroy. A large part of this unwanted heritage is gas ammunition.

In handling these gas shells, of which a large part are corroded and are leaking, accidents inevitably occur. But thanks to the good instruction of the personnel and the decontamination material used, practically all of these contamination accidents are benign.

The objective of skin decontamination is to destroy, neutralize or eliminate the liquid toxics on the skin, in order to assure the survival of the victim.

The most important point in skin decontamination is speed. If Sarin, for example, is removed from the skin within two minutes, there is a 80 % chance of survival. After five minutes only 5 % survive and after 10 minutes nobody. Normal clothing, two layers, is penetrated in 30 minutes, leather in 5 hours, a raincoat in 20 minutes. A decontaminating agent should eliminate the combat gas in less than two minutes.

Droplets of persistent war gases can be removed from the skin by chemical or physical means. Another division of the procedures is in dry and fluid processes. The dry processes are used as well for the skin as for the clothing. Several decontamination methods such as fire and strong chemicals are designed for use on equipment or to decontaminate the ground and would be far too severe for personnel.

The chemical means decompose the combat gas, but practically all used chemicals attack the eyes and the mucous fibers. Where there is a universal chemical decontaminating product for material, the same cannot be said for the skin. The chemicals used : STB, hypochlorite, lime, sodium hydroxide, phenol, chloramine B and zincchloride are not without danger.

All the wet procedures used until now represent an additional danger to the personnel. With the actual procedures : soap and shower, war gases have been washed deeper into the skin. The agent drops are distributed all over the skin and increase the danger. The use of water soluble agents increases the effect the agent has on the skin because it weakens the resistance of the skin, washing has to be done after eliminating the combat gas from the skin as is done in the US M258 skin decontaminating kit, where the droplets are first removed with plastic sticks and the skin then washed with either a phenol-sodium hydroxide solution or a chloramine B-zincchloride solution, depending on the gas used.

The half life for mustard in water is 4.5 minutes. About 20 half lives are required to reduce the concentration of an agent in solution by a factor of 10^6. Thus about 90 minutes is needed to reduce the amount of mustard in a given volume of water from 1 g to 1 mg. The half life of VX is 2.900 minutes at PH 9.5 or 140 minutes at PH 12. VX is completely miscible with water at 9.5°C, and 3 % miscible at 25°C. Obviously water alone is not a suitable decontaminant.

The physical and dry methods of decontamination do not necessarily detoxify the chemical agents, but remove them. In many Nato countries decontamination gauntlets are used. Their main content is Fullers Earth. The war gas is adsorbed and bound by this earth. The concentration dilutes, and the contact area with the skin diminishes. The adsorbing powder prevents the spreading of the liquid agent drops.

The Fullers Earth is contained in two pockets formed of a bunting tissue and a sponge tissue. A glove contains minimum 100 gram of adsorbens enough to decontaminate a person and his clothes. The decontaminant is powdered on the skin through the bunting tissue, it adsorbs the liquid gas and is wiped off with the sponge tissue on the backside of the glove.

Thus the gas is eliminated and the decontaminating agent prevents the evaporation of the war gas.

Tests were made in our laboratory to determine the effect of the decontaminating powder on the protection time of different tissues and materials, and a general result is that after an immediate decontamination no gas remains by decontaminating 15 minutes later, the penetration time of the gas is doubled.

Addition of small amounts of certain chemicals improves the decontamination. Such chemicals are for example : magnesium-oxyde, calciumoxyde, titaniumdioxyde. We are working on the addition of silverions.

The ever returning question is : the military are protected, what about the civilians ? Protection against gas war means two things :

1. *Information.*

2. *Decontamination material.*

1. Information : know the danger. In some countries : Canada, Sweden, Switzerland the public is informed and measures are taken. But these countries are exceptions. So we have to apply the proverb ; help yourself so help you God. Recently an excellent book (in french - flemish later) appeared informing the public about what to do to survive an NBC war. So far for information.

2. As for material, most of the products I talked about are not on the civilian market, not that our industry is not willing to put it on the market, but only because there are no specifications from the Ministry.

In case of an attack with persistent war gases : get under cover. A single plastic sheet gives you protection during 20 minutes. If you have a polyurethane coated sheet the protection is unlimited.

If there are parts of the body contaminated with droplets ; dust them with flour and wipe it off with tissue paper.

Correlation between some physical parameters and alkaline hydrolysis rate constants of organophosphorous compounds

by V. VOJVODIC

Military Medical Academy,
11000 Beograd, Yugoslavia

V. VELJKOVIC, I. COSIC and D. LALOVIC

Laboratory for Theoretical Physics
Boris Kidric Institute. P.O. Box 522. 11001 Beograd, Yugoslavia

SUMMARY.

In the present work was shown that there is significant correlation between alkaline hydrolysis rate constant of organophosphorous compounds and average valence number of these compounds and their radicals. On the basis of these findings, a theoretical criterion for prediction of the alkaline hydrolysis rate constant was established.

INTRODUCTION.

A large number of insecticides and pesticides is presently in commercial use. Some of these compounds, due to their high toxicity, are potentially dangerous for humans. In order to prevent introduction of these supertoxic compounds [LD50 (mouse) 0.5 mg/ kg, SC (1)] in every-day use, it is necessary to detect them early enough, before the industrial production starts. Biological tests currently in use for testing the toxicity of newly synthetized chemicals, are as a rule expensive and time-consuming. Even worse is the fact that one needs to examine a large number of these compounds, before the one, that satisfies all the prerequisites for commercial use, is reached.

This problem could be made less acute if one would be able to formulate a theoretical criterion for preselection of compounds In respect of their toxicity. The theoretical criterion would enable us to analyse a large number of compounds in very short time.

Recently, we have demonstrated that it is possible to estimate supertoxicity of organo phosphorous compounds (OPC) on the basis of the electron-ion interaction potential (EIIP) (2). In the present paper we analyse the correlation between the EIIP of OPC and their stability. The chemical stability of OPC, besides their toxicity, is an important factor in their use.

METHOD.

The potential of the electron-ion interaction in any condensed system with delocalized electrons can be expressed in momentum representation, by :

$$< \vec{K} + 2 |W| \vec{K} > = \frac{W(0)}{\varepsilon(2)} \qquad (1)$$

where $\vec{q}$ is the change of the momentum of the delocalized electron in the interaction with potential W, $\vec{K}$ is the momentum of the delocalized electron, W(0) is potential of free ion in the molecule, and $\varepsilon(q)$ is the dielectric function describing the screening of the previous component with the gas of free delocalized electrons.

In our analyses we will use the general model pseudopotential (GMP) (3, 4) of the form :

$$W(q) = A_1 Z \sin(2\pi A_2 \eta)/2\pi \qquad (2)$$

where Z is the average number of delocalized electrons, $\eta = q/2K$, K is maximal momentum of delocalized electrons, A_1 and A_2 are constants given in ref. 3.

The main characteristic of this potential is that it includes the screening of delocalized electrons (3). Thus one does not have in mind that the application to molecular system this problem may be severe. The pseudopotential (1) is obtained through the fit to semiempirical Heine-Abarenkov potential (4). It is shown by the authors of ref. 5 that this form of the potential may be obtained directly from the scattering theory.

Another important characteristic of the pseudopotential (1) is that it does not contain any free parameter, in contrast to all the other pseudopotentials used in physics of condensed matter, but it depends exclusively on the average valence number (AVN). Due to this, the GMP can be used, within pseudoatomic approximation, for the analysis of electronic properties of the complex inter-

metallic compounds (6-8). Here, the average valence number is determined by :

$$Z = \sum_{i=1}^{n} C_i Z_i \qquad (3)$$

where C_i is the concentration of the i-th component of the molecule, Z_i is the number of the valence electrons of i-th component of the molecule, and n is the number of components in the molecule.

The EIIP (2) depends only on the AVN, and it has been shown (9, 10) that there is significant correlation between AVN and biological properties of organic molecules. For this reason we have undertaken to investigate possible correlation between AVN and chemical stability of OPC.

RESULTS.

As a parameter characterizing chemical stability of OPC we have taken alkaline hydrolysis rate constant (AHRC) (11). We have found that there is a significant correlation between this constant and AVN, which can be expressed by the following relations :

$$K = 338.816(Z/Z_3) - 797.266 \qquad \text{for } Z(R_1) \quad Z(R_2) \qquad (4)$$
$$\log K = 3.466(Z/Z_3) - 8.861 \qquad \text{for } Z(R_1) = Z(R_2) \qquad (5)$$

where the numeration of radicals corresponds to their position as presented in fig. 1.

$$
\begin{array}{ccc}
R_1 & & O \\
 & \diagdown \quad \diagup\!\diagup & \\
 & P & \\
 & \diagup \quad \diagdown & \\
R_2 & & R_3
\end{array}
$$
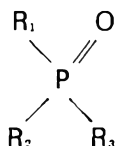
FIG. 1.

In relations (4) and (5) Z is the AVN of the whole molecule, while Z_3 is the AVN of R_3 radical (valence number for each halogen element is equal 1) (10).

In table I the values of K, calculated by the relations (4) and (5), are compared with experimental values. For the purpose of comparison, in the same table are presented the values of K from ref. 11, calculated by a method which has into account many characteristics of OPC molecules (orbital energies, bond order, dipole momentum, etc.). As one can see, there is a good

TABLE I

Alkaline hydrolysis rate constants of some OPS

R_1	R_2	R_3	Z/Z_3	K_{exp}	$K/11/$	K (our res.)
CH_3O	CH_3	F	2.67	106	148	107
C_2H_5O	CH_3	F	2.53	61	62	60
C_3H_7O	CH_3	F	2.44	26	30	29
CH_3O	C_2H_5	F	2.53	49	72	60
C_3H_7O	C_2H_5	F	2.38	9	11	9
CH_3O	C_2H_5	Cl	2.53	98	378	60
C_3H_7O	CH_3	$NO_2C_6H_4O$	0.86	2	0	0
CH_3O	CH_3O	F	2.92	18	13	18
C_2H_5O	C_2H_5O	F	2.63	2	2.5	2
C_3H_7O	C_3H_7O	F	2.48	1	1	1
CH_3O	CH_3O	Cl	2.92	2	5023	18
C_2H_5O	C_3H_5O	$NO_2C_6H_4O$	0.86	0	2	0

agreement between our values and the experimental values. It is interesting to note that our values agree with experiment better than the values from ref. 11.

CONCLUSION.

We have shown that there is a significant correlation between the AHRC and AVN. It should be pointed out that the criterion of chemical stability of OPC, based on values of AHRC calculated by the relations (4) and (5), should not be considered as an absolute and decision criterion, but rather as a statistical pre-selective criterion which enables us to predict stability of OPC with certain probability.

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Detection of mustard gas in biological material

by G. MACHATA and W. VYCUDILIK

Institute of Forensic Medicine, Chemical Department,
University of Vienna, Vienna, Austria

SUMMARY.

*Mustard gas (2,2'-Dichloroethylsulfide) can be detected in bio-
logical material, especially in urine after extraction with organic
solvents. A chromatographic column (silica gel) was used for
purification followed by specific detection (GC/MS). The detection
limit for the molecular ion was approximately ng/ml urine.*

Classical methods for chemical warfare agents analysis use main-
ly colour or precipitation spot tests with test paper strips or gas
test tubes. Only a few modern analytical identification methods
using UV- or IR-spectrophotometry have been published and
information concerning their determination in biological material,
which requires very sensitive analytical procedures, is scarce.

In one case showing the classical symptoms of mustard gas
poisoning, the required identification was carried out by urine
analysis, despite the poisoning accident took place already one
week earlier.

The urine samples were extracted twice with ether, purified via
a silica gel column and concentrated under a nitrogen atmosphere
to a smaller volume. The whole procedure is shown on the dia-
gram. The saturation of the urine with NaCl is essential for
the recovery. The cleanup by a silica gel column can be replaced
by a batch procedure, leaving the sample at least one hour
with occasional stirring. Combined GC/MS showed characteristic
fragments (m/e 109 and 158) in two of the urine samples, equiva-

lent to 0.15 Microgram and 0.10 Microgram mustard gas/100 ml urine respectively.

In addition to the urine samples, two submitted probes of suspicious material were also investigated. Substance I, a dark brown oily liquid with the characteristic mustard smell was dissolved

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┌─────────────────────────────────┐
│        20 ml URINE              │
│        (sat. NaCl)              │
├─────────────────────────────────┤
│               |                 │
├─────────────────────────────────┤
│   5 ml ETHER (Extraction)       │
│        VORTEX                   │
├─────────────────────────────────┤
│               |                 │
├─────────────────────────────────┤
│  Evaporate Solvent to 10 µl     │
│  (Nitrogen, Room temperature)   │
├─────────────────────────────────┤
│               |                 │
├─────────────────────────────────┤
│   1 ml Methylene Chloride       │
│     + Silicagel (100 mg)        │
├─────────────────────────────────┤
│               |                 │
├─────────────────────────────────┤
│   Evaporate Solvent to 10 µl    │
├─────────────────────────────────┤
│               |                 │
├─────────────────────────────────┤
│           GC/MS                 │
│   m/e 109 Basis Peak            │
│   m/e 158 Molecular Ion         │
└─────────────────────────────────┘
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in ether to a pale yellow solution. Substance II, a dark brown solid with the same odour, dissolved only partially in ether, leaving a pale yellow sandy residue behind, and giving also a pale yellow solution.

Both ether extracts were analyzed by GC (OV-1, 110° isotherm-sequential FID, NPD and PD) and compared with standard samples. Substance I showed a mustard gas content of 88 %, substance II (solid) : 29 %. The verification of the agent was done by GC/MS. The results are shown in the following table.

Selective GC detectors (NPD and PD) indicated no organic contamination with phosphorus or nitrogen containing substances.

The GC-separation shows the main components (S-lost, Yperite, Mustard gas) and also at least three minor compounds. The identification by GC/MS makes the presence of

— bis (2-Chloroethylthio)-ethane,
— bis (2-Chloroethylthioethyl)-ether,
— bis (2-Chloroethyl)-disulfide.

very probable. These substances can be useful for the investigation of the origin and preparation procedures.

TABLE

TIC : RT = 3.9 min, RR = 1

	RR	%	m/e (EI, 70 eV)
S-LOST	1.0	88.2	63, 95, 109, 123
X	2.3	2.7	60, 63, 109, 120 123, 182, 218
Y	3.0	7.5	60, 63, 88, 104 109, 118, 123, 136
Z	1.6	1.1	63, 92, 94, 109, 111 127, 129, 141, 143 155, 157, 190, 192

X : 1.2 bis-(2-chloroethylthio)-ethane.
Y : Bis-(2-chloroethylthioethyl)-ether.
Z : Bis (2-chloroethyl)-disulfide.

Emission spectroanalysis of the ether insoluble residue showed mainly silicium, smaller amounts of calcium, magnesium and iron and traces of zinc, manganese, aluminium, sodium, titanium and cadmium. The residue appears to be some type of carrier, used to improve the ballistic properties of the bomb and also added for the dispersal of the toxic material.

Mycotoxin production
by thirteen fusarium isolates
from Thailand

by R. GREENHALGH*, J.D. MILLER*, G. NEISH*
and H.B. SCHIEFER**

SUMMARY.

*Information of the toxigenic potential of fusaria from S.E. Asia
is lacking. Plant and soil collections were made in Thailand in
1982, from which 13 isolates of fusarium, including f. solani, f.
moniliforme, f. subglutinans, f. equiseti and f. semitectum were
identified and lyophilized. It seemed of interest to evaluate their
toxigenic potential in fermentation using both chemical analysis
and bioassay. Each isolate was grown in four fermentation
systems, known to produce large quantities of trichothecenes
with toxigenic isolates. These include rice (Greenhalgh et al., 1983,
Appl. Environ. Microbiol. 46:625), gyep (Miller et al., 1983, Can. J.
Microbiol. 29:1171), czapekpeptone (Uenoet et al., 1970, Chem.
Pharm. Bull. 18:304) and sucrose/glycerol (Greenhalgh et al., 1984,
J. Agric. Food Chem., in press), all at 28°C. In the case of liquid
culture fermentations, the beer was freeze-dried and made
up with water (20 ml). The aqueous solution was extracted
with ethyl acetate using Clin-Elut columns and analyzed by GC/ECD
and GC/MS. Portions of the concentrated beer were used for a
rabbit skin bioassay and in mouse gavage studies. In addition some
of these isolates were tested in barley fermentation (h.b.s.) and
in vermiculite cultures (d.c., Cullen et al., 1982, Appl. Environ.
Microbiol., 44:371).*

*Only f. equiseti 496 produced any trichothecenes (DAS 684 $\mu g/l$,
MAS 243 $\mu g/l$, Fusarenone X 197 $\mu g/l$) detectable chemically
(GC/MS). The extract from the relevant liquid culture fermenta-*

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* Agriculture Canada, Ottawa, Ontario K1A OC6, Canada.
** Toxicology Research Centre, University of Saskatchewan, Saskatoon, S7N OWO, Canada.

tion (gyep) showed a positive skin bioassay equivalent to 2.5 ppm t-2 and in gavage studies the concentrated beer caused the death of mice with histopathology, consistent with trichothecenes poisoning. As would be expected, other isolates produced toxic factors, as evidenced by both skin bioassay and mice gavage studies, but which did not correspond to known trichothecenes. The data show that an isolate of f. equiseti from Thailand can produce trichothecenes, be it in low yields as compared to Cambodian and Japanese isolates. In addition, the results show the need to use many fermentation systems to determine the toxigenic potential of fusaria.

INTRODUCTION.

Reports on the use of mycotoxins as biological weapons in South-East Asia (1, 2) have raised many issues. Among these is the question as to whether Fusarium species from this region can produce mycotoxins. Although it has been known for many years that Fusaria occur in South-East Asia (3), information on their toxigenic potential is lacking (4). This question is addressed with this paper and results are reported on experiments with

ning their toxigenic

potential on both rice and in liquid culture.

MATERIALS AND METHODS.

The experimental protocol for testing the Thai isolates is shown in table I. Samples were collected in Thailand in February 1982, including material from a banana plant, various soil samples and one leaf sample with a yellow spot. The fungi were isolated and characterized and found to contain various spp. of Fusarium, including *F. equiseti, moniliforme, subglutinans, semitectum* and *solani.* The isolates were then cultured on both a solid matrix (rice) and in liquid cultures. Three different media were used which Fusarium spp. have been shown to produce myco-toxins (5, 6) i.e., GYEP, MYRO and peptone-Czepek. The fungi on the rice matrix were extracted with 10 % methanol in water, further clean-up was carried out by extraction with ethyl acetate and column chromatography for analysis by GC/ECD (table II).

The liquid cultures were also extracted with ethyl acetate for chemical analysis (table III). In addition, extracts were also analy-

TABLE I

Protocol

```
┌──────────────────────────────────────┐
│        THAI SAMPLES, 1982            │
│  Various leaves, banana plant, soil, │
│        Yellow spot on a leaf         │
└──────────────────────────────────────┘

   ┌────────────────────────────────────────┐
   │                ISOLATES                 │
   │ F. equiseti, F. semitectum, F. solani  │
   │   F. moniliforme, F. subglutinans       │
   └────────────────────────────────────────┘

   ┌────────────────────────────────────────┐
   │                CULTURE                  │
   │            Solid:  Rice                 │
   │      Liquid: GYEP, MYRO, Cz-P,          │
   │  13 Thai Isolates, 5 positive control   │
   └────────────────────────────────────────┘

   ┌────────────────────────────────────────┐
   │                ANALYSIS                 │
   │  Bioassay:  Skin test, Mouse gavage     │
   │        Chemical:  GC/ECD                │
   └────────────────────────────────────────┘
```
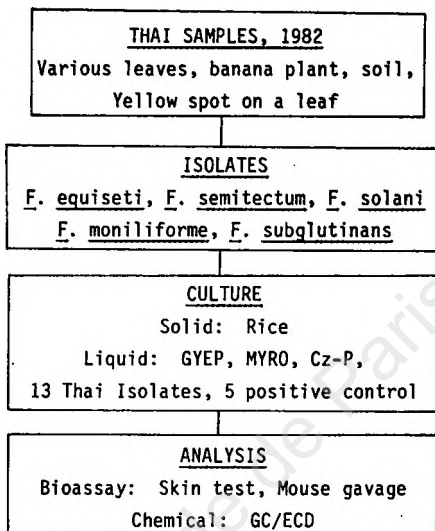
TABLE II

Analytical procedure for rice matrix

```
   ┌──────────────────────────┐
   │          BLEND           │
   │  10% MeOH/H₂O (200ml)    │
   └──────────────────────────┘
      Centrifuge ─────────────── Discard
   ┌──────────────────────────┐
   │         EXTRACT           │
   │  Clin. Elect EtAc (5ml)   │
   └──────────────────────────┘
                     ─────────── Aqueous
        CH₂CL₂
   ┌──────────────────────────┐
   │         CLEAN-UP          │
   │  Silicagel (4% H₂O)       │
   └──────────────────────────┘
                     ─────────── 5% Acetone/Toluene
     5% MeOH/CH₂CL₂
   ┌──────────────────────────┐
   │    ANALYSIS, GC/ECD       │
   │      HFB Deriv.           │
   └──────────────────────────┘
```
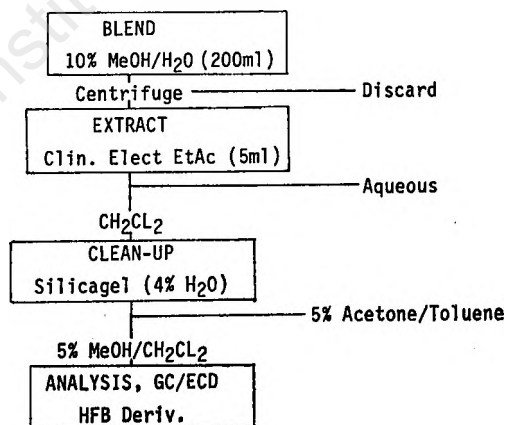
zed by bioassay using rabbit skin tests and mouse gavage with subsequent histological examination of organs.

In order to ensure the viability of the four culture systems, control studies were done with 5 Fusarium species from Canada,

TABLE III

Analytical procedure for liquid cultures

```
                    ┌─────────────────────────────────┐
                    │           FERMENTATION           │
                    │   28°C GYEP, MYRO, Cz-peptone    │
                    └─────────────────────────────────┘
                                   Filter
     ┌──────────────────────┐                    ┌──────────────┐
     │       Extract        │                    │  Lyophilize  │
     │  20ml ClinElut EtAC   │                    └──────────────┘
     └──────────────────────┘                            │ H2O
              │                                  ┌──────────────┐
     ┌──────────────┐                            │   Bioassay   │
     │   Residues   │                            │  Skin Test   │
     └──────────────┘                            │ Mouse Gavage │
        CH2CL2                                    └──────────────┘
              │
     ┌──────────────────────┐
     │  Analysis GC/ECD      │
     │  HFB Derivative       │
     └──────────────────────┘
```
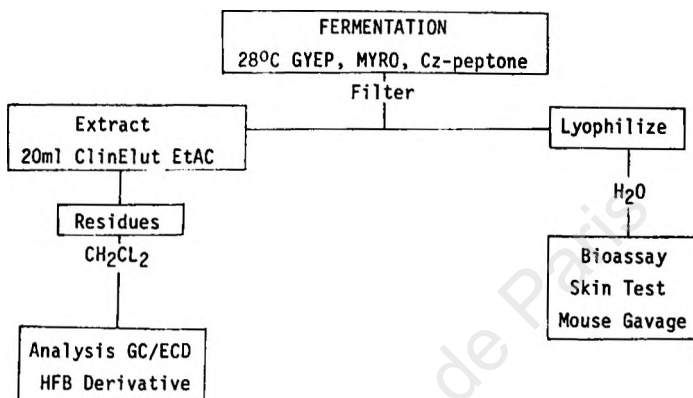
England and Japan. Both *F. culmorum* and *F. roseum* produce 3-oxygenated trichothecenes (6) and can be classified as 4-deoxy-nivalenol (DON) producing fungi (7). In liquid culture they gave 3-AcDON, Isoverracarol, diacetylhydroxydeoxynivalenol and culmorum. On rice the main produce was DON. Three isolates of *F. sporotrichioides* resulted in copious production of neosolaniol, T-2 and some HT-2.

RESULTS.

Of the Thai strains isolated, only *F. equiseti* isolated from a yellow spot on a leaf produced any detectable amounts of trichothecenes, namely DAS, MAS and Fusarenon-X, all of which were confirmed by MS. The highest yields 684,243 and 197 µg/l, respectively, were obtained in liquid culture with GYEP medium. The concentrated beer from the GYEP fermentation showed a positive skin bioassay equivalent to 2.5 ppm T-2. In the mouse gavage test, the beer caused clinical symptoms (depression) in mice after 48 h. The histopathological findings in thymus, spleen and duodenum were consistent with trichothecene poisoning. Other

strains also produced toxic factors as illustrated by the bioassay tests, but lesions did not compare with those produced by tricho-thecenes.

These results show that an isolate of *F. equiseti* extracted from samples collected in Thailand has toxigenic capability when cultured under specific conditions.

The work will be reported in greater detail in a forthcoming publication.

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Comparative study of two different field tests for the detection of Yperite in the atmosphere, applied on biological samples of gased soldiers

by A. HEYNDRICKX, H. DE PUYDT and J. CORDONNIER

Department of Toxicology, State University of Ghent,
Hospitaalstraat 13, 9000 Ghent, Belgium

SUMMARY.

In spite of the Convention of Geneva upon the use of chemical and biological weapons, modern armies should keep being aware of the possible use of war gases.

For most of the classical war gases, methods prooving their presence in the atmosphere exist, but generally they are too complicated and also too much time consuming to be useful in the field hospital. Therefore there is a need for simple but reliable field tests, that can also be performed and interpreted without analytical background. Such test kits are commercially available nowadays.

Two different army field tests for the detection of Yperite (Mustard Gas) in the atmosphere were compared. One test is only suitable for tracing Yperite, whereas the other one is a more general screening test for different war gases. The suitability of these kits for the detection of Yperite and its metabolites in biological samples was also evaluated : one of these kits proved to be a reliable screening method for Mustard Gas intoxication.

We investigated the tests for the detection of Yperite, since it has been used recently as a warfare agent in the Middle East. Although it is not a very sophisticated technique, it has proved to be very effective for the purpose it has been used for.

INTRODUCTION.

Mustard gas or Yperite, an old and formerly well known chemical warfare agent was recently used against human beings in Iran. Some of the victims were transferred to different European University Hospitals for intensive treatment. They all showed vesication of the skin and also the typical symptoms of severe damage of the respiratory mucosa.

Different kinds of attacks have been reported, but in all of them chemical and microbiological weapons were used. We had the evidence of at least three different agents : Mustard gas, mycotoxins or « Yellow Rain » and the cholinesterase inhibiting organophosphoric esters, the nerve gases. We had to screen all the samples we received from the victims on these different compounds. In this paper the screening method we developed for Yperite is described.

As Yperite is hydrolyzed in an aequeous medium to thiodiglycol, which is an important metabolite of the molecule in the human organism as well, generally this thiodiglycol is looked after to screen a patient on a possible Yperite intoxication. For this purpose different GC/MS methods are described in literature.

As we daily received a great number of samples, we had to look for a quicker and simpler method than GC/MS to be able to treat such an amount of samples. We developed an easy screening method, providing us a good indication about the severity of the intoxication.

PRINCIPLE.

Different commercial firms are producing field tests to screen the air on volatile chemical weapons. We received two different kits, one with a very general screening test, whereas the other one, with two different reagent sets, is only suitable for Yperite or for nerve gases.

In vivo Yperite does not show the metabolization we would expect when only considering the degradation reactions in water at 37°C.

The two step hydrolysis seen in water (1), is illustrated in figure 1.

In the human body the fraction that only undergoes this hydrolysis is less than 25 %. We also see the formation of the sulfoxyde and the sulfonderivative. As a strong alkylating agent Yperite will react with different functional groups of fysiologically important molecules (2). Beside this the molecule is conjugated and excreted in the urine (3). The reaction mechanism is however much more complicated as we found in the samples of the intoxicated soldiers.

Yperite

$$S \begin{cases} CH_2\text{-}CH_2\text{-}Cl \\ CH_2\text{-}CH_2\text{-}Cl \end{cases}$$

$$\mid H_2O$$

$$S \begin{cases} CH_2\text{-}CH_2\text{-}OH \\ CH_2\text{-}CH_2\text{-}Cl \end{cases} + HCl$$

$$\mid H_2O$$

Thiodiglycol

$$S \begin{cases} CH_2\text{-}CH_2\text{-}OH \\ CH_2\text{-}CH_2\text{-}OH \end{cases} + HCl$$
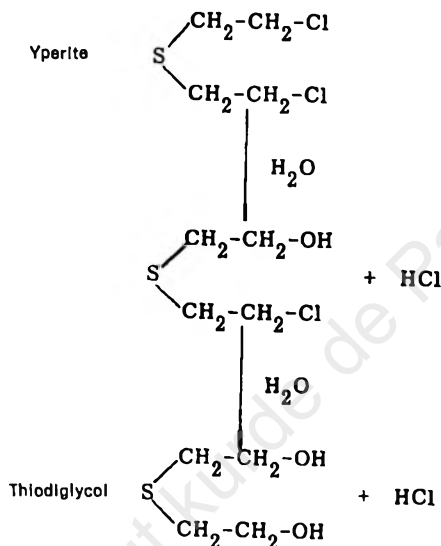
FIG. 1. — Possible two step hydrolysis of Yperite in water.

Description of the test kits.

1. KANAG   ADETOX   ADETOSS

ALN-NSA-NDE 6665 757 0600

This is a double kit, containing two series of materials and reagents, one for the detection of Yperite and another for the detection of the cholinesterase inhibiting organophosphate esters.

Material :

— a hand pump with a stroke volume of 100 ml,
— test discs to adsorb the Yperite in the air passing trough,
— one reagent to heat the disc by giving an exothermic reaction with the aluminium package.

Instructions :

— fix a disc to the pump,
— put one drop of the first reagent on the disc,
— aspirate twenty volumes of the air to be tested,
— place the disc in the original package and bring one drop of the heating reagent on each side of it, leave the disc inside for two minutes,
— bring one drop of the second reagent on the disc,
— if positive, the originally white disc will turn violet blue.

2. NSN 6565-21-87-6740

    DETECTOR KIT
  CHEMICAL AGENT
        C2

This kit is a very general test to screen the atmosphere for a whole series of common war gases. The test for Yperite is rather simple to perform.

Material :

— a hand pump with a stroke volume of 100 ml,
— glass tubes containing an adsorbent to captate the Yperite in the air passing through.

Instructions :

— fix an adsorbent tube to the pump,
— aspirate twenty pump volumes of air through it,
— add one drop of the coloring reagent,
— if positive, the originally white crystals in the tube will turn blue.

The test kits for the detection of Yperite in the air being simple as well as quite sensitive, we tried out if they could be applicable for the detection of Yperite or its metabolites in biological fluids.

To apply them on aequeous solutions instead of on a gaseous phase we developed an apparatus as shown in figure 2.

A bubble flask (sinter glass P2) with a content of 100 ml is connected to the test disc or tube of one of the kits. This is fixed to the pump by means of glass tubing. The connections were tightened with rubber rings.

Twenty millimeters of the solution to be tested is brought into the bubble flask, put in a water bath at 90ºC and heated during

15 minutes. Every minute one pump volume of air is aspirated gently through the solution and at the end of those 15 minutes another 5 pump strokes are aspirated. Volatile compounds, such as the original Yperite and its metabolites, are captated when passing through the test disc giving a positive reaction afterwards.

Only with the first kit we got positive results with an aequeous solution of 100 μg of Yperite in 1 l water at 37°C. It is obvious
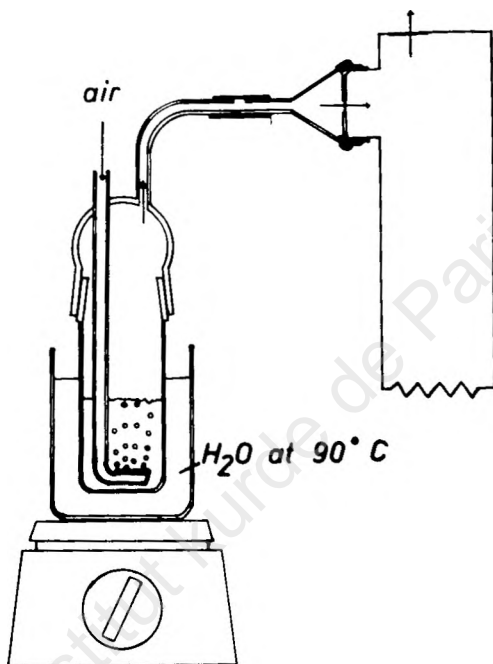
FIG. 2. — Scheme of apparatus used to detect Yperite or its metabolites in biological fluids.

that the positive reaction is possibly due to the degradation products. We also performed the test with urine samples found to be positive for Yperite, by screening them with the GC/MS method for thiodiglycol (4) and saw positive reactions again. Since Yperite is known to hydrolize in the presence of water, we tested if we could obtain positive results after a more or less long period.

In this experiment we brought 50 μg of Yperite in water (20 ml), human urine (20 ml), dry sand (20 g) and wet sand (20 g of dried sand + 2 ml of water).

We performed our test immediately, after one hour and after one week.

Simultaneously a strongly positive urine sample from an **Iranian** intoxicated soldier was also tested.

The results are summarized in table I.

TABLE I

Results of the stability test of mustard gas (Yperite)

Sample	Testing after		
	10 minutes	One hour	One week
Water	+++	+++	+
Dry sand	+++	+++	+++
Wet sand	+++	+++	++
Human urine (spiked)	+++	+++	+
Strongly positive urine (from Iranian soldier)	++++	++++	+

Note :   ++++ Very strongly positive.
           +++ Strongly positive.
            ++ Positive.
             + Weakly positive.
Sensitivity of the test : 50 µg mustard/l water.

RESULTS.

We applied this method on all the urine samples of the Iranian patients we received from the different European hospitals. The results are listed in table II.

TABLE II

Results obtained from the first analysis of Iranian soldiers' urine samples

Hospital	Total number of patients	Positives	Negatives
Vienna (Austria)	8	6	2
Hamburg (W. Germany)	2	0	2
Lausanne (Switzerland)	2	2	0
Munich (W. Germany)	3	1	2
Recklinghausen (W. Germany) . . .	10	1	9
Ghent (Belgium)	5	2	3

In Vienna and Lausanne, where the patients arrived sooner after the attacks, we found the highest percentage of positives. This could be expected since there is an active metabolization of Yperite in the Human organism. We also followed up the different patients for more than three weeks and saw negative results in all patients at least three weeks after the attacks with the chemical agents (see table III).

TABLE III

Results obtained from the consecutive analysis of Iranian soldiers' urine samples

Hospital	Patient number	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Vienna	1.		+					+	—	D													
	2.		++					+++	+	—													
	3.							—	—												—		
	4.							++++	++														
	5.							+++	+														
	6.							++	—												—		
	7.							—	—														
	8.							++	+												—		
Hamburg	1.															—							
	2.															—							
Lausanne	1.											+											
	2.											+											
Munich											+												
											++												
											—												
Recklinghausen	1.														+								
	2 10.														—								
Ghent	1.			—	—	—	D																
	2.			—	—	—	—	—															
	3.			++	+	+	—	—															
	4.			—	—	—	—	—															
	5.			+	+	—	—	—															

Days after the attack

Note : Only the days we received samples are mentioned in this table.

  D : Patient died.

++++ Very strongly positive.

 +++ Strongly positive.

  ++ Positive.

   + Weakly positive.

   — Negative.

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The test was also performed with twenty urine samples from other patients in the Intensive Care Unit of our University Hospital, also being in a condition of heavy medical treatment or intoxication. No positives were found.

The test was performed as well on twenty urine samples from healthy Iranian soldiers, living in the same conditions as the victims. No positives were found either.

DISCUSSION.

This method only has to be considered as a quick screening method, not as a real proof of the use of Yperite. Therefore a confirmation with GC/MS has to be performed. It is only a good indication for an eventual Yperite intoxication since it can be used easily in difficult conditions e.g. in the laboratory of a field hospital, where the samples can be analysed very soon after the attack. It is of utmost importance to detect the intoxication with the Yperite as soon as possible, in order to start the specific treatment that has proven to be effective if started in time. Obviously the test will also be much more sensitive when performed e.g. some hours after the attack instead of days after.

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Methemoglobinemia in patients attacked by chemical and microbiological warfare agents

by A. HEYNDRICKX and M. VAN STEENBERGE

Department of Toxicology, State University of Ghent,
Hospitaalstraat 13, 9000 Ghent, Belgium

SUMMARY.

Five Iranian soldiers, victims of a chemical and microbiological warfare attack, were hospitalized in the University Hospitals of Ghent.

Their methemoglobinemia was determined by the spectrophotometric method of Kiese. This technique is based on the difference in absorption at 577 nm between methemoglobin and carboxyhemoglobin.

All patients showed increased levels of methemoglobin.

High doses of Vitamin C and acetylcystein were administered intravenously ; the toxicological results are discussed.

INTRODUCTION.

Five Iranian soldiers, attacked by chemical and microbiological warfare agents, were hospitalized in the University Hospitals of Ghent. All of them showed symptoms of cyanosis.

Increased levels of methemoglobinemia in the blood samples of all patients were observed.

Methemoglobin is an oxidative derivative of hemoglobin in which the ferrous iron is oxidized to the ferric form (1).

For the reversible binding and release of oxygen, the iron atom should be in the ferrous state.

Spontaneous formation of methemoglobin by auto-oxidation occurs naturally.

According to Jaffé, 1981 (1), an erythrocyte NADH-methemoglobin reductase system provides methemoglobin reduction to maintain the methemoglobin concentration at a level of less than 1.5 % (fig. 1).

Various drugs, metabolites and chemicals can enhance the oxidation rate of the ferrous ion to the ferric form, thereby exceeding the capacity of the enzymatic reducing system, leading to an increased methemoglobinemia (2).

E_1 : Cytochromo b_5 reductase
E_2 : Glyceraldehyde-3-phosphate dehydrogenase
E_3 : Lactate dehydrogenase
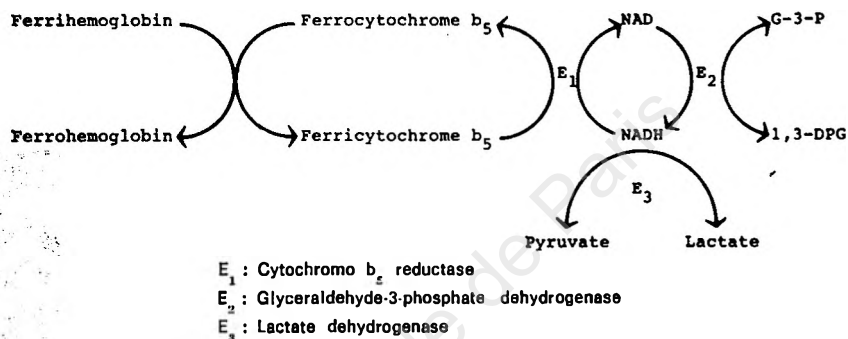
FIG. 1. — Reduction of ferrihemoglobin in red cells.
Mechanism of electron transport from NADH to ferrihemoglobin.

METHODS.

Principle of the method.

The methemoglobin content of the blood is determined by means of the spectrophotometric method of Kiese, 1947 (3).

This technique is based on the difference in absorption at 577 nm between methemoglobin and carboxyhemoglobin.

The bloodsample, containing a certain amount of methemoglobin together with a great excess of hemoglobin is diluted to hemolyse the red cells.

This solution is saturated with carbonmonoxide (CO), in order to convert the hemoglobin into carboxyhemoglobin, the methemoglobin remaining unchanged.

The absorbance of the formed carboxyhemoglobin is measured at 577 nm.

In a next stage sodiumdithionite is added to reduce the methemoglobin to hemoglobin. The latter is also converted to carboxy-

hemoglobin by means of the excess of carbonmonoxide, which is still present in the solution.

The absorbance of the total amount of carboxyhemoglobin is measured again and the rise in absorbance is equivalent with the methemoglobin concentration in the blood.

Spectrophotometric procedure.

0.1 ml blood is added to 40 ml distilled water. After hemolysis 5.0 ml phosphate buffer (pH 6.8) are added. This solution is further diluted to 50 ml with distilled water.

During 5 minutes carbonmonoxide gas is bubbled through the solution. Finally the solution is centrifuged (5 min. at 3,000 r.p.m.).

Two 2-cm cuvettes are filled with the centrifuged solution.

After addition of about 10 mg sodiumdithionite to one of the cuvettes, the optical density (A_1) of the solution with dithionite is measured at 577 nm, using the solution without dithionite as a blank. The optical density (A_2) of the dithionite containing solution is again measured at 577 nm using distilled water as a blank.

Calculation

$$\% \text{ methemoglobin} = \frac{A_1 \times 1.77 \times 100}{A_2}$$

The methemoglobin level is expressed as a percentage of the total amount of the hemoglobin content of the blood.

RESULTS AND DISCUSSION.

The evolution of the methemoglobin levels of the five Iranian patients is shown in figure 2. In each case a high level was observed in the bloodsample taken on the first day.

During 15 days 500 mg of vitamin C was given intravenously every 4 hours in combination with intravenous injections of 150 mg of acetylcystein every 3 hours. A rapid fall of the methemoglobinemia was observed.

During the therapy however, the methemoglobin levels were higher than normal.

A remarkable rise of the methemoglobin levels was also observed after hemoperfusion.
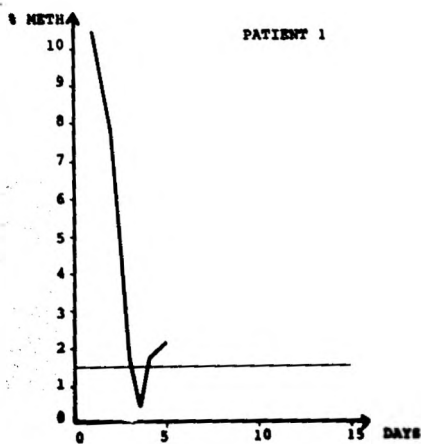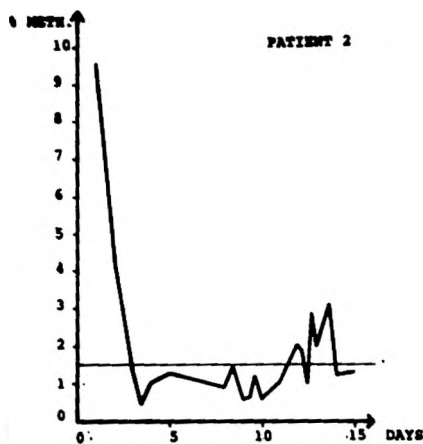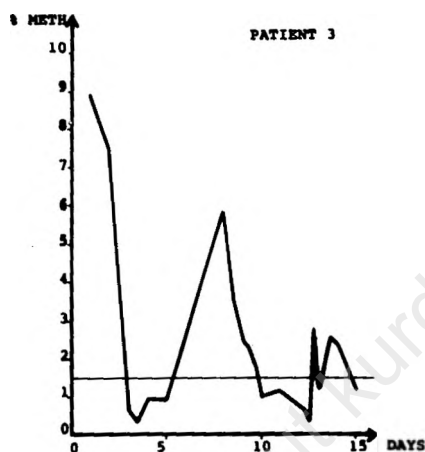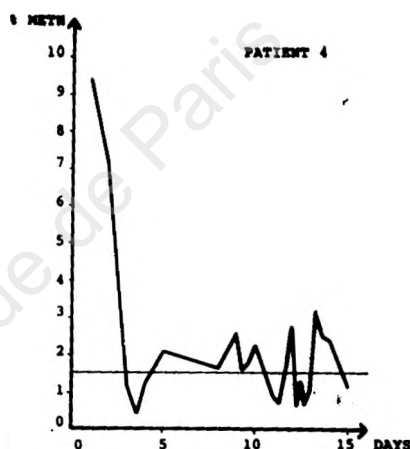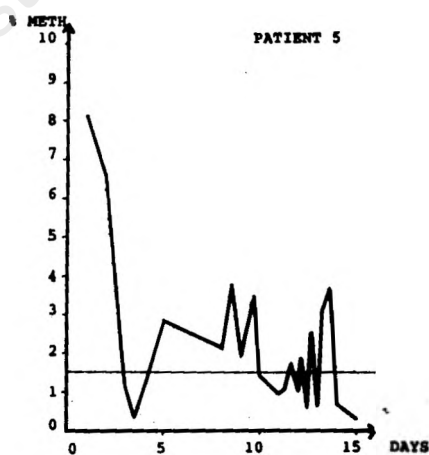
FIG. 2. — The evolution of the methemoglobinemia in the five Iranian patients. Patient 1 died five days after admission to the hospital.

Regardless the way in which the methemoglobin was produced, the therapy with vitamin C and acetylcystein has proven to be successful. Much higher concentrations of vitamin C and acetyl-cystein IV may even be used.

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Biological detection of chemical warfare agents

by WEN JU-FANG

Institute of Defence, Beijing, China

SUMMARY.

The existence of chemical warfare agents can be detected by means of sensitive responses of living animal and plants, which may be used as « alarming organisms ». As an auxiliary method, biological detection of toxic agents is still of considerable importance though analytical techniques have been highly developed nowadays. This paper presents some of our works on the biological detection of chemical warfare agents.

The chemical warfare agents tested include sarin, VX and Lewisite. All of them are synthesized in laboratory and of purity greater than 95 %. Biological materials include animals, such as fishes, land leeches and some other animals and plants. The experiments with fishes were carried out in aqueous solution of sarin, while other animals were tested by exposure to the vapor. Droplet spraying was used to contaminate the test-plants.

Fry of grass carp (Ctenopharyngodon idellus), silver carp (Hypophthalmichtus molitrix), variegated carp (Aristichthys nobilis), carp (Cyprinus carpio), dace (Cirrhina molitorella) and some small wild fishes were used in the sarin poisoning experiments. The toxic signs in fishes tested appeared in such an order as excitation, violent motor activity, loss of balance, convulsion, swim on side, upside down, sinking down the bottom, and death. The sensitivity of the fry of grass carp appeared to be highest, and next to it were the fry of silver carp, variegated caro and dace. The survival time of the fry of four species in relation to the concentration of sarin in the contaminated water are shown in table I. Figure 1 shows the concentration-survival time curves thus obtained, which fit well to a double logarithmic curve by a PS-80 computer. It is suggested that this method may be used to examine water suspected to be contaminated by sarin and may be taken as a security precuation for drinking water.

The responses of some small animals such as land leeches, insects and spiders, etc. to sarin vapor of different concentrations have been tested. The results are given in table II. It is shown that the sensitivity of land leech (Haemadipsa sp.) to sarin is much higher than other animals ; the leeches survived 1.5-2.0 µg/l for only 0.3-1.1 min. During intoxication, land leeches exibited extensive swaying of head, secreting mucus fluid, rapid crawling at first, and then slow movement, wriggling at a single place, contracturing and thickening, finally curling up into a ball to die. Therefore, the land leech turned out to the animal of choice for tests and survival time was estimated by exposure to different concentrations of sarin. Figure 2 shows the concentration-survival time curve thus obtained. The sensitivity of land leeches to sarin vapor lies in between chemical and enzyme detector tubes. Kept in ampules, bamboo tubes or glass tubes, land leeches may be carried to detect sarin vapor of low concentration and be used to ascertain the contaminated areas.

Flowers and leaves of some plants have been tested with chemical warfare agents. Color changes took place at the contaminated sites, as shown in figure 3 to figure 18. Mostly the color turned a bright to a dull tint, for instance, the flowers of egg-plant (Solanum melongena L. var. esculenta) and cowpea (Vigna sinensis) turned yellowish green from violet when contaminated by VX, and turned red from violet when contaminated by sarin or Lewisite. Leaves of eggplant and pumpkin (Cucurbita moschata) showed brown spots at sites of Lewisite contamination. The color change of these flowers and leaves after contamination are helpful in finding out contaminated areas and the kinds of toxic agents. The mechanism concerned are to be studied.

––––––

INTRODUCTION.

The existence of chemical warfare agents can be detected by means of sensitive responses of living animals and plants, which may be used as « alarming organisms ». J.H. Northrop (1947) investigated the detection of low concentradions of mustard gas and Lewisite with dogs and rats by conditioned-reflex methods. Later on, other American scientists continued the studies on the detection of some other toxic agents, such as tabun, sarin soman, CS, BZ, etc. with similar methods. J. Epstein (1956) also reported the detection of sarin-polluted water with sensitive

fishes. As an auxiliary method, biological detection of toxic agents is still of considerable importance though analytical techniques have been highly developed nowadays. This paper presents some of our results on the studies of biological detection of chemical warfare agents.

MATERIALS AND METHODS.

The chemical warfare agents tested included sarin, VX, Lewisite and musterd gas. All of them were synthesized in laboratory and of purity greater than 95 %. Biological materials included animals, such as fishes, land leeches and some other animals and plants. The experiments with fishes were carried out in aqueous solution of sarin, while other animals were tested by exposure to the vapor. Droplet spraying was used to contaminate the tested plants.

RESULTS AND DISCUSSION.

1. Fishes. Fry of carp *(Cyprinus carpio)*, grass carp *(Cteno-pharyngodon idellus)*, silver carp *(Hypophthalmichthus molitrix)*, variegated carp *(Aristichthys nobilis)*, dace *(Cirrhina molitorella)* and some small wild fishes were used in the sarin poisoning experiments. The toxic signs in fishes tested appeared in such an order as excitation, violent motor activity, loss of balance, convulsion, swim on side, and upside down, sinking down the bottom and death. The sensitivity of the fry of grass

TABLE I

Survival time of four species of fishes in various concentrations of sarin solutions

Concen-trations of sarin solutions (mg/l)	Survival time (min.)			
	Grass carp (Ctenopharyngodon idellus) Body length 4.1 ± 0.1 cm	Silver carp (Hypophthalmichthys molitrix) Body length 3.7 ± 0.1 cm	Variegated carp (Aristichthys nobilis) Body length 4.5 ± 0.1 cm	Dace (Cirrhina molitorella) Body length 3.1 ± 0.2 cm
0.01	139.6 ± 5.1*	207.0 ± 24.6	246.4 ± 20.3	538.0 ± 120.7
0.02	58.2 ± 8.8	88.8 ± 12.6	116.2 ± 5.9	240.4 ± 40.0
0.04	32.6 ± 4.6	47.8 ± 4.4	57.6 ± 6.4	111.0 ± 25.3
0.08	19.6 ± 2.1	29.8 ± 3.7	24.4 ± 3.5	54.2 ± 3.2
0.16	11.4 ± 1.8	17.2 ± 1.8	15.0 ± 1.6	27.4 ± 3.8
0.32	7.0 ± 2.3	11.6 ± 2.4	9.0 ± 1.6	14.0 ± 1.9
0.50	4.4 ± 0.6	7.0 ± 2.3	6.2 ± 0.4	

* Five fishes in each group.

carp appeared to be the highest, and next to it were the fry of
silver carp, variegated carp and dace. The survival time of the fry
of four species in relation to the concentration of sarin in the con-
taminated water are shown in table I. Figure 1 shows the con-
centration-survival time curves thus obtained. It is suggested that

A, Grass carp *(Ctenopharyngodon idellus)*.

B, Silver carp *(Hypophthalmichthys molitrix)*.

C, Variegated carp *(Aristichthys nobilis)*.

D, Dace *(Cirrhina molitorella)*.
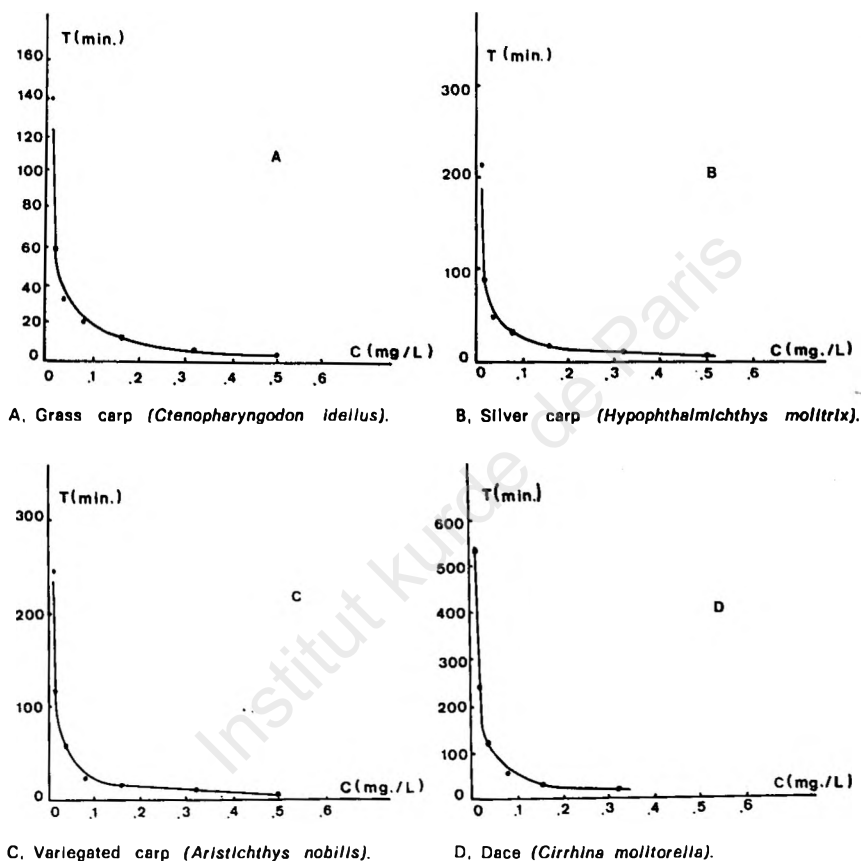
FIG. 1. — Relation between concentration and survival time of fry in sarin solution.

this method may be used to examine water contaminated by sarin
suspiciously and may be taken as a security precaution for drink-
ing water.

2. Invertebrates. The responses of some small animals, such as
land leeches, insects and spiders, etc. to sarin vapor of different
concentrations have been tested. The results are given in table II.
It is shown that the sensitivity of land leech *(Haemadipsa sp.)*
to sarin is much higher than other animals. During intoxication,

TABLE II

Sensitivities of some invertabrates to sarin vapor

Animals	Responses* (min) — Sarin concns (µg/l)			
	1.5 -- 2.0	4.0 -- 4.5	8 -- 10	20
Small land leech	0.3 -- 1.1 (+)	1 -- 1.2 (+)	0.6 (+)	0.4 -- 0.5 (+)
House fly	—	—	2 -- 6 (+)	0.7 -- 4.5 (+)
Butterfly	—	—	2.7 -- 3.5 (+)	1.5 -- 2.5 (+)
Dragonfly	18 (±)	—	6 -- 9.4 (+)	3.3 -- 3.5 (+)
Honeybee	—	15 (±)	—	3.3 (+)
Hornet	—	28 (±)	—	—
Mantis	—	—	18.1 (+)	—
Cicada	19 (—)	—	—	—
Katydid	19 (—)	—	—	—
Locust	—	18.5 (—)	—	—
Red ant	—	18.5 (±)	—	—
Cricket	50 (—)	—	—	—
Black spider	50 (—)	18.5 (—)	—	—
Colored spider	—	18.5 (—)	18.5 (—)	—
Mole cricket	—	19.5 (+)	—	—

* + ___ dead ; — ___ alive ; ± ___ dying.
  Data denote exposure time.
  Room temperature 22-27° C.

TABLE III

Survival time of land leeches (Haemadipsa sp.) in various concentrations of sarin vapor

Concentrations of sarin (µg/l)	Survival time (sec.)
1.5	82
2.4	61
3.4	66
4.4	57
5.2	75
7.4	42
8.4	40
9.7	70
10.5	36
11.8	36
14.5	32
15.7	36
20	30

* Body length 10 -- 15 mm ; 2 -- 3 leeches in each group.
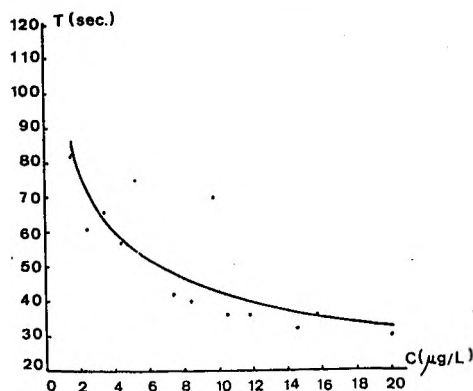
FIG. 2. — Relation between survival time of land leeches (Haemadipsa sp.) and concentrations of sarin vapor.

TABLE IV

Comparison of color changes among some plants after contamination of CWA

Contamined sites	Color changes			
	VX	L	GB	H
Flowers :				
Eggplant *(Solanum melongena var. esculenta* (violet)	Yellowish green	Red	Red	None
Cowpea *(Vigna sinensis)* (violet)	Yellowish green	Red	Red	Reddish
Kidney bean *(Phaseolus vulgaris)* (violet)	Yellow	None	None	None
Oleander *(Nerium indicum)* (red)	Yellow	None	Discolored	None
Chinese rose *(Rosa chinensis)* (red)	Yellowish green	—	—	Reddish
Leaves :				
Eggplant	None	Brown	None	None
Pumpkin *(Cucurbita moschata)*	None	Brown	None	None

TABLE V

Color-change of various species of plants after contamination of CWA

Agents	Flowers		
	Responses Tests	Responses time (min.)	Color changes
VX	33/56	< 0.3	Violet, red to blue, green, yellow, violet ; white to yellow, green ; yellow, blue to green
GB	12/37	< 1.5	Blue, violet, yellow to red
H	11/77	< 3	Yellow to green ; blue to red ; violet to red, yellow
L	27/50	< 0.4	White to violet, red, yellow, brown ; red to violet, green ; yellow to white, brown

Agents	Leaves		
	Response Tests	Response time (min.)	Color changes to
VX	5/115	—	—
GB	11/138	0.5 -- 2.5	Yellow, grey, brown, violet
H	10/36	1.5 -- 3	Green, grey, blue, brown
L	107/115	< 3	Brown (usually)

land leeches exibited extensive swaying of head, secreting mucus fluid, rapid crawling at first, and then moving slowly, wriggling at a single place, contracturing and thickening, finally curling up into a ball to die. Therefore, the land leech turned out to be the animal of choice for tests and the survival time was estimated by exposure to different concentrations of sarin. Results are given in table III and figure 2. The sensitivity of land leeches to sarin vapor lies in between the sensitivies of chemical and enzyme detection methods. Kept in ampules, bamboo tubes or glass tubes, land leeches may be carried anywhere to detect sarin vapor of low concentrations and be used to ascertain the contaminated areas.

3. Plants. Flowers and leaves of some plants were tested with chemical warfare agents. Color-change took place at the con-taminated sites, as shown in tables IV and V.

CONCLUSION.

1. The small land leech *(Haemadipsa sp.)* and fry of grass carp *(Ctenopharynodon idellus)* are very sensitive to sarin and can be used to detect and determine sarin in polluted air or water. They can also be extensively applied for the detection of some other nerve gases.

2. The flowers of some plants, such as eggplant *(Solanum melon-gena* var. *esculenta)*, cowpea *(Vigna sinensis)*, Kidney bean *(Pha-seolus vulgaris)*, Chinese rose *(Rosa chinensis)*, oleander *(Nerium indicum) etc.* will change their colors when they are contaminated by some chemical warfare agents, such as VX, sarin, Lewisite and mustard gas. Leaves of eggplant and pumpkin *(Cucurbita moschata)* will show brown spots at the sites of Lewisite conta-mination. The color-change of those flowers and leaves after con-tamination is helpful in finding out contaminated areas and the type of toxic agents. The mechanism concerned is to be studied.

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Collaborative studies on warfare chemicals by small laboratories

by M.C. DUTT

Department of Scientific Services,
Ministry of Health, Outram Road, Singapore 0316

SUMMARY.

The spectre of chemical and biological warfare and their devastation is of special concern to small countries as their entire population will be exposed to these weapons if unleashed during hostilities. Small national laboratories in such countries, though equipped with basic laboratory facilities will lack experience in identifying the chemical or biological agents. The paper outlines proposals for collaboration with centres of excellence through a co-ordinating body which will, inter alia, disseminate chemical and biological warfare agents to participating labs, collate information and establish testing protocols.

INTRODUCTION.

Chemical and/or biological weapons (CW, BW) unleashed against small countries, even at the battle front, will inevitably affect the entire population. Such countries must be particularly concerned of their ability to rapidly identify such weapons in times of war or an emergency and deal with them appropriately. Although the chemistry, dispersion characteristics, tactical use and protection against CWs known before World War II is well documented (1-3), very little is known of the esoteric ones such as the mycotoxins and binary weapons, most of which are shrouded in secrecy. What are these substances? Where does one look for them in an emergency? How does one analyse them? What are their metabolites? These are some of the questions

that a modest forensic toxicology laboratory would face in the absence of a specialized CW or BW laboratory when the use of these chemicals against the country is suspected.

With the rendering of many of the older war gases to obsolescence and the development of extremely potent new ones such as the almost odourless and colourless nerve gases, e.g. Tabun, Sarin and Soman, new methods of detection are needed. Although field detection methods have been proposed these are only applicable when such gases are at high levels of concentration and even then the results are tentative (4).

A modest forensic toxicology laboratory would normally be equipped with all the basic instrumentation, in some cases up to the GC/MS level. It would have considerable experience in handling toxicological exhibits and specimens, drugs of abuse and possibly industrial and agricultural chemicals. However, without information of the chemical or biological characteristics of new CWs and BWs, there is every likelihood of especially missing them when employing standard analytical methodology. There is, consequently, a need for small laboratories, not normally conversant with CWs and BWs, to collaborate with centres of excellence in this specialized field so that they attain a state of preparedness during emergencies, notwithstanding the strenuous efforts presently being made to supplement the 1925 Geneva Protocol on the use of toxic gases and the 1972 Biological Weapons Convention with chemical disarmament (5). The alleged recent use of CWs and BWs underscores this need (6, 7).

IDENTIFICATION.

Literature on identifying all known CWs developed after World War II is thin for the obvious reason that they are not commonly used nor are they common contaminants in food, water or air that demands routine screening. There is, apparently, no widespread interest in establishing methods of analysis for new CWs or BWs and, more importantly, precautions in handling the more deadly of these in the lab.

The problems that would confront a laboratory, uninitiated in CWs and BWs, in an emergency would be the following :

1. **Symptoms / CW relationship.**

Any CW (or BW) insidiously unleashed on a population, would produce symptoms, suddenly, on a mass-scale and provide valuable clues to the identity of the substance used. Symptoms such as difficulty in focussing near objects, acute frontal headache, running nose, choking and tightness of the chest, nausea and vomiting have been ascribed to nerve gases (4). But what about symptoms, such as stinging sensation on legs, back and eyes, photophobia, the blistering and blackening of skin and so on as reported in recent hostilities (7) ? What about other symptoms such as convulsion and diarrhoea on a mass scale ? What do they relate to ?

2. **Field tests.**

Any outbreak of mass symptoms in which CWs (or BWs) are suspected would require immediate field tests. Here, if a cannister or some other delivery system cannot be located for collection of valuable CW or BW agents air-sampling has to be resorted to. For this a comprehensive but short list of sampling and testing devices are needed to cover all known CWs. Are air aspirators, charcoal tubes, silica gel, for instance, sufficient for collection ? Are the Andrew Bournique or Lever apparatus and field tests proposed with their use comprehensive enough (8) ? Are Draeger tubes the answer ? Do they cover the entire range of CWs ? What about BWs ?

The possibility of a CW being highly volatile, as in the case of sarin, or having short half-lifes, as low as 10 minutes as in the case of T-2 (6), exacerbates the problem of detection in an emergency. Are the use of animals the best approach for screening, despite the fact that certain chemicals which are highly toxic to man are weakly so to them (5) ? Then there is the problem of sensitivity of chemical tests. Toxicities of the organo-phosphorous nerve gases range from 1 mg for sarin to 0.4 mg for Vx and for Botulinium toxin it is as low as 1 ng/kg (9). These require highly sensitive tests which may not be possible to apply in the field.

3. **Laboratory tests.**

Again, failure to get a positive response to the field screening tests (if such tests could indeed be devised) need not necessarily imply the absence of CWs (or BWs). The agent(s) could be in very low concentrations or a new one may have been used and missed detection. More sophisticated methods of testing are therefore needed and this can only be achieved in the laboratory. But then, sufficient amounts of the agent can be collected in the first instance and an appropriate generalized scheme of analysis applied so that as many classes of CWs or BWs are covered without losing valuable samples ?

Do such schemes exist and what instrumentation and accessories (e.g. GC, HPLC columns and detectors) should a lab be equipped with ? What are special precautions needed for such tests ?

The problem of devising schemes for such analysis needs considerable research so that no CW or BW is missed. Even in the case of the T-2 toxin and nivalenol implicated in yellow rain their presence would have gone unnoticed if the GC/MS technique to identify them had not been previously refined by advanced laboratories (5). What more of inexperienced labs handling such agents at toxic doses below 100 p.p.b. ?

4. **Biological specimens.**

Even with the availability of established methodologies for CW and BW agents failure to detect these in an attack could mean that the agent is highly volatile. Recourse has to be taken in analysing body fluids and perhaps tissues from those afflicted. Here, the chance of a miss is even greater in the absence of clues as to what is being looked for and the scheme for general unknowns applied. Additionally, the specimen chosen may not be the most appropriate one for the particular CW that was used. As instrumental techniques will inevitably be applied TLC, UV, IR, HPLC and mass-spectra data for all known agents has to be available to the lab. As some CWs (particularly the organophosphorous nerve gases) are likely to metabolise rapidly, the data should include those of metabolites. These are basic information which any lab would require to identify CWs in the lab. The BW agents may pose additional problems and these have to be resolved.

LABORATORY CAPABILITY.

The rarity of CW and BW usage will, understandably, find such agents relegated as of low priority in the activities of especially small labs. Consequently, such labs may find themselves inadequately prepared to cover all CWs and BWs in times of an emergency. Yet, the spectre of their usage in hostilities cannot be confidently ignored and national laboratories, especially those in front-line states, would, from time to time, be daunted with the problem of rapid and unequivocal identification of these agents.

The shroud of secrecy that must hang over an ever-growing stockpiled list of warfare chemicals and toxins in various parts of the world, makes the task of preparedness an open ended one, even for advanced laboratories. Genetic engineering, for example, though currently in its infancy, has tremendous prospects in spawning a whole range of new and deadly agents for which perhaps more sophisticated methods of detection have to be constantly devised (11), so that they are not missed by an unsuspecting laboratory.

In planning to deal with chemical and biological agents in an emergency the objective of national laboratories should be to rapidly collect and identify them. It could be too late for such labs to despatch samples to advanced laboratories. However, for unequivocal identification, samples could be submitted to such centres for confirmation or even assistance sought in case the agent(s) remains unidentifiable.

COLLABORATION.

A state of preparedness to deal with CWs or BWs calls for collaboration between laboratories so that an universal scheme could be established. The scheme should include the following :

Emergency Tests.

1. Preliminary atmospheric tests — methodology (and list of CWs and BWs covered), protection for lab personnel in the field.

2. Preliminary ground tests — most appropriate locations, methodology.

3. Laboratory tests — methodology, instrumentation, appropriate biological specimens, interpretation of data.

Confirmation.

1. Collection and transmission of samples and specimens to collaborating labs — selection of these, packaging, mode of transmission, special precautions.

2. List of collaborating labs — accredition of centres of excellence, mode of communication during an emergency.

Protection.

1. Shelter — types of shelter for group protection during and subsequent to attack, safety levels of agents for all clear signalling.

2. Food and Water — precautions to be taken, methodology for determining residual agents, safety levels, water treatment.

3. Clothing — special clothing for protection.

4. Decontamination — methods and materials for decontamination of houses, offices, etc.

5. Treatment — medical diagnosis, antidotes, mass treatment.

6. Long-term effects — safeguards against mutagenic, teratogenic and carcinogenic effects.

Proposals.

To achieve a high state of preparedness it is proposed that :

1. A co-ordinating body be established to collate and disseminate all information and data on CWs and BWs to collaborating laboratories.

2. The co-ordinating body to stock samples of all known CWs and BWs in existence, in centres of excellence on CWs and BWs, identified by the co-ordinating body.

3. The co-ordinating body arranges for the distribution of spiked and control samples, periodically, to collaborating laboratories, provides them with testing methodology (if necessary) and assesses their results. (The capability of collaborating labs in handling CWs and BWs has to be cleared first.)

4. The co-ordinating body establishes testing protocols from re-
sults returned from collaborating laboratories.

5. The co-ordinating body receives samples from a CW or BW -
victim country for immediate distribution to centres of excel-
lence for analysis.
(Samples from the victim-country could be despatched direct-
ly to such labs, while keeping the co-ordinating body infor-
med.)

CONCLUSION.

The limited resources and the extremely low frequency of use
of chemical and biological warfare agents will find many labora-
tories inadequate in rapidly identifying them in an emergency
situation. Such a capability is vital especially where large civilian
populations are exposed. A state of preparedness is, therefore,
necessary for such laboratories and only by collaborating with
advanced laboratories could this be achieved.

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Yellow rain : chemical warfare or natural phenomenon ?

by M. MESELSON

Department of Biochemistry and Molecular Biology,
Harvard University, Cambridge, Massachusetts, USA

We do not rule out the theory that mycotoxin warfare has occurred in Southeast Asia, but the evidence for it is unconvincing and becomes more doubtful with continued investigation and analysis. Our letter published in *Science* last October (1) pointed out that there were no adequate controls to test the possibility that the reported mycotoxins were produced naturally by toxic fungi in foods and in the environment. This shortcoming in the evidence remains, but is partly superceded by newer evidence casting doubt on whether the reported mycotoxins were even present, at least at the time the samples were collected.

The numerous samples of alleged chemical warfare agent from Laos which we and others have examined, including the ABC News sample reported to contain T2 and other mycotoxins, are almost certainly the natural feces of Southeast Asian honey bees (1, 2, 3). We know of no sample claimed to be a chemical warfare agent from a site of alleged attack in Southeast Asia which has been shown to be anything other than bee feces.

Out of 18 groups of Hmong refugees from Laos to whom we showed bee feces on leaves during March 1984 in Thailand, only one individual in one group identified the material as insect droppings. Several of the Hmong said that the fecal spots were « chemie », their term for the purported chemical warfare agent. We know that massive showers of bee feces do occur, having been caught under one near Chaing Mai. Although standing about 200 meters from a large tree with many bee nests, we heard no bees and saw none overhead.

We know of no reason to believe that bee feces pose a toxic hazard to people. How then are we to interpret refugee

claims that showers of this material cause illness ? It is possible that the Hmong, Kampucheans and Thais who have made such claims, not knowing the correct explanation of the shower of yellow spots and being aware of stories of poison from the sky recurrent in Southeast Asia for more than 20 years, have mistaken bee defecation for chemical warfare and have associated it with real or rumored illness. Almost all interviews have been done with persons self-selected in advance for believing themselves to be chemical warfare victims, so that such stories would be over-represented in the available reports.

A precedent for widespread but almost certainly mistaken attribution of illness and death to unusual materials from the sky exists in a US National Academy of Sciences study of the effects of herbicides in Vietnam done for the Department of Defense published in 1974 (4). The Academy reported widespread claims by Vietnamese highlanders of diarrhea, abdominal pain, vomiting, skin rash, fever, dizziness, coughing blood and massive deaths attributed to herbicide spraying. However, the 10 villages studied by the Academy were not all exposed to the same herbicide and a parallel Academy study of lowland Vietnamese did not find major illness or deaths. None of the herbicides used would be expected to cause such serious effects. It therefore seems likely that the illness and death which the Vietnamese highlanders attributed to herbicide spraying was partly of natural origin and partly exaggeration and rumor. We have spoken with Hmong leaders in the US who expressed similar reservations about the yellow rain stories.

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Total arsenic determination in urine and faeces of soldiers after a gas attack by atomic absorption spectrophotometry using the MHS-20 hydride generating system

by A. HEYNDRICKX and N.E. PARISIS

Department of Toxicology, State University of Ghent,
Hospitaalstraat 13, 9000 Ghent, Belgium

SUMMARY.

Biological samples are decomposed in a mixture of nitric-per-chloric-sulfuric acid with final additions of hydrogen peroxide.

Aliquots of the diluted digests are taken for analysis by AAS.

During the hydride generating a silanized glass tube is used in order to conduct the arsine from the generating chamber to the quartz cell.

Argon, containing 1 % of oxygen, is used as a carrier gas.

A 3 N hydrochloric acid solution serves as a rinsing agent for the generating chamber.

The optimal conditions of the assay have been determined.

The method has been evaluated by determining the total arsenic content in urine and faeces of Iranian soldiers, which survived a chemical attack. The standard calibration graph is linear from 0.102 ng to 38 ng of As^{5+}. A detection limit of 102 pg, which corresponds to a concentration detection limit of 10 ng/l for a 10 ml sample volume (0.01 ppb), and a sensitivity of 73 pg As^{5+}/ 0.0044 absorbance units (1 % absorption) are obtained.

INTRODUCTION.

Arsenic, which is probably the most extensively distributed element in environmental and biological samples, has been known for centuries as a famous poison.

It has had a great agricultural, industrial and medicinal application for men and animals.

Therefore in case of intoxication with an unknown poison, arsenic determinations have to be included in the general screening

$$R - As = 0 + 2\ HS - R' \longrightarrow R - As \begin{array}{c} S - R' \\ S - R' \end{array} + H_2O$$

$$\begin{array}{c} R \\ R \end{array} As - OH + HS - R' \longrightarrow \begin{array}{c} R \\ R \end{array} As - S - R' \\ + H_2O$$
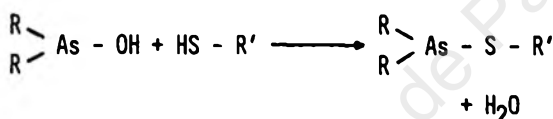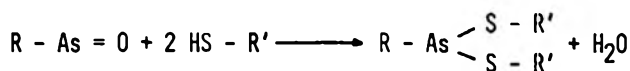
FIG. 1. — Mono- and di-substituted arsenical reactions.

$$R - As = 0 + \begin{array}{c} HS - C - \\ HS - C - \end{array} \longrightarrow R - As \begin{array}{c} S - C - \\ S - C - \end{array} + H_2O$$
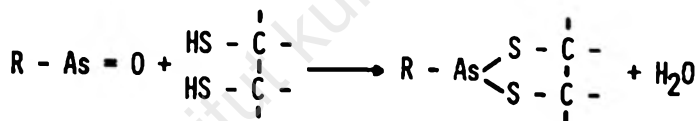
FIG. 2. — General arsenical reaction giving a stable 5-membered ring compound.

procedure. The mechanism by which a trivalent arsenical exerts its toxic effects is to some extent based on the interaction with tissue sulfhydryl and disulfide groups.

According to Squibb *et al.*, 1983 (1), disubstituted arsenicals will only react with single SH groups, while monosubstituted arsenicals rapidly react with two SH groups (fig. 1). As described by Peters, 1955 (2), particularly stable compounds are formed between monosubstituted arsenicals and dithiols when the SH groups are so arranged that a 5- or 6-membered ring can be formed (fig. 2).

As an example it was shown by Massey *et al.*, 1962 (3), that the reaction of arsenical Lewisite with the cyclic dithiol cofactor for pyruvate oxidase, lipoic acid, or with two closely held thiol residues in the lipoic acid dehydrogenase molecule, is particularly stable because of the formation of a 6-membered ring (fig. 3).

$$H_2C - SH$$
$$H_2C$$
$$H C - SH \quad + \quad CL_2As - CH = CH - CL \longrightarrow$$
$$(CH_2)_4$$
$$CO_2H$$

$$H C - S$$
$$H_2C \quad \diagdown \quad As - CH = CH - CL$$
$$H C - S \quad \diagup$$
$$(CH_2)_4 \qquad\qquad + 2 \ HCL$$
$$CO_2H$$

LIPOIC ACID   LEWISITE
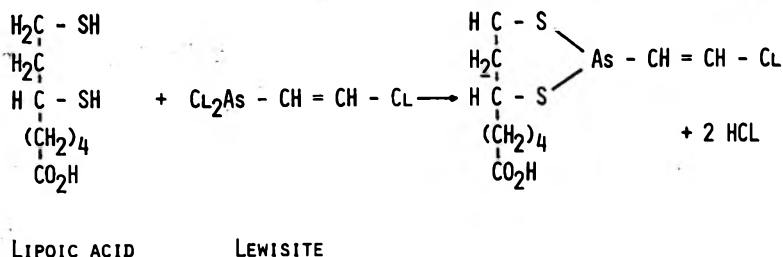
FIG. 3. — Reaction of lipoic acid with Lewisite giving a stable 6-membered ring compound.

$$CH_2 - SH$$
$$CH - SH \qquad + \qquad \begin{matrix} CL \diagdown \\ \\ CL \diagup \end{matrix} AsCH = CHCL \longrightarrow$$
$$CH_2 - OH$$
$$(BAL) \qquad\qquad (LEWISITE)$$

$$CH_2 - S \diagdown$$
$$CH \ - S \diagup AsCH = CHCL + 2 \ HCL$$
$$CH_2 - OH$$

FIG. 4. — Detoxfication mechanism.

QUARTZ CELL

GLASS TUBE

ARSINE GENERATING CHAMBER

3% NaBH₄ in 1% NaOH
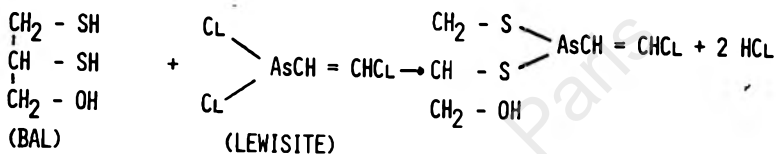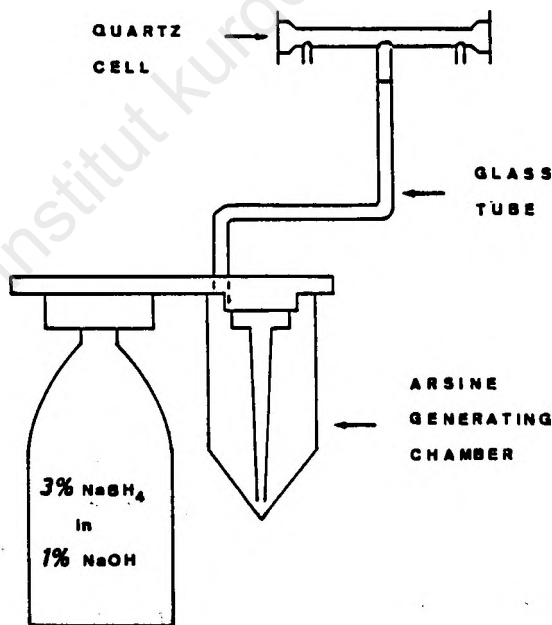
FIG. 5. — Hydride system.

This means that the high sensitivity of these enzyme systems to arsenic *in vivo* is due to the greater stability of the As-S$_2$ complex formed. This is also the reason why British Anti-Lewisite (BAL ; 2,3-Dimercaptopropanol) can antagonize the action of arsenic on these enzymes, as illustrated in figure 4.

Heyndrickx, 1957 (4, 5), has studied the complexity constants of metals and -SH and -S-S-groups. These studies show the very difficult reaction mechanisms with human plasma, serum, globulin, albumin, etc.

At present the most reliable methods for the toxicological determination of arsenic are based on the evolution of the arsine gas e.g. the Gutzeit, the Cribier, the silver diethyl-dithiocarbamate and the atomic absorption spectrophotometric methods.

As shown in figure 5, the latter method involves the volatile hydride generating of the element, by treating the digested specimens with sodium borohydride and subsequent atomization of the hydride in the light path of an atomic absorption spectrophotometer.

EXPERIMENTAL.

Hydride generating assembly.

The hydride generating device is a Perkin Elmer MHS-20 Mercury/Hydride system. It is equipped with a high purity quartz cell, which is closed at both ends by quartz windows. The cell, which can be replaced very easily, can be heated up to 1,000°C.

This atomization device, which is mounted in the sample compartment of the spectrometer, is carefully aligned in the light beam to give maximum light transmission.

The electronic system allows to select the desirable duration of purge and reaction between sample and reagent solution.

AAS system.

The MHS-20 system is installed in a Perkin Elmer model 372 atomic absorption spectrophotometer.

Results are shown on a 4-digit electronic display. An arsenic electrodeless discharge lamp was used with a Perkin Elmer EDL power supply.

Digestion apparatus.

Fifty milliliters quartz Kjeldahl digestion flasks are used for the acid decomposition of biological samples.

Generating chambers and glassware.

Polypropylene vessels, which are used as arsine generating vessels and other classical glassware are cleaned by staying over-night in a mixture of potassium dichromate and sulfuric acid and further rinsed with tap water, 3 N nitric acid and bidistilled water before drying in an oven. The arsine generating vessels are furthermore filled with diluted hydrochloric acid (1 to 1) and kept in that condition for one week. According to Moody *et al.*, 1977 (6), this procedure is considered as very essential in order to remove all traces of ions from the walls.

Reagents.

— Sodium borohydride solution — 3 %.
   Dissolve 30 g analytical grade $NaBH_4$ (Merck) and 10 g ana-lytical grade NaOH (UCB) in 400 ml bidistilled water, dilute up to 1 liter and filtrate. Store in the refrigerator where it remains stable for one week.
— Sulfuric acid working solution — 1.5 %.
   The solution is obtained by diluting the appropriate volume of 96 % suprapur sulfuric acid (Merck) to 1 liter.
— Hydrochloric acid rinsing solution — 3 N.
   The solution is prepared by diluting the appropriate volume of 37 % suprapur hydrochloric acid (Merck) to 1 liter.
— Nitric acid 65 %, suprapur (Merck).
— Perchloric acid 70 %, suprapur (Merck).
— Sulfuric acid 96 %, suprapur (Merck).
— Hydrogen peroxide solution 30 %, selectipur (Merck).
— Arsenic standard solution (As_2O_3), Titrisol (Merck).
— Arsenic stock solution. Dilute the Titrisol standard solution up to 1 l with 0.3 M hydrochloric acid in order to obtain a 1 mg/ml As^{5+} solution.
— Intermediate standard. Pippet 50 µl stock solution in a vol-umetric flask and further dilute with bidistilled water to 50 ml in order to obtain a final solution of 1 µg/ml As^{5+}

— Working standard. Take 1 ml of the intermediate standard and dilute with 4 ml bidistilled water in order to obtain a final solution containing 0.2 ng/μl As^{5+}.

— Carrier Gas. High purity argon (L'Air Liquide Cargal 1), containing 1 % of oxygen, is used to purge the system.

Destruction procedure.

For the decomposition of biological samples, a wet digestion procedure is used according to Agemian *et al.*, 1980 (7), who emphasize the necessity to use a mixture of nitric, perchloric and sulfuric acid in order to obtain a satisfactory recovery of arsenic from p-arsanilic acid, benzenearsonic acid, monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and triphenylarsine oxide. 1 ml urine or 2 g faeces are placed in 50 ml Kjeldahl flasks. One adds 10 or 25 ml concentrated nitric acid.

The solution is slowly heated at about 75°C for 1 hour, subsequently at 100°C for 1 hour and finally at 140°C for another 1 hour.

At the end a clear colorless solution is obtained. The destruction is completed by adding 1 ml concentrated sulfuric acid, 3 ml concentrated perchloric acid and by finally increasing the temperature to the boiling point, where it is kept for 45 minutes. When sulfur dioxide fumes are evolving, the flask is cooled, 2 ml of hydrogen peroxide are added and the mixture is boiled again for 5 minutes. As As^{3+} and As^{5+} do not give the same peak area when determined by AAS, the same procedure is repeated with another 2 ml of hydrogen peroxide in order to obtain a complete oxidation of the trivalent arsenic to the pentavalent form.

According to Evans *et al.*, 1979 (8), the determination of the total arsenic content has to be done in the As^{5+} form, because it requires less time to oxidize the trivalent arsenic rather than to reduce the pentavalent form. After cooling, the content of the Kjeldahl flask is quantitatively transferred into a 50 ml volumetric flask and further diluted with bidistilled water.

A portion of the diluted digest (up to 5 ml) is brought into the polypropylene vessel, diluted to 10 ml with bidistilled water and submitted to a total arsenic determination.

$$MH_x \quad + H^{\cdot} \longrightarrow MH_{x-1} + H_2$$
$$MH_{x-1} + H^{\cdot} \longrightarrow MH_{x-2} + H_2$$

$$\cdot$$
$$\cdot$$
$$\cdot$$

$$MH \quad + H^{\cdot} \longrightarrow M \quad + H_2$$
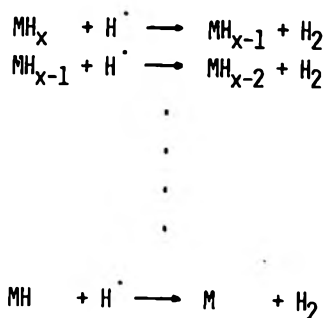
FIG. 6. — Atomisation of volatile hydride forming elements due to collisions with free H⁻ radicals.

$$H^{\cdot} + O_2 \rightleftharpoons OH^{\cdot} + O^{\cdot}$$
$$O^{\cdot} + H_2 \rightleftharpoons OH^{\cdot} + H^{\cdot}$$
$$OH^{\cdot} + H_2 \rightleftharpoons H_2O + H^{\cdot}$$
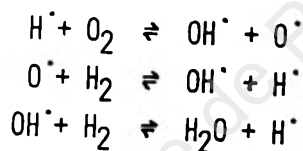
FIG. 7. — Formation of H⁻ radicals by reaction with oxygen.
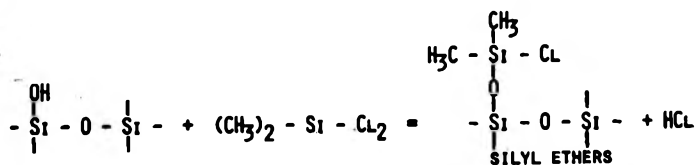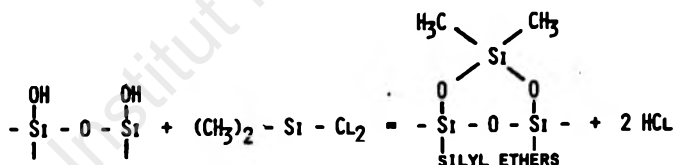
FIG. 8. — Reaction between surface hydroxy groups and DMCS (dimethyl-dichlorosilane).

Atomization mechanism.

The method of determination is based on the theory of hydrogen radicals formation as first proposed by Dedina and Rubeska, 1980 (9). The reaction principle was further investigated by Welz and Melcher, 1983 (10).

They concluded that the atomatization of the volatile hydride forming elements in a heated quartz cell must be due to collisions with free hydrogen radicals (fig. 6).

The formation of hydrogen radicals by chemical reaction with oxygen, as proposed by the same authors, is illustrated in figure 7.

In an attempt to apply this theory in practice and to establish a new sensitive method for the routine determination of arsenic in biological material, many difficulties arose, such as instability of the peak height, high blank values and memory effects. During the experiments it was noticed that the different kind of plastic tubes, used to conduct the gases from the reaction vessel to the quartz cell, had an important influence on the final results.

The introduction of a silanized glass tube improved both sensitivity and reproducibility. The glass tube was initially cleaned according to the general procedure used for cleaning glassware. After drying, the glass tube was filled with a 5 % solution of dimethyldichlorosilane in toluene. The tube was closed at both ends and warmed for 30 minutes in an oven or waterbath at 50°C. After rinsing with toluene and methanol, nitrogen was blown through the tube in order to remove the solvent.

The reactions between the surface hydroxy groups and the dimethyldichlorosilane are shown in figure 8.

When not used, the silanized glass tube had to be kept in a desiccator, because it is well known that moisture destroys the silanized glass surface. After the tube had been used for more than 500 determinations within a period of 2 weeks, the silanization process had to be repeated.

Determination procedure.

The instrumental parameters are listed in figure 9.

Before analysing the digested samples, the sensitivity of the instrument has to be controlled with standard solutions containing 5 and 10 ng As^{5+} in 10 ml of 1.5 % sulfuric acid.

The influence of the sulfuric acid concentration on the sensitivity is illustrated in figure 10.

All samples are analysed in triplicate, the arsenic content is calculated by the method of standard additions.

Spectrophotometer
Lamp power : 8.0 watt
Light source : EDL
Wavelenght : 193.7 nm
Spectral slit width : 0.7 nm
Mode : absorption
Reading time : 20 sec
Measuring mode : peak area
Background correction : none
MHS-20
Quartz cell temperature : 880° C
Purging gas : Argon + 1 % O_2 : 1.6 barr
Purge I : 40 sec
Reaction time : 4 sec
Purge II : 20 sec
Reaction volume : 10 ml
Sample dilution liquid : H_2O bidestillated
Standard dilution liquid : 1.5 % H_2SO_4.

FIG. 9. — Instrumental parameters.
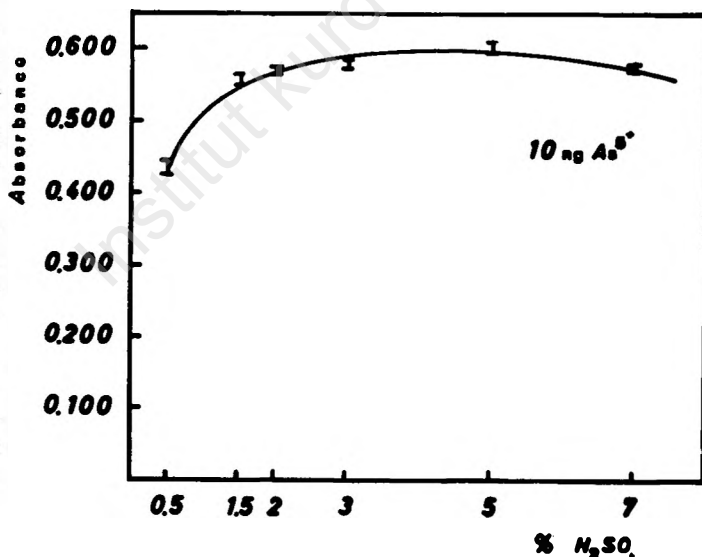
FIG. 10. — Influence of the sulfuric acid concentration on the sensitivity.

During the determination only one polypropylene vessel is used. After each measurement the vessel is rinsed with 20 ml of a 3 N hydrochloric acid solution.

This is an important step : it eliminates the memory effect and stabilizes the baseline.

RESULTS AND DISCUSSION.

With the proposed method, a calibration curve, which is linear from 0.102 ng to 38 ng of pentavalent arsenic, can be established (fig. 11).
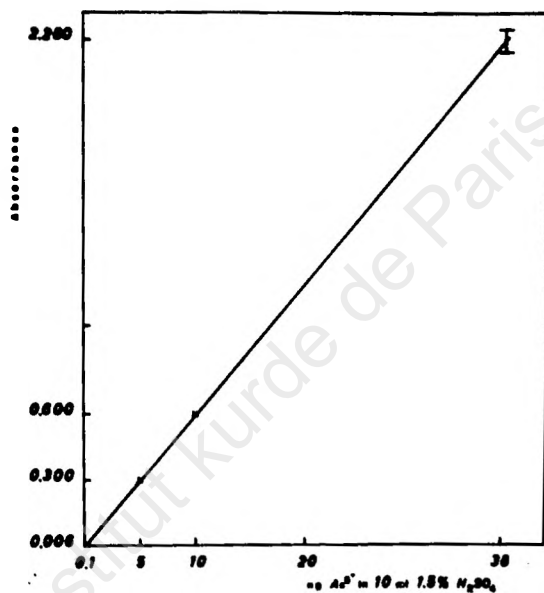
FIG. 11. — Standard calibration graph.

The range can be improved by increasing the reaction time between the sodium borohydride and the sample from 4 to 9 sec. This is attended with a slight loss in sensitivity as shown in figure 12.

The detection limit is 102 pg, which corresponds to a concentration detection limit of 10 ng/l for a 10 ml digested sample volume, or 0.01 ppb. The calculated sensitivity amounts 73 pg of As^{5+} for a 1 % absorption, or 0.0044 absorbance units in optical transmission at the 193.7 nm absorption line.

With this method the total arsenic content in urine and faeces of several Iranian patients, flown in from the Iranian battlefields and treated in 6 European Hospitals, was evaluated.
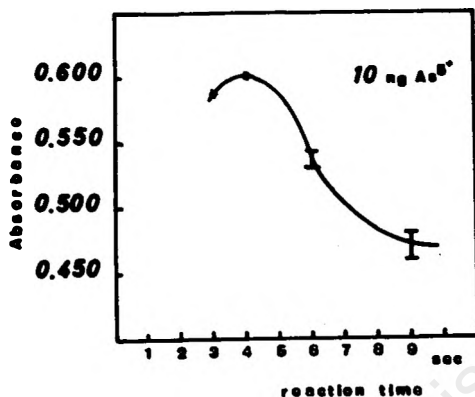
FIG. 12. — Influence of the reaction time on the peak absorbance for 10 ng As⁺⁺.

TABLE I

Arsenic levels in urine of 23 patients from 5 different hospitals

Hospital	η	Mean + SD µg/l	Range µg/l	D_1	D_2
1	10	12.8 ± 5.9	6.0 — 26.1	19/3	20/3
2	3	29.1 ± 17.5	16.3 — 49.1	15/3	19/3
3	2	19.9 ± 19.4	6.2 — 33.6	16/3	19/3
4	2	34.5 ± 20.5	20 — 49		30/3
5	6	21.1 ± 10.8	7.1 — 36.0	11/3	15/3
Total	23	19.6 ± 12.6	6.0 — 49.1		

D_1 : date of sampling
D_2 : date of determination

TABLE II

Arsenic levels in blood, urine and faeces from 2 patients

Patient	Urine, µg/l	Blood, µg/100 ml	Faeces, µg/100 g
A	12.7	2.0	44.8
B	10.3	1.6	1.1

Control sample from Iran.
As in urine : 34.7 µg/l.

The samples were analysed the day they arrived in the laboratory.

When urine samples, which were spiked with standard Lewisite, were treated in exactly the same way, a 100 % recovery of the arsenic added was obtained.

TABLE III

Arsenic levels in urine from 5 patients
University Hospitals Ghent

Patient	Arsenic in urine
1	12.7 µg/l
2	27.2 µg/l
3	20.6 µg/l
4	8.3 µg/l
5	28.1 µg/l

Day of determination : 20-03-84
Day of sampling : 19-03-84

From the results in table I, II and III it can be concluded that all patients showed normal arsenic values, which means that there was no arsenic intoxication in these patients and that the use of any war gas, containing arsenic (e.g. Lewisite), was excluded.

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Chromatographic procedures for the toxicological determination of bis (2-chloroethyl) sulfide (mustard gas, Yperite) in environmental and human biological samples

by A. HEYNDRICKX, J. CORDONNIER and A. DE BOCK

Department of Toxicology, State University of Ghent,
Hospitaalstraat 13, 9000 Ghent, Belgium

SUMMARY.

Two chromatographic procedures for the toxicological determination of the vesicant Yperite are described.

A primary methylene chloride extract is submitted to a simple clean-up procedure by passing through a Sep-Pak column. For qualitative and semi-quantitative analysis, thin layer chromatography (TLC) is applied (war gases, field samples). Visualization of the TLC spots is accomplished using a $PdCl_2$ reagent, which allows the detection of Yperite in submicrogram amounts. The quantitative determination of Yperite is done by capillary gas chromatography with electron capture detection, using a direct injection technique. Without the need for extensive clean-up procedures, the electron capture detector provided excellent stability and sensitivity. These toxicological procedures can be used to detect trace quantities of Yperite in soil, vegetation, water and spiked human biological fluids. Machata and Vycudilik used a similar method and confirmed their results also by mass spectrometry (15).

INTRODUCTION.

Bis (2-chloroethyl) sulfide was first synthesized by Despretz in 1822. Although the toxic effects of mustard gas were known since

1866 (Meyer), Lommel and Steinkopf looked for other synthesis methods (1915-1925). As vesicant for military purposes, it was first used during World War I in leper (Ypres, Belgium, 1917) (1).

Although gas chromatography/mass spectrometry (GC/MS) is the method of choice for the identification of Yperite, several other analytical procedures have been reported. Colorimetry (2, 3), thin layer chromatography (4, 5), gas liquid chromatography (7-10), and high performance liquid chromatography (6) are still widely used as toxicological routine methods.

However the need still exists for an analytical procedure, which allows the detection of trace quantities of sulfur mustard in soil, vegetation, water, biological tissues and fluids, without the need of extensive clean-up procedures.

Thin layer chromatographic studies, as reported elsewhere (4, 5), did not mention the simultaneous chemical detection of Yperite and some organophosphorous insecticides. The TLC procedure, developed here, is based on the use of a silica gel chromatoplate and a $PdCl_2$ reagent for visualization. The detection sensitivity of the proposed method is less than one microgram.

Several scientists employed gas chromatographic methods, using packed columns (7-10).

The somewhat limited separation performance of a packed column, however, is the main disadvantage of these systems. In order to screen a large number of possible substances in environmental or biological materials, analyses on twin-columns seem to be necessary to increase the separation capacity ; capillary gas chromatography (GC^2) was chosen as the method of choice for toxicological routine work (11, 12). In this study a capillary fused silica column, combined with a direct injection technique, is used. At the same time electron capture detection (ECD) significantly improves the specificity and sensitivity (picogram amounts) of the procedure.

Prior to capillary gas chromatography however, a further purification of the extract has to be done. The proposed procedure, which is based on the liquid liquid extraction principle, meets the requirements of the GC detection system. The quantitative results are calculated from calibration curves, which are established by treating spiked samples in exactly the same way.

EXPERIMENTAL.

Equipment.

A 25 m × 0.32 mm i.d. CP *tm* Sil 5 fused silica capillary column is used (Chrompack, Middelburg, The Netherlands).

CP *tm* Sil 5 is a non-polar poly-dimethyl siloxane gum phase.

Considering the polarity this column is equivalent to a number of well known and frequently used phases such as SE 30, OV 1, OV 101, DC 200, SE 96 and SP 2100.

The column was installed in a Perkin Elmer Sigma 2B gas chromatograph, equipped with a ^{63}Ni electron capture detector (ECD). A direct injection technique was applied, using a 1.0 μl Hamilton Syringe (Hamilton, Switzerland).

A 0.8 mm i.d. capillary glass insert liner is used to connect the column to the injector.

Purified argon/methane (90/10) is used as carrier and make-up gas at a pressure of 0.5 bar.

Reagents and materials.

All solvents were Merck analytical or pesticide grade. An Yperite stock solution containing 1 mg/ml was prepared in n-hexane. An external standard solution containing 100 ng/ml of pentachloroethane in n-hexane was also prepared.

Custom-made quartz conical test tubes were obtained from Vel (Belgium) and the silica Sep-Pak cartridges originated from Waters Associates.

Thin layer chromatography.

Glass plates (20 × 20 cm), pre-coated with a 0.25 mm layer of silica gel 60 F_{254} (Merck), were used. The mobile phase consisted of methylene chloride. Prior to chromatography the TLC chamber was saturated for 30 minutes with the developing solvent.

Gas chromatography.

The temperature of the injection port was set at 260°C. The detector was heated at 300°C. A temperature program was initiated

at 55°C for 5 minutes, increased up to 120°C at 5°C/min., and finally increased up to 300°C with a rate of 10°C/min., where it was kept for another 10 minutes.

Sample preparation.

A 10 g or 10 ml sample amount was placed into a 16 $\times$ 200 mm test tube. After addition of 0.5 ml of a saturated NaCl solution, an extraction was accomplished by adding 10 ml of CH_2Cl_2, shak-
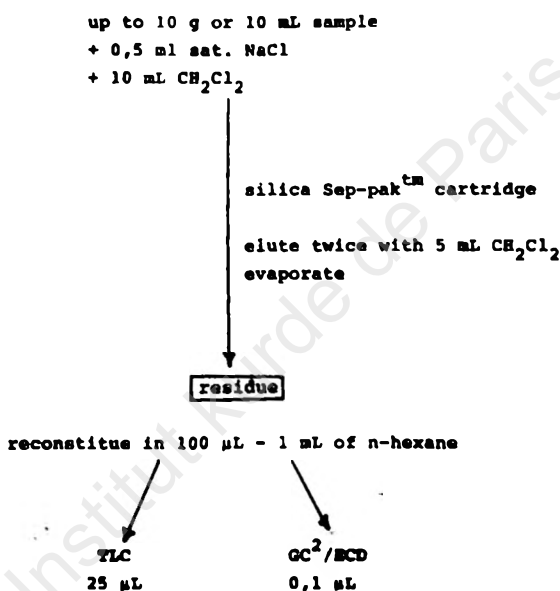
FIG. 1. — Extraction procedure.

ing the resulting mixture for 15 minutes at high speed on an automatic shaker, and centrifuging for 5 minutes at 2,000 rpm. The organic layer was then passed through a silica Sep-Park cartridge. In order to obtain a complete elution 2 $\times$ 5 ml of CH_2Cl_2 were added.

The eluant was collected in a conical test tube and evaporated to dryness at room temperature under a gentle stream of nitrogen. The residue was reconstituted with 100 to 1,000 μl of n-hexane, containing pentachloroethane (100 ng/ml) as the external standard (fig. 1).

A 25 μl of the redissolved residue was applied on the TLC plate. The plate was allowed to develop over a 15 cm distance, removed from the chromatographic chamber and air dried. After spraying with a $PdCl_2$ reagent (2 % in 10 % HCl), Yperite appeared as a yellow spot with an R_F value of 0.77. Another 0.1 μl aliquot of the reconstituted residue was injected onto the capillary column. A standard calibration curve, evaluated for spiked plasma samples, yielded excellent results in the subnanogram range. However, the detector response was no longer linear once the
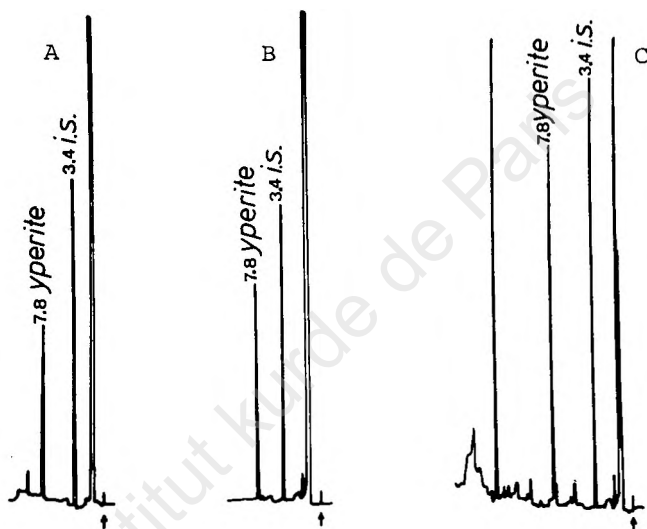
FIG. 2. — Chromatograms of the standard yperite (A), a spiked plasma sample (B) and a suspected soil sample (C).

concentration exceeded 5 ng/0.1 μl injection volume. Chromatograms obtained from the injection of a spiked plasma sample extract and an extract of a suspected soil sample are shown in figure 2.

RESULTS AND DISCUSSION.

Relatively pure sample extracts, containing a sufficient amount of the poison, can be analysed by TLC, the latter technique being useful for identity confirmation and semi-quantitation. Using methylene chloride as the developing solvent (R_F 0.77), the $PdCl_2$-reagent allows the simultaneous chemical detection of Yperite and

some organophosphorous insecticides. PdCl$_2$ has about the same sensitivity (1 µg) as the NBP reagent [4-(p-nitrobenzyl)-pyridine], mentioned in literature (4).

For a quantitative analysis of low concentrated samples of Yperite, capillary gas chromatography has to be preferred. The main advantages of the use of a fused silica capillary column are the flexibility, which is due to the material itself and the dimensions of the column, and the great inertness, characterized by a low metal oxide content (less than 1 ppm) (13). In spite of the easy contamination and the loss of resolution, the direct injection technique, which allows the exact quantitation of
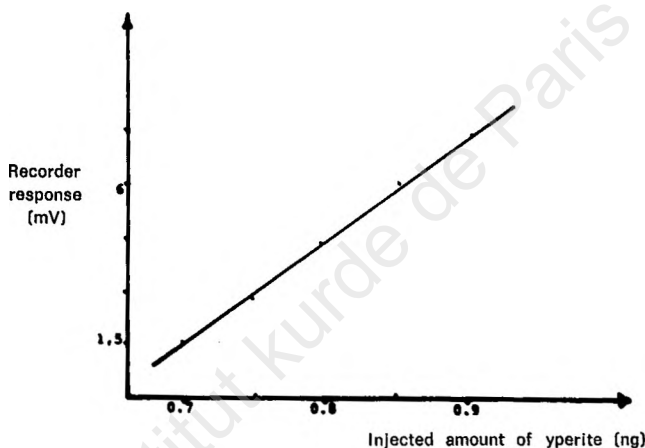
FIG. 3. — Calibration graph.

small amounts, is still the most suitable technique for trace analysis. The capillary column is permanently deactivated (p.d.) at a high temperature (300°C), in order to allow the siloxane polymer to react with the free silanol groups on the surface of the column (14). The electron capture detector allows the determination of Yperite in the picogram range. The sulfur mustard is still detectable below 100 pg, with an acceptable signal to noise ratio, a stable baseline and a good linearity (fig. 3), y = 0,7395 - 8,833 x.

The reproducibility of the procedure is evaluated from the results obtained after a five fold analysis of a simulated Yperite sample (spiked plasma samples containing 4.5 µg/ml of Yperite) and two suspected samples taken from Iran (soil and bomb content). The five determinations were carried out at the same day (table I).

TABLE I

Reproducibility data (within run)

Sample number	Concentration found		
	Simulated plasma yperite sample (µg/ml)	Yperite soil sample from Iran % (w/w)	Yperite bomb sample from Iran % (w/w)
1	4.2	13.9	60.2
2	3.8	14.3	58.4
3	4.6	14.5	57.9
4	3.9	14.2	59.8
5	4.4	13.7	60.4
Average	4.18	14.12	59.34
SD	0.33	0.32	1.12
S$\bar{x}$	0.15	0.14	0.50
CV %	8.01	2.26	1.89

The chloride ions, added before extraction, stabilize the sulfur mustard by slowing down the formation of the dialkylsulfonium ion in the presence of water (4). The silica Sep-Pak^{TM} extraction - purification procedure is based on the liquid liquid extraction principle. The organic extraction solvent (CH_2Cl_2) is poured into the silica Sep-Pak^{TM} cartridge, prepacked with an inert matrix of large surface area. The impurities are retained in the matrix, while the compounds of interest pass freely through the cartridge. As compared to classical extraction procedures, the proposed silica Sep-Pak purification and extraction method offers the advantage to yield extracts, which are pure enough for direct analysis, with few interfering peaks and a good recovery and reproducibility.

CONCLUSION.

The described column extraction and purification procedure, followed by TLC for qualitative and semi-quantitative analysis and capillary GC for the quantitation of Yperite, enables the toxicologist to screen environmental samples within a short time and at a high sensitivity range. The same method was applied on human biological samples of Iranian soldiers attacked by gases, in agreement with Machata and Vycudilik, who also used a similar method for the analysis of biological materials. According to their reports, mass spectrometry was used to confirm their results.

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Gas chromatography and toxicological determination of trichothecenes in biological and environmental materials : applicability to environmental samples associated with « Yellow Rain »

by A. HEYNDRICKX, N. SOOKVANICHSILP
and M. VAN DEN HEEDE

Department of Toxicology, State University of Ghent.
Hospitaalstraat 13, 9000 Ghent, Belgium

SUMMARY.

Trichothecenes have been found as contaminants in foods and feeds. Recently, they were detected in samples associated with « Yellow Rain ».

Methods for the analysis of mixed trichothecenes in a variety of biological and environmental materials are presented. Substrates were extracted with acetone, chloroform, acetonitrile, or a methanol/water (1/1) mixture. Washing the extracts with a ferric chloride gel or n-hexane or re-extracting with chloroform was necessary for some substrates prior to a further clean-up on XAD-2 or alumina columns. The eluates were derivatized with heptafluorobutyrylimidazole (HFBI). The HFB-esters were separated on an SE-52 WCOT glass capillary column and quantitated with a ^{63}Ni electron capture detector. The detection ability varied from 0.02 to 2 ppm, depending on the types of trichothecenes and substrates. Recoveries for the less polar trichothecenes were rather high, whereas those for the more polar ones were low.

INTRODUCTION.

The trichothecenes are a group of fungal metabolites produced by various species of Fusarium, Myrothecium, Trichoderma, Trichothecium and other species of imperfect fungi. There are more than 50 known derivatives of trichothecenes including those found in laboratory cultures (1). The basic chemical structure of the trichothecene family is shown in figure 1. As most naturally occur-
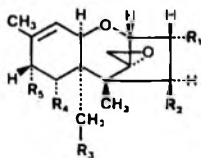
Compound	R_1	R_2	R_3	R_4	R_5
T-2 toxin	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
HT-2 toxin	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
Diacetoxyscirpenol	OH	OAc	OAc	H	H
Verrucarol	H	OH	OH	H	H
T-2 tetraol	OH	OH	OH	H	OH
Fusarenon-X	OH	OAc	OH	OH	=O
Deoxynivalenol	OH	H	OH	OH	=O
Nivalenol	OH	OH	OH	OH	=O

FIG. 1. — The basic structure of the trichothecene family.

ring compounds contain an epoxide ring at C-12,13 and a double bound at C-9,10, they are characterised as 12,13-epoxytrichothec-9-ene. Ueno, 1977 (2), classifies trichothecenes according to their chemical structures, while Pathre and Mirocha, 1977 (3), divide them according to their solubility : group A, including T-2 toxin, HT-2 toxin, Diacetoxyscirpenol and Verrucarol, is highly soluble in most aprotic solvents such as ethyl acetate, acetone, chloroform, methylene chloride and diethyl ether, while groupe B, including T-2 tetraol, Deoxynivalenol, Nivalenol and Fusarenon-X, is soluble in either very polar solvents or protic solvents such as methanol and ethanol.

Naturally occurring trichothecenes are found as contaminants in foods and feeds associated with human and animal toxicosis,

e.g. Taumelgetreide toxicosis (Siberia), alimentary toxic aleukia (USSR), red mold toxicosis (Japan) and moldy corn toxicosis (USA) (2). Trichothecenes are not only toxic to animal cells but also to higher plants (4). Since about 1975, many reports on the use of chemical warfare agents in Southeast Asia have been documented. This kind of chemical warfare is known as « Yellow Rrain » (according to its appearance as a yellowish, wet or sticky semisolid of relatively large particle size, with a rainlike sound when being sprayed). The most characteristic symptoms of Yellow Rain intoxication are including bloody vomit and bloody diarrhea. Fatalities occur among 10 to 20 % of the population directly exposed to the attack. Recent analysis of samples (e.g. leaves, urine, blood, tissues) from Laos and Kampuchea, associated with Yellow Rain, showed that some samples contained trichothecenes, e.g. T-2 toxin, HT-2 toxin, Diacetoxyscirpenol, Deoxynivalenol and Nivalenol (5, 6). Therefore the aim of the present work is to develop a sensitive detection technique for the analysis of various trichothecenes in different samples.

MATERIALS AND METHODS.

Apparatus and materials.

1. Gas chromatograph.

   A Sigma 2B gas chromatograph (Perkin Elmer), equipped with a ^{63}Ni electron capture detector was used. The chromatographic column was a 25 m x 0.5 mm, i.d. SE-52 WCOT glass capillary column. Operating conditions were : column temperature, initiated at 180°C for 5 min., increased at 2°C/min. to 200°C, then increased at 5°C/min. to 240°C, where it was kept for 5 min. ; temperature of the injection port, 260°C ; detector temperature, 300°C ; carrier gas (argon/methane, 90/10), 5 ml/min.

2. Centrifuge : maximum 5,000 rpm (Ecco-Alpha 1).

3. Vortex Mixer (Lab-Line).

4. Grinder.

5. Chromatographic columns, for XAD-2 column clean-up (20 cm x 1 cm i.d.).

6. Chromatographic columns, for Al_2O_3 column clean-up (upper part 10 cm x 1.6 cm i.d., lower part 10 cm x 0.6 cm i.d.).

7. Glass stopped tubes.

Reagents.

1. Standard trichothecenes : T-2 toxin, HT-2 toxin, Diacetoxy-scirpenol, Verrucarol, T-2 tetraol, Verrucarin A and Roridin A (obtained from Sigma) ; Fusarenon-X, Deoxynivalenol and Niva-lenol were gifts.

   Dissolve each standard, except Deoxynivalenol and Nivalenol, in ethyl acetate and make a working solution of 4 μg/ml. Make a working solution of Deoxynivalenol and Nivalenol in ethanol at the same concentration.

2. Methoxychlor (Poly Science).

   Prepare a working solution of 4 μg/ml in pentane, for using as the internal standard solution.

3. N-Heptafluorobutyrylimidazole, HFBI (Pierce).

4. Sodium bicarbonate solution, 5 % in water.

5. XAD-2 resin (Fluka AG), 20-50 mesh.

   Place approximately 25 g of resin in a beaker and rinse it re-peatedly with 50 ml methanol until clear. Then rinse it twice with 50 ml 5 % sodium chloride solution and twice with 50 ml 1 % sodium bicarbonate solution. Finally rinse the resin 5 times with 100 ml distilled water. Store the resin in a refrigerator under a methanol/water mixture (30/70).

   Prepare the column as follows : insert a glass wool plug into the bottom of a chromatographic column and add a slurry of XAD-2 resin in order to obtain a bed volume of about 5 ml. Wash the resin 10 times before use with a volume of dis-tilled water equal to twice the bed volume of the resin at a rate of 4-5 ml/min. The XAD-2 resin can be reused repeated-ly after applying a proper washing and rehydration procedure.

6. Ferric chloride gel (pH 4.6).

   Prepare a 15 % solution of $FeCl_3.6H_2O$ (Merck) in water. Ad-just the pH of the solution to 4.6 with 1 N and 0.1 N NaOH and make up to 6 times the volume with distilled water (e.g.

25 ml of a 15 % $FeCl_3.CH_2O$ solution has to be made up to 150 ml) ; recheck the pH after adding the water.

7. Alumina, activity Super I-IV (10 % w/w water).

Weigh 100 g alumina activity Super I (basic Al_2O_3, type W 200-Woelm). Deactivate by adding 10 ml water and shake vigorously until lumps can no longer be observed. Let it stand for 24 hours.

Place a glass wool plug into the conical end of a chromatographic column. Fill the column with 2 g deactivated alumina under continuous vibration and top with about 200 mg anhydrous Na_2SO_4. Wash the column with 10 ml n-hexane before use.

8. Acid washed sand.

Wash the sand several times with water and once with conc. H_2SO_4 ; discard every washing. Add again conc. H_2SO_4 and let it stand for 24 hours. Discard the acid and wash the sand with water until the washings are free from acid. Heat the acid washed sand in a furnace at 500°C for 24 hours. After being cool, wash the sand with n-hexane and dry in an oven.

9. Saturated sodium chloride solution in water.

10. Anhydrous sodium sulfate.

11. Trifluoroacetic anhydride (Merck).

12. Benzene, pentane, acetonitrile, methanol, chloroform and n-hexane (analytical grade, Merck).

13. Parafilm.

14. S & S filter paper circles, diam. 100 mm, nr 595 (Schleicher & Schüll GmbH, Western Germany).

Derivatization.

Bring the following amounts of individual standards into a glass stopped tube : T-2 toxin 6 μg, HT-2 toxin 2 μg, Diacetoxyscirpenol 4 μg, Fusarenon-X 1 μg, Verrucarol 0.5 μg, T-2 tetraol 1 μg, Deoxynivalenol 1 μg, Nivalenol 1.5 μg and Methoxychlor 2 μg ; evaporate to dryness under nitrogen. Dissolve the residue in 0.5 ml benzene by agitating with a Vortex Mixer. Add 350 μl HFBI. Seal the stopper with parafilm and mix vigorously for 1 min. Heat the tube during

1 hour in a water bath maintained at 60°C. Let the content cool to room temperature. Add 2.5 ml 5 % NaHCO₃ solution and shake for 2 min. with the Vortex Mixer. Centrifuge at 2,500 rpm. Dilute 50 µl of the supernatant to 500 µl with pentane. Inject 0.5 µl into the gas chromatograph with a 1 µl Hamilton syringe.

Extraction and clean-up.

Biological fluids and tissues

Blood : add 10 ml acetone to 5 ml blood, mix well and filter through a filter paper. Wash the precipitate 3 times with 3 ml acetone. Evaporate the filtrate to dryness under nitrogen. Dissolve the residue in 150 µl methanol, add 2.5 ml water and pass through a prepared XAD-2 column. Wash the column with 10 x 5 ml water and discard the eluate. Elute the trichothecenes with 15 ml of a methanol/water mixture (9/1). Evaporate the eluate to dryness and dissolve the residue in 0.5 ml benzene. Do the derivatization as mentioned before.

Urine : extract 10 ml urine with 3 x 20 ml chloroform. Dry the chloroform layer over anhydrous Na₂SO₄ and filter. Evaporate the chloroform layer to dryness. Dissolve the residue in 150 µl methanol and add 2.5 ml water. Clean up on the XAD-2 column and derivatize.

Muscle (cow) and heart (pig) : weigh 6 g of the tissue and homogenize with 5 x 20 ml acetonitrile using a mortar and sand. Dry the acetonitrile over anhydrous Na₂SO₄ and filter. Concentrate the filtrate to about 3 ml. Add 15 ml water and 1 ml saturated NaCl solution. Wash the mixture with 3 x 15 ml n-hexane and discard the n-hexane layer. Warm the aqueous phase to get rid of n-hexane traces and cool. Clean up on the XAD-2 column and derivatize.

Liver (pig) and brain (human) : weigh 6 g of the tissue and proceed upon the same manner as mentioned under « Muscle and heart » for homogenization and washing with n-hexane. Extract the n-hexane washed aqueous phase with 3 x 10 ml chloroform. Dry the chloroform layer over anhydrous Na₂SO₄ and filter. Evaporate the chloroform layer to dryness. Dissolve the residue in 150 µl methanol and add 2.5 ml water. Clean up on the XAD-2 column and derivatize.

Environmental materials

Water : filter 30 ml water through a filter paper. Wash the residue on the paper with 3 x 3 ml methanol/water (1/1). Concentrate the filtrate to about 20 ml. Clean up on the XAD-2 column and derivatize.

Pebble : extract 25 g pebbles with 5 x 10 ml methanol/water (1/1), filter and concentrate to about 5 ml. Add 15 ml water. Clean up on the XAD-2 column and derivatize.

Salt : extract 15 g sea salt with 3 x 20 ml acetonitrile, filter and concentrate to about 3 ml. Add 15 ml water and extract with 3 x 10 ml chloroform. Dry the chloroform layer over anhydrous Na_2SO_4 and filter. Evaporate the chloroform layer to dryness. Dissolve the residue in 150 μl methanol and add 2.5 ml water. Clean up on the XAD-2 column and derivatize.

Leaf : extract 3.5 g of dry leaves with 5 x 20 ml acetonitrile, filter and concentrate to about 5 ml. Add 25 ml $FeCl_3$ gel solution, mix and let it stand for a while. Filter through a fluted filter paper. Concentrate the filtrate to about 15 ml. Extract with 3 x 10 ml chloroform and filter over anhydrous Na_2SO_4. Evaporate the filtrate to about 1 ml and pass through a prepared alumina column. Elute the trichothecenes with 15 ml chloroform and 20 ml methanol/chloroform (1/9). Evaporate the eluate to dryness, dissolve the residue in 0.5 ml benzene and derivatize.

Bark : extract 3 g bark with 5 x 10 ml acetonitrile, filter and concentrate to about 5 ml. Proceed upon the same manner as described under « Leaf » for cleaning up with $FeCl_3$ gel and re-extracting with chloroform. Evaporate the chloroform extract to dryness. Dissolve the residue in 150 μl methanol and add 2.5 ml water. Clean up on the XAD-2 column and derivatize.

Rice : extract 20 g rice with 5 x 20 ml acetonitrile. Do the following steps as described for liver and brain.

Recovery experiments.

Standards

Bring the amounts of all trichothecenes into a test tube and evaporate to dryness as mentioned in the derivatization procedure.

a) Dissolve the residue in 150 µl methanol, add 2.5 ml water and bring onto the XAD-2 column. Do the clean-up procedure. After evaporating the eluate to dryness, add 2 µg Methoxychlor and evaporate the content to dryness again. Dissolve the residue in 0.5 ml benzene and derivatize.

b) Dissolve the residue in 3 ml acetonitrile, add 15 ml water, wash with 3 x 15 ml n-hexane and discard the n-hexane layer. Warm the aqueous phase to get rid of traces of n-hexane, cool and bring onto the XAD-2 column. Do the clean-up and derivatization as mentioned under *a.*

c) Dissolve the residue in 3 ml acetonitrile, add 15 ml water and extract with 3 x 10 ml chloroform. Evaporate the chloroform to dryness, add 2 µg methoxychlor and evaporate the content to dryness again. Dissolve the residue in 0.5 ml benzene and derivatize.

Spiked samples

Muscle (cow) spiked with individual standards :

Add an individual trichothecene standard (in an amount as mentioned under « Derivatization ») to 6 g muscle and evaporate the solvent. Proceed upon the same manner as described under « Extraction and Clean-up » procedure for homogenization, washing with n-hexane and clean-up on the XAD-2 column. Add 2 µg Methoxychlor to the residue obtained after evaporation of the eluate and evaporate to dryness again. Dissolve the residue in 0.5 ml benzene and derivatize.

Muscle (cow) spiked with the standard mixture :

Add the trichothecene mixture (containing amounts of individual standards as mentioned under « Derivatization ») to 6 g muscle. Do the following steps as described under « Muscle spiked with individual standards ».

Spiked blood, urine, heart, liver, brain, pebble, salt, leaf, bark and rice :

For non-fluid samples, add the trichothecene mixture and evaporate the solvent before homogenization or extraction with the appropriate solvents; for blood as well as for urine, evaporate the solvent of the standard solutions to dryness, then add a suitable amount of blood or urine and mix well. Do the following steps as

described in the corresponding extraction and clean-up procedure, add 2 µg Methoxychlor, evaporate to dryness and dissolve the residue in 0.5 ml benzene before derivatization.

Sample analysis.

The samples analysed were water, pebbles (red), salt (grey), leaves (4 samples), bark (2 samples, crude and extracted) and rice.

All samples were prepared according to the corresponding « Extraction and clean-up » procedure.

In case of bark, dissolve the extract in chloroform, evaporate a known portion to dryness, redissolve the residue in 5 ml acetonitrile and do the following steps for clean-up with $FeCl_3$ gel, chloroform extraction, clean-up on the XAD-2 column and derivatization, as described for bark.

RESULTS AND DISCUSSION.

Derivatization.

Heptafluorobutyryl esters of trichothecenes can be hydrolyzed back to the corresponding alcohols in the presence of an acid; however, derivatization with HFBI does not form an acid as a by-product. Romer *et al.*, 1978 (7), found that T-2 toxin and Diacetoxyscirpenol required 15 sec. at room temperature for derivatization, while Scott *et al.*, 1981 (8), found that Deoxynivalenol had to be heated for 1 hour at 60°C. In the present study, all the trichothecenes used could be derivatized after heating at 60°C for 1 hour. Since the derivatized products are not so stable at room temperature or even in a refrigerator, the analysis should be done soon after derivatization.

Verrucarin A and Roridin A, which are more complicated in their chemical structures (fig. 2), could not directly be derivatized with HFBI. Both had first to be hydrolyzed with 1 N NaOH in an aqueous-alcoholic medium. Then they had to be extracted with chloroform, dried, dissolved in benzene and derivatized.

Benzene seems to be an excellent derivatizing medium. As it can interfere with electron capture detection (7), it was diluted with pentane. The internal standard solution (Methoxychlor dissolved in pentane) could be used for making dilutions of the deriva-

tized toxins. However, Methoxychlor was added to the toxin mix-
ture just before derivatization in order to control any evaporation
of benzene while heating for 1 hour at 60°C. The results obtained
after derivatization with TFAA (trifluoroacetic anhydride) were less
satisfactory than those obtained when using HFBI. This was mainly
due to the high volatility of the TFA derivatives giving rise to
inaccuracies during the quantitation of the derivatized products.

Verrucarin A : R = —CCH(OH)CH(CH₃)CH₂CH₂OCCH=CHCH=CHC—

Roridin A : R = —CCH(OH)CH(CH₃)CH₂CH₂OCHCH=CHCH=CHC—
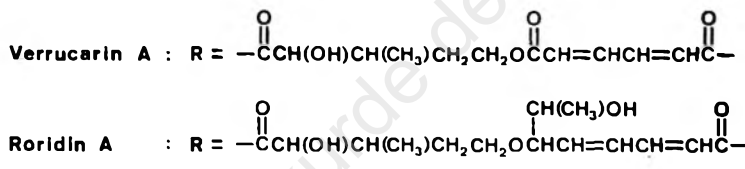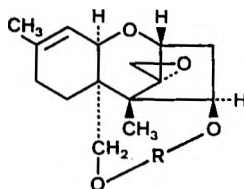
FIG. 2. — Examples of macrocyclic trichothecenes.

Extraction.

According to the solubility and the group division as described
by Pathre and Mirocha, 1977 (3), trichothecenes of group A are
efficiently extracted from the substrates with aprotic solvents,
whereas those of group B are extracted with methanol, aqueous
methanol, aqueous acetonitrile, or water. As mentioned by Watson
et al., 1983 (5), acetone was used for precipitating the blood pro-
teins and as the solvent for extracting the trichothecenes. For
tissues, salt, leaves, bark and rice, acetonitrile was used as the
extracting solvent, as it had been mentioned that acetonitrile ap-
peared to be one of the favourable solvents used for the extraction
of group A trichothecenes from corn and mixed feeds (3). Although
ethyl acetate can be used for extraction as well as acetonitrile,
it gives rise to extracts requiring an extensive purification (3). In
favour of this finding (3), the same results were obtained in this
study. Watson *et al.*, 1983 (5), also used acetonitrile to extract

some trichothecenes of both groups from leaf and tissue samples. A mixture of methanol/water (1/1) was also proposed for the extraction of T-2 toxin and Diacetoxyscirpenol from corn and mixed feeds (7), and for Deoxynivalenol from wheat and other grains (8). Kamimura *et al.,* 1981 (9), used methanol/water (95/5) to extract Nivalenol, Deoxynivalenol, Fusarenon-X, T-2 toxin, Diacetoxyscirpenol, HT-2 toxin, etc. in cereals, grains and foodstuffs. In this study, methanol/water (1/1) was used to extract the toxins from pebbles, since it was found that the extract could be passed directly through the XAD-2 column after being diluted with water. In addition, one had been trying to extract some toxins, e.g. : T-2 toxin, Diacetoxyscirpenol, HT-2 toxin, Verrucarol and Nivalenol from the toxin adsorbing charcoal and it was found that 100, 75, 55, 53 and 3 % of these toxins, respectively, could be extracted with chloroform.

Clean-up.

A variety of columns can be used for clean-up, depending upon the types of extracts and the methods of extraction, e.g. : XAD-2 (5), silica gel (7, 8), florisil (9) and XAD-4 (9). Using XAD-2 columns it was found that the per cent recoveries of group A trichothecenes were rather high (82 to 101 %), while those of group B toxins were low (32 to 64 %) (table I). This might be due to

TABLE I

Per cent recoveries of trichothecenes using different methods

Trichothecene	XAD-2[a]	n-hexane + XAD-2[b]	CHCl$_3$[a]
T2 toxin .	95	74	102
HT-2 toxin	101	99	89
Diacetoxyscirpenol .	82	91	84
Fusarenon-X	64	61	64
Verrucarol	91	90	82
T 2 tetraol	32	23	—
Deoxynivalenol	57	42	23.5
Nivalenol	45	28	—

a, b, c as described under • Recovery experiments • for standards.

the loss of group B toxins by washing with water prior to elution. The suitability of silica gel and florisil was also checked and it was found that different eluents were needed to elute all the toxins of the mixture; the eluates also contained much more im-

purities as compared to an XAD-2 clean-up. Initial washing or re-extracting before column clean-up sometimes seemed to be necessary. In the present study it was found that trichothecenes of group B were poorly re-extracted into chloroform and that T-2 tetraol and Nivalenol, at the amounts spiked, could not be detected (table I). Washing the extract with n-hexane after a previous addition of a saturated NaCl solution in order to lower emulsion formation, not only reduced impurities, but also caused a marked reduction in recovery of some toxins (table I). For leaves, both FeCl₃ gel and alumina columns were needed. However, these procedures also caused a marked reduction in recovery of group B toxins, which means that T-2 tetraol, Deoxynivalenol and Nivalenol could no longer be detected (table III). Similar results were found for bark.

Recovery experiments.

What the addition of single standards or a standard mixture to muscle tissue is concerned, the recoveries found were not extremely different (table II). Per cent recoveries from all kinds of

TABLE II

Comparison of the per cent recoveries of trichothecenes for muscle spiked either with individual standards or with a standard mixture

Trichothecene	Spiking with individual standards	Spiking with a standard mixture
T-2 toxin	73	75
HT-2 toxin	74	78
Diacetoxyscirpenol	84	82
Fusarenon-X	54	61
Verrucarol	81	72
T-2 tetraol	15	16
Deoxynivalenol	27	29
Nivalenol	28	25

spiked substrates with group A trichothecenes were rather high, whereas those for group B trichothecenes were much lower (table III). However, due to the high sensitivity of the proposed gas chromatographic procedure, the described methods are suitable for the simultaneous detection of low levels of both toxin groups in all samples, which means saving of time. Some examples of chromatograms obtained during the recovery studies are shown in figure 3.

A
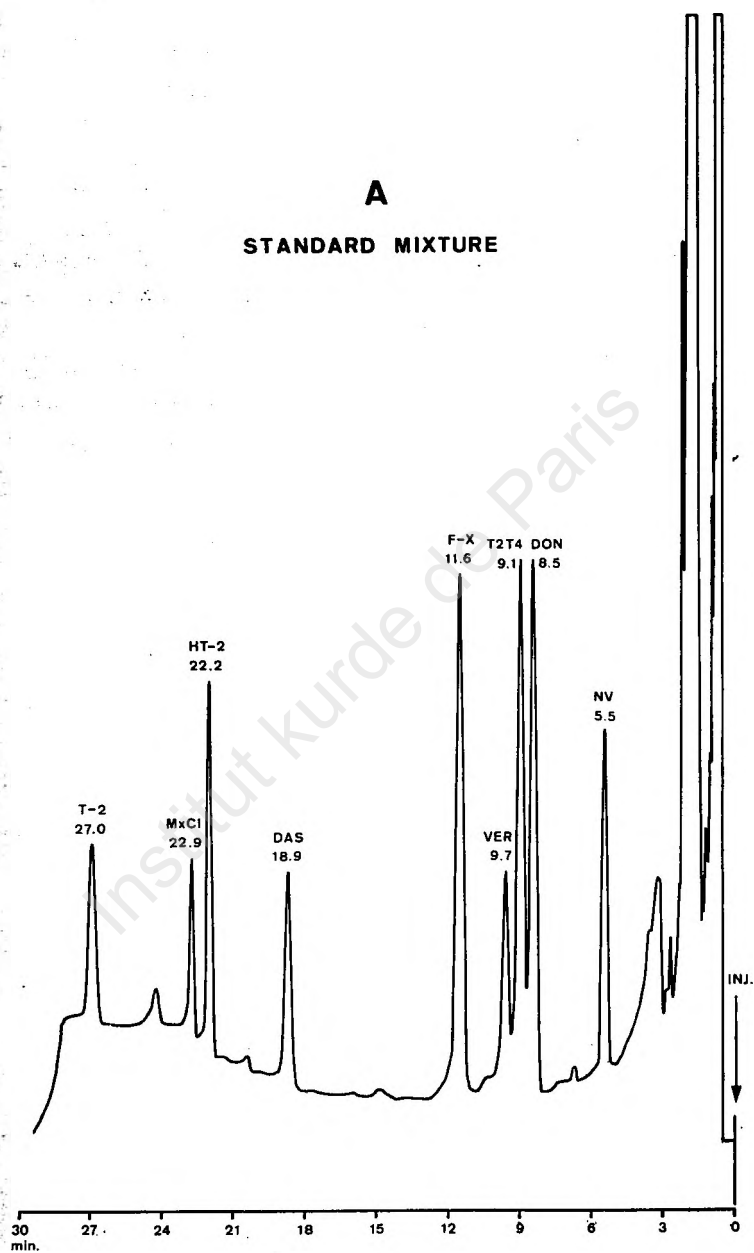
STANDARD MIXTURE

FIG. 3. — Chromatograms of the standard mixture (A)
and spiked sample extracts (B, C and D).

B

SPIKED BLOOD SAMPLE

C

SPIKED URINE SAMPLE
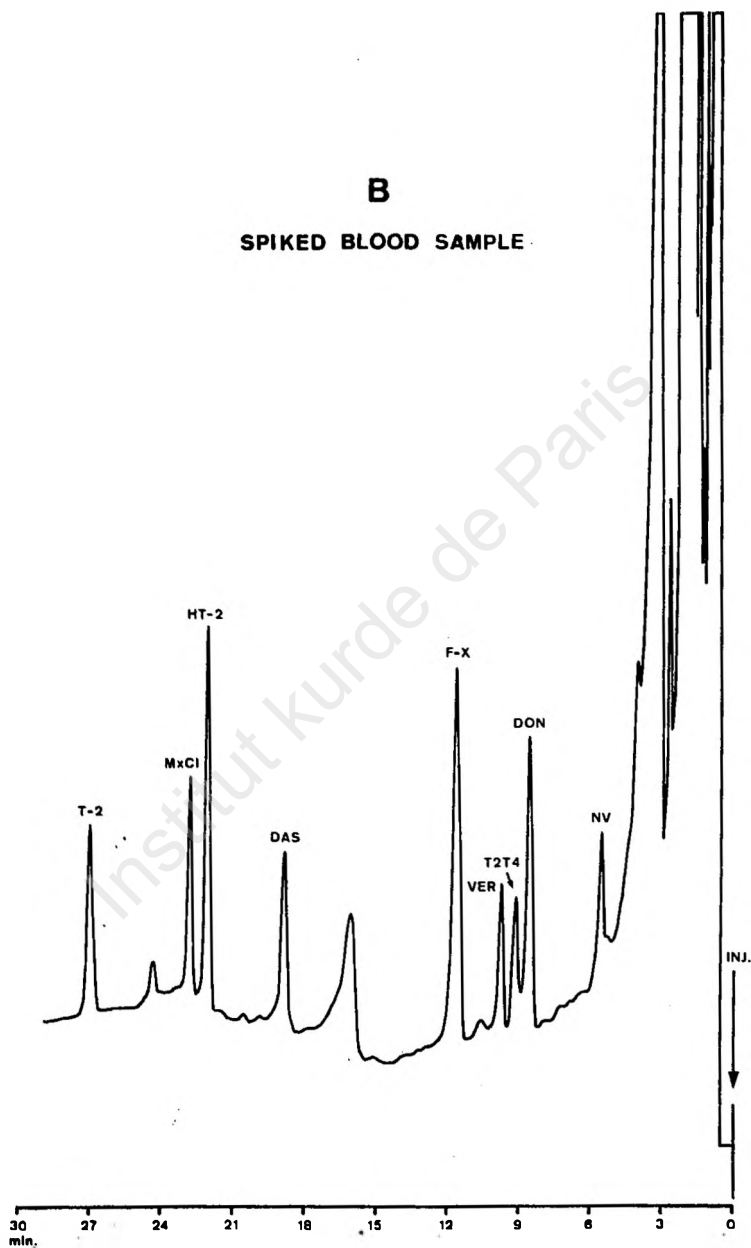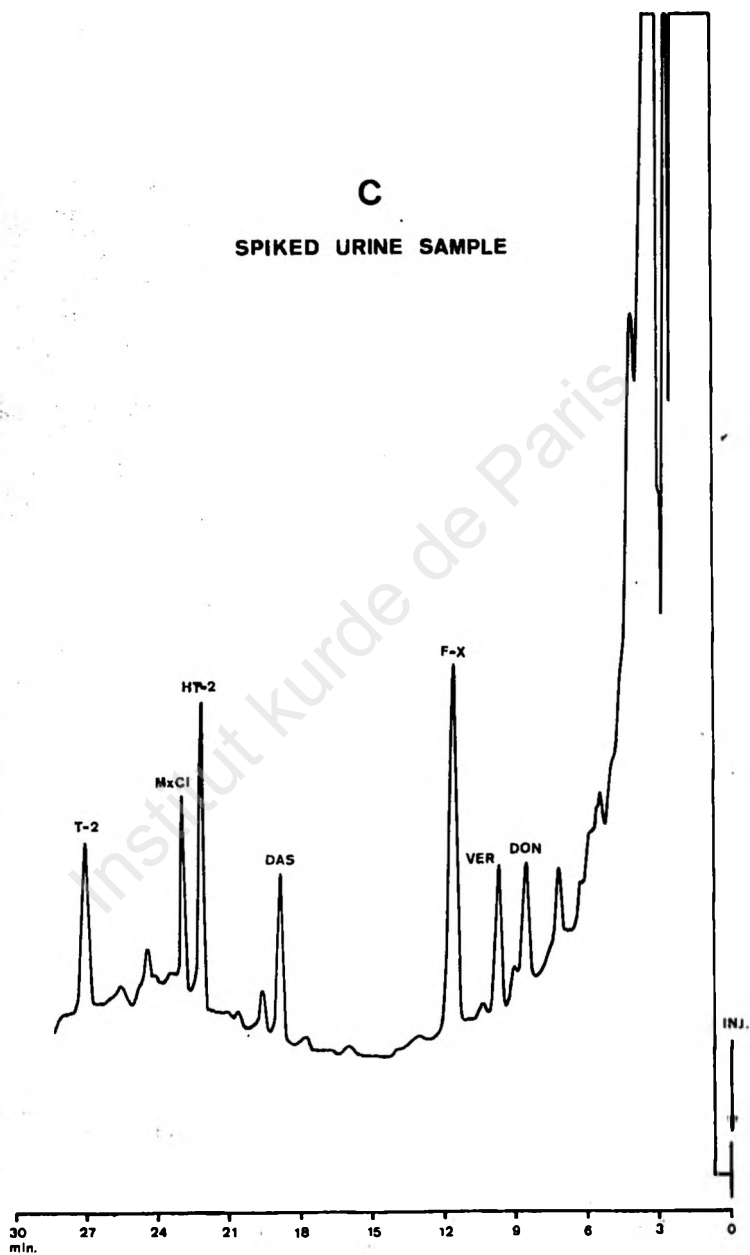
D

SPIKED MUSCLE SAMPLE
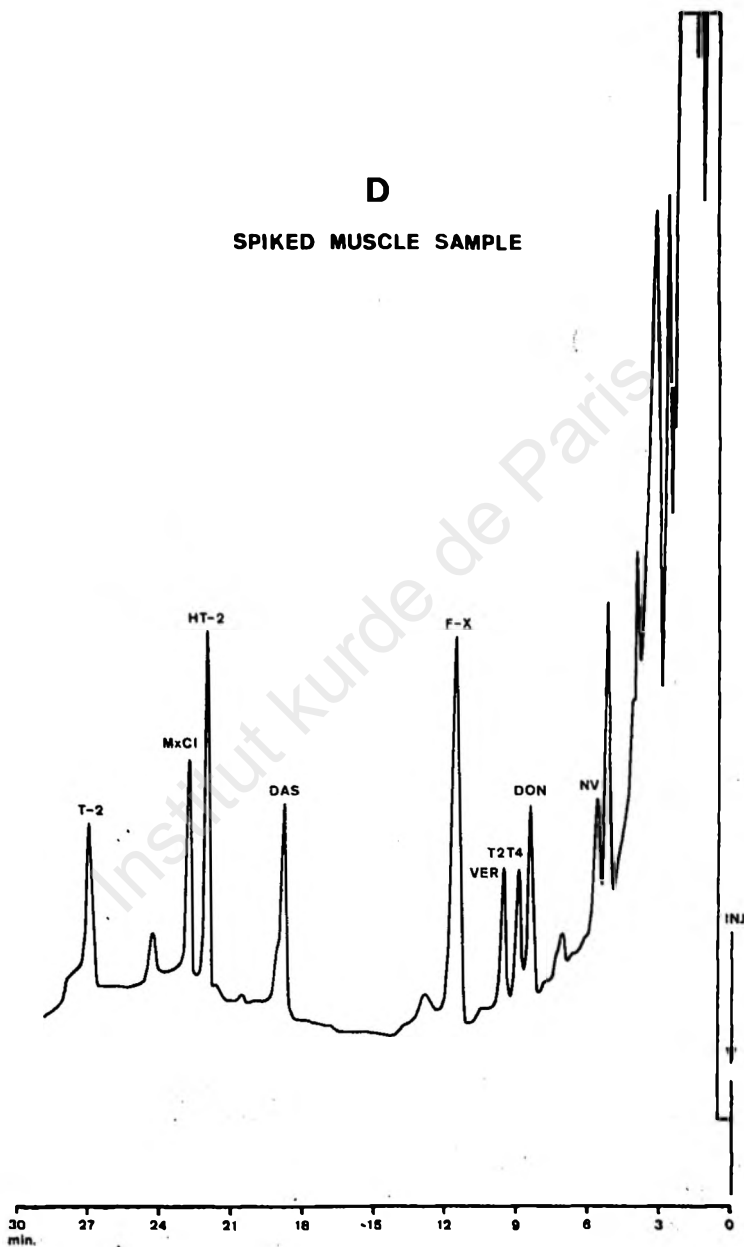
TABLE III

Per cent recoveries of trichothecenes
for various biological and environmental materials

	T-2	HT-2	DAS	F-X	VER	T_2T_4	DON	NV
Blood . . .	89 (1.2)	82 (0.4)	73 (0.8)	59 (0.2)	78 (0.1)	20 (0.2)	44 (0.2)	33 (0.3)
Urine . . .	88 (0.6)	73 (0.2)	72 (0.4)	63 (0.1)	82 (0.05)	—	19 (0.1)	—
Muscle . . .	75 (1)	78 (0.33)	82 (0.07)	61 (0.17)	72 (0.08)	16 (0.17)	29 (0.17)	25 (0.25)
Heart . . .	77 (1)	79 (0.33)	68 (0.07)	55 (0.17)	64 (0.08)	15 (0.17)	41 (0.17)	—
Liver . . .	72 (1)	87 (0.33)	70 (0.67)	41 (0.17)	67 (0.08)	—	18 (0.17)	—
Brain . . .	78 (1)	70 (0.33)	88 (0.67)	37 (0.17)	65 (0.08)		15 (0.17)	—
Stone . . .	84 (0.24)	82 (0.08)	82 (0.16)	61 (0.04)	82 (0.02)	23 (0.04)	64 (0.04)	34 (0.06)
Salt . . .	107 (0.4)	82 (0.13)	76 (0.27)	54 (0.07)	81 (0.03)	—	51 (0.07)	—
Leaf . . .	103 (1.7)	81 (0.57)	75 (1.1)	20 (0.29)	78 (0.14)	—	—	—
Bark . . .	91 (2)	86 (0.67)	80 (1.3)	31 (0.33)	74 (0.17)	—	—	—
Rice . . .	95 (0.3)	82 (0.1)	74 (0.2)	57 (0.05)	71 (0.03)	—	17 (0.05)	—

DAS   Diacetoxyscirpenol.   F-X   Fusarenon-X.
NV   Nivalenol. T_2T_4 T-2 tetraol.
VER   Verrucarol.
DON   Deoxynivalenol.

Values in parentheses indicating detection ability (μg trichothecene/g or ml material).

Analysis of environmental samples.

None of the sample chromatograms exhibited peaks corresponding to those of standard trichothecenes (fig. 4). If a positive peak is found, a further confirmation with gas chromatography/mass spectrometry can be applied. The yellow spots, which were present on the leaves and the bark, were found to contain pollen grains (microscopic examination using a magnification of 100 x 12.5). However, the analysis of 32 other environmental samples as leaves, water, pebbles, bark of trees and soil (Heyndrickx's private communication), revealed that 4 samples were positive for trichothecene mycotoxins (table IV). The greyish spots on these positive samples were submitted to a microscopic examination. Pollen grains were not detected. These samples were furthermore analysed by mass spectrometry, according to the procedure earlier

STONE SAMPLE
24.2 g

INJ.

30 min. 27 24 21 18 15 12 9 6 3 0

. A.
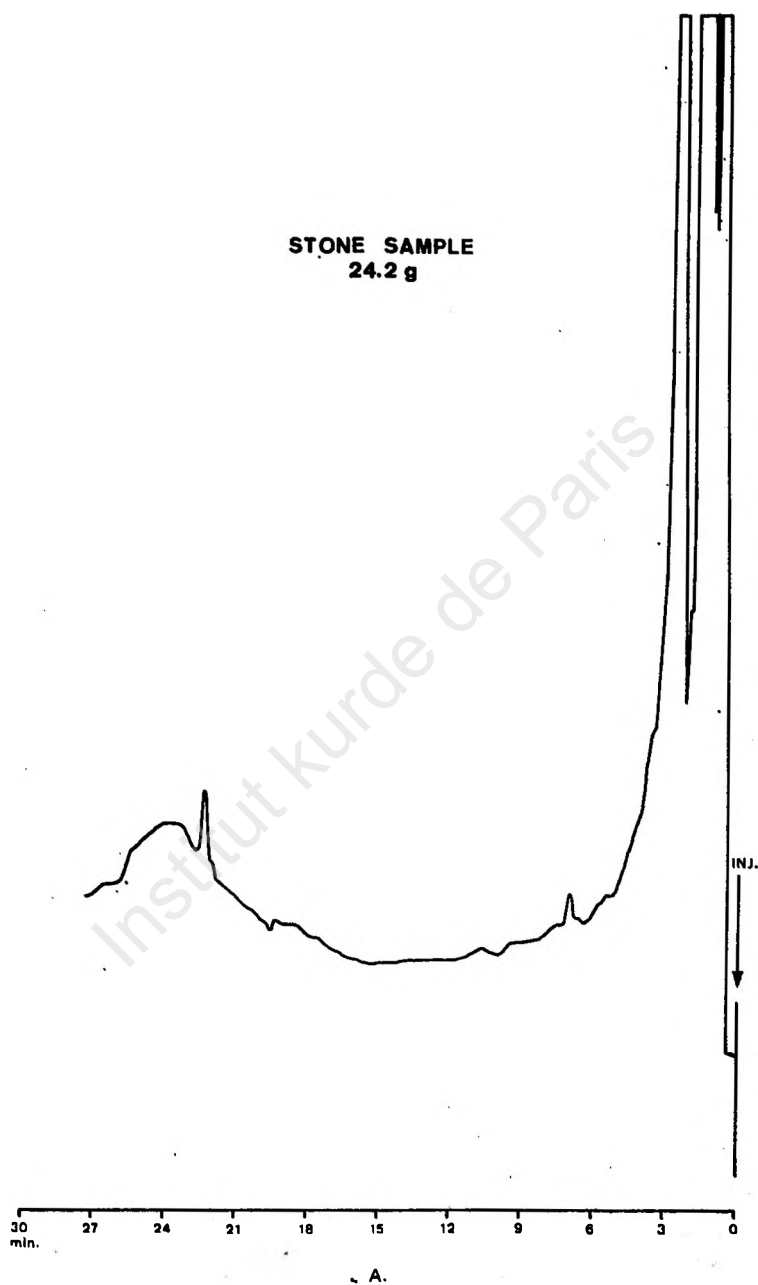
FIG. 4. — Chromatograms of sample extracts.

LEAF SAMPLE
3.5 g

INJ.

30   27   24   21   18   15   12   9   6   3   0
min.

B.

RICE SAMPLE
20 g

C.

BARK SAMPLE
0.603 g

INJ.

| 30 | 27 | 24 | 21 | 18 | 15 | 12 | 9 | 6 | 3 | 0 |

D.
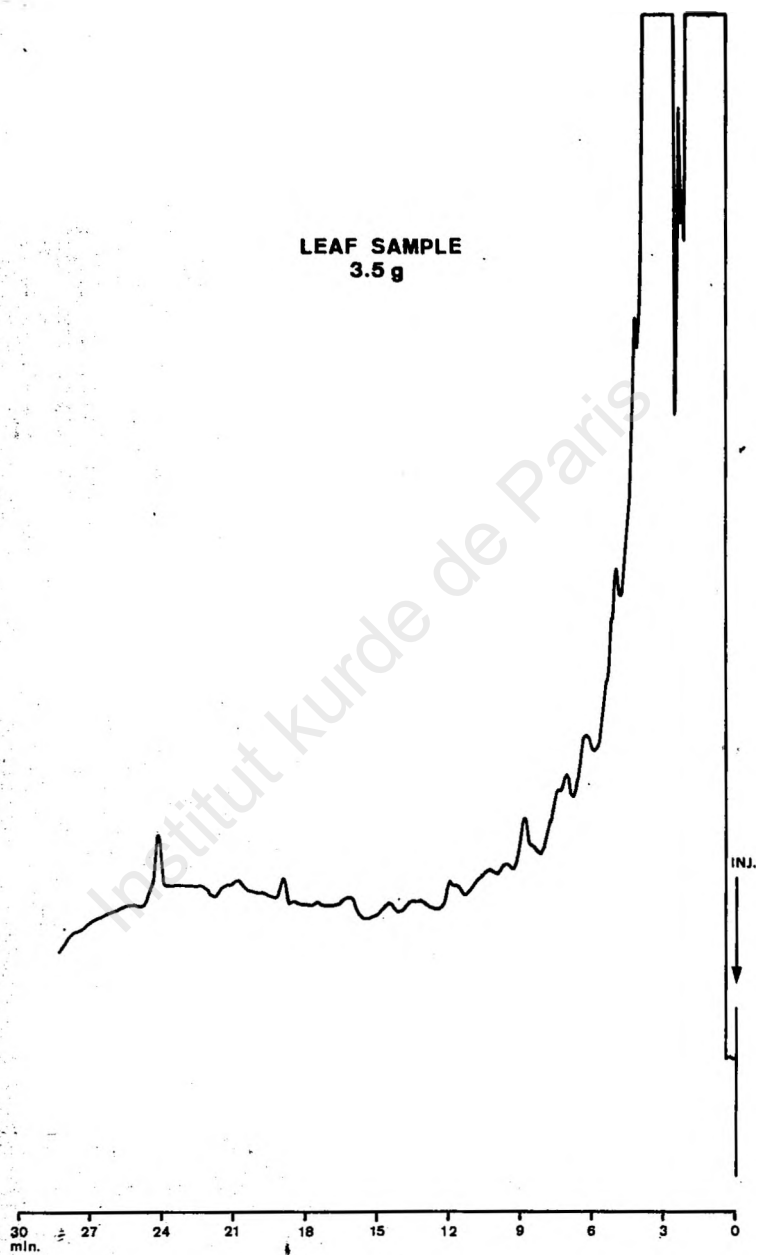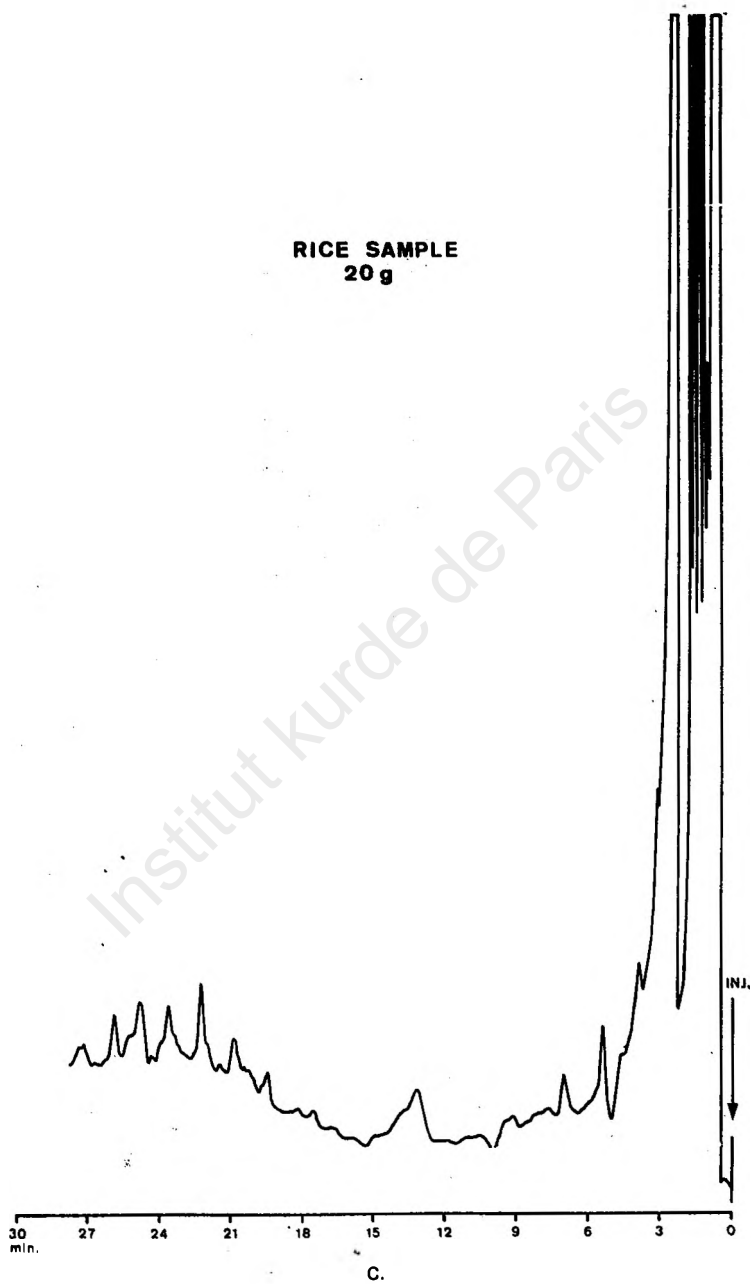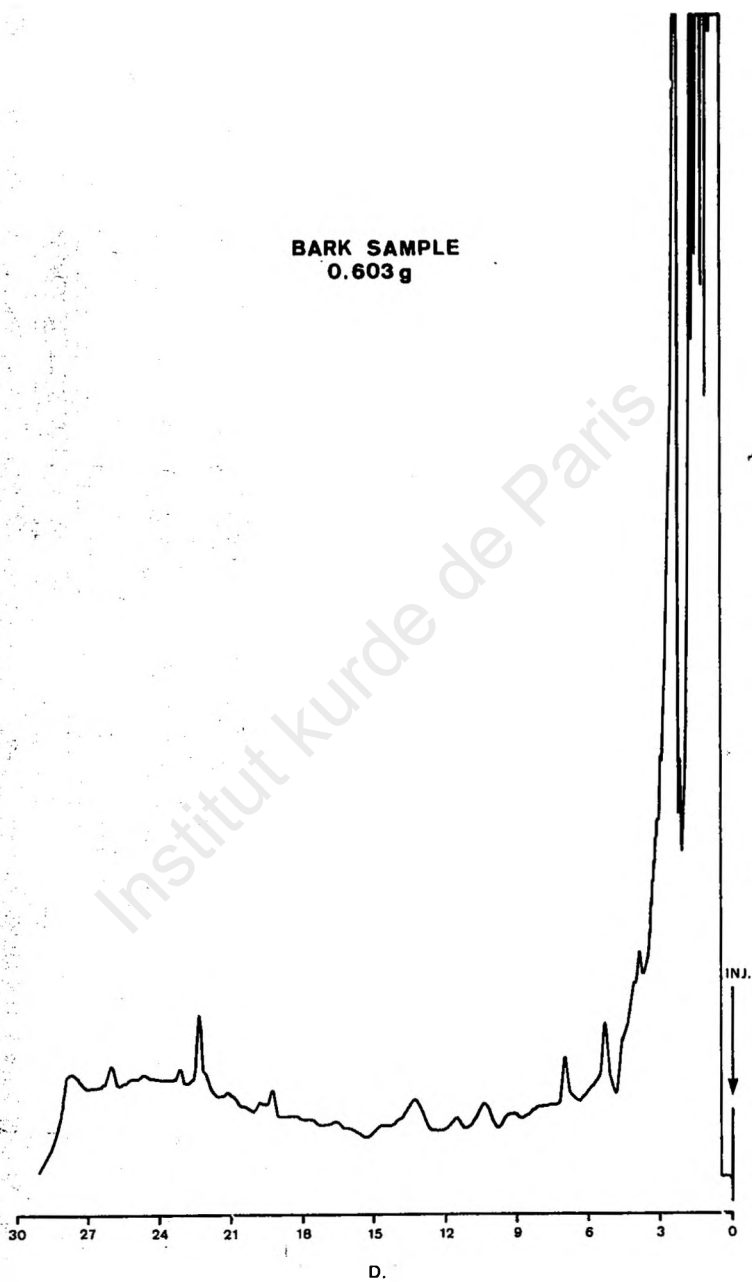
TABLE IV

Trichothecenes detected in environmental samples (ppm)

Sample	T-2 toxin	Nivalenol	Deoxynivalenol
Pebble (spots)	2.3	118	36
Bark (tree) (0.3 g)	98	—	—
Leaf (dry) (0.5 g)	25	16	—
Leaf (dry) (0.5 g)	4.2	92	64

described by Mirocha *et al.*, 1983 (10). It was found that the TMS derivatives of the trichothecenes studied yielded much better spectra than the HFB ones. The positive results, obtained by gas chromatography, as mentioned, showed no negative mass spectra and vice versa. As usual in forensic toxicology, in between analytical determinations the necessary blank samples were run in order to check possible impurities; no interferences were detected.

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A gas chromatographic procedure for the toxicological determination of trichothecenes in human tissues and body fluids

by A. HEYNDRICKX, N. SOOKVANICHSILP
and M. VAN DEN HEEDE

Department of Toxicology. State University of Ghent,
Hospitaalstraat 13, 9000 Ghent, Belgium

SUMMARY.

*Post-mortem human tissues and body fluids, which were col-
lected from two Iranian soldiers, were analyzed on trichothecene
mycotoxins, because of their reported use in chemical warfare.
The samples were extracted with acetonitrile, acetone, chloroform,
or ethyl acetate, and according to the nature of the sample a
further purification had to be performed by washing the extracts
with n-hexane or by re-extracting with chloroform. The XAD-2 resin
was chosen for the final clean-up of all extracts. The purified ex-
tracts were analyzed by gas chromatography.*

*Heptafluorobutyrylimidazole (HBFI) was considered as the most
suitable derivatizing agent for preparing compounds which are
detectable in the pg range by means of a ^{63}Ni electron capture
detector. The use of a CP ^{tm} Sil 5 fused silica capillary column
allowed an acceptable separation of all the heptafluorobutyrates
studied.*

INTRODUCTION.

Since various documents have reported that trichothecene my-
cotoxins, which are known as « Yellow Rain », have been used
as chemical warfare agents in Southeast Asia, a suspicion of
using such compounds as chemical weapons in other countries
has been gradually increased. Recently, Iranian soldiers were atta-

cked with chemical weapons by the Iraqis and several intoxicated soldiers were sent to different hospitals in Europe. Some victims died and in two cases the autopsy materials were sent to our Department for toxicological screening on chemical warfare agents including trichothecene mycotoxins.

MATERIALS AND METHODS.

Instrumentation.

A Perkin Elmer Sigma 2B gas chromatograph, equipped with a ^{63}Ni electron capture detector, was used. The chromatographic column was a 25 m x 0.32 mm i.d. CP ^{tm} Sil 5 WCOT fused silica column. The injection port and detector temperatures amounted 260 and 300°C respectively. A column temperature program was initiated at 170°C for 5 min., increased to 200°C at 2°C/min. and finally increased to 250°C at 5°C/min. Purified Argon/methane (90 : 10) was used as the carrier gas at a pressure of 0.8 bar. A direct injection technique, using a 1.0 μl Hamilton syringe, was applied.

Reagents.

All solvents (Benzene, pentane, acetonitrile, methanol, chloroform, n-hexane and ethyl acetate) were Merck analytical grade.

Standard trichothecenes : T-2 toxin, HT-2 toxin, diacetoxyscirpenol, verrucarol, T-2 triol and T-2 tetraol were obtained from Sigma® Chemical Company (St Louis, USA). Neosolaniol, Fusarenon-X, Nivalenol and Deoxynivalenol were gifts. Except for Nivalenol and Deoxynivalenol, stock solutions (1 mg/ml) of each toxin were prepared in ethyl acetate. For Nivalenol and Deoxynivalenol, a stock solution of the same concentration was prepared in ethanol. Working solutions, containing 4 μg/ml of each of the toxins, were prepared by dilution either with ethyl acetate or ethanol.

Methoxychlor (Internal Standard) was obtained from Polyscience Corp. (Evanston, Illinois, USA). A working solution containing 4 μg methoxychlor/ml of pentane was prepared.

N-Heptafluorobutyrylimidazole (HFBI) was purchased from Pierce Chemical Company (Illinois, USA).

Sodium bicarbonate solutions : 1 % and 5 % w/v in water.

Sodium chloride solutions : 5 % w/v in water and aqueous saturated solution.

Amberlite XAD-2, 20-50 mesh, was purchased from Fluka A.G. (Buchs, Switzerland). A 25 g amount of resin was placed in a beaker and repeatedly rinsed with 50 ml methanol until a clear supernatant was obtained. The resin was further rinsed with 2 x 50 ml 5 % sodium chloride solution and 2 x 50 ml 1 % sodium bicarbonate solution. Finally the resin was washed 5 times with 100 ml distilled water and stored in a refrigerator under a methanol/ water mixture (30 : 70). To prepare the column a glass wool plug was inserted into the bottom of a 10 mm i.d. glass column, which was subsequently filled with a slurry of XAD-2 resin in order to obtain a bed volume of about 5 ml. Before use the resin was eluted 10 times with a volume of distilled water equal to 2 times the bed volume of the resin (Rate 4-5 ml/ min.). When a proper washing and rehydration procedure was applied, the XAD-2 column could be used repeatedly.

Acid washed sand. An amount of sand was washed several times with water and once with conc. sulfuric acid. After rejection of the sulfuric acid, the sand was covered for 24 hours with another fraction of concentrated sulfuric acid. After discarding the acid, the sand was washed again with water until neutral reaction.
The acid washed sand was subsequently heated for 24 hours in a furnace at 500°C. After being cool, the sand was extracted with n-hexane and finally dried in an oven at 100°C.

S & S filter paper circles, diam. 100 mm, nr 595 (Schleicher & Schüll GmbH, W-Germany).

Derivatization.

In order to prepare a standard mixture the following amounts of individual standards were placed into a glass stoppered tube : 6 µg T-2 toxin, 2 µg HT-2 toxin, 1 µg T-2 triol, 3 µg Neosolaniol, 4 µg Diacetoxyscirpenol, 1 µg Furasenon-X, 1 µg Verrucarol, 1 µg T-2 tetraol, 1 µg Deoxynivalenol, 1,5 µg Nivalenol and 2 µg Methoxychlor. The solvent mixture was evaporated and the final residue was redissolved in 0.5 ml benzene by agitating with a Vortex Mixer. After addition of 350 µl HFBI, the tube was stoppered and

vigorously mixed for 1 min. The tube was further heated for 1 hour in a water bath at 60"C. When the content of the tube was cooled to room temperature, 2.5 ml of a 5 % NaHCO₃ solution were added. The tube was shaken for another 2 min. on the Vortex Mixer and centrifuged at 2,500 rpm. A 50 μl amount of the supernatant was diluted to 500 μl with pentane and 0.1 μl was injected into the gas chromatograph with a 1 μl Hamilton Syringe.

Sample preparation.

The samples analysed were : kidney, liver, lung, muscle, blood, urine, ascites, fluid of the pleural cavity and bile. From the first autopsy only kidney, liver and lung were available.

Biological fluids

Blood : to a 5 ml blood sample, 10 ml acetone were added. The mixture was thoroughly shaken by agitating with a Vortex Mixer and filtered through a filter paper. The precipitate was washed three times with 3 ml acetone. The filtrate was evaporated to dryness with nitrogen and the obtained residue was dissolved in 150 μl methanol, diluted with 2.5 ml water and passed through a prepared XAD-2 column. The column was first washed with 10 x 5 ml water and the trichothecenes were eluted with 15 ml of a methanol-water mixture (9 : 1). The eluate was gently evaporated to dryness. The final residue was dissolved in 0.5 ml benzene and derivatized as mentioned before.

Urine, ascites and fluid of the pleural cavity : in case of ascites and fluid of the pleural cavity a 10 ml sample amount was used. From the second autopsy only 2.5 ml of urine was available. The fluids were extracted with 3 x 20 ml chloroform. The chloroform was dried over anhydrous Na₂SO₄, filtered and gently evaporated to dryness. The residues were dissolved in 150 μl methanol, diluted with 2.5 ml water and transferred to the XAD-2 column.

Bile : 3 ml of bile were diluted with 10 ml water and extracted with 3 x 15 ml ethyl acetate. The extract was filtered over anhydrous Na₂SO₄ and evaporated to dryness. The residue was redissolved in 0.5 ml methanol, diluted with 10 ml water and extracted with 3 x 15 ml chloroform. The extract was filtered over anhy-

drous Na$_2$SO$_4$ and carefully evaporated to dryness. The residue was dissolved in 150 μl methanol, diluted with 25 ml water and subsequently cleaned up using the XAD-2 column.

Tissues

Six grams of each sample from the first autopsy were prepared, while 25 g sample amounts from the second autopsy were analyzed.

Muscle : the tissue was homogenized in a mortar with purified sand and acetonitrile and extracted 3 times with 50 ml of this solvent. The extract was dried over anhydrous Na$_2$SO$_4$, filtered and concentrated to about 3 ml. To this concentrate 15 ml water and 1 ml saturated NaCl solution were added. The resulting mixture was partitioned with 3 x 15 ml n-hexane, each n-hexane fraction being discarded. After being warmed up to get rid of n-hexane traces, and cooled, the aqueous phase was further cleaned up on the XAD-2 column.

Kidney, liver and lung : the tissues were subjected to an acetonitrile extraction and a partitioning with n-hexane as described for muscle. The hexane washed aqueous phase was further re-extracted with 3 x 10 ml chloroform. The chloroform extract was dried over anhydrous Na$_2$SO$_4$, filtered and evaporated to dryness. The residues were dissolved in 150 μl methanol, diluted with 2.5 ml water and subsequently cleaned up using the XAD-2 column.

RESULTS AND DISCUSSION.

With the exception of the bile, the human autopsy specimens, originating from the two Iranian soldiers, could be prepared according to the methods as described earlier (1). Bile had to be extracted with ethyl acetate prior to chloroform extraction. A direct extraction of bile with chloroform resulted in the formation of an emulsion. A direct clean-up of the ethyl acetate extract on XAD-2, led to an eluate which contains many impurities. The finding of Pathre and Mirocha, 1977 (2), that ethyl acetate, although one of the most efficient extracting solvents, gave an extract requiring an extensive purification, could confirm our experience.

Gas chromatography with electron capture detection seems to be a very sensitive method for the detection of trichothecenes.
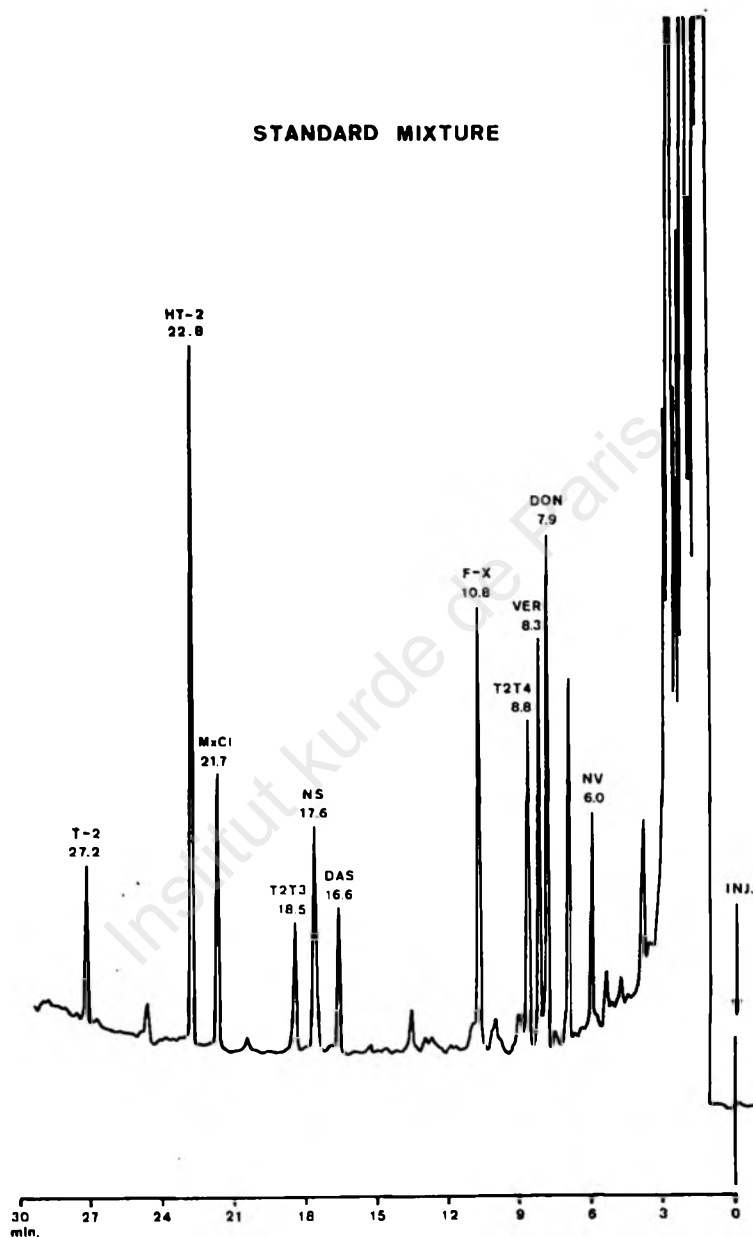
STANDARD MIXTURE

FIG. 1. — Chromatogram of a standard mixture using a 25 m CP^{tm}Sil 5 WCOT fused silica column with a column temperature program initiated at 170° C for 5 min., increased to 200° C at 2° C/min. and finally increased to 250° C at 5° C/min.
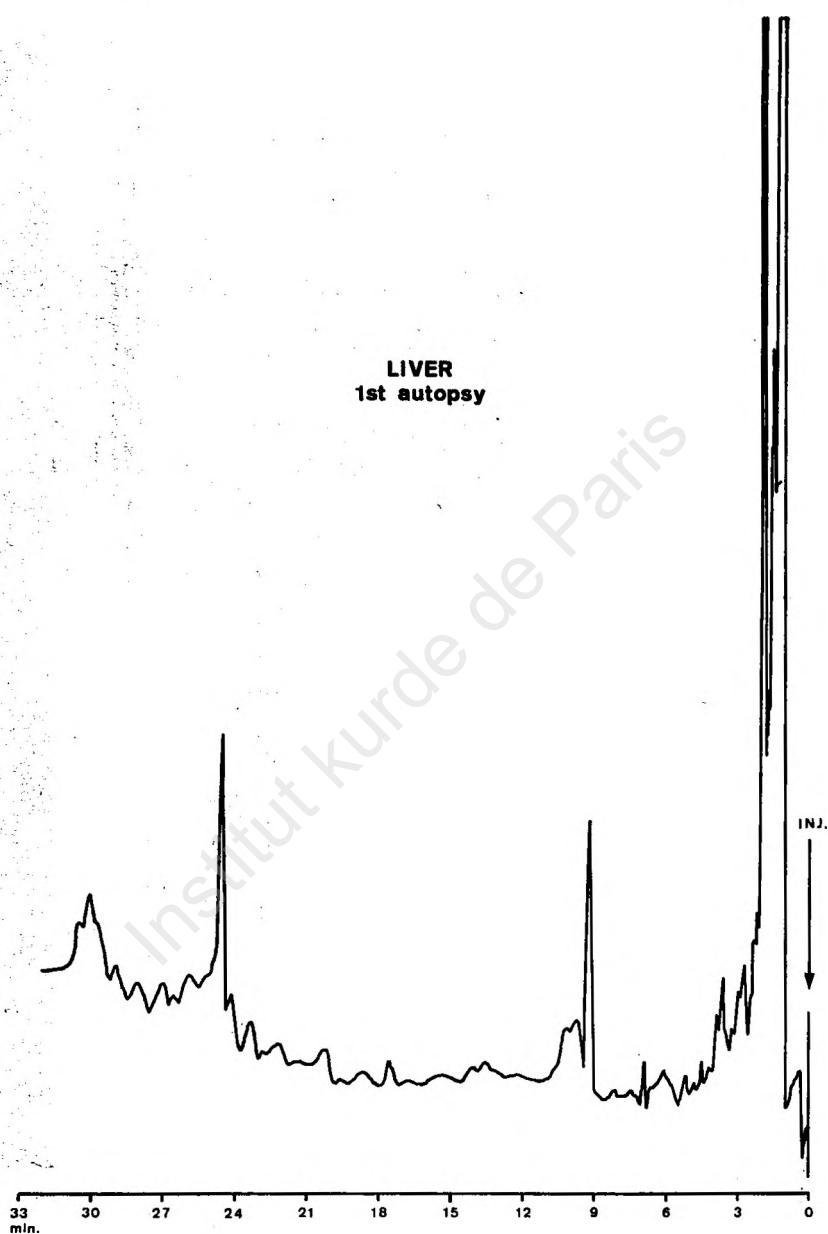
FIG. 2 A.

FIG. 2. — Examples of chromatograms obtained from the injection of different sample extracts.

LUNG
1st autopsy

INJ.

33 min. 30 27 24 21 18 15 12 9 6 3 0

FIG. 2 B

ASCITES
2nd autopsy

FIG. 2 C.

KIDNEY
2nd autopsy

INJ.

| 33 min. | 30 | 27 | 24 | 21 | 18 | 15 | 12 | 9 | 6 | 3 | 0 |
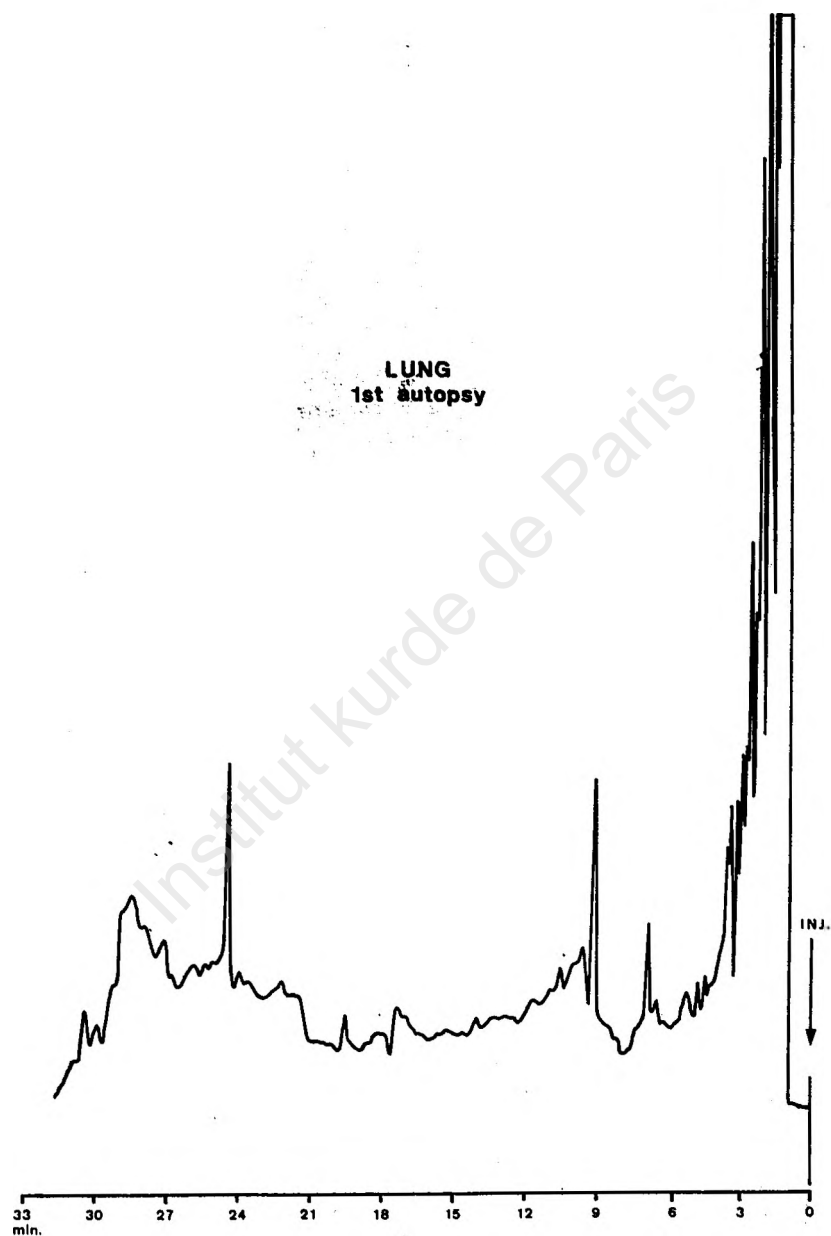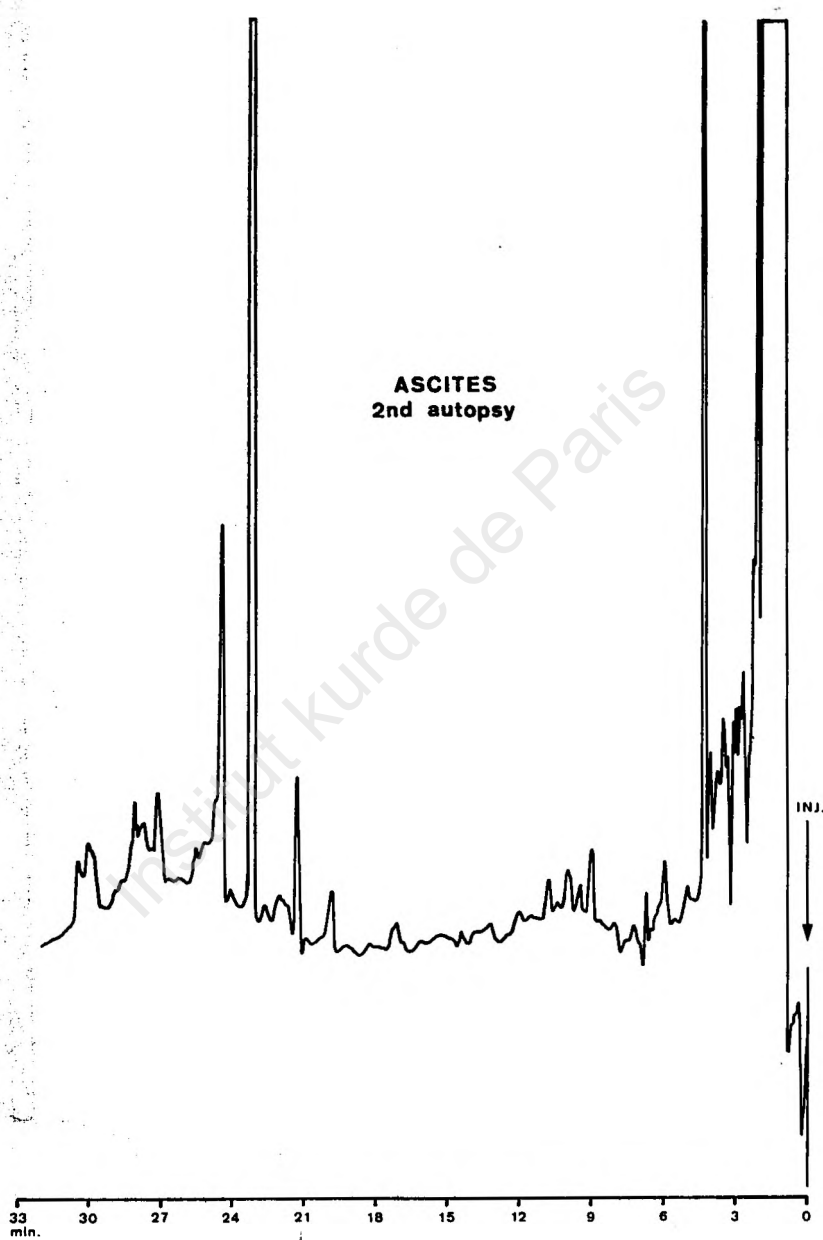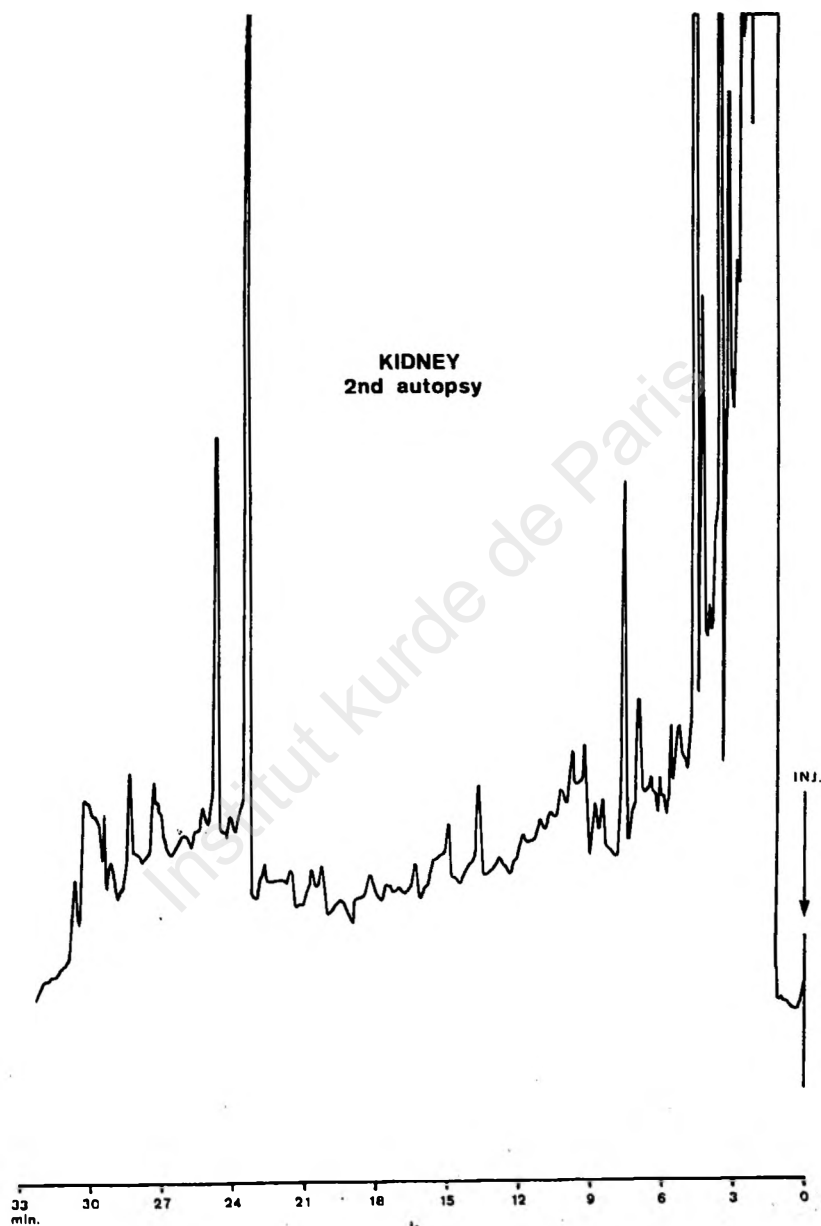
FIG. 2 D.

It was mentioned by Naoi, 1983 (3) that in comparison with the flame ionisation detector, the trichothecenes, which had no C=O (Carbonylgroup) at the C-8 position, exhibited about 100 times more sensitivity to the electron capture detector, whereas the trichothecenes, which possess the C=O function, showed a sensitivity which is about 2,500 times higher. The use of a CP *tm* Sil 5 fused silica capillary column, under the conditions as mentioned before, provided a good separation between Diacetoxyscirpenol, Neosolaniol and T-2 triol (fig. 1). The latter three compounds could not be separated when a 25 m SE-52 glass capillary column was used (1). Watson *et al.*, 1983 (4) and Mirocha *et al.*, 1983 (5) analysed biological samples from Southeast Asia. Various amounts of different trichothecenes were detected in post mortem samples of human origin. In the present analysis of the autopsy samples, originating from the two Iranian soldiers however, none of the sample chromatograms exhibited peaks corresponding to those of the trichothecene standards (fig. 2).

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Detection of trichothecene mycotoxins (Yellow Rain) in blood, urine and faeces of Iranian soldiers treated as victims of a gas attack

by A. HEYNDRICKX, N. SOOKVANICHSILP
and M. VAN DEN HEEDE

Department of Toxicology, State University of Ghent,
Hospitaalstraat 13, 9000 Ghent, Belgium

SUMMARY.

Blood, urine and faeces, originating from 30 Iranian soldiers, which were all victims of a gas attack, were analysed for tricho- thecene mycotoxins, using capillary gas chromatography with electron capture detection. Blood and urine were respectively extracted with acetone and chloroform. Faeces were first extracted with acetonitrile. The acetonitrile was partially evaporated and diluted with water in order to allow a first purification, by re- extracting with chloroform. Prior to gas chromatography a final clean-up of all sample extracts was performed on XAD-2 columns.

We concluded that some sample extracts contained T-2 toxin, HT-2 toxin, Diacetoxyscirpenol, Verrucarol and/or Nivalenol in con- centrations ranging from 0.07 to 0.70 ppm.

INTRODUCTION.

In the spring of 1984, Iranian soldiers, victims of a gas attack near the Majnoun Islands, were transferred to different European Hospitals for treatment. They were all heavily burned by a vesi- cant identified as sulfur mustard (1, 2). According to their state- ments, it became clear that bombs and shells containing diffe- rent agents, had been used at the same time. Therefore the use of trichothecene mycotoxins, better known as « Yellow Rain », was also suspected, because of their reported use as warfare agents in Southeast Asia and Afghanistan. Five Iranian patients were

treated in the University Hospitals of Ghent (Belgium). **Blood,**
urine and faeces samples were daily analysed for the presence
of sulfur mustard (Yperite), acetylcholinesterase inactivators and
mycotoxins. At the same time samples, originating from patients
treated in Austria (Vienna), Western Germany (Hamburg, Munich
and Recklinghausen) and Switzerland (Lausanne) were also analy-
sed (3, 4, 5, 6, 7, 8, 9, 10).

MATERIALS AND METHODS.

All details about equipment, standards, reagents, materials and
sample preparation (blood and urine) can be found in other arti-
cles (3, 4). Faeces (5 to 10 g) were homogenized and extracted
with 3 x 20 ml acetonitrile. The extract was filtrated and evapo-
rated to about 3 ml. After addition of 15 ml H_2O, the acetoni-
trile/water mixture was extracted with 3 x 10 ml $CHCl_3$. The
chloroform extract was dried on anhydrous Na_2SO_4 and evaporated
to dryness. The residue was redissolved in 150 μl methanol, di-
luted with 2.5 ml water and transferred onto an XAD-2 column,
as already described before (3, 4).

All extracts were analysed by GC using a 25 m x 0.32 mm i.d.
CP ^{tm} Sil 5 fused silica capillary column and a ^{63}Ni electron cap-
ture detector. Prior to gas chromatography the final extracts were
treated with HFBI.

RESULTS AND DISCUSSION.

The proposed methodology was applied on blood, urine and
faeces samples originating from 30 Iranian patients. The results
are shown in table I. At the same time, blood and urine samples,
originating from Iranian soldiers, which were fighting on the same
battlefield, eating the same food but not being attacked by chem-
ical or microbiological warfare agents, were analysed. The chro-
matograms of those blank sample extracts exhibited no peaks
indicating the presence of trichothecene mycotoxins.

One of us (Heyndrickx A.) went to the battlefield to take the
necessary samples. He was eating the same food as the soldiers.
He did not show any symptoms of intoxication. His blood and uri-
ne samples were negative on mycotoxins (trichothecenes), which
excluded any backgrounds from those compounds, coming from
environmental origin. These samples were controlled by gas chro-

TABLE I

Mycotoxin concentrations found in blood, urine and faeces of Iranian soldiers
(results of Yperite tests and cholinesterase activity determinations are also given)

N°	—	Date	—	Origin Nature Patient N°	NV	VER	DAS	HT-2	T-2	Sulfur mustard (Yperite)[*]	Acetyl-cholinesterase activity (%)[**]
00	—	063	—	Vb 1	0.15	0.33	—	—	—	—	46.1
00	—	063	—	Vu 1	—	0.10	—	—	0.18	Slightly positive	—
00	—	063	—	Vf 1	—	0.30	—	—	—	—	—
04	—	113	—	Vb 1	—	0.07	—	—	—	—	—
14	—	113	—	Vu 1	—	0.14	—	—	—	Slightly positive	—
00	—	063	—	Vb 2	0.11	0.29	—	—	—	—	59.4
00	—	063	—	Vu 2	—	0.07	—	0.07	—	Positive	—
74	—	193	—	Gb 3	—	—	0.54	—	—	—	67.6
52	—	163	—	Lu 4	0.09	—	—	—	—	Slightly positive	—
79	—	193	—	Gu 5	—	—	—	0.13	—	Positive	—
48	—	153	—	Mb 6	0.15	—	—	—	—	—	55.3
02	—	113	—	Vb 7	—	—	0.54	—	0.41	—	69.3
08	—	113	—	Vb 8	—	—	0.38	—	—	—	73.7
13	—	113	—	Vu 9	0.16	—	—	—	—	Strongly positive	—
39	—	133	—	Vu 10	—	—	0.22	—	—	—	—
57	—	163	—	Vb 10	—	—	0.70	—	—	—	—

Symbols : V (Vienna), G (Ghent), L (Lausanne), M (Munich), b (Blood), u (Urine), f (Faeces), NV (Nivalenol), VER (Verrucarol), DAS (Diacetoxyscirpenol), HT-2 (HT-2 toxin), T-2 (T-2 toxin).

[*] Yperite was detected according to the procedure of Heyndrickx et al. (1). Using this method, still positive results could be found with liver, kidney and muscle of an Iranian soldier, who died two weeks after a gas attack. The samples (25 g) were tested about three weeks after death.

[**] Acetylcholinesterase activity was determined according to the method of Morand and Laborit, 1947) (11). Reference value : 50 ± 10 %.

matography and mass spectrometry, using the techniques as mentioned.

A correlation between the mycotoxin concentrations found and the symptomatology observed in those patients could not be made because it was very difficult to find out if some particular symptoms were due either to mycotoxins, sulfur mustard or Tabun (12).

About 6 weeks after the first samples came in, some original samples, kept all the time in the refrigerator ($\pm$ 4°C), were extracted again according to the same procedure. The sample chromatograms exhibited no peaks anymore corresponding to those of the trichothecene standards. When the same original samples were analysed by GC/MS after a previous conversion, either to HFB-esters or to TMS derivatives, no peaks corresponding to those of the standards were observed in the Total Ion Chromatogram (TIC). We proved that trichothecene mycotoxins are not stable when present in biological tissues and body fluids : ipv ; deterioration *in vitro* at 4°C and metabolization in man (3, 4). These findings also proved that the chromatographic peaks, as observed during the first analysis, were not due to interfering co-extracted materials.

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Recherches toxicologiques
de gaz neurotoxiques
dans les prélèvements de soldats intoxiqués

par A. HEYNDRICKX et B. HEYNDRICKX

Laboratoire de Toxicologie, Université de l'Etat à Gand,
Hospitaalstraat 13, 9000 Gand, Belgique

RESUME.

Les derniers mois, suite aux intoxications massives qui se sont produites en Iran, le problème du diagnostic se posait. Au début aucune analyse ni diagnostic ne pouvaient être faits, comme la toxicité des gaz sur l'homme est mal connue ces dernières années. Heureusement depuis la dernière Guerre Mondiale 1940-45, nous n'avons plus connu des intoxications dues à l'usage militaire de produits chimiques ou microbiologiques en Europe. Les médecins en Iran étant incapables de faire un diagnostic précis, plusieurs soldats intoxiqués furent envoyés en Europe et au Japon. Les premiers malades qui sont arrivés en Europe furent accueillis en différents hôpitaux universitaires. Là aussi, les médecins étaient incapables de faire un diagnostic précis, on parlait même de « brûlures ». Dans un des pays européens, finalement on suggérait la Lewisite, parce qu'on avait retrouvé des quantités d'arsenic — à notre avis minimes et normales — dans les liquides biologiques humains. Finalement des échantillons des patients des hôpitaux universitaires de Vienne nous furent envoyés à Gand sur demande de nos collègues autrichiens. Nous faisions un screening toxicologique général comme nous pratiquons d'habitude en toute expertise toxicologique. Nous étions à même de démontrer l'Ypérite ou le gaz moutarde, des constituants de la Pluie Jaune (mycotoxines) et une inhibition de l'activité acétylcholinestérasique du plasma sanguin chez plusieurs de ces soldats. Nous discutons les neurotoxiques et l'identification de ce groupe de poisons.

SUMMARY.

Toxicological analysis of neurotoxic war gases in biological fluids
  of intoxicated soldiers.

*The last few months, Gas Warfare was used by Iraq in Iran,
where the question arose to have diagnostic facilities for those
patients. When the soldiers were flown over to different univer-
sity hospitals in Europe, the physicians were unable to diagnos-
tisize what was happening, the case was described as being
« burns ». Also in one of the european hospitals a wrong con-
clusion was drawn, saying Lewisite had been used. The ar-
senic contents however in the biological fluids of blood and
urine were as normal as for every human being. It was only on the
second arrival when we received blood, urine and faeces from the
university hospital of Vienna, that we could identify mustard gas
or Yperite, mycotoxins or Yellow Rain and a lowered cholineste-
rase activity of the plasma in some patients. The differences we
found in the soldiers coming from the same chemical warfare
attack is evaluated.*

INTRODUCTION.

Plusieurs méthodes pour déterminer l'activité acétylcholinesté-
rasique du plasma sanguin ont été décrites les dernières années.
La Belgique étant un pays rural, nous avons connu des intoxica-
tions massives, surtout en Flandre. Ainsi nous avons eu la possi-
bilité d'évaluer ces différentes méthodes de détermination, et de
comparer leurs applications en toxicologie. Quoique plusieurs de
ces techniques soient parfaitement valables, nous avons dû cons-
tater que la discussion et la conclusion clinique étaient pratique-
ment impossibles. Les résultats sont tellement divergents d'après
la personne et l'activité individuelle, que des valeurs normales
sont indispensables pour conclure si l'ouvrier agricole présente
une inhibition cholinestérasique dans le plasma. En plus, diffé-
rentes substances chimiques actives des groupes des produits
phytopharmaceutiques résultant en une inhibition de l'activité
acétylcholinestérasique du plasma tout à fait différent, de sorte
qu'une interprétation clinique devient impossible, ne se basant
que sur ces valeurs-là. Nous avons constaté par exemple, dans
le cas d'une intoxication par le Diazinon avec une inhibition à
100 % de l'activité acétylcholinestérasique, que les symptômes

chez l'ouvrier intoxiqué se limitaient à quelques maux de tête. Il s'en remettait très facilement après quelques jours. Au contraire, si les intoxiqués, avec la même activité acétylcholinestérasique dans le plasma de 0 %, étaient retrouvés après emploi du Parathion, le malade fut prêt à succomber. L'activité acétylcholinestérasique dans le plasma n'est qu'un effet secondaire et non primaire de l'intoxication. Nous avons décrit ces symptômes à plusieurs reprises (1).

Dans les cas des intoxications à origine inconnue en Iran et en Europe, une évaluation totale de l'analyse toxicologique était nécessaire. Suite aux résultats positifs mentionnés (Ypérite ou gaz moutarde, mycotoxines (Pluie Jaune) et l'inhibition acétylcholinestérasique) nous concluons à un mélange de trois gaz, employé par l'Iraq en Iran lors d'une attaque. Ces résultats coïncident partiellement avec les expertises faites par les Nations Unies (2). Les experts avaient d'ailleurs trouvé des échantillons de gaz moutarde et de tabun, ce dernier étant un gaz neurotoxique. Restaient les mycotoxines dont nous avons décrit les méthodes analytiques et les conclusions toxicologiques (3-10).

ANALYSE.

Plusieurs auteurs allemands font état de l'utilisation d'une méthode biologique dans des expertises judiciaires (11). Nous avons utilisé, pour la détermination de l'activité acétylcholinestérasique plasmatique, la technique publiée par P. Morand et H. Laborit (12) et inspirée des travaux de White et Stedman (13).

I. Réactifs.

a) Solution NaOH à 0,1 N (exactement titrée).

b) Solution NaOH à 0,01 N obtenue extemporanément par la dilution de la précédente avec l'eau bidistillée fraîchement bouillie.

c) Solution à 0,01 N, approximativement, de chlorhydrate d'acétylcholine. On utilise une ampoule commercialisée contenant 100 mg de chlorhydrate d'acétylcholine. Dissoudre ce produit dans l'eau bidistillée, et amener la solution à 50 ml. Y ajouter 0,5 ml d'une solution d'acide acétique à 10 %. Agiter et conserver au frigo à 4°C. Temps de conservation : maximum 1 mois.

d) Solution de rouge de Crésol à 0,2 % dans l'alcool à 60°.

2. **Mode opératoire.**

Deux ml de sang, hépariné, sont centrifugés pendant 20 minutes à 3.000 tours. Le plasma ne peut être coloré en rouge, la présence d'hémoglobine gênant considérablement l'observation du virage. Au bout de ce laps de temps, on prépare un flacon Erlenmeyer en y versant 0,5 ml de plasma sanguin, 10 ml de la solution d'acétylcholine, ensuite 2 gouttes de solution de rouge crésol. Aussitôt après on ajoute, goutte à goutte, à la burette, de la solution NaOH à 0,1 N, jusqu'à ce que la zone violette, ainsi provoquée au sein du liquide, ait tendance à s'étendre (+ 1,5 ml) après quoi on ajoute, avec précaution, de la solution à 0,01 N, jusqu'au virage au rose pâle stable, sans dépasser cette teinte. On met aussitôt au thermostat (35°C + 1°), et on met en marche le chronomètre.

Toutes les cinq minutes, on titre, à l'aide de la solution à 0,01 N d'hydroxyde sodique, l'acidité apparue, en s'arrêtant au début du virage, c'est-à-dire lorsque le liquide, de jaune serin qu'il était, passe au jaune d'or avec reflets violets, ce qui s'observe le mieux par réflexion sur fond blanc. On note donc toutes les cinq minutes le volume de NaOH à 0,01 N utilisé, et ceci, pendant 60 minutes.

On additionne les 12 volumes obtenus. La quantité totale de solution utilisée pour la titration, exprimée en 1:10 de ml, donne directement l'activité acétylcholinestérasique, telle que définie par les auteurs de la méthode. Valeur normale (moyenne) 50 % ± 10 %.

RESULTATS DES ANALYSES TOXICOLOGIQUES.

Comparant la méthode de Morand et Laborit (12) et celle de Nenner (14), nous savons par expérience que la méthode de Morand et Laborit est certainement la plus commode et la plus facile à interpréter en ce qui concerne l'état clinique et l'intoxication. Sa grande valeur est qu'elle soit simple, facile à en conclure, les valeurs normales étant de 50 % ± 10 %. Appliquant cette méthode, la conclusion n'exige pas la valeur normale individuelle du patient avant l'intoxication. Dans la méthode de Nenner les valeurs normales sont tellement dispersées, déjà pour chaque individu, qu'il est pratiquement impossible, lors des intoxications moins graves ,d'en déduire les conclusions cliniques

et toxicologiques. Pour cette raison, dans ces cas-ci, nous avons appliqué cette ancienne méthode qui a fait preuve de son utilité. Le jour même où les malades sont arrivés dans les différents hôpitaux en Europe, nous avons reçu les échantillons et nous avons fait les déterminations nécessaires (tableau I).

Si nous évaluons ces différentes valeurs, nous trouvons certains patients qui présentent des résultats positifs : une diminution de leur valeur acétylcholinestérasique. D'autres ne la pré-

TABLEAU I

Noms	Cliniques	Dates	% ACH
H.D.M.	Gand	19 mars 1984	59,6
A.S.H.	Gand	19 mars 1984	79
A.I.	Gand	19 mars 1984	67,6
C.M.H.	Gand	19 mars 1984	45,4
M.G.	Gand	19 mars 1984	66
M.G.	Munich	15 mars 1984	55,3
S.A.	Munich	15 mars 1984	62,5
H.F.	Munich	15 mars 1984	33,7
O.J.	Recklinghausen	19 mars 1984	39
A.G.	Recklinghausen	19 mars 1984	46
A.H.	Recklinghausen	19 mars 1984	74
H.S.M.	Recklinghausen	19 mars 1984	48
T.A.R.	Recklinghausen	19 mars 1984	58
N.K.	Recklinghausen	19 mars 1984	62
K.A.	Recklinghausen	19 mars 1984	52
Y.H.	Recklinghausen	19 mars 1984	47
M.A.A.	Recklinghausen	19 mars 1984	37
A.M.R.	Recklinghausen	19 mars 1984	58
A.S.H.	Hamburg	22 mars 1984	45* 46**
J.H.M.	Hamburg	22 mars 1984	59
N.H.	Lausanne	16 mars 1984	35
R.M.S.	Lausanne	16 mars 1984	30
E.A.R.	Vienne	11 mars 1984	73,7
N.M.	Vienne	06 mars 1984	59,4
M.J.	Vienne	06 mars 1984	46,6
T.M.	Vienne	08 mars 1984	46,1
A.B.N.	Vienne	11 mars 1984	25,2
A.A.R.	Vienne	11 mars 1984	69,3
F.A.	Vienne	11 mars 1984	30,1
M.	Vienne	11 mars 1984	63,4

Ces soldats ont été attaqués par des gaz de guerre le 27 février 1984 à l'exception de ceux qui ont été hospitalisés à Gand dont la date de l'attaque se situe au 9 mars 1984.

% ACH : activité acétylcholinestérasique du plasma sanguin.

* 45 % avant hémoperfusion.

** 46 % après hémoperfusion.

sentent pas. Le point d'interrogation subsiste : ces soldats étaient
dans le même combat, la même attaque donc des gaz de guerre.
Comment expliquer ces résultats ?

Allant sur place, nous avons constaté après un examen du
champ de bataille, que plusieurs bombes et obus avaient été
employés. Les soldats interrogés nous ont très bien expliqué
que les attaques par avion se faisaient par différentes bombes
— dont le contenu est différent — d'un même avion. Le mélange
ne se fait qu'après explosion. Il suffit que les soldats soient
dispersés à une certaine distance, pour que les concentrations
relatives soient différentes. Une bombe ne contient donc pas
les trois substances en même temps. Ceci explique facilement
les différentes concentrations que nous avons retrouvées dans
le sang, les urines et les matières fécales : des mycotoxines, de
l'Ypérite et les valeurs d'activité acétylcholinestérasique. N'ou-
blions pas que ces analyses ont été faites environ 2 à 3 semai-
nes après l'attaque, c'est-à-dire après l'arrivée des soldats in-
toxiqués dans les différents hôpitaux européens. Nous n'avons
pas pu démontrer le tabun tel quel, dans ces échantillons biolo-
giques humains. Ceci n'a rien d'étonnant, vu le temps entre
l'attaque et les analyses.

Lors de notre examen sur place, sur le champ de bataille,
nous avons reçu des témoignages qu'il y a eu aussi des atta-
ques, non par mélange mais par un produit seulement. La de-
scription des morts et l'examen des cadavres dans la morgue
de Téhéran nous ont démontré que ces thèses étaient exactes.
Aussi, plusieurs soldats sont morts avant de pouvoir mettre leur
combinaison protectrice ou de pouvoir s'injecter avec les ampou-
les au sulfate d'atropine et le Toxogonin®, qui étaient mis à leur
disposition. S'ils survivaient, la régénération en général par le
tabun se fait après 3 ou 4 jours. En ce qui concerne le mélange,
la pathologie est naturellement tout à fait différente, vu l'effet
synergétique de ces différents gaz. Nous nous trouvons ici devant
des cas primeurs sur le plan mondial. Il y a des malades que
nous avons vus à Téhéran dans les différents hôpitaux dont les
symptômes ne correspondent pas aux gaz mentionnés plus haut.
Il s'agit donc d'un quatrième gaz que nous n'avons pas pu mettre
en évidence ou d'un mélange de gaz. Même après des semaines,
les malades étaient dans un état comateux avec un regard hallu-
ciné. La peau n'était nullement attaquée. Les médecins traitants
prétendent que lors d'attaques précédentes, plusieurs de ces ma-
lades montraient des symptômes neuropathologiques même après

des mois. Il subsiste encore un grand point d'interrogation en ce qui concerne l'origine de ces gaz toxiques.

CONCLUSION.

En employant une méthode simple qui peut être appliquée dans tout laboratoire même sur un champ de bataille, il est possible de faire un diagnostic sur les produits neurotoxiques employés dans le combat. La diminution de l'activité acétylcholinestérasique du plasma chez le soldat intoxiqué, nous a permis de faire un diagnostic valable et d'appliquer un traitement. Naturellement, comme nous l'avons décrit, des doses massives de sulfate d'atropine doivent être employées, et que le Contrathion® est un réactivateur beaucoup plus intéressant et efficace que le Toxogonin®. Nous savons que les concentrations de Toxogonin® sont moins élevées et plus facilement applicables sur le champ de bataille que le Contrathion®; mais en clinique où les possibilités et facilités sont plus larges, le Contrathion® est certainement plus valable. D'ailleurs, ce produit-là, que nous employons et avec lequel nous avons une longue expérience, est bien préféré au Toxogonin®, étant un réactivateur lors des intoxications constatées chez les fermiers avec une bien plus grande efficacité. Dans certains cas nous allons jusqu'à 50 et même 100 mg de sulfate d'atropine par voie intraveineuse en 24 heures, nous employons 2 ampoules de 200 mg de Contrathion® (voie intraveineuse) toutes les 2 heures.

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Extraction of the cartridge used during the hemoperfusion therapy of the intoxicated soldiers

by A. HEYNDRICKX, N. SOOKVANICHSILP and B. HEYNDRICKX

Department of Toxicology, State University of Ghent,
Hospitaalstraat 13, 9000 Ghent, Belgium

According to previous work (1), we tried to extract some toxins, e.g. T-2 toxin, diacetoxyscirpenol, HT-2 toxin, verrucarol and nivalenol from the toxin adsorbed charcoal, and we found that 100, 75, 55, 53 and 3 % of these toxins, respectively, could be extracted with chloroform.

We therefore used chloroform for the extraction of the cartridges.

The upper parts (1-2 cm deep) of the two cartridges obtained after hemoperfusion of the two Iranian soldiers (2) (whose blood and/or urine were found to contain compounds exhibiting peaks corresponding to peaks of some trichothecenes), intoxicated by chemical and biological warfare, and admitted at the University Hospitals in Ghent, were used. Thirty ml H_2O were added and extracted with 30 ml chloroform. The liquid phases (both water and chloroform) were transferred into a separator. The layers were separated. The cartridges were extracted again with 2×30 ml chloroform. The combined chloroform was dried over anhydrous Na_2SO_4, evaporated to just dryness. The residues were dissolved in 150 μl methanol ; 2.5 ml water were added. The clean-up on the XAD_2 was done according to our method described earlier. None of these cartridges were found to contain any compounds exhibiting peaks corresponding to those of any trichothecenes.

Activated charcoal was used in the treatment of the intoxicated soldiers. Trichothecenes were found in the faeces before the application. As soon as the activated charcoal was given, no trichothecenes could be found back in the stools.

The extraction of the activated charcoal yielded no results.

By standing, trichothecenes disappear very quickly (± one week) *in vitro*, when present in blood of man. In urine however, up to one month after standing, they could be detected.

The deterioration takes place much faster in some biological fluids of man than in other ones.

It is also possible that trichothecenes could not be re-extracted from the charcoal or that they are completely deteriorated.

Anyway, many intoxicated soldiers could be saved from death, applying the hemoperfusion (2).

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Treatment of Iranian soldiers attacked by chemical and microbiological war gases

by A. HEYNDRICKX and B. HEYNDRICKX

Department of Toxicology, State University of Ghent,
Hospitaalstraat 13, 9000 Ghent, Belgium

SUMMARY.

Different treatments on soldiers intoxicated with nerve gases (Tabun), mycotoxins (Yellow Rain) and mustard gas (Yperite) were worked out and compared after treatment in different European Hospitals : Austria, Western Germany, Sweden, the Netherlands, Great Britain, Switzerland, Belgium : 40 patients.

A. GENERAL TREATMENT.

a) Skin.

1. After the attack in the field. Washing procedure : rinse abundantly immediately, wash with fluid soap for two minutes, rinse 2 minutes, wash again for another 2 minutes and again at last rinse 10 minutes. Do not use detergents ! Patients who jumped directly into the water after the attack on the islands, had no skin lesions (mustard gas). The ones who jumped in contaminated water showed worse lesions.

2. Afterwards bring on the wounds every 2 hours towels soaked in a 2‰ solution of chloramine-T during the first day (Dakin solution, treatment First World War 1914-18 in Belgium).

3. Treat the wounds afterwards twice a day with an ointment of silver-sulfadiazine at 1 %. (Constituents of the O/W ointment : polysorbate 60, polysorbate 80, glycerine, monostearate, cethylalcohol, liquid parafine, propyleneglycol, water).

b) **Oral.**

1. 40 g activated charcoal, repeat three times every 4 hours.
2. Administration of a mixture of 25 g $MgSO_4$ and 1 g KCl in 100 ml of water.
3. Administration of a 10 % solution of mannitol, fluid food, milk diet, etc.

c) **Intravenously.**

— 1 g vitamin C, three times a day.

— 300 mg acetylcystein four times a day (up to 150 mg/kg).

— 2 g Thienamycine, twice a day. (note : first of all perform an antibiogram).

— 21 g piracetam a day (Nootropil UCB, some Hospitals applied it, to avoid strange psychic reactions after the attack : fourth unknown gas or unknown mixture ?).

— During 7 days an hemoperfusion of 3 hours/day (single needle) in serious cases we found high concentrations of methemoglobin (« black colour » of patient, etc.).

d) **Oral spray.**

— Thimerosal (thiomersal), an organic mercury derivative, in ethanol.

— Or 0.2 ‰ hexomedine.

— Or sulfonamides.

e) **Eye treatment.**

— A 0.2 ‰ solution in water of mercuryoxycyanide with methylene blue (as a dye).

— Or 15 % sulfacetamide in hydroxypropylmethylcellulose.

— Or chlorotetracycline eye ointment (Aureomycine) (5 mg/g or 10 mg/g).

B. TREATMENT IN VIENNA.

[(A.a)1) (A.b)1,2,3) (A.c),d),e)]]
Same as mentioned under general treatment :

a) Skin.

— Treat with Actisorb twice every day, leave it on the skin.
— Wash off Actisorb every day twice with 1.5 L mother milk during three weeks.

b) Oral.

— 1.5 L mother milk every day.

c) Intravenously.

— Same as mentioned under general treatment.

d) Nerve gases (tabun).

— Atropine sulfate : 7 x 2 mg every 2 minutes I.V. Repeat until dilatation of pupils, in some cases up to 100 mg/I.V./day.
— Contrathion® : 2 x 2 ampoules of 200 mg every 2 hours. Dissolve every ampoule in 50 ml water and inject I.V. : better results than applying Toxogonin®.

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Toxicological properties of T-2 toxin and related trichothecenes

by Y. UENO, A. MUTO and J. KOBAYASHI

Department of Toxicology and Microbial Chemistry, Faculty of Pharmaceutical Sciences,
Tokyo University of Science, Tokyo 162, Japan

SUMMARY.

More than 60 kinds of trichothecene mycotoxins are known to be produced by fungi as Fusarium, Trichoderma, Myrothecium, Stachybotrys *and others. These mycotoxins are classified into four types : type A (T-2 toxin, diacetoxyscirpenol, etc.) ; type B (nivalenol, deoxynivalenol, etc) ; type C (crotocin, etc.) ; and type D (verrucarins, roririns and satratoxins), depending on their fungal producers and chemical characteristics.*

Originally, some trichothecenes such as trichothecin and crotocin were isolated as antifungal antibiotics, and diacetoxyscirpenol was isolated as a phytotoxic compound.

Thereafter, many trichothecenes were isolated as causative toxicants in association with moldy food- and feed-induced intoxication of man and livestocks : T-2 toxin in moldy corn toxicosis in USA ; nivalenol and deoxynivalenol in red-mold toxicoses in Japan ; deoxynivalenol in vomiting and feed refusal problems in USA ; satratoxins in Stachybotryotoxicose in Central Europe. The acute symptoms were characterized by dermal toxicity, nausea, vomiting, refusal of feeds, diarrhea, anemia and leukopenia.

The LD_{50} values (mg/kg) : (T-2 toxin) ; 6-week old mice 10.5 (po), 5.2 (ip), 2.1 (sc) and 4.2 (iv), new born mice 0.15 (sc), 4-week-old mice 1.6 (sc) ; cats 0.5. (sc) ; swine 1.21 (iv). (nivalenol) mice 4.1 (ip) and 6.3 (iv). (deoxynivalenol) mice 46 (po). (Satratoxin G) mice 1.23 (ip). (Satratoxin H) 5.69 (ip).

Postmortem examination revealed an extensive hemorrhage in intestine, thymus, spleen, ovary and cellular destruction in bone marrow.

Inhalation toxicity test revealed that all mice died 5 days
after 160 min.-exposure to the culture filtrate of Fusarium sporo-
trichioides (33 ppb T-2 toxin) and 3 days after 30 min. of T-2 toxin
(140 ppb) in 3 % propylene glycol.

All trichothecenes possess a high dermal toxicity, but the ef-
fective doses of the mycotoxins widely ranged from 10^{-11} to 10^{-7}
mol. T-2 toxin was the highest in toxicity and followed by HT-2
toxin, diacetoxyscirpenol, and verrucarin A. Nivalenol and deoxy-
nivalenol were extremely low in dermal toxicity.

Pretreatment of mice with cysteamine (100 mg/kg ip) or gluta-
thinone (100 mg/kg) 2 hr before sc injection of T-2 toxin
(1-3 mg/kg) decreased neither the number of lethal cases nor
elongated the lethal time. The modification of acute toxicity of
T-2 toxin to mice by chlorpromazine, indomethacin, prednisolone
and other chemicals will be discussed.

INTRODUCTION.

In the early stage of trichothecene problem, crotocin, trichothe-
cin, verrucarins and others were found as potent antifungal me-
tabolites. After finding T-2 toxin as a causative toxin in moldy
corn toxicosis in USA, more than 60 kinds of the trichothecene
derivatives are detected in the fungal and plant metabolites.
The major trichothecene-producing fungi, *Fusarium graminearum*
and *F. sporothrichioides*, are widely distributed in cereal grains
such as barley, wheat and corn, and produce T-2 toxin, nivalenol,
and deoxynivalenol, which cause food- and feed-borne toxicoses
in animals and man.

The trichothecenes possess a potent cytotoxicity and induce
severe cellular damages in hematopoietic tissues. In this paper,
the authors summarized the toxicological features of these toxi-
cologically important fungal metabolites, in addition to pre-
vention and prophylaxis.

TRICHOTHECENE-PRODUCING FUNGI.

The most important fungi which are responsible for the pro-
duction of trichothecenes are the genus *Fusarium*. The other ge-
nera of fungi such as *Trichothecium*, *Trichoderma*, *Myrothecium*,
and others also produce the trichothecenes, as summarized in

table I. Mycotoxicologically important fungi are *F. graminearum* (*Gibberella zeae* in perfect stage), *F. sporotrichioides*, and *Stachybotrys atra*. *F. sporotrichioides* (= *F. tricinctum*) produces T-2 toxin, HT-2 toxin and related compounds, which are presumed to be causative toxicants in alimentary toxic aleukia (ATA) and

TABLE I

Trichothecene-producing fungi

Groups	Fungi	Major trichothecenes
(A)	*Fusarium sporotrichioides*	T-2 toxin, HT-2 toxin, neosolaniol, diacetoxyscirpenol
	Fusarium semitectum	Diacetoxyscirpenol
	Fusarium equiseti	Diacetoxyscirpenol
	Fusarium heterosporum	3'-hydroxy T-2 toxin 3'-hydroxy HT-2 toxin
(B)	*Fusarium graminearum*	Nivalenol, deoxynivalenol, fusarenon-X
	Fusarium culmorum	3-acetyldeoxynivalenol
(C)	*Cephalosporium crotocinigerum*	Crotocin
	Trichothium roseum	Crotocin
(D)	*Myrothecium roridum*	Roridin A, D, E
	Myrothecium verrucaria	Verrucarin A, B
	Stachybotrys atra	Satratoxin F, G, H
	Verticimonosporium diffractum	Vertisporin

others. *F. graminearum* produces nivalenol, deoxynivalenol and their derivatives, and these toxins are frequently detected in barley, wheat, corn and other cereals. These moldy cereal grains sometimes induce severe intoxications such as Akakabi (red-mold) disease in Japan, vomiting and feed-refusal in USA, and others.

Stachybotrys atra is often detected in cellulose-rich substrates such as hay, straw and others. This fungus produces satratoxin H, G, and other macrocyclic trichothecenes, which induce satratoxicoses in farm animals in Central Europe. Currently, the mycology and chemistry of trichothecenes are reviewed in several books and literatures (1-3).

CHEMISTRY OF TRICHOTHECENES.

The trichothecenes belong to sesquiterpenoid compounds and are characterized by 12,13-epoxy-trichothecene nuclei. Biogenetically,

the ring system arises from cyclization of farnesyl pyrophosphate followed by two 1,2-methyl group shifts. More than 60 kinds of the trichothecenes are grouped into four categories according to the similarity of functional groups and producing fungi (4), as shown in fig. 1 and table I.

The first (A) is characterized by a functional group other than a ketone at C-8. This is represented by T-2 toxin, HT-2 toxin, diacetoxyscirpenol and others. The second category (B) has a carbonyl

(A) T-2 toxin

(B) Nivalenol   R=OH
    Deoxynivalenol   R=H

(C) Crotocin

(D) Satratoxin H
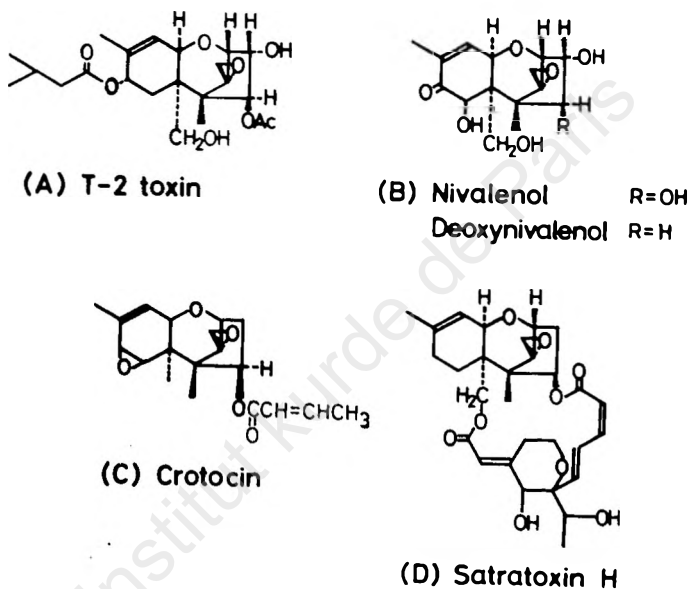
FIG. 1. — Major trichothecenes.

function at C-8, and nivalenol, deoxynivalenol and their esters are representatives of this group. The third (C) is characterized by a second epoxide at C-7,8 (crotocin) or C-9,10. The last category (D) includes those containing a macrocyclic ring between C-4 and C-15 with two ester-linkages. Verrucarins, roridings, satratoxins and other derivatives are included in this group, as shown in fig. 1.

The natural metabolites are soluble in polar solvents such as chloroform, acetone and ethyl acetate. But, the highly hydroxylated trichothecenes such as nivalenol, deoxynivalenol and T-2 tetraol are sparingly soluble in these solvents, and soluble in more polar solvents such as methanol and water.

The 12,13-epoxy ring is extremely stable to extramolecular nucleophilic attack. This sterically hindered epoxide is slowly attacked by various acids at elevated temperature. Ammoniation of the trichothecenes results in a deacylation of esters to lead the hydroxylated derivatives. The decontamination or removal of trichothecene mycotoxins are very difficult at present.

Another unreported procedure for degradation of the mycotoxins is to use « super acids » such as trifluoromethane-sulfonic acid and magic acid (hydrofluoroboric acid plus antimony pentafluoride (HBF$_4$ + SbF$_5$). Triphenylphosphine is also expected to remove the oxygen from the epoxide ring to yield the corresponding olefin under relative mild conditions (5).

TOXICOLOGY.

Acute toxicity.

The LD$_{50}$ values of major trichothecenes are summarized in table II. The po LD$_{50}$ (mg/kg) of T-2 toxin is 10.5 in mice, 3.06 in guinea pigs, 5.2 in rats, and 6.1 in trouts, indicating no marked species difference in susceptibility to the trichothecenes. From the data that the LD$_{50}$ (mg/kg) of fusarenon-X (4-acetylnivalenol)

TABLE II

LD$_{50}$ values (mg/kg) of the trichothecenes

Groups	Trichothecenes	Animals	Routes	LD$_{50}$ (mg/kg)
(A)	T-2 toxin	Mouse	Ip	5.2
		Rat	po	5.2
		Swine	Iv	1.2
	Diacetoxyscirpenol	Mouse	Ip	23.0
(B)	Nivalenol	Mouse	Ip	4.1
	Fusarenon-X	Mouse	Ip	3.4
		Rat	po	4.4
	Deoxynivalenol	Mouse	po	46.0
(C)	Crotocin	Mouse	po	1000
(D)	Roridin A	Mouse	Iv	1.0
	Verrucarin A	Mouse	Iv	1.5
		Rat	Iv	0.87
	Verrucarin B	Mouse	Iv	7.0
	Verrucarin J	Mouse	Iv	0.5
	Satratoxin G	Mouse	Ip	1.23
	Satratoxin H	Mouse	Ip	5.69

in mice is 3.4 (iv), 3.4 (ip), 4.2 (sc) and 4.5 (op), the acute toxicity of trichothecenes is similar irrespective of administration routes.

Age difference is one of characteristics of the toxicity of tri-chothecenes. The LD_{50} (mg/kg) of T-2 toxin in mice is 0.15 (a day old), 1.6 (4 week old) and 2.1 (6 week old). Another characteristic toxicity is the high susceptibility of animals, when exposed by inhalation route. Exposure of mice to the culture filtrate (33 ppb of T-2 toxin) of *F. sporotrichioides* or the purified T-2 toxin (140 ppb in 3 % propylene glycol) for a short period resulted in the killing of exposed mice several days after the inhalation (6). Therefore, the acute lethal toxicity of T-2 toxin is relatively high when the toxin is administered through the cutaneous and mucous membranes.

Chemical structures of the trichothecenes give a great influen-ce on their toxicity. As shown in table II, the ip LD_{50} (mg/kg) in mice is 0.5 (verrucarin J), 1.23 (satratoxin G), 3.4 (fusarenon-X), 5.2 (T-2 toxin), 9.0 (HT toxin), 23.0 (diacetoxyscirpenol), 70 (de-oxynivalenol) and 810 (crotocin). It is very likely that the macro-cyclic trichothecenes (group D) possess the highest toxicity fol-lowed by group A and B toxins.

Pharmacological effects.

The trichothecene mycotoxins are highly toxic to animal tissues, but not specific to muscle and nervous elements. The toxin indu-ces hypothermia and decreased respiratory rate shortly after the administration. Diarrhea is observable 36-60 hours after the admin-istration of fusarenon-X in rats. The toxin may attack the vessel wall permeability system, and thereby causes leakage of plasma contents into the intestinal lumen to lead severer diarrhea (7).

The dermal toxicity test was frequently employed for the screen-ing of toxigenic *Fusarium* spp. and their metabolites. This dermal toxicity, characterized by red spot on the painted skin and inflam-mation reaction is induced by doses widely ranged from 10^{-11} (T-2 toxin, verrucarin A) to 10^{-7} (deoxynivalenol) mole (6). Incre-ment of vascular permeability is one of this dermal toxicity of trichothecenes, and the potent cytotoxicity of trichothecenes in-duces the cellular damage of tissues.

Vomiting is one of the characteristic symptoms of farm animals and man that consumed the trichothecene-containing cereals, and deoxynivalenol was identified as a causative toxin under the na-me of « vomitoxin ». All the trichothecenes possess this pharma-

cological action. The minimum vomiting doses (mg/kg, sc) are as follows : T-2 toxin, 0.1 (cat); diacetoxyscirpenol, 0.2 (duckling), and 2.4 (man, iv); nivalenol, 1.0 (duckling, sc); deoxynivalenol 13.5 (duckling) and 0.05 (swine). Since the prior administration of metaclopramide (0.5 mg/kg) or chloropromazine (1.0 mg/kg) protected the fusarenon-X (0.3 mg/kg, iv)-induced vomiting. the trichothecenes is presumed to attack the chemoreceptor trigger zone in the medulla oblongata (7).

Systemic effects.

T-2 toxin and other trichothecenes have produced alterations in various organs and tissues. The tissues with the most severe alterations are those with a component of rapidly dividing and growing cells. Thus, the lymphoid tissues, hematopoietic tissues, intestinal mucosa and gonads are most affected when these toxins are given to animals.

Cellular degeneration and necrosis of bone marrow were observed in mice and rats given T-2 toxin and others. Lymphoid organs such as the thymus, spleen and lymph nodes were decreased in size after the toxins. Pathologically, necrosis of lymphoid cells was observed in the lymph nodes, Pyer's patches, and peribronchiolar and palpebral lymphoid fillicles of guinea pigs given by gavage 2.5 and 5.0 mg/kg of T-2 toxin (8). In cats, given multiple oral doses of T-2 toxin developed leukopenia, enlarged hemorrhagic mesenteric lymph nodes, necrosis of lymphoid cells in the spleen and lymph nodes (9). Such lesions were observed in rats and mice given nivalenol or fusarenon-x[10]).

Cellular lesions of other organs such as liver, kidneys are far less than those of hematopoietic organs and intestines. However, foci of hemorrhages were observed in the heart of pig, and myocardial hemorrhages were noted in mice given fusarenon-x[10]). In rats given single doses of T-2 toxin, the heart lesions consisted of intestinal oedema, focal cellularity and damages of myocytes shortly after the administration. In rats killed 1 or 2 months after the last of 10 daily injections of T-2 toxin, cardiomyopathy-like changes were seen with hypertrophy, focal fibrosis and other lesions. Based on these findings, T-2 toxin is an non-specific cardiotoxin (11).

In the central nervous system, meningeal hemorrhage, petechial hemorrhage, subarachnoidal hemorrhage were observed in cats, rabbits others after the trichothecene exposure.

Malfunction of bone marrow.

In the ATA developed in USSR, a marked decrease of circulating white blood cell numbers, aleukia, was one of the characteristic symptoms of man exposed to moldy cereals. Experimental toxicology with T-2 toxin, a major mycotoxin of *F. sporotrichioides* (table I), revealed that aleukia developed in cats after multiple administration of T-2 toxin (9). Table III summarizes the dose-response relationship in experimental animals.

TABLE III

Experimental toxic aleukia induced by the trichothecenes

Trichothecenes	Animals and man	Routes	Doses (mg/kg)	Duration
T-2 toxin	Cat	sc	0.05	1 week
	Chicken	po	0.9	3 weeks
	Mouse	po	3	3-5 days
	Monkey	po	0.5 - 1.0	15 days
Diacetoxyscirpenol	Rat	iv	0.15 - 0.3	1-5 weeks
	Dog	iv	0.25	4-5 weeks
	Man	iv	4.1 mg/m	4-8 hours × 24 days
Verrucarin A	Rat	iv	0.25	4 weeks
	Dog	iv	0.08	2-4 weeks
	Monkey	iv	0.08	2-4 weeks

Since the trichothecene compounds possess a potent cytotoxic action on mammalian cells, several derivatives were evaluated as antitumor agents. Diacetoxyscirpenol (=Anguidine) was used as an antileukemic in man, and case reports cited aleukia after 4-8 hours infusion/day for 4-5 weeks. It is presumed from the data of cell culture experiments that stem cells of bone marrow are severely affected by the trichothecene mycotoxins.

Immunosuppressive effects.

The immunosuppressive effects of trichothecenes have been investigated by *in vivo* and *in vitro* methods in regard to antibody formation, allograft rejection, delayed hypersensitivity, and blastogenic response to lectins.

All these data revealed that T-2 toxin and others possess a potent immunosuppressive action. The immuno responses are regulated by several kinds of T-cell, B-cell and related lymphoid

cells. An important point is the selective sensitivity of these cells to the trichothecenes. In delayed hypersensitivity experiments, T-2 toxin was presumed to interfere the proliferation of suppressor T cells which appear in DH-tolerant mice (12). For antibody formation, both IgE and Igl antibody response to DNP-OVA were suppressive when mice were repeatedly treated with fusarenon-X.

Such immunosuppressive action of trichothecenes are closely related to the depression of defence potential to infection. Chickens received T-2 toxin caused an increment of mortality when inoculated with *Salmonella*. Model experiments with virulent *Mycobacterium bovis* (Raventel) also demonstrated that the survival of mice infected with this virulent strain was decreased when 0.1 mg/kg of T-2 toxin was administered, and this T-2 toxin action was higher than those of cortisone (5 mg) (13).

BIOTRANSFORMATION AND TOXICITY.

T-2 toxin and others are widely biotransformed by microbes and animal tissues. In animal tissues, T-2 toxin is deacetylated by hepatic microsomes into HT-2 toxin, and non-specific esterase (s) catalyses this enzymatic reaction (14). Further detailed analysis revealed, as shown in fig. 2, several acyl residues are removed stepwisely via neosolaniol, 4-deacetylneosolaniol, to end T-2 tetraol. Other pathway is 3'-hydroxylation of T-2 toxin and HT-2 toxin. Tracer experiments revealed these 3'-hydroxylated metabolites remained in circulating blood with a longer life time in comparison with the deacylated metabolites (15). Therefore, these 3'-hydroxylated T-2 toxin and HT-2 toxin are presumed to be very important in T-2 toxin-exposed animals.

Table IV summarizes the acute toxicity of T-2 toxin and its metabolites in mice. The LD_{50} value of 3'-hydroxy T-2 toxin is comparable to the parent compound. While, the deacylated metabolites such as HT-2, neosolaniol and T-2 triol are less toxic than T-2 toxin, pathological and histological observations revealed that all these metabolites possess a common feature characterized by cellular destruction of actively dividing cells.

Currently, the author isolated several soil bacteria which are capable to assimilate T-2 toxin as a sole carbon and energy source. One of these bacteria, *Curtobacterium* sp. biotransformed T-2 toxin into HT-2 toxin and this metabolite was further transformed

into T-2 triol. No other trichothecene-like compound (s) was detected in the culture. Therefore, it is conceivable that T-2 toxin is ultimately biotransformed into non-toxic agent (s) by soil bacteria (16).
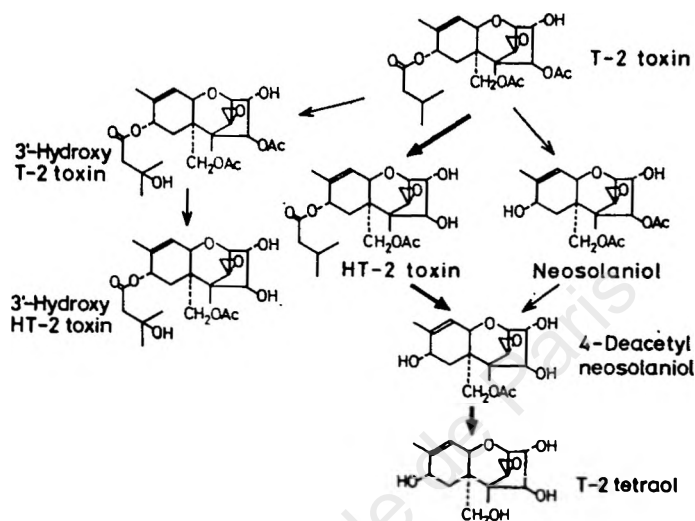
FIG. 2. — Metabolic pathway of T-2 toxin.

TABLE IV

LD$_{50}$ values of T-2 toxin and the biotransformed products

Trichothecenes	Animals	Routes	LD$_{50}$ (mg/kg)
T-2 toxin .	Mouse	ip	5.2
HT-2 toxin	Mouse	ip	9.0
Neosolaniol	Mouse	ip	14.5
T-2 triol .	Mouse	ip	68.0
3'-Hydroxy T-2 toxin	Mouse	ip	4.63
3'-Hydroxy HT-2 toxin .	Mouse	ip	22.8

PREVENTION AND PROPHYLAXIS.

Prevention of the trichothecene exposure and treatment or prophylaxis of the trichothecene toxicosis are of great concern to the health authorities. There are several ways of prevention. For example, the cutaneous exposure is reduced by a simple shelter, sheeting and others. Since the cutaneous toxicity of T-2 toxin and others is higher than that of oral route, these procedures are effective.

Trichothecenes are very stable compounds in normal conditions, and therefore, it is very hard to develop a usable way for decontamination of the toxins. Uptake of orally ingested toxic substances sometimes can be reduced by ingesting either specific binding agents or non-specific materials. However, some pharmaco-kinetic data revealed the rapid distribution in the body. Furthermore, as shown in fig. 2, T-2 toxin is rapidly metabolized into deacylated and hydroxylated compounds, which still possess a potent toxicity. Enzymatic detoxication is expected only when the enzymatic mechanism of decomposition of an epoxide ring of the trichothecenes has been clarified. However, this epoxide is not attacked by usual epoxide hydrase and GSH-transferases. The hydroxylation at C -3' of isovaleroyl residues of T-2 toxin and HT-2 toxin is presumed by catalyzing by cytochrome P-450 system. Inducers of metabolic enzyme systems may increase the rate of transformation of T-2 toxin into these hydroxylated metabolites. But, these metabolites possess the toxicity comparable to the parent T-2 toxin, as shown in table III.

Two types of prophylaxis-immunological and drug-therapeutic are specifically considered as means for providing protection against trichothecenes, but there are limitations on the effectiveness of both procedures.

The injection of antibodies that specifically bind with trichothecenes could have practical. Currently, the author prepared monoclonal antibody to T-2 toxin, and co-administered to mice (ddy male, 6 weeks old) in doses of 3.8 mg of T-2 toxin (sc) and 250 mg of the antibody (ip) per kg. No protective effect of the antibody was observed as far as the lethal toxicity is concerned. From the evidence that this monoclonal antibody was effective by *in vitro* cell culture system, it is presumed that this antibody is unstable in *in vivo* system, or the metabolites such as 3'-hydroxy T-2 toxin exhibit the toxicity.

No specific treatment of trichothecene-induced toxicosis is known at present. Potential clinical manifestations are believed to occur after exposure to trichothecenes. As mentioned in the section of toxicology, the trichothecenes induce dermal lesion, diarrhea, vomiting, and shock. These acute symptoms are expected to be depressed by clinical drugs such as antihistamines, inhibitors of prostaglandin synthesis, central depressants, and anti-inflammatory drugs.

However, the pretreatment of rabbits with promethazine (5 mg/kg iv) did not protect the increment of vascular permeability in-

duced by fusarenon-X (6). Trichothecene-induced vomiting in dogs was depressed by prior administration of chlorpromazine (1.0 mg/kg) or metaclopramide (0.5 mg/kg) (7). Therefore, it is effective to use these central depressants for prophylaxis of trichothecene-induced vomition or emesis.

It is well known that a rapid and significant elevation of circulating white blood cell counts takes place after T-2 toxin and other trichothecenes (10). It means that the toxins induce shock state in animals. In this respect, the authors examined protective effect of anti-inflammatory drugs to T-2 toxin toxicoses in mice. T-2 toxin (1.8 mg/kg) was sc administered to male mice (ddY, 4 weeks old) before or after treatment with drugs daily for 3 days. This dose corresponds to about LD_{50}-LD_{80} value, and most of the T-2 toxin-treated mice died 1-3 days after the sc injection.

As shown in table V, the pretreatment of mice with prednisolone or hydrocortisone in doses of 100 mg/kg for 3 days caused the suppression of lethal toxicity of toxin. Macroscopically, he-

TABLE V

Effect of anti-inflammatory drugs on the lethal toxicity of T-2 toxin in mice

Drugs	Doses (mg/kg × day)	Died/used	%
Exp. A			
Control	—	8/10	80
Prednisolone	100 × 3	3/9	33**
Exp. B			
Control		9/10	90
Hydrocortisone	100 × 3	5/10	50**
Phenylbutazone	50 × 3	10/10	100

morrhage of the intestines and diarrhea are also much less than the control mice received T-2 toxin alone. In mice posttreated with hydrocortisone, the lethal toxicity of T-2 toxin was also decreased, but prednisolone and phenylbutazone were not effective.

Other chemicals such as glutathione (1 g x 3 days), chlorpromazine (20 mg/kg x 3 days), indomethacin (5 mg/kg x 3 days), and others were not protective for T-2 toxin toxicosis when the lethality is employed for evaluation of protection.

The present data are primitive and no conclusive explanation for the protective effect of hydrocortizone could be presented. However, the authors are convinced that these basic approaches stimu-

late the development of specific therapeutic agents for the treatment of acute trichothecene toxicosis.

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Presence of mycotoxins and a man-made material in a « Yellow Rain » sample

by J.D. ROSEN

Department of Food Science, Cook College, New Jersey Agricultural Experiment Station,
Rutgers University, New Brunswick, N.J. 08903 USA

SUMMARY.

A yellow rain sample obtained by the American Broadcasting Company (ABC) was analyzed in our laboratory by selected ion monitoring. T-2 toxin, diacetoxyscirpenol, deoxynivalenol and zearalenone were present in concentrations of 48, 42, 58 and 265 ppm, respectively. Because of the manner in which these analyses were conducted and because of the fact that no recovery studies could be performed on the substrate, the quantities of mycotoxins reported represent minimum values. Further analysis of the sample revealed the presence of polyethylene glycol, a man-made material. Polyethylene glycol can be used as a carrier for dispersion of organic chemicals and is also used as a reactant in the synthesis of emulsifiers. There has been speculation that polyethylene glycol was present in the ABC News sample as a result of « polyethylene glycol bloom », a phenomenon in which the chemical leaches out of synthetic rubber stoppers. We will present evidence that this did not occur with the sample we analyzed.

In March 1981, a team of Hmong resistance fighthers scraped a yellow powder from vegetation several kilometers southeast of Ban Long Tien, a village in the Phou Bia area of Laos. The powder was placed in a glass vial and brought back to the Ban Vinai refugee camp in Thailand. The vial and its contents were turned over to Charles Whitney, the Ban Vinai hospital administrator, who had previously supplied the vial to the Hmong after

thoroughly washing and sterilizing it. The sample was brought back to the United States by Whitney, who (at the request of the American Broadcasting Company News Division) then gave it to Arthur D. Little, Inc., a consulting firm hired by ABC News to analyze the sample. A technician at Arthur D. Little then subdivided the sample so that two different analyses could be performed. Analysis for macrocyclic trichothecenes was to be done by Dr. Bruce Jarvis at the University of Maryland, while analysis for the simpler trichothecenes was to be performed at Rutgers University. The subsamples were packaged in glass vials that were covered with rubber-teflon sandwich type stoppers. The stoppers were held in place by an aluminum crimp. The rubber stopper that had been used to cover the original vial was discarded.

The sample we received was analyzed by gas chromatography/mass spectrometry/selected ion monitoring. Complete details of the techniques used and the results obtained have been published (1). The sample was found to contain 48 ppm T-2 toxin, 42 ppm diacetoxyscirpenol (DAS), 58 ppm deoxynivalenol (DON, vomitoxin) and 265 ppm zearalenone. The latter was confirmed by a full-scale mass spectrum, which in litigation in American courts of law involving food and drug adulteration as well as environmental contamination carries the same weight of evidence as fingerprints do in criminal proceedings. Full scale mass spectra of T-2 toxin, DAS and DON exhibited m/e values identical to those obtained from mass spectra of authentic standards, but the spectra also exhibited m/e values from materials present in the sample and poorly resolved by gas chromatography. By the use of selected ion monitoring, however, we were able to confirm the presence of T-2 toxin, DAS and DON. Although this technique is not considered as confirmatory as identical mass spectra, it has been calculated (2) that under the conditions we employed our chances of making a misidentification were about 1 in a million. We also obtained unequivocal evidence for the presence of polyethylene glycol (PEG), a man-made synthetic chemical used, among other things, for spraying insecticides from aircraft and for the synthesis of emulsifiers such as Tween-60. Emulsifiers serve the purpose of incorporating water-insoluble materials (such as the trichothecenes) into aqueous media. If organic solvents were to be used to spray toxins from an aircraft, there would be a good possibility that the aircraft itself would be destroyed in an explosion.

It was subsequently shown that the ABC News sample also contained pollen and that some, but not all, of this pollen was disseminated by bees (3). However, it is quite possible that during the pollinating season (when this sample was collected) the sample became contaminated with pollen after a yellow rain attack. Alternatively, the yellow rain could have landed on pollen previously present. The latter possibility has also been proposed by Ashton *et al.* (3).

Those who prefer to believe that yellow rain is a natural phenomenon can not accept two facts. One fact is that T-2 toxin was found on a partially decontaminated gas mask taken from a dead Soviet soldier in Afghanistan. The second fact is that a synthetic substance was found in a yellow rain sample. Numerous mental exercises have been used to explain away the PEG finding. The first explanation involved extraction of PEG by the sample from the rubber stopper. When it was pointed out that this was highly unlikely, attention was focused on possible PEG bloom in the rubber stopper provided by Arthur D. Little. When it was shown that the portion of the stopper facing the sample was made of Teflon (4) (there is no PEG in Teflon) and that a photograph of the original rubber stopper indicated no PEG bloom (5), the explanation was shifted to contamination by PEG somewhere between Laos and our laboratory. Even laboratory contamination could « not be ruled out » (6). These explanations are as absurd as the explanation that the « Iranians are gassing themselves » (7). It is difficult to imagine the presence of PEG or commercial emulsifiers in a primitive area of Laos and it is inconceivable that anyone without chemical expertise would open up a vial containing what was believed to be toxic material. It is also inconceivable that PEG contaminated the sample in our laboratory because all our glassware was washed with chromic acid and distilled water before use. It is easy to explain away data by claiming contamination. It is far more difficult to suggest a reasonable source for this contamination. A more reasonable objection to our findings is that no other laboratory has found PEG. This argument falls apart when it is realized that there was only one other sample large enough to look for PEG and that the other analysts did not use the same analytical procedures that we used.

Those who prefer a natural explanation for our findings also conveniently ignore the fact that we found trichothecenes at concentrations at least one order of magnitude higher than concentrations previously reported te be due to natural phenomena. This

is even more incredible when one considers the hypothesis that the toxins were produced on bee feces, a substrate not particularly good for growth of fusaria (fungi which need substrates containing a minimum of 21 % water) let alone enough nutrients to support the production of secondary metabolites such as the mycotoxins, especially under temperature conditions higher than those known to give optimum mycotoxin yield. Also conveniently overlooked is that we found DON in combination with T-2 toxin and DAS. Species of fusaria that produce DON do not produce T-2 toxin or DAS, while species that produce the latter two toxins do not produce DON. In all analyses of naturally-occurring mycotoxin outbreaks, DON has never been found together with T-2 toxin and DAS, except in one case (8). This case involved finding 20 parts per billion (ppb) of T-2 toxin together with appreciable quantities of DON. No mass spectral confirmation was provided. In addition, the analyses were performed by thin layer chromatography, a technique which does not have the capability of identifying 20 ppb T-2 toxin.

Finally, let us not forget that similar concentrations of a highly unusual combination of trichothecenes were identified by Mirocha (9) in a sample obtained in Spring 1981 from an area in Kampuchea approximately 600 km south of the area from which the ABC News sample was obtained. While bees may have visited both areas, a more plausible connection is that fighting involving allies of the Soviet Union was occurring in both areas and that reports of symptoms consistent with trichothecene poisoning was common to both areas.

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Pollen : evidence and controversy

by H. COHEN* and G. NEISH**

SUMMARY.

During the last few years, there has been much controversy related to pollen associated with trichothecenes in samples from Southeast Asia in the so-called « Yellow Rain » phenomenon. Evidence has shown that pollen and trichothecenes have been found together in only two samples. The controversy raises the following questions : Does pollen exist in S.E. Asia ? Do bees defecate ? Does pollen have a natural nutrient that encourages trichothecene production when invaded by fusaria. The first two questions will be outlined regarding the literature published. To answer the last question a series of experiments have been conducted using bee pollen (from Quebec) and various Fusarium isolates. These isolates are : Fusarium graminearum DAOM 180378 (Canada) ; Fusarium sporotrichioides DAOM 175516 (Canada) ; Fusarium equiseti DAOM 183570 (Thailand) ; Fusarium semitectum DAOM 183470 (Thailand - Kampuchea border).

Inoculum : Potato sucrose agar plug, 8 mm diameter, containing mycelium. Incubation conditions : $25 \pm 1°C$ in the dark except for brief periods of observation. Incubation period : three to four weeks.

Experiments : 1° autoclaved rice/unwashed pollen (20 g rice or unwashed pollen pellets — 5 g vermiculite — 20 ml distilled water ; autoclaved 30 min.) ; 2° autoclaved washed pollen (20 g moist washed pollen cake — 5 g vermiculite — 15 ml distilled water ; autoclaved 15 min.) ; 3° unsterilized unwashed pollen (20 g pollen pollets — 5 g vermiculite — 20 ml distilled water) ; 4° propylene oxide sterilized unwashed pollen (20 g pollen pellets

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* Food Production and Inspection Branch, Laboratory Services Division, Agriculture Canada, Ottawa, Ontario. K1A OC6.

** Biosystematics Research Institute. Research Branch, Agriculture Canada, Ottawa. K1A OC6.

— 5 g vermiculite — 20 ml distilled water). The rice cultures are positive controls. The extraction was performed using chloroform: ethanol:water (80:20:10) 150 ml — Purification was carried out using a silica gel Sep-Pak followed by a Cyano (CN) extraction column. The qualitative and quantitative analysis using HPLC, or GC Mass Spectra. Details of method will be given. Under laboratory conditions the production of DON, T$_2$, HT$_2$, 15 AcDON is three times lower in pollen in relation to rice. No zearalenone pro- duction was observed on pollen — the strains from Thailand did not produce significant amounts of toxin in rice. Bee pollen is apparently inferior to rice for mycotoxin production. No fusaria have been isolated from yellow spot and it also should be noted that neither bee droppings nor bee pollen are lethal. Considering the illness and fatalities subsequent to the yellow rain, a concur- rent phenomenon should occur.

INTRODUCTION.

First of all, I would like to express my appreciation to Profes- sor Heyndrickx and his colleagues for inviting me to the First World Congress on « New Compounds in Biological and Chemical Warfare ».

In recent years, there have been numerous reports of ongoing chemical and biochemical warfare in South East Asia and Afgha- nistan. It has been alleged that subsequent to such attacks of « Yellow Rain », there is a rapid onset of illness with characteristic symptomatology and in many instances the victims have died (1-10).

The biochemical agent that could account for this pathology has been identified and quantitated by techniques which are reliable and relevant (11-12) to blood, urine, tissues and other substrates, as being trichothecenes, which are mycotoxins produced by *Fusa- rium* species and some other fungi. Mycotoxins are secondary metabolites produced by a fungus after it has, to a considerable degree, digested the substrate on which it is growing. In nature, when these fungi produce mycotoxins, they do so in small quan- tities (at levels of a few ppm). In the laboratory, mycotoxins are produced easily in quantity, either through appropriate selection of the growth medium or by modifying the strain (genetic muta- tion, for example). One can thus produce large quantities of my- cotoxins over long periods of time.

As you are probably aware, under laboratory conditions the production of mycotoxins can reach gram per liter quantities. If one considers that these products may be toxic at the microgram level or lower, mycotoxins have the potential to be extremely potent weapons.

Normally, high concentrations of *Fusarium* mycotoxins over a widespread area would be indicative of human intervention. For example, in field conditions, the average level of vomitoxin in the Ontario white winter wheat crop was only 0.18 µg/g in 1981, and 0.70 µg/g in 1982. However, at Agriculture Canada, vomitoxin has been produced in Ontario, Canada in large quantities for toxicological studies by inserting a toothpick with fungal mycelium growing on it into an ear of corn. Levels of 300 µg/gram were achieved using this method.

While it is not easy to distinguish between the effects of natural contamination and man-made contamination, there is a distinction between the two. It is a matter of speed of onset, degree of illness and number of victims.

When an entire group of people suddenly takes severely ill or dies within a matter of a few hours, the likelihood of natural contamination is very remote. Natural contamination (13) is a slow-moving process and is unlikely to affect large groups of people on a sudden basis. One may therefore suggest that the reports received from various agencies of widespread illness with a very rapid onset, after attacks of so called « Yellow Rain », tend to mitigate against natural contamination.

Two samples, one of which was tested by Dr. Mirocha and one by Dr. Rosen contained both trichothecenes and pollen. Numerous other yellow spots alleged to be « Yellow Rain » have also proved to be predominantly pollen but were apparently free of mycotoxins. Extrapolating from these facts, a theory has recently been proposed to the effect that the so called « Yellow Rain » is no more than extensive and heavy showers of bee droppings. This theory includes a hypothesis that bee pollen and droppings can be colonized occasionally by fusaria which can then proceed to produce mycotoxins in this substance. The original theory was elaborated on the basis of what may be referred to as the « Chinese Paper » (14).

I will be discussing this theory, first by presenting and analyzing the data set out in the Chinese Paper (based on an official translation by the Secretary of State - Translation Bureau, Ottawa).

Secondly I would like to share with you the results of some recent experiments.

The Chinese paper cited by Dr. Meselson describes the falling of so-called « Yellow Rain » in the region south of the Yang River, describing it as a viscous liquid which falls into the ground, on leaves and roofs. It always happens in the same area and contains a few to 160 drops per square meter and always occurs at mid-day or in the afternoon, and lasts between 5 to 20 minutes.

The Chinese authors put forward the theory that the « Yellow Rain » was bee feces and in an effort to explain the presence of pollen in bee excrement, they noted that :

1. Pollen is constituted by a double layered wall, the external wall being very solid. This outer wal is resistant to both acid and alkali and will not decompose when heated to temperatures of up to 300°C.

2. Bees lack enzymes to decompose the pollen wall. The nutritive constituents of the pollen, i.e., starch, proteins and fats are inside the pollen wall.

3. The nutrients either pass through the germ pores or there is a split in the pollen wall. The nutrients are digested in the mid-gut of the bee, leaving in many areas the structure of the ornamentation of the cell wall and, in particular, the outer wall remains intact. This undigested material is later discharged from the body during flight.

From these observations some easy calculations are worth attention :

a) The Chinese paper described the descent of yellow rain for 20 mins, covering 20 acres with a viscous liquid at about 160 spots of liquid per square meter implies 1.37×10^7 drops per 20 acres (assuming 1 dropping/bee that is 13.7 million bees).

b) Since the fluid is viscous and the size of the spot is a few mm in width — let us assume an average, radius of 0.2 cm with a height of 0.2 cm (liquid viscous). Then the total volume of the material will be about 330 litres and, if the density was unity, the total weight was 330 kg.

The material is surmised to be fecal material from bees. If the average bee weighs 500 mg and can defecate 5 % of its body weight then the « Yellow Rain » in this case was caused by about 12 million bees.

If the « Yellow Rain » in the case of South East Asia was similarly caused by bees, surely the victims of « Yellow Rain » would have noticed the millions of bees that would have been responsible. In addition, quite apart from the Chinese paper, if in fact the victims of so called « Yellow Rain » attacks in S.E. Asia were bombarded with large amounts of bee droppings it is difficult to imagine that they would not have noticed the presence of some bees. After all, as we are all well aware, bees fly fairly close to the ground and as such are quite visible. None of the refugees mention bees or having been stung. Bees also make a sound wich is quite characteristic. It is likely that the refugees would have reported any unusual sounds.

In pollen the nutrients are in compact form, both proteins and starches are very large molecules and fats are insoluble in water; it is therefore difficult to imagine that these substances would pass through the pores, unless there was a mechanical split of the outer wall or the wall had been rendered porous by some enzymatic process. It is to be noted that bees do not have the necessary enzymes in their digestive tract.

In conclusion, the Chinese authors state that 12.1 % of the grains of pollen in bee excrement are damaged and the same proportion of damaged pollen is found in the so-called « Yellow Rain » (12.6 %).

« Yellow Rain » (Chinese) also contains algae, fungal hyphae and bacteria. This is hardly surprising or significant since soil and vegetation samples from just about anywhere could be expected to contain such contaminants.

The only relationship between the Chinese paper and the situations described from S.E. Asia is the use of the term « Yellow Rain ». Obviously the term is used in the Chinese paper simply as an expression to describe the colour of bee excrement and pollen. However, there is nothing in the Chinese paper concerning illness or death as a result of the « yellow » droplets. No analyses as to toxicity were performed for the obvious reason that no toxicity was present. This is a totally different picture to that experienced in Kampuchea and Laos. (15-19).

Analyzing yellow spots on leaves in our laboratories, we did not find any mycotoxins or a significant amount of fungal growth. The samples were fairly clean and consisted primarily of pollen. In open field conditions, one cannot associate the natural presence of fungi or pollen with a phenomenon which results in sudden and widespread death and illness of animals and man. The most

important point remains, that after the so-called « Yellow Rain » attacks people and animals fell ill and indeed there were fatalities (20-23). Certainly this is not caused by pollen. There must therefore be a concurrent phenomenon in operation.

Also, if *Fusarium* is present on plant material, it can sometimes be detected by direct microscopic examination, e.g. orange sporodochia containing masses of macroconidia might be found, pink mycelium bearing characteristic conidia might be found, perithecia might be found and so on.

In any case, if a *Fusarium* isolate has sufficiently digested a substrate — be it a leaf, pollen, rice or what have you — to the point where it is producing high levels of trichothecenes, the presence of the fungus, and the highly degraded state of the substrate, will be obvious. The substrate will be moldy.

As I stated earlier, it has been suggested that pollen is a source of nutrients for the fungi and that the trichothecene contamination of pollen found in S.E. Asia is of natural origin. Therefore, the object of our recent experiments was to obtain data on the effectiveness of pollen as a substrate for mycotoxin production by *Fusarium* species under laboratory conditions.

We wanted to determine whether *Fusarium* species can produce mycotoxins when grown on bee-collected pollen and, if so, to determine whether bee-collected pollen is a substrate that is superior to autoclaved rice as a promotor or enhancer of mycotoxin production by these fungi, or whether pollen acts as an inhibitor of such production.

For the following series of experiments four isolates have been used.

Fusarium graminearum Schwabe (DAOM 180378). This fungus was isolated from a maize caryopsis from a maize plant grown near Ottawa, Ontario. Prior to this study it was known to be a producer of zearalenone, DON and acetyl-DON (24-25).

Fusarium sporotrichioides Sherbakoff (DAOM 175516). This fungus was isolated from a maize caryopsis obtained from a maize plant grown near Ottawa, Ontario. Prior to this study it was known to be a producer of T-2 toxin (25-26).

Fusarium equiseti (Corda) Sacc. (DAOM 183570). This fungus was isolated either from a leaf or from *soil* adhering to the leaf that also had a pollen spot (« Yellow Rain ») on it. This leaf from the Pong Namron area of Thailand was received from H.B. Schiefer in 1982. The identity of the leaf was not determined; it is to be

noted that the fungus was *not* isolated from the pollen spot but from another part of the leaf.

Fusarium semitectum Berk. & Rav. (DAOM 183470). This fungus was isolated from a piece of banana shoot received from H.B. Shiefer who collected it at a Khmer-Rouge camp on the Thailand-Kampuchea border (approx. 13°27'N, 102°24'E, near Poipet) in 1982.

DESIGN OF THE EXPERIMENTS.

The experiments were set up using the following :

— Rice (Uncle Ben's).
— Vermiculite.
— Washed pollen.
— Bee pollen pellets (Labonté Pollen de fleurs en grains, Les Miels Labonté Inc. Victoriaville, Quebec, Canada).

A series of experiments was set up as follows :

a) Autoclaved rice/unwashed pollen.
b) Autoclaved washed pollen.
c) Unsterilized unwashed pollen.
d) Cold sterilisation using propylene oxide.
e) Conidial inoculum/low temperature incubation.

The rice cultures were positive controls.

Inoculum.

Inoculum preparation (experiments a-d).

Lyophilized cultures were plated out onto potato sucrose agar. They were incubated at 25°C for a few days until sufficient growth was obtained and then stored in a refrigerator. The inoculum in each case was an 8 mm potato sucrose agar plug containing mycelium. The identity of the fungal isolates was re-verified by examining slides cultures set up from the same cultures that were used to set up the experiments.

a) *Autoclaved Rice/Unwashed Pollen Experiment.*

Twenty grams of either Uncle Ben's rice or unwashed Labonté bee-collected pollen pellets were mixed with 5 g vermiculite and 20 mL distilled water in a 250 mL flask capped with aluminum

foil and autoclaved for 30 min, the flasks were cooled to room temperature and then stored at 5°C overnight.

Prior to inoculation the pollen plus vermiculite was scored and broken up using a sterile spatula because the pollen cakes during autoclaving. The rice plus vermiculite was treated similarly. Each of the four isolates was inoculated into three rice plus vermiculite cultures and three pollen plus vermiculite cultures. After inoculation the cultures were incubated at 25 ± 1°C.

On rice the relative growth rates and the pigmentation of the isolates were consistent with what would be predicted from their growth rates and pigmentation in potato sucrose agar (PSA) or potato dextrose agar (PDA). For example, both the *F. graminearum* and the *F. sporotrichioides* isolates rapidly invaded the rice and produced the pink-red pigments that they normally produce on PSA or PDA. The *F. equiseti* and *F. semitectum* isolates grew slightly more slowly and produced no pink-red pigments. All isolates grew more slowly in the pollen. For the most part the three replicates for each isolate/substrate combination did not show much variation with respect to growth, colour, aerial mycelium production.

One replicate for each isolate/substrate combination was analysed for mycotoxins after an incubation period of three weeks; the remaining duplicates were incubated four weeks.

b) *Autoclaved Washed Pollen Experiment.*

To set up this experiment 100 g pollen pellets were placed in a filter in a Buchner funnel and washed within excess of one litre of hot distilled water. For 100 g pollen pellet, after washing and vacuum filtration, the moist cake weighed 115 ± 2 g. The colour of the elutant changed from dark brown at the beginning of the wash to pale yellow by the time the wash was finished. Twenty grams of the moist cake was placed in a 250-mL flask and mixed with 5 g vermiculite and 15 mL distilled water prior to autoclaving for 15 min. After autoclaving the pollen/vermiculite cake was scored and broken up using a sterile spatula and the flasks were then inoculated (three replicates per isolate) immediately and incubated at 25 ± 1°C.

The general impression, overall, was that the fungi grew more rapidly over the washed pollen than they did over the unwashed pollen. The cultures were incubated for 29 days.

c) *Unsterilized Unwashed Pollen Experiment*.

The method used in this experiment was the same as that for the autoclaved unwashed pollen experiment, except that the flasks were not autoclaved prior to inoculation of two replicates with either *F. graminearum* DAOM 180378 or with *F. semitectum* DAOM 183470. During incubation fungi other than the ones that we inoculated could be observed but these were not identified except for an obvious mucoraceous fungus in one flask.

d) *Propylene Oxide Sterilized Unwashed Pollen Experiment*.

A vial containing 2 ml propylene oxide was double-wrapped in tightly sealed plastic bags together with two 250 ml flasks. Each flask contained 20 g unwashed pollen pellets, 5 g vermiculite, and 20 ml distilled water. After 23 hours the bags were then slit open and left in a fume hood for another hour or so before they were inoculated in the same way as the previous cultures except for the scoring and breaking of the cake step which was unnecessary. Each of the four isolates was inoculated into four flasks. Two other flasks were used as uninoculated controls. Six days later no growth of any kind could be observed in any of the cultures and the reason for this has yet to be determined. Possibly the fungi in the inoculum blocks were killed by propylene oxide residues; possibly the cold sterilized pollen contained antifusarial substances that prevented the cultures from growing. We first attempted to deal with the problem by irrigating the blocks with 5 ml sterile distilled water. Unfortunately this was unsuccessful. Success was finally achieved by re-inoculating the cultures two days later. Microscopic examination made prior to delivering the cultures for analysis revealed that at least two of the cultures (one *F. graminearum* culture and one *F. sporotrichioides* culture) were obviously contaminated with a mucoraceous fungus similar to the one seen previously in the unsterilized material. It is not known whether this contamination was the result of inadequate sterilization of the flasks or the result of subsequent contamination after the propylene oxide sterilization. There was no sign of contamination in the uninoculated controls. Another feature of the propylene oxide treated pollen was the strong amine-like odor produced. This odor was noted only in the inoculated flasks; the uninoculated controls did not have an unusual odor. The cultures were incubated for four weeks after they were re-inoculated.
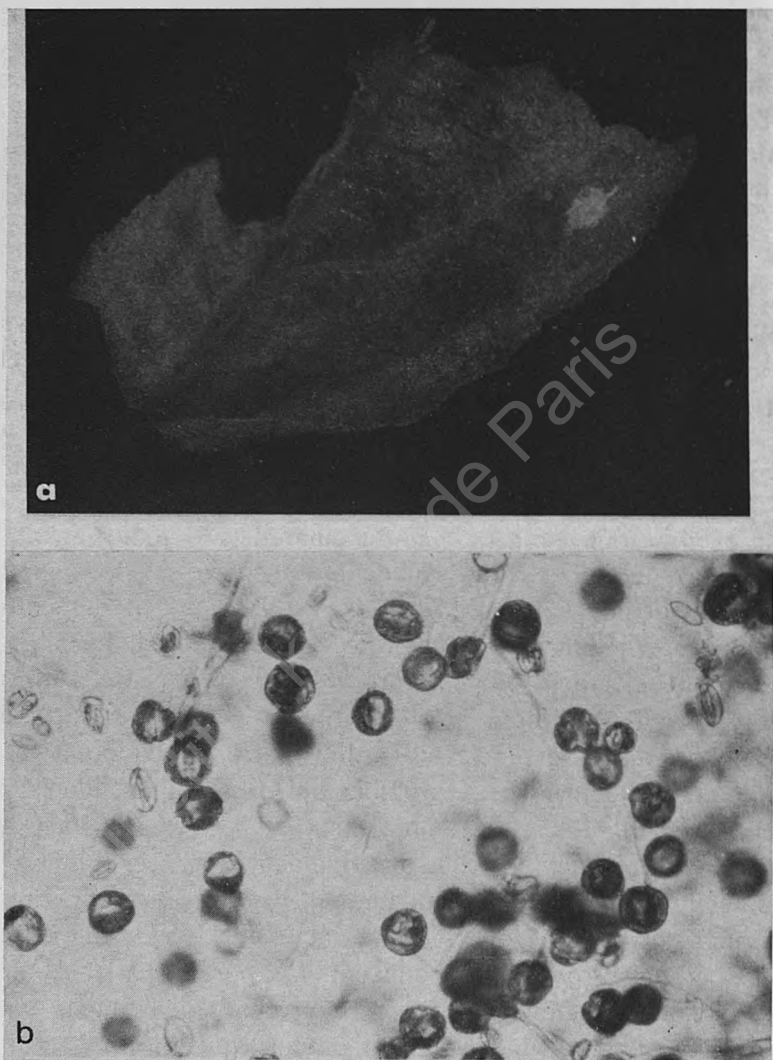
PLATE 1. — (a) Leaf with yellow spot on it from the Pong Namron area of Thailand. This leaf was received from H.B. Schiefer in 1982 (Photograph courtesy of H.B. Schiefer) ; (b) photomicrograph of material removed from the yellow spot (water mount, × 420).
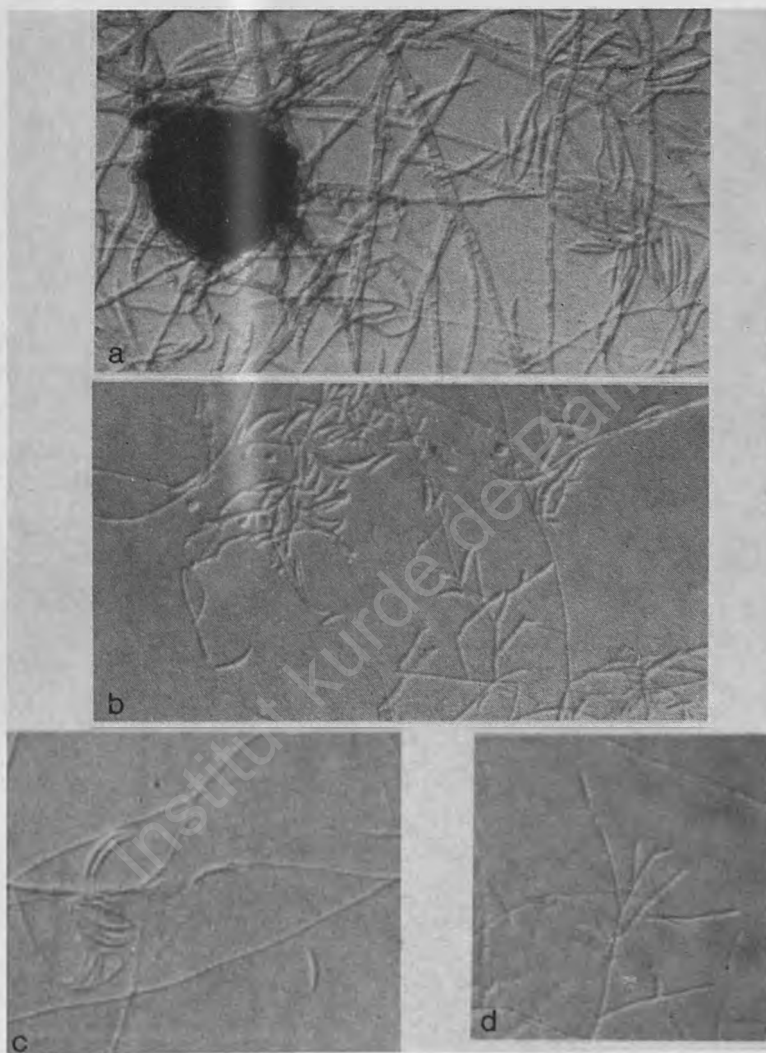
PLATE II. — Photomicrographs of slide cultures of (a) *F. graminearum* DAOM 180378, (b) *F. sporotrichioides* DAOM 175516, (c) *F. equiseti* DAOM 183570, and (d) *F. semitectum* DAOM 183470 mounted in lactophenol-cotton blue. Interference contrast (× 380).
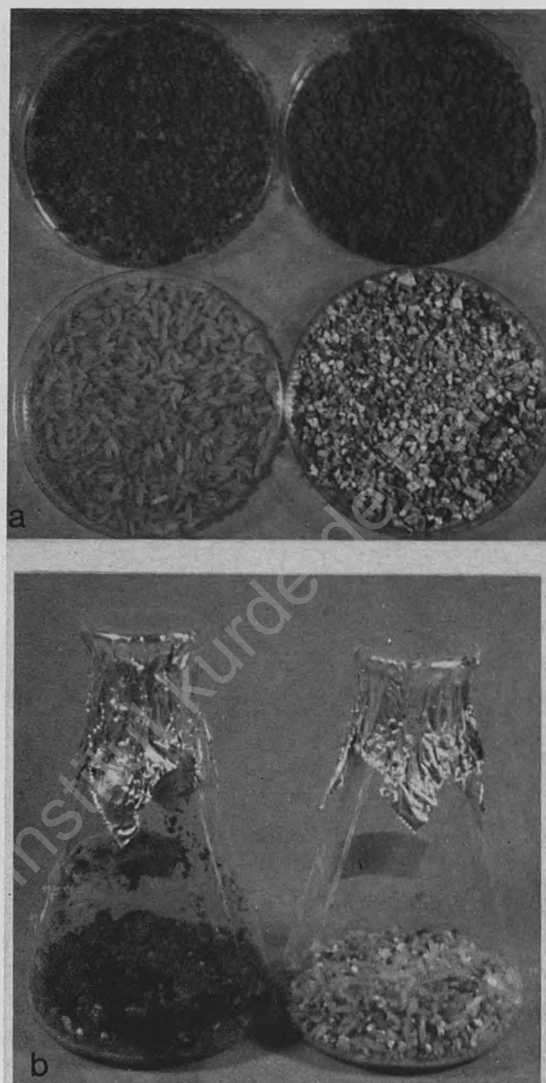
PLATE III. — (a) bee-collected pollen pellets (upper left), washed pollen (upper right), rice (lower left), and vermiculite (lower right); (b) autoclaved uninoculated unwashed pollen/vermiculite (left) and rice/vermiculite (right).
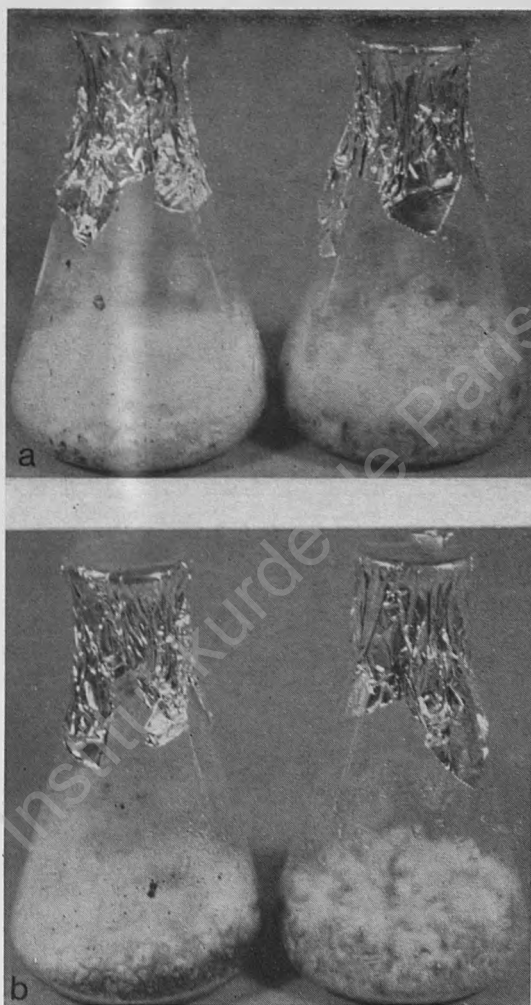
PLATE IV. — Autoclaved unwashed pollen (left) and rice (right) cultures
20 days after inoculation and Incubation at 25° C.
(a) *F. graminearum* DAOM 180378 ; (b) *F. sporotrichioides* DAOM 175516.
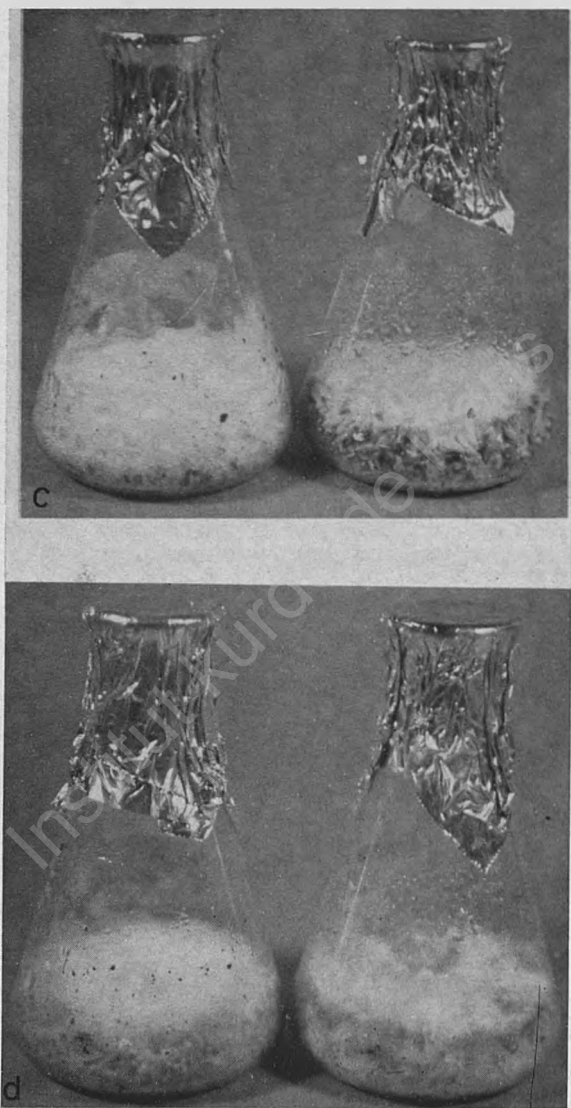
PLATE IV. — (cont'd) (c) *F. equiseti* DAOM 183570 ; (d) *F. semitectum* DAOM 183470.
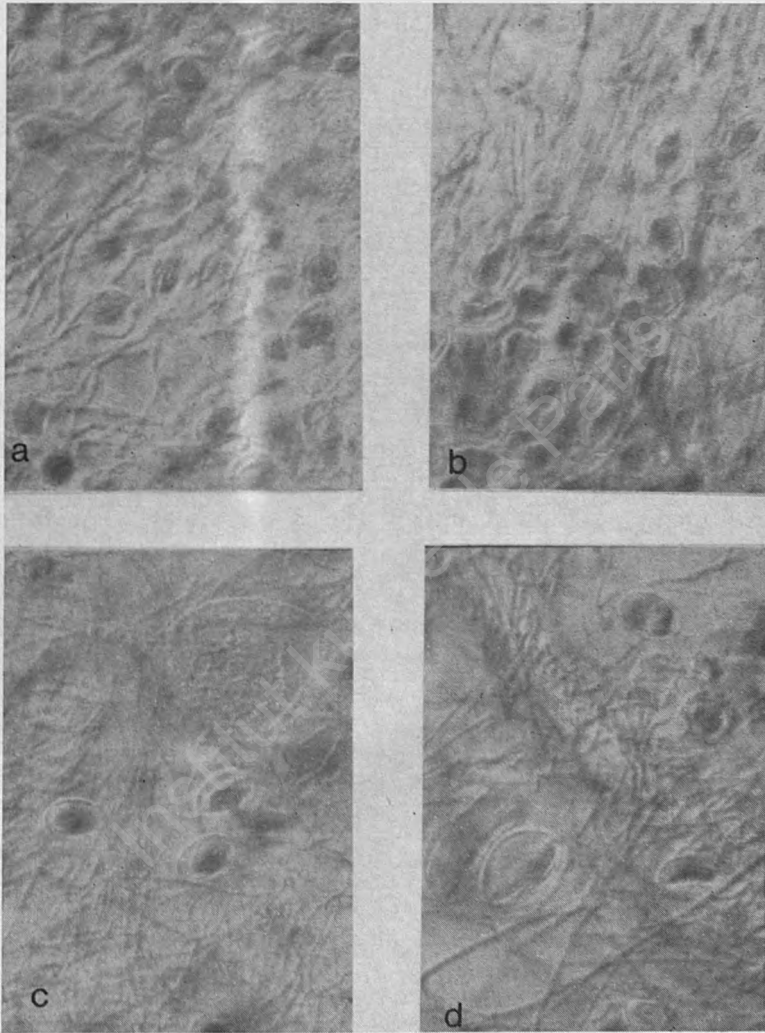
PLATE V. — Photomicrographs of material removed from autoclaved unwashed pollen cultures of (a) *F. graminearum* DAOM 180378, (b) *F. sporotrichioides* 175516, (c) *F. equiseti* DAOM 183570 and (d) *F. semitectum* DAOM 183470. Lactophenol-cotton blue. Interference contrast (× 380).

e) *Conidial Inoculum/Low Temperature Incubation Experiment.*
Rice/vermiculite or unwashed pollen pellets/vermiculite media
were prepared using methods similar to those described for the
autoclaved rice/unwashed pollen experiment (a). Two flasks con-
taining rice/vermiculite and three flasks containing pollen
pellets/vermiculite were each inoculated with approximately
17×10^4 *F. sporotrichioides* (DAOM 175516) conidia suspended in
0.5 mL sterile distilled water. The cultures were incubated at
$25 \pm 1°C$ for 12 days followed by incubation for an additional
15 days at $10 \pm 1°C$; during days 3-7 of the incubation period
the cultures were thoroughly shaken several times to break up
the mycelium.

Extraction and analysis.

All samples were extracted with 200 mL of a mixture of chlo-
roform-ethanol-water (80 + 20 + 10). The samples were homo-
genized using a Polytron mixer. The extract was then filtered
through a bed of Celite 545 (30 g) and then rinsed with 100 mL
of chloroform-ethanol (80 + 20). The organic solvent was then
evaporated to dryness on a rotary evaporator. The extracts were
resolubilized in 50 ml of chloroform-ethanol (80 + 20) and fil-
tered using Whatman 41 filter paper.

Purification of the samples for trichothecene analysis (see flow chart).

1 ml of the above filtrate equivalent to 0.4 g of samples is
evaporated to dryness on a water bath using a gentle stream of
nitrogen. The residue is then dissolved in 1 ml of methylene
chloride. Quantitatively transfer methylene chloride solution to
a silica gel Sep-Pak™ attached to a 20 ml glass syringe. A total
of 7 ml of methylene chloride is collected through the Sep-Pak.
This fraction is discarded. The trichothecenes are then eluted with
15 ml of methylene chloride-methanol (95 + 5) followed by 10 ml
of chloroform-methanol (1 + 1). This fraction is collected in a
50 ml round bottom flask and then evaporated to dryness.
The fraction containing the trichothecenes is further purified
using a Cyano™ extraction column. Dissolve residue from the
Sep-Pak in 1 ml of chloroform-hexane (1 + 1) and quantitatively
transfer to the Cyano column. A total of 15 ml is collected in a

15 ml centrifuge tube. This fraction (1) contains T-2, DAS and 15A-DON. Now elute Cyano column with 5 ml chloroform followed by 10 ml chloroform-ethanol (98 + 2). This fraction (2) contains DON, HT-2, 15A-DON. Finally add 10 ml of chloroform-ethanol (90 + 10). This final fraction (3) contains T-2 tetraol.

Evaporate the three fractions to dryness and add 1 ml of to-luene-acetonitrile (95 + 5) and vortex to solubilize residue.

The underivatized fractions are then analysed by capillary gas chromatography/mass spectroscopy using negative chemical ioni-sation.

Zearalenone analysis.

Zearalenone analysis was done on the initial extract (chloro-form-ethanol 80 + 20). An aliquot of the sample extract (50 ml) is analyzed directly by HPLC equipped with a fluorescence de-tector set at 274 nm excitation and 418 nm emission. HPLC con-ditions according to Cohen and Lapointe (27).

Extraction and purification of sample (rice or pollen)

```
20 g sample
200 ml CHCl₃ (8) + EtOH (2) + H₂O (1)
Polytron (5 minutes)
        ↓
Filter
        ↓
Evaporate to dryness
        ↓
Bring to 50 ml volume
        ↓
Take 4 gm aliquot for purification
        ↓
Sep Pak
        ↓          — Discard 7 ml methylene chloride
Collect     — 15 ml CH₂Cl₂/CH₃OH (95 + 5)
        ↓          — 10 ml CHCl₃/CH₃OH (1 + 1)
Evaporate to dryness
        ↓
Cyano Cartridge
        ↓
15 ml CHCl₃/hexane (1 + 1)                    Fraction 1
        ↓
5 ml CHCl₃ + 10 ml (HCl₃/EtOH (98 + 2)        Fraction 2

10 ml CHCl₃/EtOH (90 + 10)                    Fraction 3
```

GC/MS conditions for mycotoxin analysis.

Mycotoxin samples were analysed using a Finnigan 4500 GC/MS instrument equipped with a Townsend discharge (TD) ionization

source. Samples were introduced into a 15 m DB-5 0.32 mm fused silica column (J & W) with on-column injection mode. The GC oven is programmed to go from 130°C to 205°C at 20°C/min then to 280°C at 10°C/min; hold at 280°C for 2 min.

Ionizing electrons were produced using TD negative chemical ionization with reagent gas O_2. This method of ionization shows high sensitivity to underivatized mycotoxins and it can use O_2 as reagent gas.

The mycotoxins monitored were :

(1) DON
(2) 15A-DON
(3) DAS
(4) HT-2
(5) T-2

For DON the M-1 (295) and a fragment ion (248) were monitored. The M-1 (337) ion was monitored for 15A-DON. For DAS, HT-2, and T-2 the M + O_2 ions were monitored which is characteristic of TD O_2 ionization. The relative RT were as follows :

DON	5.4 min	HT-2	8.7
15A-DON	5.6		
DAS	6.0	T-2	9.6

RESULTS AND DISCUSSION.

a) This experiment was set up using *Autoclaved Rice/Unwashed Pollen* incubated for 3 weeks or 4 weeks at 25°C ± 1°C. The samples were inoculated with four different isolates using an 8 mm PSA plug containing mycelium. In the samples analyzed after 3 weeks *F. graminearum* (DAOM 180378, Canada) was found to produce DON and 15A-DON in both rice and pollen. Pollen results were ca. 3 times lower than rice. The rice sample also produced zearalenone (table I). There was no zearalenone production in pollen. *F. sporotrichioides* (DAOM 175516, Canada) was found to produce high levels of T-2 in rice and substantially lower levels in pollen. Lower levels of HT-2 were also found in both pollen and rice. *F. equiseti* (DAOM 183570, Thailand) and *F. semitectum* (DAOM 183470, Thailand) were found not to produce any mycotoxins. After 4 weeks *F. graminearum* (DAOM 180378, Canada) produced DON and 15 A-DON in rice but only DON in pollen.

TABLE I

Autoclaved rice / unwashed pollen

Mycotoxin	Fusarium graminearum DAOM 180378 Canada		Fusarium sporotrichioides DAOM 175516 Canada		Fusarium equiseti DAOM 183570 Thailand		Fusarium semitectum DAOM 183470 Thailand	
	Rice	Pollen	Rice	Pollen	Rice	Pollen	Rice	Pollen
DON	20.3	6.0						
15 A-DON	3.5	1.0						
DAS								
T-2			100.0	37.5				
HT-2			11.3	3.3				
Zearalenone	240							

Incubation period : 3 weeks.   Inoculum : 8 mm PSA plug containing mycelium.
All data expressed as ppm using GC/MS.
All data are the average of 1 replicate.

TABLE II

Autoclaved rice / unwashed pollen

Mycotoxin	Fusarium graminearum DAOM 180378 Canada		Fusarium sporotrichioides DAOM 175516 Canada		Fusarium equiseti DAOM 183570 Thailand		Fusarium semitectum DAOM 183470 Thailand	
	Rice	Pollen	Rice	Pollen	Rice	Pollen	Rice	Pollen
DON	14.9	0.4						
15 A-DON	8.5							
DAS								
T-2			54.0	6.0				
HT-2			8.9	1.0				
Zearalenone	1050				15			

Incubation period : 4 weeks.   Inoculum : 8 mm PSA plug containing mycelium.
All data expressed as ppm using GC/MS.
All data are the average of 2 replicates.

Zearalenone was also produced in the rice sample (table II).
F. sporotrichioides (DAOM 175516, Canada) was found to produce
lower levels of T-2 in pollen and rice than those found in table I.
HT-2 levels were also lower in both pollen and rice than those in
table I. *F. equiseti* (DAOM 183570, Thailand) under this longer
incubation period did produce a low level (15 ppm) of zearalenone
in rice but not in pollen. No other mycotoxins were detected.
No mycotoxins were detected in rice and pollen inoculated with
F. semitectum (DAOM 183470, Thailand).

TABLE III

Autoclaved washed pollen

| Mycotoxin | Fusarium graminearum DAOM 180378 | | Fusarium sporotrichioides DAOM 175516 | | Fusarium equiseti DAOM 183570 | | Fusarium semitectum DAOM 183470 | |
| | Canada | | Canada | | Thailand | | Thailand | |
	Rice	Pollen	Rice	Pollen	Rice	Pollen	Rice	Pollen
DON	14.9	23.0						
15 A-DON	8.5	1.8						
DAS								
T-2			54.0	78.6				
HT-2			8.9	101.7				
Zearalenone	1050				15			

Incubation period : 4 weeks.   Inoculum : 8 mm PSA plug containing mycelium.
All data expressed as ppm using GC/MS.
All pollen data are the average of 3 replicates.

TABLE IV

Propylene oxide sterilized unwashed pollen

| Mycotoxin | Fusarium graminearum DAOM 180378 | | Fusarium sporotrichioides DAOM 175516 | | Fusarium equiseti DAOM 183570 | | Fusarium semitectum DAOM 183470 | |
| | Canada | | Canada | | Thailand | | Thailand | |
	Rice	Pollen	Rice	Pollen	Rice	Pollen	Rice	Pollen
DON	14.9	9.6						
15 A-DON	8.5							
DAS								
T-2			54.0	8.3				
HT-2			8.9	6.5				
Zearalenone	1050				15			

Incubation period : 4 weeks.   Inoculum : 8 mm PSA plug containing mycelium.
All data expressed as ppm using GC/MS.
All pollen data shown are the average of 3 replicates.

TABLE V

Fusarium sporotrichioides DAOM 175516 Canada

Mycotoxin	Rice	Pollen
DAS	100.0	9.2
HT-2	1.0	2.9
T-2	301.0	441.0

— Incubation period (12 days at 25° C, 15 days at 10° C).
— Inoculum : ca. 17 × 10^1 conidia/replicate.
— All data expressed as ppm using GC/MS .
  Rice data are the average of two replicates.
  Pollen data are the average of three replicates.

b) This experiment was set up using *Autoclaved Washed Pollen* only (table III). The incubation period was just over 4 weeks and results are an average of 3 replicates. Rice data are from table II and are only shown for comparison. *F. graminearum* (DAOM 180378, Canada) produced the highest levels of DON when compared to other pollen experiments. The levels were also higher than in the rice. Also a small amount of 15 A-DON was produced. *F. sporotrichioides* (DAOM 175516, Canada) produced higher levels of T-2 and HT-2 in comparison with the rice cultures. Washing the pollen enhanced production of DON, HT-2 and T-2 as compared to the unwashed pollen. *F. equiseti* (DAOM 183570, Thailand) and *F. semitectum* (DAOM 183470, Thailand) were found not to produce any mycotoxins.

c) *Unsterilized Unwashed Pollen* was also investigated using *F. graminearum* (DAOM 180378, Canada) and *F. semitectum* (DAOM 183470, Thailand). No mycotoxins were detected using this technique. Samples in this experiment were done in duplicate.

d) This experiment was set up using *Propylene Oxide Sterilized Unwashed Pollen* (table IV). The incubation period was 4 weeks and all pollen data shown are the average of 3 replicates. Again, as in table III, rice data are included for comparison. *F. graminearum* (DAOM 180378, Canada) produced only DON. The level compared to table I is higher but lower than for the unwashed pollen in table III. *F. sporotrichioides* (DAOM 175516, Canada) also produced low levels of T-2 and HT-2 when compared to washed pollen in table III. *F. equiseti* (DAOM 183570, Thailand) and *F. semitectum* (DAOM 183470, Thailand) were found not to produce any mycotoxins.

e) The results cited in table V must be interpreted with a good deal of caution for the following reasons :

1. We are dealing with only one experiment using *F. sporotrichioides* (DAOM 175516, Canada).

2. These results were obtained in laboratory conditions and such cannot be transposed into the natural environment as explained previously.

3. These results were obtained in an experiment in which the three following elements were present :

a. Inoculations were done with 17 x 10^4 conidia;
b. The cultures were subject to shaking;

c. The cultures were incubated 12 days at 25° ± 1°C followed by 15 days at 10° ± 1°C.

The rice experiment was done in duplicate while the pollen experiment was done in triplicate.

With both substrates the results obtained were far higher for T-2 toxin than was the case when the agar plug inoculum was used. However, the results obtained on pollen were extremely surprising. There was an almost exclusive production of T-2 toxin (441 ppm as opposed to 9.2 ppm DAS and 2.9 ppm HT-2).

On rice, we found the following results : 100 ppm DAS, 1 ppm HT-2 and 301 ppm T-2.

When the same fungus was used with the agar plug inoculum on both rice and pollen, no DAS was detected, and the levels of the two other toxins were lower. In this case, the temperature was maintained at 25° ± 1°C throughout the incubation and the cultures were not shaken.

The results obtained seem to suggest that using a simple technique, it is possible to produce in laboratory conditions a high concentration of toxin in both rice and pollen (with pollen being, in this instance, the superior substrate); under natural conditions, it seems unlikely that such concentrations could be obtained.

The temperature modification from 25° ± 1°C to 10° ± 1°C may be the key factor in promoting the production of the toxin. Further research is needed to confirm this.

In addition to DON, 15 A-DON, DAS, HT-2 and T-2 all the samples were screened for Verrucarol, T-2 Tetraol, Neosolaniol, Nivalenol, T-2 Triol and 3-acetyl DON. None of these trichothecenes were produced in amounts detectable by the GC/MS.

In conclusion the Meselson Theory can be summarized as follows : « Yellow Rain » is bee feces and is comprised primarily of pollen. Its presence in heavy concentrations in certain areas is the result of « cleansing flights » by large numbers of bees. Bee feces is usually non-toxic but can be invaded occasionally by trichothecene-producing fungi (probably *Fusarium* spp.) which then proceed to produce trichothecenes in the pollen.

There is no question that bees defecate and that some yellow spots found in South East Asia may be bee feces. However these spots are free of trichothecene contamination.

In our experiments, we found that it is possible for fusaria to produce trichothecenes in bee-collected pollen. However to establish the underlying equation present in the Meselson theory, that bee feces and fusaria and trichothecenes come out to a natural phenomenon, certain specific conditions must be present.

1. In order for fusaria to produce toxins in bee-collected pollen, the substrate apparently must be heavily invaded and the presence of the fungus obvious. If something similar to what we observe in the laboratory were to happen in nature one might expect, based on our experiments, that pollen overgrown by *Fusarium* (hence more likely to be contaminated by trichothecenes) might not be yellow but some other colour (e.g., off-white) characteristic of the mycelium rather than the pollen. One might also expect that the appearance of the spot would be fluffy or matted rather than powdery, again a reflection of the morphology of the mycelium rather than the pollen.

2. In our study only the Canadian isolates produced trichothecenes in either rice or pollen. The fusaria from S.E. Asia did not produce any trichothecenes.

3. In our study, no mycotoxins were produced on pollen when *F. graminearum* had to compete for the substrate with other microorganisms. Example : in the experiment done with unwashed, unsterilized pollen, no mycotoxins were detected.

4. In our study the fungi were supplied with a relatively large amount of pollen, with ample moisture, and, with the exception of two experiments, were incubated at a relatively constant temperature (25° ± 1°C), in the dark and free from competition.

5. On pollen, trichothecenes were produced in both autoclaved unwashed and washed pollen and also in cold sterilized pollen using *F. graminearum* and *F. sporotrichioides*. No zearalenone was produced on pollen by any of the isolates.

6. In our study on pollen and rice inoculated with *F. sporotrichioides* conidia, a considerable amount of T-2 was produced in pollen and rice and a lesser amount of DAS in rice. Conditions included a drop in temperature from 25° ± 1°C to 10° ± 1°C and shaking of the cultures.

7. No fusaria were isolated when bits of a yellow spot were plated out onto a medium that is selective for *Fusarium* species.

Those who promote the theory that trichothecene contamination of pollen is natural should find this pollen and demonstrate the presence of both the fungi and the trichothecenes in them. The onus is on those who have claimed a natural origin to demonstrate its occurrence.

There are additional points which, though less obvious, may be relevant in this regard. Let us assume that we do have bee feces contaminated naturally by fusaria. For such contamination to become the source of dietary intoxication, the bee feces would have to reach the food cycle. One can safely assume that Hmong tribesmen do not eat bee feces. If the material were to reach the dietary chain by seepage into the water or food supply, you would have a slow-moving phenomenon and very widespread illness. In short the whole population in an area would fall ill, and we would find the contamination in the blood and tissues of the whole population. This is not what has occurred. Some people fell ill suddenly after an attack and persons in the nearby area who were not subjected to the attack have no symptoms or evidence of intoxication.

There is also no evidence that fusaria can spontaneously colonize the mid-gut of te bee and produce the toxin which is supposedly excreted during the « cleansing flight ». If the bee were to absorb contaminated pollen with high concentrations of trichothecenes, the bee would not survive. (This is what could literally be described as « bee droppings » !).

*
* *

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A P P E N D I X I

Isolates :

Fusarium graminearum DAOM 180378 (Canada).
Fusarium sporotrichioides DAOM 175516 (Canada).
Fusarium equiseti DAOM 183570 (S12-6CB) (Thailand).
Fusarium semitectum DAOM 183470 (SO3-5) (Thailand-Kampu-chea border).

Inoculum : potato sucrose agar plug, 8 mm diameter, containing mycelium.

Incubation conditions : 25 ± 1°C in the dark except for brief periods of observation.

Experiments :

I. Autoclaved Rice/Unwashed Pollen (20 g rice or unwashed pollen pellets + 5 g vermiculite + 20 ml distilled water ; autoclaved 30 min.).

II. Autoclaved Washed Pollen (20 g moist washed pollen cake + 5 g vermiculite + 15 ml distilled water ; autoclaved 15 min.).

III. Unsterilized Unwashed Pollen (20 g pollen pellets + 5 g vermiculite + 20 ml distilled water).

IV. Propylene Oxide Sterilized Unwashed Pollen (20 g pollen pellets + 5 g vermiculite + 20 ml distilled water ; pairs of 250 ml flasks exposed to 2 ml propylene oxide while sealed for 23 h inside two tightly sealed plastic bags).

APPENDIX II

Pollen identification of bee pollen pellets

Colour	Pollen/100 grains
Orange Brown 48. v. O.	*Taraxacum* 100 %
Orange Brown 50. s. O.	*Taraxacum* 50 % ; Circa 50 % *Sambucus*
Orange Brown 55. s. Br	*Trifolium* 85 % ; *Medicago* 10 % ; *Epilobium* 3 % ; Undetermined 2 %
Orange Brown 55. s. Br	*Trifolium* 99 % ; Undetermined 1 %
Orange Brown 56 deep Br	*Trifolium* 90 % ; Rosaceae 10 %
Orange Yellow Yellowish Brown 74. s.y Br	*Medicago* 90 % ; *Epilobium* 5 % ; Undetermined 5 %
Orange Yellow Yellowish Brown 74 s.y Br	*Medicago* 95 % ; Undetermined 5 %
Orange Yellow Yellowish Brown 66. v. OY	*Taraxacum* 100 %
Orange Yellow Yellowish Brown 68 s. OY	*Gentianella* ? 100 %
Orange Yellow Yellowish Brown 78. d.y Br	*Epilobium* 60 % ; *Medicago* 30 % ; *Viburnum* 10 %
Yellow Olive Brown 84. s.Y	*Medicago* 95 % ; Undetermined 5 %
Yellow Olive Brown 84. s.Y	*Medicago* 99 % ; Undetermined 1 %
Yellow Olive Brown 87. m. Y	*Viburnum* ? 100 %
Yellow Olive Brown 90. g. y Y	Rosaceae 100 %
Yellow Olive Brown 91 d. gy.Y	Rosaceae 95 % ; Undetermined 5 %
Yellow Olive Brown 94. 1.01 Br	*Vaccinium* 99 % ; Undetermined 1 %
Yellow Olive Brown 95. m.01 Br	*Trifolium* 99 % ; Undetermined 1 %
Greenish Yellow Olive 105. gy.gY	Rosaceae 90 % ; Undetermined 10 %
Yellow Green Olive Green 128.d.gy.01G	*Trifolium* 50 % ; *Epilobium* 50 %

Colour values ISCC-NBS Colour-Name Charts were assigned to 19 various randomly selected pellets which accounted for most of the colour variation of the pellets.

C.W. Crompton, Biologist.
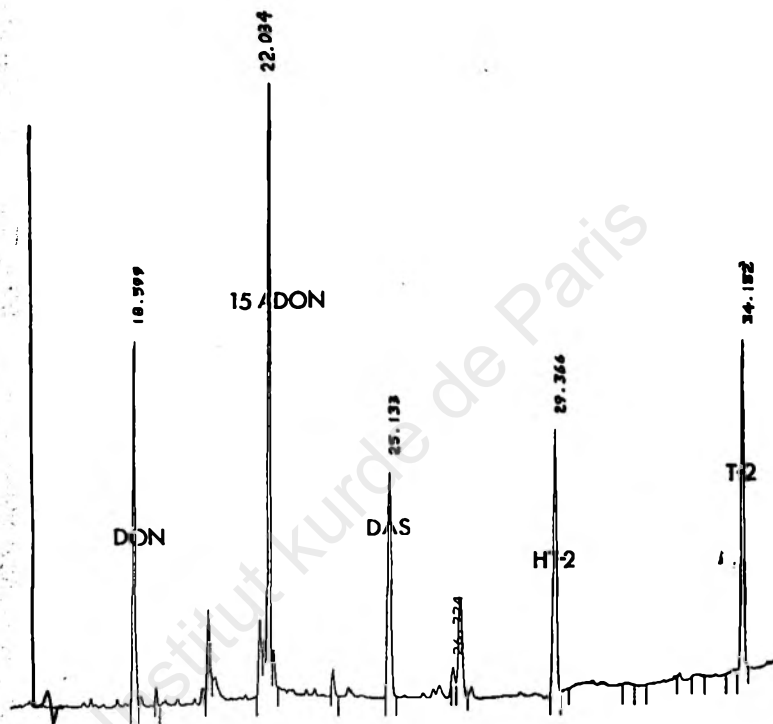
FIG. 1. — Std. — 20 pg DON
— 90 pg 15 A-DON
— 250 pg DAS
— 40 pg HT-2
— 250 pg T-2

All as their respective HFBI derivates.
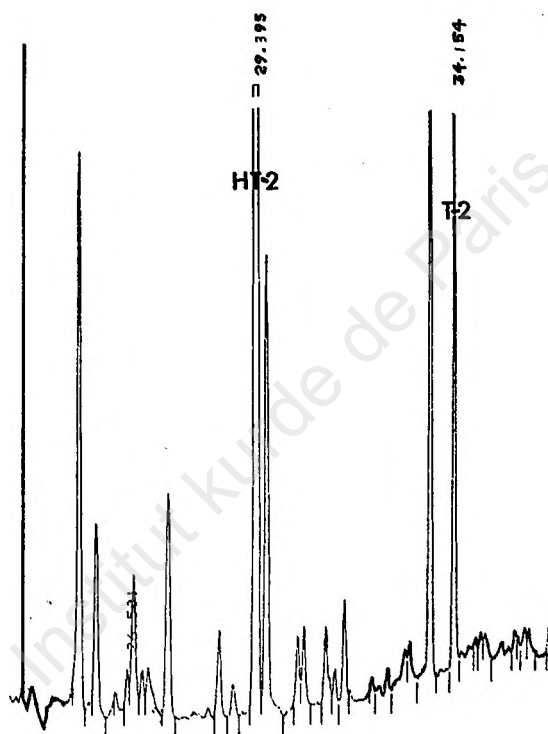
FIG. 2. — Washed pollen sample showing 101.7 ppm HT-2 and 78.6 ppm T-2. Isolate 175516.
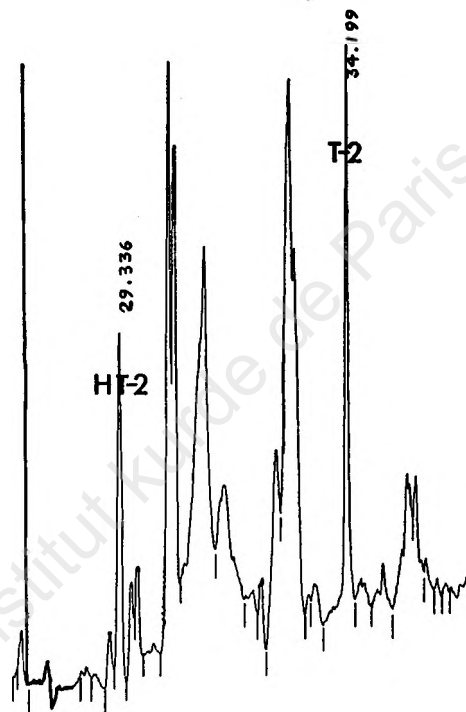
FIG. 3. — Rice control sample showing 8.9 ppm HT-2 and 54.0 ppm T-2. Isolate 175516.
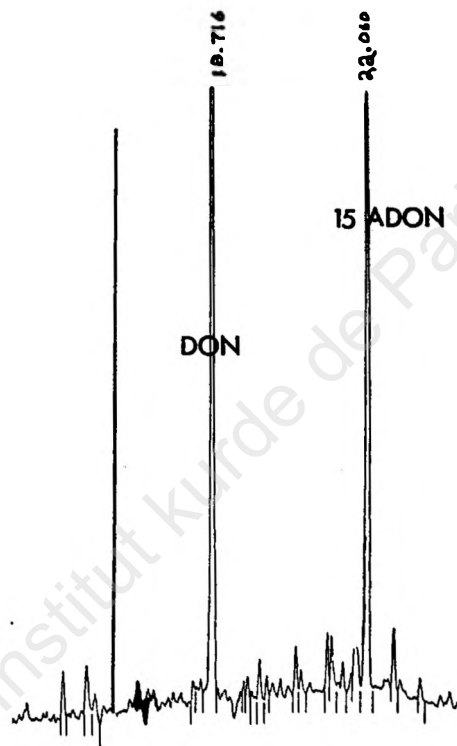
FIG. 4. — Unwashed pollen sample showing 6.0 ppm DON and 1.0 ppm 15 A-DON.
Isolate 180378.
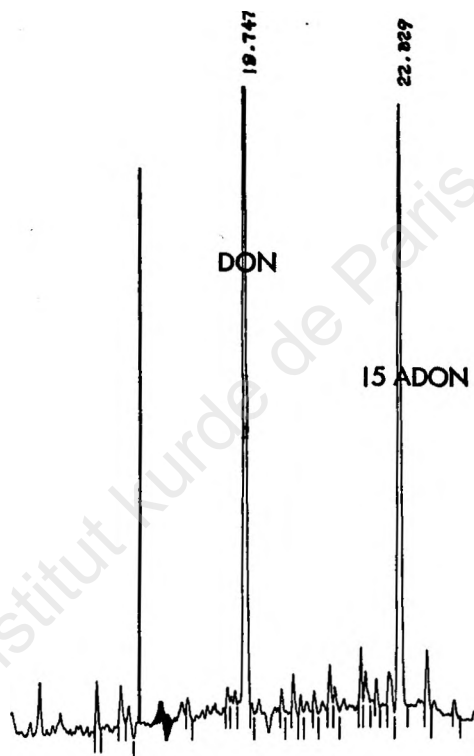
FIG. 5. — Rice control sample showing 14.9 ppm DON and 8.5 ppm 15 A-DON.
Isolate 180378.
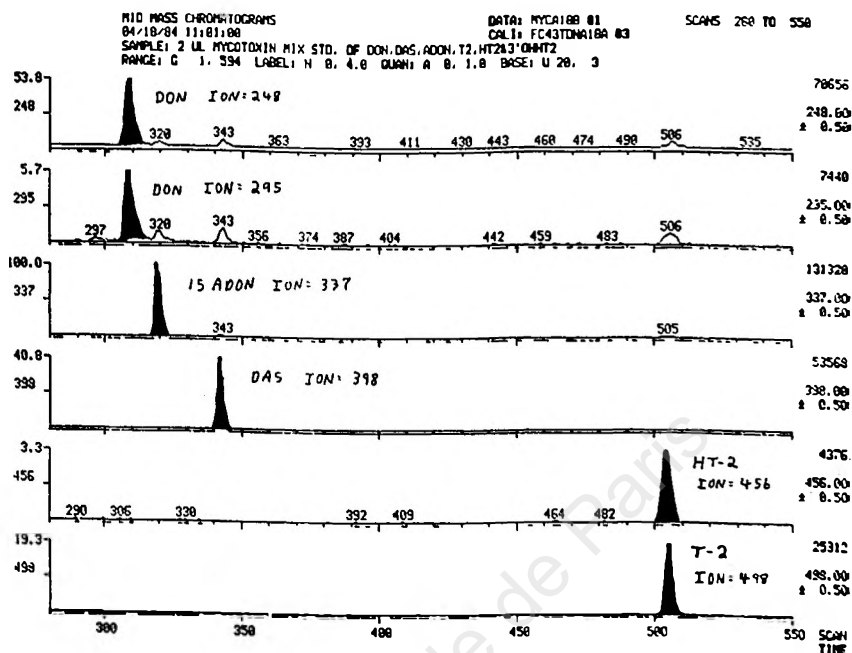
FIG. 6. — MID Mass Chromatograms showing ions for DON, 15 A-DON, DAS, HT-2 and T-2.
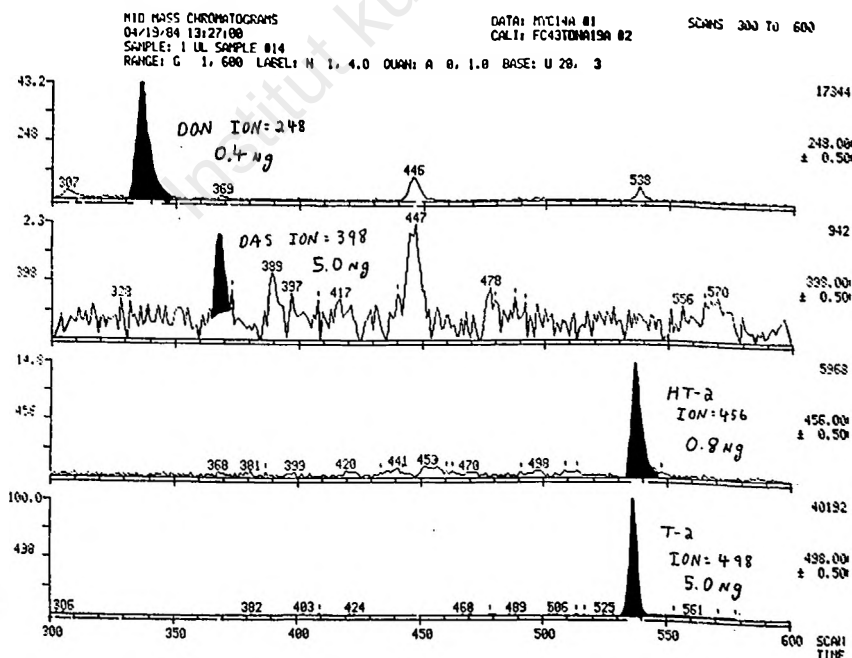
FIG. 7. — Unwashed pollen sample analyzed by GC/MS using MID Mass Chromatograms. The sample shows presence of DON, DAS, HT-2, and T-2 as indicated by their respective ions.

Analytical methodology, detection of trichothecenes from Southeast Asian samples and their residue in animal tissue

by C.J. MIROCHA, R.J. PAWLOSKY and K. CHATTERJEE

Department of Plant Pathology, University of Minnesota,
St. Paul, MN 55108, USA

SUMMARY.

T-2 toxin, diacetoxyscirpenol, deoxynivalenol, nivalenol and zea-ralenone were found in environmental samples obtained from alleged chemical attack areas in South East Asia. Moreover, T-2 and HT-2 were also found in blood and urine samples from victims. The method of extraction and cleanup varied depending on the substrate of analysis but XAD-2 or CHEM-ELUTE columns were used in the cleanup procedures. Resolution of the mixture was obtained on 30 meter 0.25 mm bonded phase DB5 capillary columns. T-2, DAS were resolved as their trifluoroacetate derivatives using trifluoroacetic acid anhydride as the reagent. DON, NIV and Zearalenone were resolved as trimethylsilyl ether derivatives. Detection and quantitation was done on a Hewlett-Packard 5987 combination GS/MS in electron impact or positive and negative chemical ionization modes. Quantitation was done with external standards.

Intoxication of cats, monkeys, rats or swine with either T-2, DAS, MAS or TAS resulted in toxic residue in tissues of the heart, kidney, and lung as well as detection of metabolites in blood and urine. The metabolism of T-2 toxin varies in different animals.

INTRODUCTION.

The trichothecenes are a group of natural products produced by various species of the genus *Fusarium* as well as species of

Trichothecium and Trichoderma. Those trichothecenes produced by *Fusarium* are among the most toxic of the many derivatives and are best represented by T-2 toxin [4,15-diacetoxy-8-(3-methyl-butyryloxy)-3-hydroxy-12,13-epoxy-trichothec-9 -ene].

The intentions of this presentation are threefold : (1°) describe some of the methodology used in analysis; (2°) present data from analyses of environmental and human samples from Southeast Asia and (3") present data showing analyses of tissue from animals dosed with T-2 toxin. The latter was done in an effort to understand the kinds of metabolites of T-2 toxin to expect in various tissues in an effort to corroborate the residue found in a victim of alleged biological-chemical warfare in Southeast Asia.

Some of the data found in this presentation has already been published (Mirocha *et al.*, 1983). Rosen and Rosen (1982) have also published on their findings of a yellow powder sample collected in an area of Laos subjected to chemical attack. The latter have also found polyethylene glycol in their sample and are reporting their findings in these proceedings.

ANALYSIS OF BLOOD SAMPLES.

Two methods were tested for the analysis of T-2 and its metabolites in the blood of a cow. The general description of the XAD-2 method is shown in figure 1 and the CLIN-ELUTE (also called CHEM-ELUTE) method is shown in figure 2.

The T-2 toxin extracted by this procedure was concentrated and analyzed as its trifluoroacetate derivative on a combination gas chromatograph/mass spectrometer (Hewlett Packard 5987) using a 30 meter DB-5 capillary (0.25 mm) column. The linearity of the methods was compared with an external standard of T-2 Toxin and is presented in figure 3. The Bovine blood was amended with 2.5, 12.5, and 25 and 50 nanograms of T-2 toxin and then extracted by the two methods i.e. the XAD-2 and CLIN-ELUTE methods. The quantities found were within 10 % of the external standard and both methods were found to be acceptable. However CLIN-ELUTE (CHEM-ELUTE) was found to be more linear than XAD-2 between the entire concentration range. The former method of extraction gives near perfect linearity between 2.5 and 25 ng when monitored at mass 563 with the mass spectrometer in the

Blood analysis by XAD-2 method

```
                          BLOOD SAMPLE
                                |
                                |    PRECIPITATE WITH ACETONE AND FILTER
                                |
RESIDUE                      FILTRATE
                                |
                                |    EVAPORATE
                                |
ACETONE DIST.            ACETONE CONCENTRATE

                                     DISSOLVE IN METHANOL (1 ML)

                                     ADD WATER (19 ML)

                                     CONCENTRATE TO 15 ML.

                                     LOAD ONTO XAD-2 COLUMN

                                     RINSE WITH WATER (50 ML)

                                     ELUTE WITH METHANOL, WATER (9:1)

WATER ELUATE            90% METHANOL ELUATE
```

CONCENTRATE FOR GC/MS ANALYSIS.
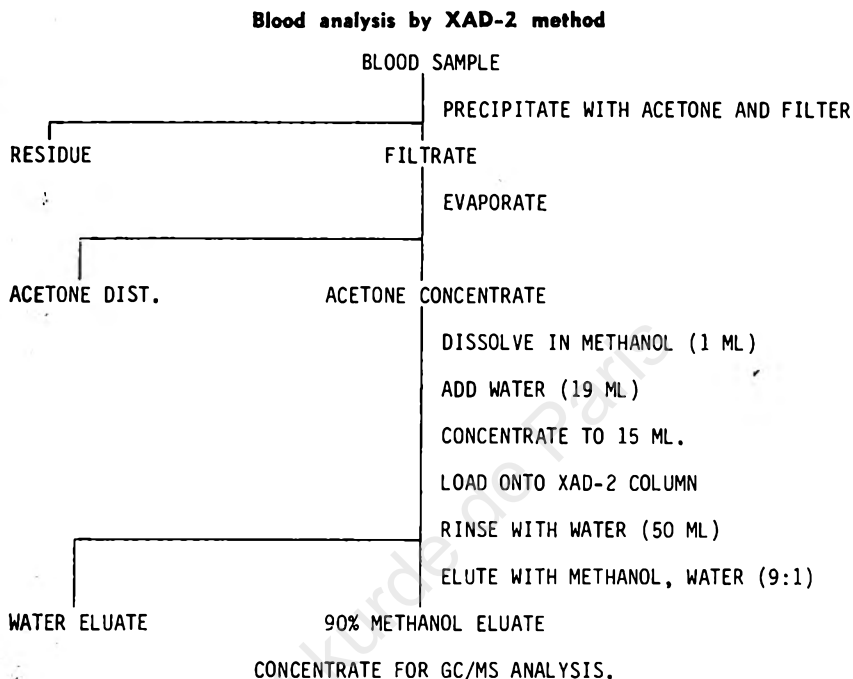
FIG. 1. — Method of analysis of T-2 toxin and its metabolites
in the blood of a cow using the XAD-2 column method.

Blood analysis clin-elute method

BLOOD SAMPLE (2 ML)

EXTRACT WITH MeOH : WATER
(30 : 70, V/V, 10 ML).
LOAD ONTO A CLIN-ELUTE
CARTRIDGE.
ELUTE WITH EtOAc (200 ML)

EtOAc ELUATE.

CONCENTRATE FOR GC/MS ANALYSIS
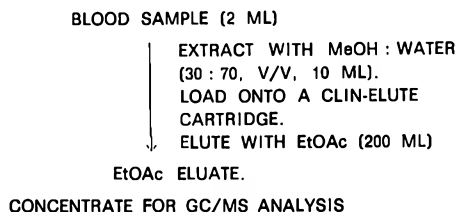
FIG. 2. — Method of analysis of T-2 toxin in bovine blood using the CLIN-ELUTE method.

chemical ionization (methane) mode. The T-2 standard **reference** line was obtained from values gained after simple **injection** of known quantities of T-2 toxin (TFA) directly into the GC/MS.
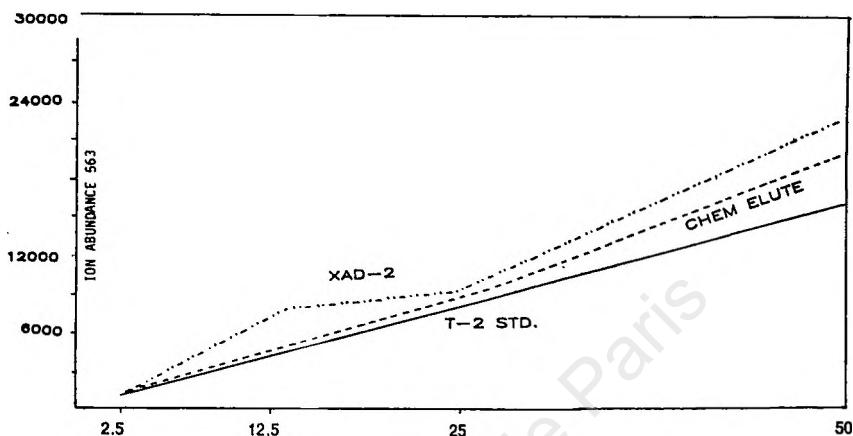
FIG. 3. — Comparison of the results obtained after amending bovine blood with 2.5, 12.5, 25 and 50 nanograms of T-2 toxin. The quantitation was done on the mass spectrometer using the abundance of the molecular ion (563) of TFA-T-2.

ANALYSIS OF SAMPLES FROM SOUTHEAST ASIA.

Tables I and II are representations of data already presented and documented in another publication (Mirocha *et al.*, 1983). The vegetation shown in Table I shows the presence of T-2 toxin, deoxynivalenol and nivalenol extracted from the surface of leaves collected in an attack area. The toxic components were simply extracted by dipping the leaves in ethyl acetate and concentrating to a minimum volume after which a suitable derivative was made for analysis by GC-MS. These toxins do not normally occur on leaves as the organism that produces them (*Fusarium roseum* « Graminearum ») does not colonize leaf surfaces of living plants. Moreover, these toxins were also found in water and on rock scrapings taken from attack areas. The yellow powder sample shown in Table II, also contains inordinate amounts of these toxins including the fungal sex hormone called zearalenone. These data lead me to conclude that the toxins were placed in the environment by the intervention of man and not through natural occurrence. Extracts made from similar leaf and water samples as well as samples of cereal grains found in the water samples as well as

TABLE I

Analysis of Fusarium toxins from samples originating in Southeast Asia

Sample	Fusarium toxins (ppm)			
	T-2	DON	NIV	DAS
Leaf # 1 (0.2 g)	3.17	59.0	109.0	—
Leaf # 2 (0.2 g)	35.7	0.0	21.7	—
Water (10 ml)	0.0	0.2	0.2	1.0 (ppb)
Tissue (33 mg) in water	0.0	66.0	0.0	0.29
Unidentified scraping from rock (< 1 mg) .	0.0	0.0	0.0	10.0

O = not detected ; (—) = not analyzed.
T-2 = T-2 toxin ; DON = deoxynivalenol ; NIV = nivalenol ; DAS = diacetoxyscirpenol.

TABLE II

Analysis of yellow and red powder samples gathered from chemical attack areas in Southeast Asia

Sample	Fusarium metabolites (ppm)						Pigments		
	T-2	HT-2	DON	NIV	DAS	ZEA	A	B	C
Yellow powder . . .	143	—	0	0	27	40	+	+	+
Fusarium roseum culture .							+	+	+
Pink powder . . .	0	0	—	—	0	0	—	—	—

O = negative ; (—) = not analyzed. 1/Yellow Fusarium pigments soluble in petroleum ether.
DON = deoxynivalenol ; DAS = diacetoxyscirpenol ; ZEA = zearalenone.

samples of cereal grains found in the environment adjacent but not in the attacked zone were negative for the above toxins.

Tables III trough VIII are compiled for purposes of illustrating the origin of diverse samples of blood obtained from alleged victims of biological-chemical warfare in Southeast Asia. The legends of the tables are well documented and I will not elaborate on them in the text. It is important to note the concentrations of T-2 and HT-2 in the blood and urine of these victims in spite of the fact that they were collected at various time intervals after exposure. The method of urine analysis was similar to that of the XAD-2 method briefly described earlier.

The presence of T-2 and HT-2 in the blood cannot be explained on consumption of food contaminated with Fusarium toxins. All samples were taken from victims who claimed to have been attacked; they do not represent those who died, except in one case, but only those who were able to walk out to refugee camps after exposure to chemicals. The presence of T-2 and other tri-

TABLE III

Bloc Her (8-year old boy), Tong Her (6-year old boy) and Xia Sue Xiong (young girl) were exposed in Laos to an agent described as yellow to reddish brown, late in March, 1982. Blood was drawn on 17 April, 1982. Bloc Her complained of bloody diarrhea and coughing of blood whereas Xia Sue Xiong complained of bloody diarrhea and abdominal pain

Results	T-2	HT-2 (ppb)
Bloc Her (Blood)	ND	ND
Tong Her (Blood)	110	296
X.S. Xiong (Blood)	46	ND

TABLE IV

Neng Xiong, a Hmong refugee in Thailand, suffered from chemical attack in Phou Bia higlands of Laos, on March 25, 1982. Her blood was sampled on 6 April, 1982. The victims of her village complained of vomiting, fever, headaches, swollen eyes and chest pain

Results	T-2	HT-2 (ppb)
Blood sample 1	101.0	8.2
Blood sample 2	32.9	34.0

TABLE V

Post mortem blood samples were obtained from a 25-year-old Hmong refugee admitted to Ban Vinai, Thailand hospital. He indicated exposure to chemical attack in Laos at some undetermined time. Blood was drawn just before death at which time the victim suffered a massive gastrointestinal hemorrhage

Results	T-2 Toxin	HT-2 Toxin (ppb)
	14.5	19.2

TABLE VI

Victims named Prek Reth and Pen Nom (Khmer Rouge Guerrilas) exposed on 13 February 1982 in Toul Chrey Kampuchea. Blood samples collected by western physicians 24 hours later in Phum Tmey camp where they sought medical assistance. They reported severe eye irritation, prolonged and repeated episodes of vomiting, respiratory problems, trembling and diarrhea

Results	T-2	HT-2 (ppb)
Prek Reth (whole blood)	18	22
Phen Nom (whole blood)	11	10
Phen Nom (urine)	Trace	18

chothecenes have not been demonstrated in their rice **rations**; trichothecenes have never been demonstrated to occur naturally in rice. Moreover, in order to find T-2 in their blood, based on the monkey as a model, they would have to consume at least 2 mg of T-2/kg of body weight, an amount that in a 68 kg man would be equal to 136 mg of T-2 toxin. Such an amount of T-2 toxin could only be found in a laboratory culture and not in food.

TABLE VII

A small aircraft sprayed a white powder near Pailin, Kampuchea on March 5, 1982. The next day 10 of a group of 15 people walked through the area and developed symptoms identical to those already described. A second artillery shell attack occurred on March 7, 1982. Blood and urine was drawn from 3 survivors on March 13, 1982

Results	Urine (ppb)	
	T-2	HT-2
Neung Hon	5.0	1.8
Chan Saran	4.0	1.3
Bun Thoem	22.0	7.4

The presence of T-2 and HT-2 in the whole blood and serum of victims a full two weeks after exposure is under investigation. Normally, laboratory data shows that the toxin should be metabolized within an hour if injected into the blood stream and will last at least 72 hrs, in some cases, if given to animals by intubation. However, recent reports indicate that T-2 toxin is metabolized into the glucuronic acid glycoside with a glycosidic linkage at C-3. Through recycling via the bile into the stomach, a sufficient quantity could conceivably be reintroduced into the blood stream. It is suggested that all future analyses incorporate hydrolysis of the glycoside with glucuronidase enzymes in order to obtain the free unbound T-2 toxin.

Table VIII shows the results of residue analysis of a victim who had died from complications due to toxin exposure. The concentrations of toxic residue found in the heart, lung, esophagus, kidney and large intestine were exceptionally high. In order to substantiate these findings, we dosed cats with T-2 toxin and analyzed the tissues to see if similar results could be obtained.

As shown in Table IX, the cats received 250 μg/kg of T-2 on the first day followed by 1.5 mg/kg on the second day. The animals were sacrificed 6 hours after the second dose. T-2, HT-2, TC-1, TC-3, and T-2 tetraol were present in heart, lung and kidney

TABLE VIII

One of the victims of the 13 February 1982 chemical attack on Tuol Chrey, Kampuchea, died on 16 March 1982. The victim had made a brief recovery on March 12 and 13 but then suffered a relapse with signs of fever and jaundice, became anuric, lapsed into coma and died. Shortly before death, the victim vomited blood. An autopsy and chemical analysis of the following tissues were made : heart, lung, esophagus, stomach, liver, kidney, and large intestine. The results are shown below a :

Sample No. FS731	Tissue	Amount g	Toxins		
			DAS	T-2	HT-2
A	Heart	7.9		—	1.2 ppm
B	Esophagus	13.5		25.1 ppb	4.02 ppm
C	Liver	9.5		—	—
D	Kidney	10.4	2.55 b/ ppm	6.8 ppb	—
E	Lung	4.5		8.5 ppb	—
F	Large Intestine	5.3		88 ppb	9.6 ppb

a) (—) = not detected. All analyses done by positive chemical ionization in methane.

b) DAS used as internal standard was overwhelmed by endogenous DAS. Victim was treated in Nong Pru Hospital.

TABLE IX

Residue analysis of the cat after T-2 intoxication. The animal received 200 ug/kg on the first day followed by 1.5 mg/kg on the next. The cat was sacrificed 6 hours after the second dose

Tissue or fluid	Toxin residues found in ppb				
	T-2	HT-2	TC-1	TC-3	T-2 Tet.
Heart	0.99	199.90	13.25	58.75	10.14
Lung	15.30	25.50	5.42	5.28	15.30
Kidney	34.07	138.58	34.07	55.88	57.57
Liver	—	2.92	—	1.00	9.56
Blood	—	0.46	—	0.82	44.50
Urine	—	0.21	—	0.34	160.88

whereas the liver, blood and urine contained HT-2, TC-3 and T-2 tetraol. Similar findings were found in the monkey and pig with T-2 intoxication and rat and cat with diacetoxyscirpenol. This substantiates are earlier observations of residue in the organs of man as based on animal models.

CONCLUSIONS AND DISCUSSION.

The data compiled in this presentation as well as that documented in other reports strongly supports the contention that biological-chemical agents had been used in Southeast Asia. Ana-

lyses of environmental samples and yellow powder collected in villages allegedly attacked contained trichothecenes that are not of natural origin. The residue found in blood, urine and body tissues of victims contained T-2 metabolites that could only be accounted for by exposure to trichothecenes other than by natural means.

A hypothesis has been advanced that suggests that perhaps trichothecenes could be accounted for by their presence in the feces of bees present in Southeast Asia. This suggestion is not seriously considered by us because of the following reasons : (1°) *Fusarium* cannot colonize bee feces to produce toxins because it cannot compete with other more aggressive fungi such as *Penicilium* and *Aspergillus* species ; (2°) the presence of trichothecenes has never been demonstrated in bee feces in Southeast Asia; (3°) the presence of trichothecenes in the blood, urine and tissues of victims cannot be explained on bee excrement as this does not comprise any part of their diet; (4°) eye witness descriptions of chemical attacks on villages followed by death or immobilization of the victims have never been correlated with bee flight patterns or excrement elimination rhythms. It is necessary to evaluate all of the data of an experiment in order to arrive at a sound scientific conclusion. It is not enough to suggest an alternative solution; serious consideration demands data and analyses that will support the hypothesis proposed.

The leaf samples analyzed and reported in this presentation did not contain yellow spots on them suggestive of bee excrement. They were ordinary leaves and petioles with no obvious signs on them except for slight fungal growth as one obtains with leaves which are placed in a chamber when moist and signs of dessication after they were dried. Leaves collected by others that contained discrete yellow spots never showed any signs of trichothecenes after chemical analyses. The latter is convincing evidence that bee feces really present no basis for consideration as the makers of « Yellow Rain ». Until such evidence is obtained, the hypothesis appears absurd.

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Diagnosis, Treatment, Influence

Effects of dietary trichothecenes on the immune system of mice

by H.B. SCHIEFER, S.C.E. FRIEND and M.K. WELCH

Toxicology Research Centre and Department of Veterinary Pathology,
Western College of Veterinary Medicine, University of Saskatchewan,
Saskatoon, Saskatchewan S7N OWO, Canada

SUMMARY.

Young male white Swiss mice were fed diets supplemented with T-2 toxin at levels of 5, 10 or 20 ppm, control diet ad libitum *or control diet at a restricted rate, for 1 to 6 weeks.*

The effect of the toxin on the immune system of these mice was assessed by counting total spleen cell numbers, and by assessing the in vitro *proliferative response of spleen cells to the polyclonal mitogens, Concanavalin A (Con-A) and lipopolysaccharide (LPS). After 3 weeks of feeding, only the spleen cell counts of mice fed 20 ppm T-2 were significantly lower. Consumption of 20 ppm T-2 for 1-4 weeks depressed spleen proliferative responses to the T-cell mitogen Con-A ; the response to LPS, a B-cell mitogen, was depressed in mice fed 10 and 20 ppm T-2 and in mice fed at a restricted rate.*

Other mice were infected with Herpes Simplex virus Type I (HSV-1). Mice on 20 ppm T-2 were highly susceptible to HSV-1 infection, and there was little or no inflammatory response in affected tissues. The immunosuppressive effects were greater than those caused by cyclophosphamide.

When latency of HSV-1 was established, feeding of T-2 for 3-6 weeks did not reactivate the virus, whereas liquid nitrogen and cyclophosphamide did reactivate the virus.

A chronic bacterial infection, with Corynebacterium kutscheri *was established in other mice. Feeding of 10 or 20 ppm T-2 or gavage with one single (sublethal) dose of T-2 toxin did not exacerbate the chronic bacterial infection, whereas corticosteroid treatment did so.*

The results demonstrate that although dietary T-2 toxin can cause immunosuppression, the response is not sufficient to re-activate or exacerbate latent/chronic infections.

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INTRODUCTION.

Although the effects of T-2 toxin on the lymphoid tissues, as gauged by morphological and hematological investigations, have been relatively well studied at least in the mouse, only a few workers have tried to assess the effects of this toxin on lymphocyte functions either *in vitro* of *in vivo*. Considering the importance of immunosuppression, with concomitant increased susceptibility to infectious agents and reactivation of latent infection, a series of experiments were conducted.

MATERIALS AND METHODS.

Mice were fed semisynthetic diets which contained 0, 5, 10 and 20 ppm of T-2 toxin for 1 to 6 weeks. The effect of the toxin on the immune system was assessed by mitogen assays (Con-A and LPS were used as mitogens), by infecting mice with Herpes Simplex virus Type 1 (HSV-1) and studying the lesions histologically, by challenging a latent HSV-1 infection with a T-2 toxin containing diet, and by challenging a latent *Corynebacterium kutscheri* infection.

RESULTS.

Consumption of 20 ppm T-2 for 1-4 weeks depressed spleen proliferative responses to the T-cell mitogen Con-A ; the response to LPS, a B-cell mitogen was depressed in mice fed 10 and 20 ppm T-2 and in mice fed at a restricted rate.

Mice on 20 ppm T-2 were highly susceptible to HSV-1 infection, and there was little or no inflammatory response in affected tissues. The immunosuppressive effects were greater than those caused by cyclophosphamide.

When latency of HSV-1 was established, feeding of T-2 for 3-6 weeks did not reactivate the virus.

A chronic bacterial infection with *C. kutscheri* was not exacerbated by feeding 10 or 20 ppm T-2 toxin or by one single (sublethal) dose of T-2 toxin, whereas corticosteroid treatment did so.

The results demonstrate that although dietary T-2 toxin causes some immunosuppression, the response is not sufficient to reactivate or exacerbate latent and chronic infections.

REFERENCES.

The work reported has been published or is in the process of being published :

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Systemic effects of topical application of trichothecenes in rodents

by H.B. SCHIEFER

Toxicology Research Centre, University of Saskatchewan,
Saskatoon, Saskatchewan S7N OWO, Canada

SUMMARY.

The skin irritation potential of trichothecene mycotoxins is well known and has been employed as a bioassay, but little attention has been paid to the systemic effects after topical application. In order to determine possible systemic effects, various trichothecenes, dissolved in DMSO, were applied to the skin of mice and rats and animals were observed for 14 days or were killed after 6, 12, 18 and 24 hours for histologic examination of tissues.

Mice receiving 20, 30 or 40 mg/kg BW of T-2 toxin died within 4-6 days while 5 and 10 mg/kg reduced the total mortality but did not prolong the time to death appreciably. With T-2 toxin being the most potent, other trichothecenes could be ranked in descending order as Verrucarin A, HT-2, DAS, Roridin A and Vomitoxin, the latter producing no effect at 40 mg/kg. Those animals surviving to 14 days developed skin lesions to varying degrees. The sequential study of internal organs showed that mitotic activity in the duodenum had ceased after 6 hrs, with subsequent appearance of necrotic debris in crypts, and by 24 hrs more or less complete necrosis of thymus and spleen was found.

Rats could only tolerate doses of 1 to 2 mg/kg of T-2 toxin, and developed, at a dose level of up to 5 mg, severe CNS, cardiovascular and enteric disturbances.

Mixtures of various trichothecenes were determined to be more potent than singular trichothecenes.

With the exception of the characteristic lesions observed histologically, in the sequential killing study (6-24 hrs), none of the postmortem finding or skin lesions were specifically indicative of trichothecene toxicity. Thus, cutaneous lesions or death after topical application of trichothecenes may not be recognized

as being caused by these toxins on the basis of clinical or post-mortem examination. On the contrary, lesions might be interpreted as being caused by other agents or as being the result of autolysis.

The most commonly occurring and observed intoxications due to trichothecenes are associated with ingestion of these toxins. This study shows that trichothecene intoxication associated with topical, and probably also inhalation application routes may not be diagnosed as such, unless physicochemical analysis of body fluids or tissues would be carried out in cases of suspected exposure.

INTRODUCTION.

The skin irritation potential of trichothecene mycotoxins is well known and has been employed as a bioassay, but little attention has been paid to the systemic effects after topical application. In order to determine possible systemic effects, various trichothecenes were applied to the skin of mice and rats.

MATERIALS AND METHODS.

Trichothecenes were dissolved in DMSO and delivered topically at a rate of 1, 2, 5, 10, 20, 30 and 40 mg/kg BW to groups of mice and rats. A patch of hair was shaved from the backs of the animals and the toxins were applied at a rate of 1 μl/g BW. Animals were observed for up to 14 days, and deaths were recorded daily ; other animals were killed after 6, 12, 18 and 24 hours for histological examination of thymus, spleen and duodenum.

RESULTS.

Within 6 hrs after application of the toxin, the animals had piloerection and huddled in a corner. Their condition progressively declined with time, and this change increased in severity and rate as the dose received increased. Mice receiving 20, 30 or 40 mg/kg BW of T-2 toxin died within 4-6 days, while levels of 5 and 10 mg/kg reduced the total mortality but did not prolong the time to death appreciably. T-2 toxin was determined to be the most potent singular trichothecene. Other trichothecenes could be ranked in descending order, i.e., DAS, HT-2, Verrucarin, Rori-

din A and Vomitoxin, the latter producing no deaths, even at 40 mg/kg.

Animals surviving up to 14 days developed skin lesions of varying intensity.

The study of internal organs showed that mitotic activity in the duodenum had ceased after 6 hrs, with subsequent appearance of necrotic debris in crypts. By 24 hrs, a more or less complete necrosis of thymus and spleen was observed. The various trichothecenes showed slightly different patterns of activity, e.g., while the duodenal mucosa appeared to be normal again 24 hrs after T-2 toxin application, other trichothecenes, such as DAS, produced longer lasting effects.

A mixture of T-2, DAS and DON (1:1:1), applied in concentrations of 5, 10, 20, 30 and 40 mg/kg BW, proved to be more potent than T-2 toxin alone and killed mice within the first 24-48 hrs.

Rats could only tolerate doses of 1 to 2 mg/kg of T-2 toxin, and developed, at a dose level of up to 5 mg, severe CNS, cardiovascular and enteric disturbances, followed by death, within 24 hrs.

DISCUSSION.

The experiments showed that topical application of trichothecenes leads to systemic effects and death, and that a mixture of 3 different trichothecenes is more toxic than individual trichothecenes.

With the exception of the characteristic lesions observed histologically, in the sequential killing study (6-24 hrs), none of the postmortem findings or the skin lesions were specifically indicative of trichothecene toxicity. Thus, cutaneous lesions or death after topical application of trichothecenes may not be recognized as being caused by these toxins on the basis of clinical or postmortem examination. On the contrary, lesions might be interpreted as being caused by other agents or as being the result of autolysis. In such cases, an etiological diagnosis cannot be established unless physicochemical analysis of body fluids or tissues would be carried out.

Note. — The results reported here are in the process of being published elsewhere, e.g.: H.B. Schiefer and D.S. Hancock. Systemic effects of topical application of T-2 toxin in mice. *Toxicol. Appli. Pharmacol.* (1984) and in further papers to be submitted for publication.

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Etude de la tcxicité aiguë de l'Eserine, VX et le Paraoxon, pour établir un modèle mathématique de l'extrapolation à l'Etre humain

by C. LEBLIC, H.M. COQ and G. LE MOAN

Department of Toxicology. CEB. Vert-le-Petit, France

SUMMARY.

The acute toxicities of Eserine, VX and Paraoxon were measured in multiple animal species, in order to extrapolate lower species data to man. It seems that the type of function that was studied ($DL_{50} = A \cdot P^{-K}$) cannot be applied to the three compounds and generalized to all the cholinesterase inhibitors. Nevertheless, it allows with reservations an interesting evaluation of tne human toxicity.

INTRODUCTION.

Le but de cette étude a été d'aborder le problème de la toxicité aiguë par des composés inhibiteurs des cholinestérases chez l'homme, où ce type de détermination toxicologique n'est en aucun cas envisageable. Certes des cas d'intoxications aiguës par des pesticides sont rapportés par la littérature mais la disparité des cas, le manque de connaissance des doses et des conditions exactes de l'intoxication ne permettent pas une exploitation toxicologique correcte. La description des symptômes, les délais de mortalité sont toutefois des compléments d'information intéressants.

Une étude rigoureuse sur un grand nombre d'espèces animales de poids croissant menée dans des conditions le plus homogène possible peut permettre d'utiliser les résultats obtenus dans une tentative d'extrapolation à l'homme.

Différentes méthodes d'extrapolation ont été proposées, celle de D.M. Maxwell et D.E. Lenz a été retenue. Les auteurs étudiaient les moyens de protection de l'homme contre les agents organophosphorés utilisés comme toxiques de guerre en recherchant une relation simple d'extrapolation humaine à partir de données DL$_{50}$ d'origine bibliographique. Ils en ont tiré une relation mathématique simple :

$$DL_{50} = A \cdot P^{-K}$$

où A est une constante quelle que soit l'espèce qui varie avec la voie d'administration

où K est une constante quelle que soit la voie d'administration.

MODE OPERATOIRE.

La détermination de la toxicité aiguë (DL$_{50}$) de trois produits inhibiteurs des cholinestérases (Eserine, VX, Paraoxon) a été réalisée sur six espèces animales (femelle) de poids croissant et selon deux voies d'injection, sous-cutanée SC et intrapéritonéale IP :

— la souris (Swiss souche CEB) : poids moyen 20 g,
— le hamster (Syrien doré) : poids moyen 70 g,
— le rat (wistar souche CEB) : poids moyen 200 g,
— le cobaye (Commun) : poids moyen 350 g,
— le lapin (Néozélandais albinos) de trois tailles :
  — Petit ou P : poids moyen 1.500 g.
  — Moyen ou M : poids moyen 2.500 g.
  — Gros ou G : poids moyen 3.500 g.
— le chien (Beagle souche CEB) : poids moyen 10 kg.

METHODE DE CALCUL.

A. *Détermination des valeurs DL$_{50}$ (dose létale 50 %).*

La méthode utilisée est la méthode simplifiée d'évaluation d'expérience dose-effet de Lichtfield et Wilcoxon, 1949. Nous avons choisi cette méthode pour différentes raisons :

— la méthode donne non seulement la dose létale 50 et la pente de la droite, mais aussi leurs limites de confiance ;

— la méthode utilise les résultats dans leurs propres **unités** ;

— le 0 et 100 % sont utilisés efficacement ;

— la méthode reconnaît l'hétérogénéité, quand elle existe, et donne les limites de confiance corrigées dans un tel cas ;

— la méthode permet la comparaison de deux droites par leur parallélisme et le calcul de la puissance relative avec ses limites de confiance.

Nous avons fait réaliser un programme sur Hewlett Packard 98-30 A qui permet d'utiliser la méthode de Lichtfield et Wilcoxon d'une façon automatique et plus efficace parce que plus rapide et plus précise. Le principe de la méthode reste le même, la démarche suivie n'est pas modifiée, toutefois, sont entrées dans le programme les valeurs qui ont servi à l'établissement des abaques et non les abaques elles-mêmes.

On introduit pour le calcul les unités dans lesquelles les doses sont exprimées, le nombre de doses utiles (il ne faut pas considérer plus d'une dose de 0 et 100 %) et, pour chaque dose, le nombre d'animaux utilisés et le nombre d'animaux réagissant. Le tracé de la courbe dose-effet se fait sur papier log-probit ; l'appareil pointe les valeurs expérimentales sauf le 0 et le 100 % qui sont soumis à correction en fonction de leur valeur attendue. L'appareil effectue les calculs, puis imprime les résultats en même temps qu'il trace la meilleure droite qu'on puisse faire passer par les points expérimentaux. La méthode nous donne la DL 50, fDL 50 (coefficient qui permet de calculer les limites de l'intervalle de confiance) et les limites de confiance à 95 %. Elle nous donne aussi la pente S, fS (coefficient permettant de calculer les limites de l'intervalle de confiance) et les limites de confiance à 95 %.

B. *Tracé des courbes DL_{50} en fonction du poids corporel.*

Ce type de tracé permet de calculer les valeurs A et K de la relation proposée par Maxwell et Lenz : $DL_{50} = A \cdot P^{-K}$. L'expression est de la forme $y = a x^n$, sa transformation en échelle logarithmique donne $\log y = \log a + (n) \log x$ où n (K) est la pente de la droite tracée en échelle log-log, une droite décroissante indiquant une pente négative. Pour connaître la valeur a (A), un point quelconque M appartenant à la droite est choisi dont les coordonnées permettent le calcul des logarithmes respectifs ; M (x,y) → (log x, log y) avec $\log a = \log y - (n)$

log x (si n négatif, log a = log y + n log x). A partir du log de a obtenu, on obtient la valeur a que l'on reporte dans l'expression mathématique.

Les résultats sont portés sur papier log-log. Les DL_{50} en mg/kg ou en γ/kg avec leurs limites de confiance à 95 % en abscisse, le poids corporel en g en ordonnée.

Un programme sur Hewlett Packard 98 — 30 A a été réalisé pour permettre un tracé optimum. On introduit pour le tracé et pour chaque espèce, le poids moyen des animaux utilisés pour la DL_{50}, la valeur DL_{50} correspondante et la valeur fDL_{50} qui permet le tracé de l'écart de confiance à 95 %. Les calculs effectués, l'appareil trace la meilleure droite compte tenu des différents points expérimentaux, donne l'expression $DL_{50} = A.P^{(K)}$ correspondante et effectue un test supplémentaire d'indépendance de la valeur DL_{50} avec le poids corporel.

— Dans le cas d'un test positif (l'hypothèse d'indépendance n'est pas à rejeter), la valeur DL_{50} semble indépendante du poids corporel, le tracé est une droite qui tend vers l'horizontale (K tend vers 0). Une valeur DL_{50} égale à A semble être valable quelle que soit l'espèce.

— Dans le cas d'un test négatif (l'hypothèse d'indépendance est à rejeter), la valeur de DL_{50} varie avec le poids.

Le signe de K indique la tendance d'évolution de la toxicité avec le poids : K positif correspond à une diminution de toxicité. K négatif correspond à une augmentation de toxicité.

C. *Comparaison des voies d'injections pour un même produit.*

Un test réalisé aussi sur un programme Hewlett Packard a pour but de vérifier si des parallèles peuvent être tracées à partir des valeurs brutes DL_{50} en fonction du poids, respectivement pour chaque voie ; dans le cas positif, les deux voies sont comparables. Une différence significative se traduit par deux parallèles distinctes. La puissance relative de l'une par rapport à l'autre est significative et égale au rapport des constantes A_1 et A_2. Une différence non significative se traduit par des parallèles très proches, la puissance relative n'est pas significative.

Ce test est intéressant car il permet de voir si dans le cadre d'une extrapolation humaine la valeur DL_{50} présumée est fortement influencée ou non par la voie d'administration.

INTERPRETATION DES TRACES DL$_{50}$ EN FONCTION DU POIDS CORPOREL.

I. ESERINE

A. *Examen des tracés.*

Pour les deux voies, la répartition des points est homogène de part et d'autre du tracé, avec des pentes positives qui indiquent une diminution de la toxicité avec le poids (fig. 1 et 2). Les équations respectives sont :

— DL$_{50}$ $_{SC}$ = 0,645 . P $^{+\ 0,211}$ avec DL$_{50}$ 60 kg = 6,56 mg/kg (3,07 — 14,0).

— DL$_{50}$ $_{IP}$ = 0,372 . P $^{+\ 0,269}$ avec DL$_{50}$ 60 kg = 7,21 mg/kg (2,25 — 23,0).

Dans les deux cas, le test d'indépendance est négatif ; la valeur DL$_{50}$ varie avec le poids corporel. Le test de parallélisme entre les voies ne montre pas de différence significative de toxicité dans le cadre d'une extrapolation.

B. *Comparaison des valeurs lapins.*

Voie SC :

— Lapin P 1.620 g (environ 2,5 mois) 4,29 mg/kg (3,79 — 4,85).
— Lapin M 2.150 g (environ 3,5 mois) 3,38 mg/kg (3,12 — 3,66).
— Lapin G 3.600 g (environ 6 mois) 3,15 mg/kg (2,77 — 3,58).

L'évolution de la toxicité par rapport au poids semble inversée par rapport au tracé général. L'examen des valeurs et de leurs intervalles de confiance montre qu'une différence existe entre le lapin P et les lapins M et G. Cette variation est faible et semble ne pas être significative sur le plan métabolique puisque le lapin P et le lapin M ont très peu d'écart d'âge.

Il semble donc que pour l'espèce lapin la DL$_{50}$ soit peu influencée par l'âge et le poids, et qu'une valeur moyenne voisine de 3,6 mg/kg peut être retenue pour la zone de poids étudiée.

Voie IP :

— Lapin P 1.660 g (environ 2,5 mois) 1,91 mg/kg (1,49 — 2,46).
— Lapin M 2.425 g (3,5 à 4 mois) 2,47 mg/kg (1,92 — 3,17).
— Lapin G 3.700 g (environ 6,5 mois) 6,47 mg/kg (5,11 — 8,19).

L'évolution de la toxicité est la même que celle observée sur le tracé général. La variation de toxicité est peu significative entre
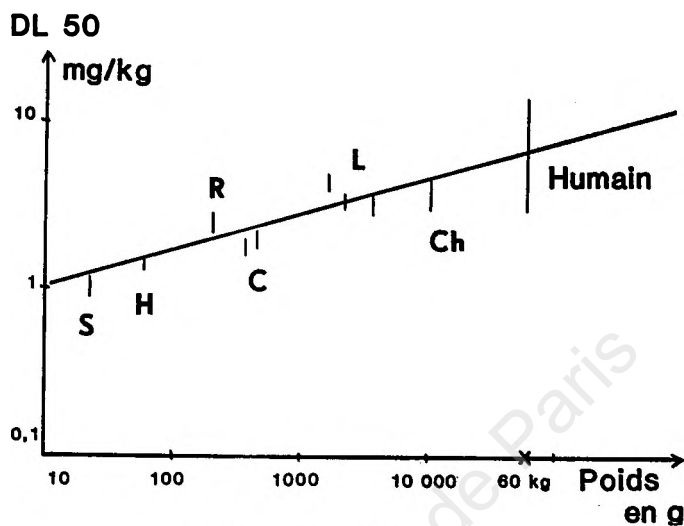
FIG. 1. — Eserine voie SC.
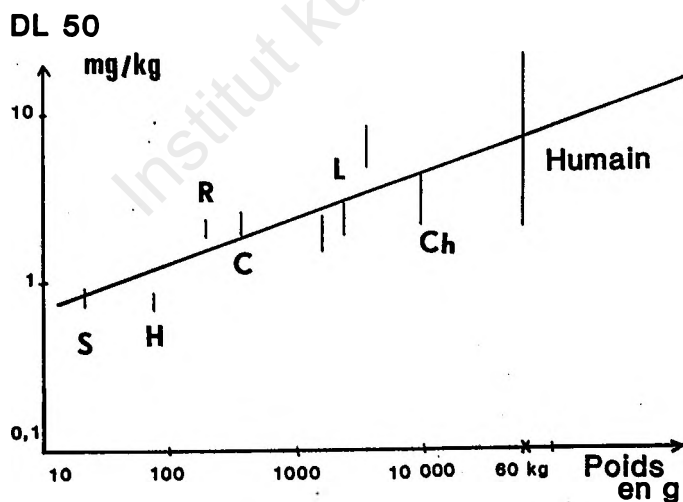
FIG. 2. — Eserine voie IP.

les lapins P et M. Une différence nette s'observe chez le lapin G, beaucoup moins sensible que les lapins P et M et que le chien ($DL_{50} = 3,05$ mg/kg).

Il semble que le phénomène de détoxification hépatique soit en rapport avec l'âge, mais il ne doit pas représenter la voie métabolique principale car cette variation n'a pas été retrouvée en voie SC, où la DL_{50} varie peu avec l'âge et le poids.

C. *Comparaison des voies d'injection.*

<div style="text-align:center">

Voie SC   *Voie IP*

$DL_{50} = 0,645 . P^{+0,211}$ $DL_{50} = 0,372 . P^{+0,269}$

Le test de parallélisme est positif

$DL_{50} = 0,570 . P^{+0,231}$ $DL_{50} = 0,463 . P^{+0,231}$

</div>

La différence de toxicité entre les voies n'est pas significative. Dans le cadre d'une extrapolation humaine les toxicités SC et IP sont du même ordre $DL_{50\ SC}$ 60 kg = 6,56 mg/kg (3,07 — 14,0), $DL_{50\ IP}$ 60 kg = 7,21 mg/kg (2,25 — 23,0).

D. *Conclusion.*

— K est positif quelle que soit la voie d'administration et ne varie pratiquement pas avec la voie ($K_{SC} = + 0,211$, $K_{IP} = + 0,269$).

— A varie mais peu avec la voie d'injection ($A_{SC} = 0,645$, $A_{IP} = 0,372$).

<div style="text-align:center">

II. **V.X.**

</div>

A. *Examen des tracés.*

Les tracés en fonction de la voie d'injection sont assez différents, c'est pourquoi ils seront étudiés séparément.

Pour la voie sous-cutanée, la répartition des points de part et d'autre du tracé est homogène et la pente négative indique une augmentation de la toxicité avec le poids corporel. Le test d'indépendance de la toxicité avec le poids, négatif, confirme cette variation. On note cependant une pente faible (fig. 3). L'équation obtenue est :

— $DL_{50} = 26,82 . P^{-0,083}$ avec DL_{50} 60 kg = 10,75 γ/kg (5,65 — 20,44).

Pour l'espèce lapin, on retrouve l'augmentation de la toxicité avec le poids. L'examen plus précis des valeurs et des intervalles de confiance à 95 % montre que la variation de toxicité non significative entre le lapin P et M, le devient entre lapin (P et M) et G.

— Lapin P 1.650 g (environ 2,5 mois) 18,55 γ/kg (16,9 — 19,9).
— Lapin M 2.310 g (3,5 mois) 17,35 γ/kg (15,7 — 19,1).
— Lapin G 3.655 g (6,5 mois) 14,06 γ/kg (13,1 — 15,1).
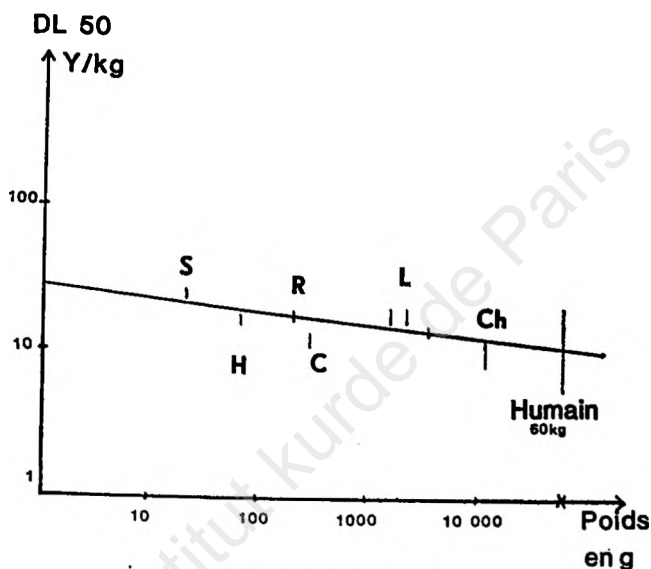
FIG. 3. — V.X. voie SC.

Il semble possible qu'un phénomène particulier, attribuable à l'âge, intervienne pour le lapin G. Peut-être existe-t-il chez celui-ci une baisse de la fonction détoxifiante du foie et/ou un phénomène de toxification périphérique lié ou non à une élimination plus lente par stockage dans les graisses ou déficience rénale.

Pour la voie intrapéritonéale, l'examen du tracé général montre une répartition assez homogène de la plupart des points, le point chien s'en éloignant assez nettement. Avec ce type de tracé le test d'indépendance de la toxicité avec le poids est positif, une valeur voisine de 47,6 γ/kg est envisageable quelle que soit l'espèce et quel que soit le poids (fig. 4). L'équation générale est :

— $DL_{50} = 47,60 \cdot P^{+0,022}$ (K tend vers 0).
La valeur extrapolée à l'humain n'est pas très précise, DL_{50} 60 kg = 60,6 γ/kg (19,2 — 191,2).
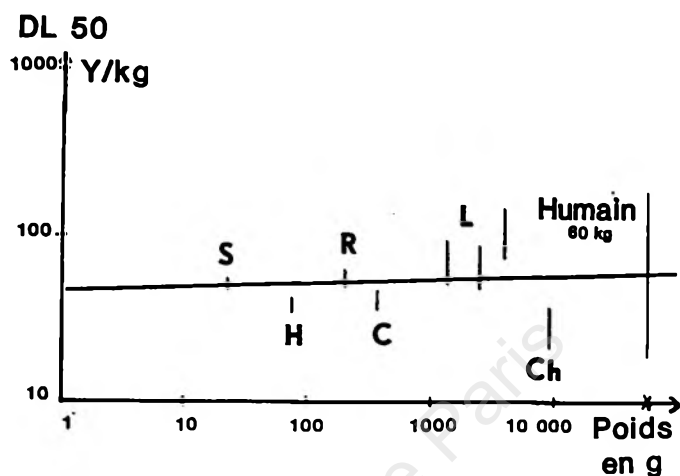
DL 50

FIG. 4. — V.X. voie IP — tracé général.
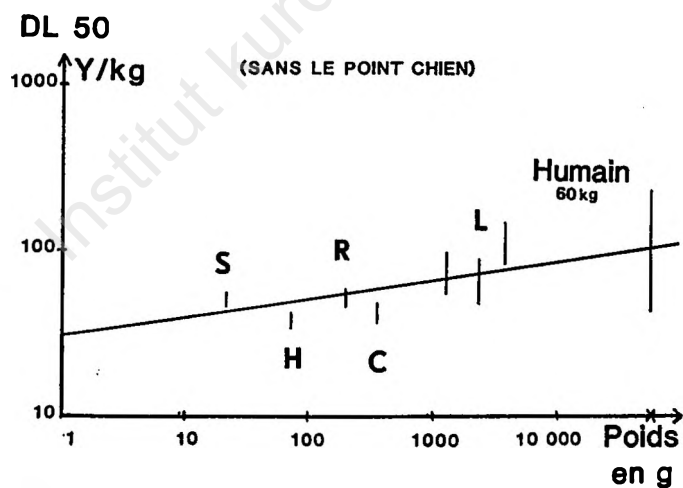
DL 50

FIG. 5. — V.X. voie IP — sans le point chien.

Un essai de tracé excluant le point chien montre une meilleure répartition des points. Le test d'indépendance devient négatif, rejetant l'hypothèse d'une valeur DL_{50} commune (fig. 5). L'équation est la suivante :

— $DL_{50} — 31,63 . P^{0,109}$ avec DL_{50} 60 kg = 105 γ/kg (47,4 — 232).

Ce second tracé semble plus satisfaisant mais ne présente guère d'intérêt dans le cadre d'une extrapolation humaine, car il va dans le sens d'une optimisation dangereuse de la toxicité. Il est cependant intéressant de noter la très grande sensibilité du chien et une investigation particulière de son métabolisme est envisageable. Le test de parallélisme réalisé entre les deux tracés, avec et sans point chien, ne donne pas de différence significative.

Pour l'espèce lapin, on n'observe pas de différence significative entre les lapins P et M mais une différence nette entre les lapins M et G, le lapin G semblant moins sensible que les lapins P et M. Au contraire de la voie SC la toxicité a tendance à diminuer dans l'espèce lapin avec le poids et l'âge.

— Lapin P 1.350 g (environ 2,5 mois) 73,55 g γ/kg (56,9 — 95,1).
— Lapin M 2.440 g (3,5 à 4 mois) 66,16 g γ/kg (48,6 — 90,1).
— Lapin G 3.870 g (7,5 mois) 107,3 g γ/kg (76,8 — 150).

Il semble donc que le phénomène de détoxification hépatique soit plus intense chez le lapin G et donc que seules les hypothèses attachées à des phénomènes périphériques peuvent expliquer la variation de toxicité SC en sens inverse du lapin G par rapport aux lapins P et M. Toutefois, dans l'hypothèse d'une indépendance de la toxicité avec le poids, une valeur moyenne de la DL_{50} peut être dégagée chez le lapin pour la zone de poids étudiée, qui serait comprise entre 76,8 et 90,1 γ/kg (zone de superposition des écarts de confiance).

B. *Comparaison des voies d'injection.*

Voie SC

$$DL_{50} = 26,82 . P^{-0,083}$$

Voie IP

$$DL_{50} = 47,60 . P^{+0,022}$$

le test de parallélisme entre les deux voies est positif

$$DL_{50} = 22,64 . P^{-0,056}$$

$$DL_{50} = 75,75 . P^{-0,056}$$

La puissance relative du groupe 1 (SC) par rapport au groupe 2 (IP) est égale à 0,3, la différence de toxicité en fonction de la

voie est significative, la voie SC étant plus toxique que la voie IP (facteur 3), traduisant l'importance du phénomène détoxifiant hépatique.

C. *Conclusion par rapport à l'équation de Maxwell et Lenz.*

— K est négatif pour la voie SC (K = — 0,083), positif pour la voie IP (K = + 0,022). Il varie peu avec la voie d'administration ; dans les deux cas la valeur K tend vers 0 (la pente tend vers l'horizontale). La valeur commune donnée par le test de parallélisme est : (K = — 0,056).

— A varie avec la voie d'injection (A_{SC} = 26,82, A_{IP} = 47,60).

Dans le cadre d'une extrapolation humaine la différence de toxicité entre les voies est significative et de l'ordre de 1 à 3, avec une supériorité de toxicité de la voie SC.

A titre indicatif, les équations données par Maxwell et Lenz pour le VX sont les suivantes :

— Voie SC DL_{50} = 22,0 . $P^{-0,04}$.
— Voie IM DL_{50} = 16,6 . $P^{-0,08}$.
— Voie IV DL_{50} = 8,30 . $P^{-0,02}$.

III. PARAOXON

A. *Voie SC.*

Le tracé d'une droite moyenne dans ces coordonnées log-log montre une tendance légère à la diminution de toxicité avec le poids. La répartition, bien qu'homogène des points, montre qu'aucun n'appartient vraiment à la droite (fig. 6).

Deux groupes se distinguent avec pour le groupe 1 « G_1 » : souris, cobaye, chien, situé au-dessus de la droite et pour le groupe 2 « G_2 » : hamster, rat, lapins, situé au-dessous de la droite. L'imprécision sur le tracé est visualisée par un grand écart de confiance sur la valeur extrapolée humaine.

L'équation générale est : DL_{50} = 0,380 . $P^{+0,079}$ avec DL_{50} 60 kg = 0,903 mg/kg (0,16 — 5,17), l'hypothèse d'indépendance de la toxicité avec le poids est suggérée.

L'examen de ces valeurs « lapin » montre que la DL_{50} ne varie pratiquement pas avec le poids et l'âge. Une valeur commune, voisine de 0,46 mg/kg, peut être retenue pour la zone de poids étudiée. On retrouve dans l'espèce lapin, la tendance exprimée

DL 50

FIG. 6. — Paraoxon voie SC — tracé général.

DL 50
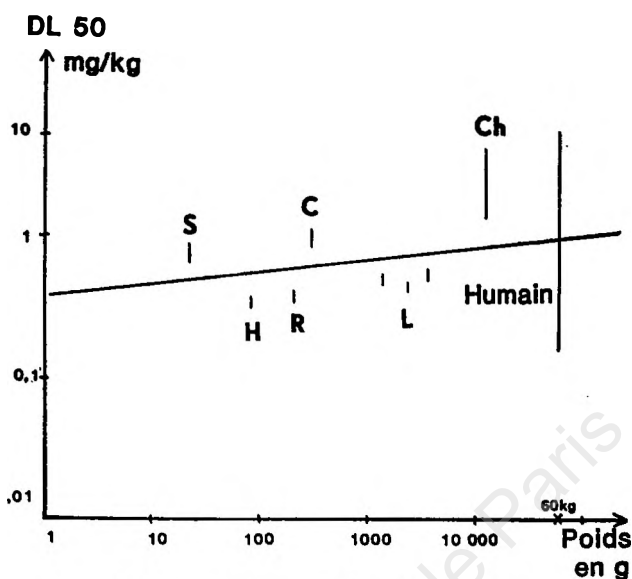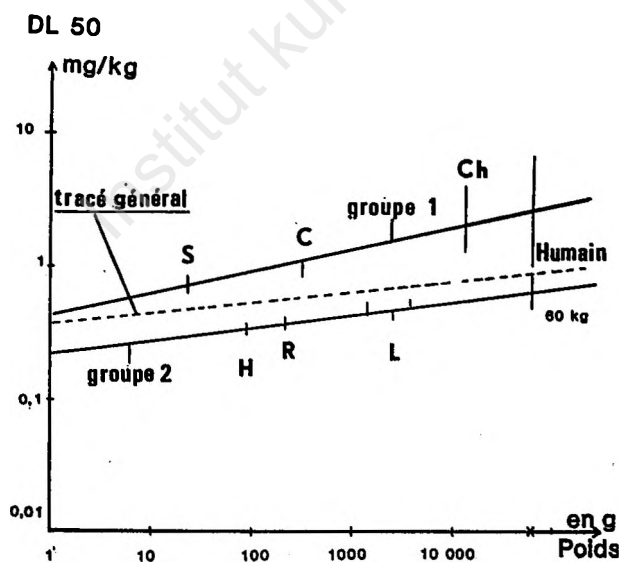
FIG. 7. — Paraoxon voie SC — tracé selon deux groupes G_1 et G_2.

sur le tracé général, d'une possibilité d'indépendance de la toxicité avec le poids corporel.

— Lapin P 1.375 g (2 à 2,5 mois) 0,49 mg/kg (0,46 — 0,56).
— Lapin M 2.375 g (3,5 à 4 mois) 0,435 mg/kg (0,41 — 0,46).
— Lapin G 3.600 g (environ 6 mois) 0,52 mg/kg (0,48 — 0,56).

Un tracé selon deux groupes d'animaux donnent les équations suivantes avec (fig. 7) :

— pour le groupe 1 (souris, cobaye, chien) : $DL_{50} = 0,443 \cdot P^{+ 0,163}$

— Pour le groupe 2 (hamster, rat, lapins) : $DL_{50} = 0,226 \cdot P^{+ 0,098}$ et respectivement comme valeur extrapolée à l'humain de 60 kg [2,65 mg/kg (1,04 — 6,73)] et [0,66 mg/kg (0,48 — 0,91)].

Le test d'indépendance est pour les deux groupes négatif, le test de parallélisme entre les deux tracés est positif. La puissance relative est pour G_1/G_2 égale à 2,8, la toxicité en G_2 étant 2,8 fois plus importante qu'en G_1 dans le cadre d'une extrapolation.

Il semble que ce type de tracés selon deux groupes soit plus satisfaisant, mais n'apporte pas de réels avantages pour l'extrapolation humaine. La valeur DL_{50} donnée par le tracé général est très voisine des limites respectivement inférieure et supérieure de G_1 et G_2. D'après le test de parallélisme entre G_1 et G_2, la valeur extrapolée humaine varierait de 1 à 2,8 selon l'appartenance de l'homme à l'un ou l'autre des groupes.

Tracé général : $DL_{50} = 0,9$ mg/kg (0,16 — 5,17).

— G_1 : DL_{50} comprise entre *1,04* et *6,73*.
— G_2 : DL_{50} comprise entre 0,48 et *0,91*.

B. *Voie IP.*

La répartition des points est homogène de part et d'autre du tracé, avec une tendance nette à la diminution de la toxicité avec le poids ; le test d'indépendance est négatif. On ne retrouve pas, de façon appréciable, les deux groupes d'animaux observés en SC (fig. 8).

L'équation est : $DL_{50} = 0,672 \cdot P^{+ 0,230}$ avec DL_{50} 60 kg = 8,42 (3,0 — 23,7).

On observe une toxicité très voisine entre les lapins P et M, légèrement supérieure pour le lapin G. La tendance à l'augmentation de toxicité avec l'âge ou le poids n'est pas significative. Une zone commune se retrouve sur les intervalles de confiance qui indique qu'une valeur voisine de 4 mg/kg peut être retenue pour l'espèce lapin, dans la zone de poids étudiée.

— Lapin P 1.712 g (2,5 à 3 mois) 4,82 mg/kg (3,76 — 7,80).
— Lapin M 2.544 g (3,5 à 4 mois) 4,64 mg/kg (3,39 — 6,36).
— Lapin G 3.373 g (5,5 à 6 mois) 3,31 mg/kg (2,56 — 4,29).
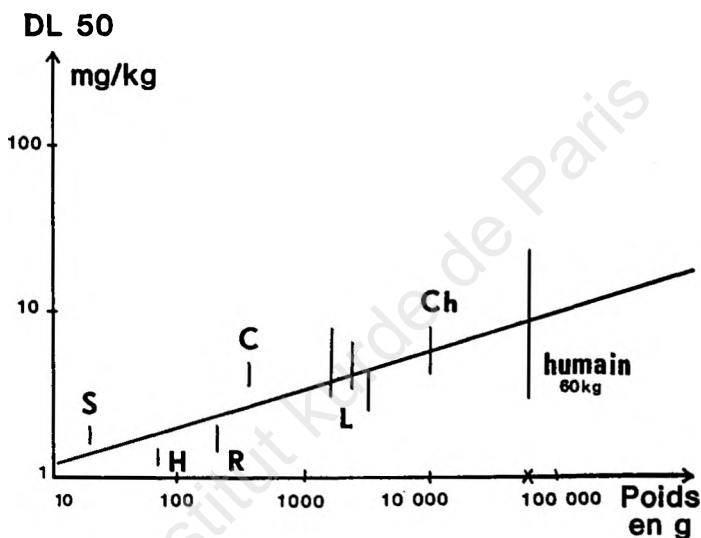
FIG. 8. — Paraoxon voie IP.

C. *Comparaison des voies d'injection.*

Voie SC
$$DL_{50} = 0,380 \cdot P^{+0,079}$$

Voie IP
$$DL_{50} = 0,672 \cdot P^{+0,230}$$

Le test de parallélisme entre les 2 voies est positif

$$DL_{50} = 0,200 \cdot P^{+0,186}$$

$$DL_{50} = 0,885 \cdot P^{+0,186}$$

La puissance relative de SC/IP = 0,226, soit de IP/SC = 1/0,226 = 4,4.

La différence de toxicité en fonction de la voie d'injection est pour les tracés généraux significative. La voie SC étant 4 fois plus toxique que la voie IP (tendance qui se retrouve nettement entre les valeurs expérimentales).

D. *Comparaison des deux groupes isolés en SC avec le tracé IP général.*

Pour le G_1 voie SC par rapport au tracé général IP, le test de parallélisme est positif et la différence de toxicité significative. La puissance relative de G_1 SC/IP égal à 0,5, la voie SC est 2 fois plus toxique que la voie IP.

Pour G_2 voie SC par rapport au tracé général IP, le test de parallélisme est positif et la différence significative, la puissance relative de G_2 SC/IP égal à 0,15, la voie SC est pratiquement 7 fois plus toxique que la voie IP.

On retrouve la différence de toxicité relative de G_2/G_1 qui était en SC voisine de 3 (G_2 2,8 fois plus toxique que G_1).

E. *Conclusion.*

— K est positif quels que soient les tracés, il varie avec la voie d'injection si l'on compare les tracés généraux ($K_{SC} = 0,079$, $K_{IP} = 0,230$), la variation est plus faible si l'on distingue les deux groupes

$$K_1 \text{ sc} = 0,163 \atop K_2 \text{ sc} = 0,098 \Big\} \quad K_{IP} = 0,230$$

— A varie peu pour les tracés généraux ($A_{SC} = 0,380$, $A_{IP} = 0,672$), la variation est aussi plus nette avec les deux groupes

$$A_1 \text{ so} = 0,443 \atop A_2 \text{ so} = 0,226 \Big\} \quad A_{IP} = 0,672$$

(surtout pour le groupe 2).

Dans le cadre d'une extrapolation humaine la différence de toxicité entre les deux voies est significative, la voie SC étant toujours plus toxique que la voie IP. Si l'on s'en tient aux tracés généraux le facteur de variation serait de 1 à 4. Selon l'appartenance de l'homme à l'un ou l'autre des groupes, le facteur serait de 1 à 2 ou de 1 à 7.

DISCUSSION.

En l'état actuel des travaux, si l'on compare les résultats acquis à ceux proposés par Maxwell et Lenz, on note que la loi énoncée ne s'applique pas dans tous ces termes pour les produits étudiés.

Pour l'Eserine.

— K est positif quelle que soit la voie, il ne varie pratiquement pas avec la voie d'injection et peut donc être considéré comme constant pour les voies étudiées.

($K_{SC} = + 0,211$, $K_{IP} = + 0,269$, $K_{\text{test de parallélisme}} = + 0,231$).

— A est constante quelle que soit l'espèce et varie avec la voie d'injection.

($A_{SC} = 0,645$, $A_{IP} = 0,372$).

Pour le VX,

— K est positif ou négatif selon la voie considérée, il varie peu avec la voie d'injection et tend pour les deux voies vers 0. Si l'on se réfère au test de parallélisme entre les deux voies, une valeur négative constante peut être retenue.

($K_{SC} = - 0,083$, $K_{IP} = + 0,022$, $K_{\text{test de parallélisme}} = 0,056$).

— A est bien constante quelle que soit l'espèce (avec une réserve pour la valeur chien IP) et varie avec la voie d'injection.

Pour le Paraoxon,

— K est toujours positif, la variation entre les voies dépend des tracés considérés mais quelles que soient les équations retenues K varie peu, il est compris entre :

$+ 0,079$ ($K_{SC \text{ tracé général}}$) et $+ 0,230$ ($K_{IP \text{ tracé général}}$).

— A varie avec la voie d'injection, d'une façon plus ou moins significative selon les tracés. Il varie avec l'espèce si l'on retient l'existence possible des deux populations suggérées pour la voie SC ($A_{G1} = 0,443$, $A_{G2} = 0,226$) ; cette variation est toutefois faible.

Il semble donc que ce type de modèle mathématique ne soit pas généralisable tel quel aux produits inhibiteurs des cholinestérases, organophosphorés et carbamates et qu'un modèle mathématique, peut être mieux adapté, serait à rechercher. Toutefois, malgré les imperfections que ce type d'étude comporte de par sa nature même, réactif biologique, nombre d'animaux parfois insuffisant (DL_{50} chiens effectuée sur lot de 5), elle nous permet une évaluation assez bonne de la fourchette de doses toxiques chez l'humain.

ESERINE   DL$_{50}$ SC : 6,6 mg/kg (3,0 — 14,0)
                    IP : 7,2 mg/kg (2,2 — 23,0)

VX   DL$_{50}$ SC : 10,7 γ/kg (5,6 — 20,4)
                    IP : 60,6 γ/kg (19,2 — 191)

PARAOXON   DL$_{50}$ SC = 0,90 mg/kg (0,2 — 5,2)
                    IP : 8,4 mg/kg (3,0 — 23,7)
              Réf. NIOSH 1979 DL$_0$ VO = 5 mg/kg

A côté de la recherche d'une méthode d'extrapolation meilleure, il serait intéressant d'approfondir certains points spécifiques comme la sensibilité particulière que semble présenter le chien au VX par voie SC ou les espèces du groupe 2 au paraoxon par voie SC, par des études sur le devenir *in vivo* du toxique dans ces espèces animales.

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Interethnic differences of human serum paraoxonase activity-relevance for the detoxification of organophosphorous compounds

by M. GELDMACHER-von MALLINCKRODT,
T.L. DIEPGEN and P.W. ENDERS

Institut für Rechtsmedizin
and Institut für Medizinische Statistik und Dokumentation
der Universität Erlangen-Nürnberg
D-8520 Erlangen, FRG

SUMMARY.

The extent to which foreign compounds are detoxified in humans often depends on the metabolic conversion. For many compounds metabolism occurs mainly in the liver, but also serum plays a role for some chemicals like paraoxon, 0,0-diethyl-0-4-nitrophenylphosphate and some other organophosphates. The activity of paraoxonase, the enzyme which hydrolyses paraoxon in human serum shows a genetically influenced polymorphism with strong interethnic differences. The serum paraoxonase genotype has a significant influence on the paraoxon clearance and consequently on the toxic action of paraoxon and some related organophosphates.

Alkylphosphates are esters or amides of phosphoric acid, phosphonic acid and phosphinic acid (Schrader, 1963).

They are strong inhibitors of acetylcholinesterase. The symptoms of poisoning are explained by the accumulation of released acetylcholin at the receptor site. The toxicity of organophosphates is very variable. This is partly caused by the fact that in warm blooded animals some compounds are more rapidly detoxicated than others. Also a toxification through metabolic conversion is observed. The velocity of the detoxification considerably depends on the activity of ester cleaving enzymes.

One of the oldest and most well known alkylphosphates is parathion (E 605). In warm blooded animals the relatively untoxic parathion is converted to the PO-analogon paraoxon which is responsible for the toxicity. Parathion and paraoxon are detoxified by hydrolysis (Poore and Neal, 1972 ; cf fig. 1).

Most of the detoxification reactions of foreign compounds take place in the liver. On the other side for some poisons also enzymes in the serum play a major role. This is especially true for
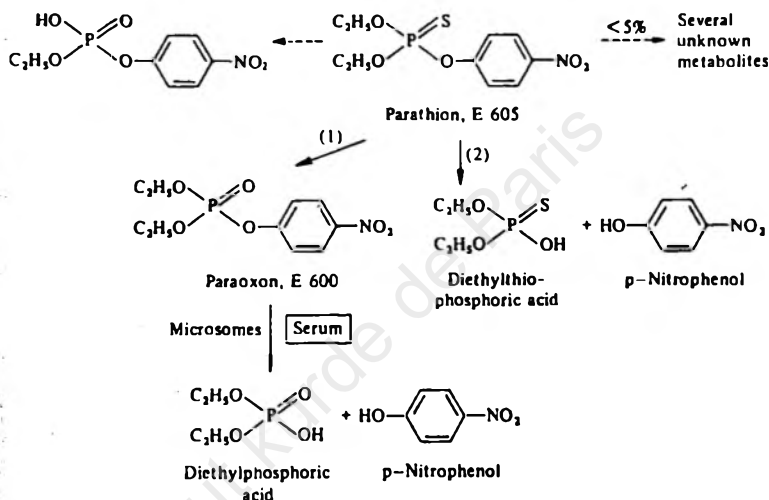
FIG. 1. — Metabolism of parathion in warm-blooded animals.

the hydrolytic cleavage of paraoxon into diethylphosphoric acid and 4-nitrophenol by the enzyme paraoxonase, first observed by Aldrige (1953 a, b) in sera from warm blooded animals.

This was shown for humans by Erdös *et al.* (1959, 1960, 1961) as well as by Skrinjaric-Spoljar and Reiner (1968). However, these authors suggested that not only one single enzyme is responsible for the paraoxon hydrolysis in serum. Most of the activity of human serum paraoxonase is found in Cohn fraction IV-1. This enzyme activity depends on the presence of calcium ions and is consequently inhibited by EDTA. A second paraoxonase independent of calcium and consequently not inhibited by EDTA is found in the albumin fraction (Cohn fraction V). According to the Enzyme Nomenclature the paraoxonase is an arylesterase.

For the EDTA sensitive part of serum paraoxonase (phosphoryl-phosphatase activity a polymorphism could be established in West Germans (Krisch, 1968 ; Geldmacher-von Mallinckrodt *et al.,* 1969, 1973, 1979 ; Zech and Zürcher, 1974). The results of Zech and Zürcher are shown in figure 2.

During extended family investigations in West-Germans we were able to show that this polymorphism is caused by genetic factors and follows a two allele model (Geldmacher-von Mallinckrodt *et al.,* 1973, 1979).
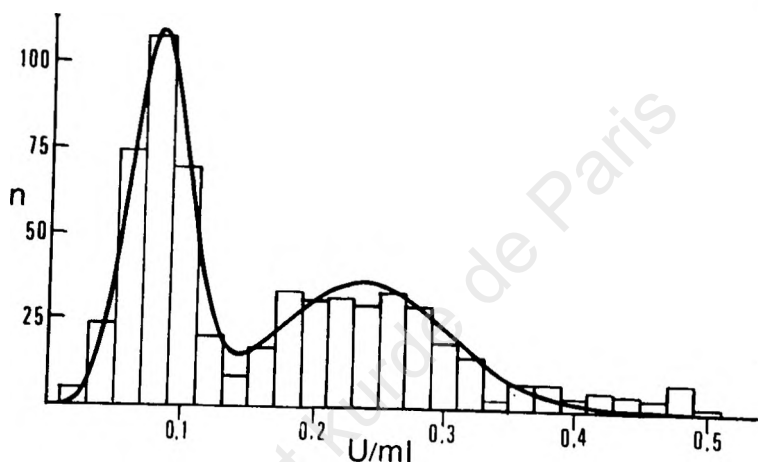
FIG. 2. — Individual phosphorylphosphatase activity in serum of 619 humans, males and females, the age varying from 4 to 78 years (Zech and Zürcher, 1974).

Playfer *et al.* (1976) confirmed this for a British (fig. 3), Eiberg and Mohr (1981) for a Danish and Carro-Ciampi *et al.* (1981) for a Canadian collective. We followed up the problem and investigated additional European collectives (Geldmacher-von Mallinckrodt *et al.,* 1983) using the method of Krisch 1968 (cf fig. 4). The distributions are very similar. More than 50 % of the probants belong to the low activity group, some 35 % to the medium group and about 6 % to the high activity group.

In different ethnic groups (Indians, Kenians, Malaysians, Nigerians, Chinese) Playfer *et al.* (1976) found different patterns of distribution of serum paraoxonase activity. The results are demonstrated in figure 5. We extended these investigations and were able to confirm Playfer *et al's* data for Mongoloides as well as for Negros (fig. 6).

In mongoloid and negroid collectives there are very similar distributions which compare to Playfer's data for Chinese. As did the European collectives, the mongoloid and negroe collectives also show a well distinguishable group with low activity. The percentage of the low activity groups was significantly lower (about 5-10 %) than in the European collectives (> 50 %). This became especially apparent when the data were analysed statis-
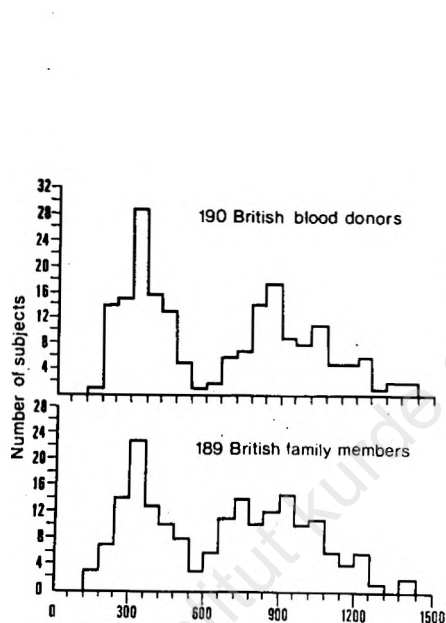
Plasma paraoxonase activity (μmol PNP/l)

FIG. 3.   FIG. 4.

FIG. 3. — Frequency distribution histograms of (i) British blood donors (ii) British families (Playfer *et al.*, 1976).
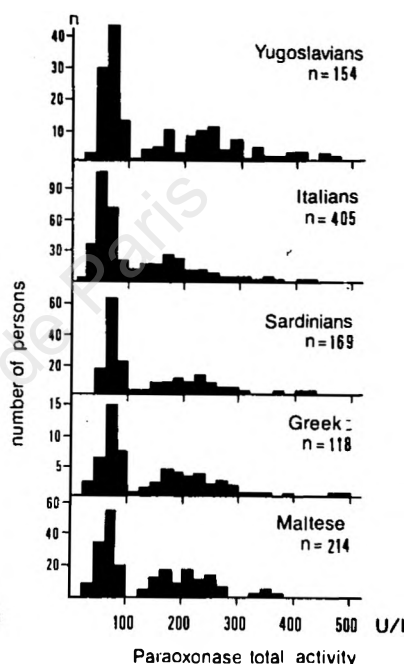
FIG. 4. — Frequency distribution histograms of different European collectives (Geldmacher - von Mallinckrodt *et al.*, 1983).

tically using the method of iteration of Hammel (Hommel *et al.*, 1978, 1980).

While the Hardy-Weinberg rule for a two allele model was exactly true for Caucasians, this was not the case for the mentioned negro and mongoloid collectives where the Hardy-Weinberg rule neither for a two nor for a three allele model could be established.

In some of the studied collectives we could not detect the low activity group (< 100 U/l) neither by eye nor by the method of
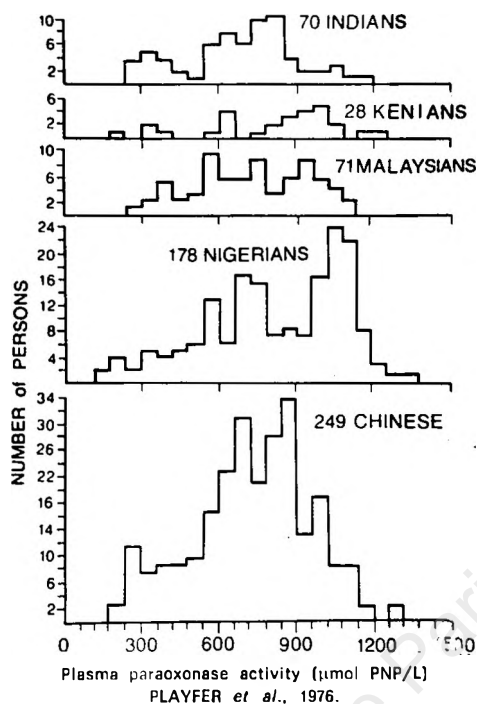
FIG. 5. — Plasma paraoxonase activity in different ethnic groups
(Playfer et al., 1976).
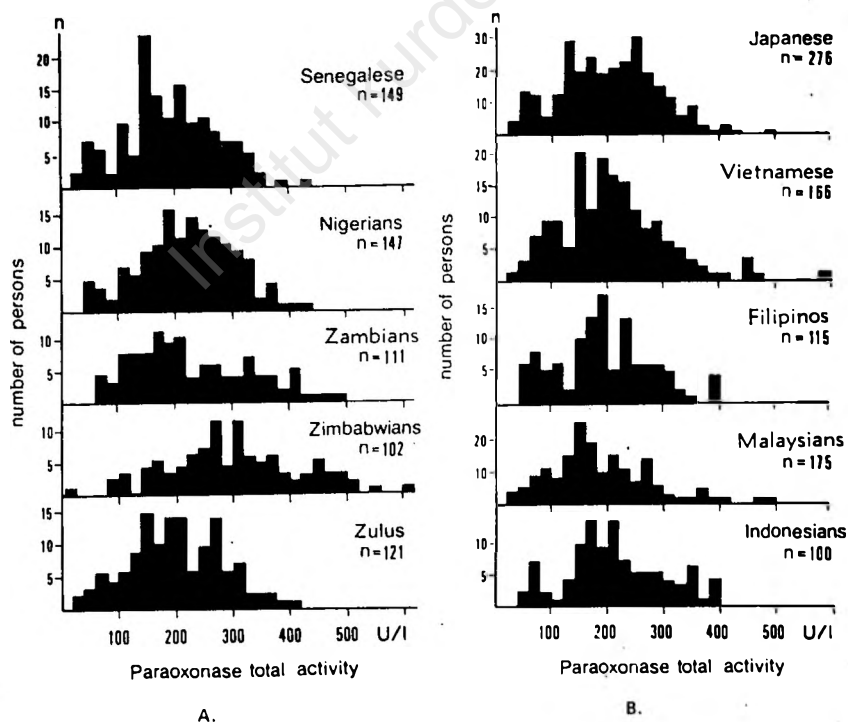
FIG. 6. — Serum paraoxonase activity in different ethnic groups (own results).
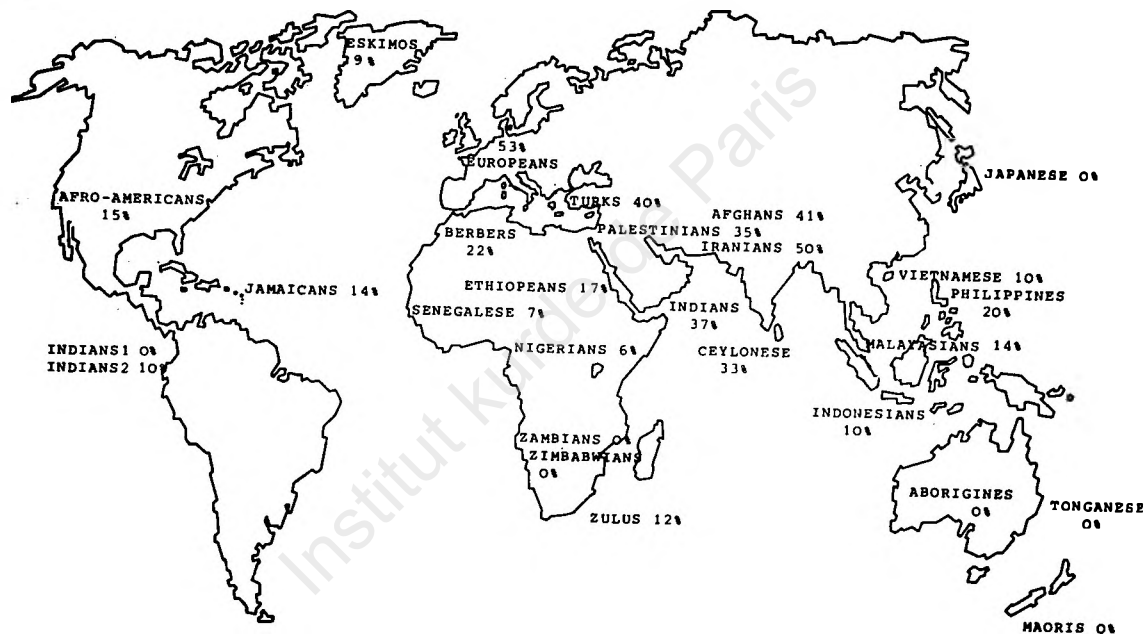
FIG. 7. — Percentages of homozygote phenotypes with low serum paraoxonase activity
in random samples taken from different ethnic groups.

iteration. This was e.g. true for some tribes in the South Pacific and in South Africa as well as in an Indian tribe from Central America.

The frequency of the low activity group is shown in a map of the world (fig. 7).

The highest percentage of the low activity group could be observed in Europe. Beginning in Europe this group decreases steadily which is possibly caused by migration and mixing of the populations.

In all collectives we also studied the distribution of the EDTA stable paraoxonase activity. The activity was much lower (between 5 and 70 U/l measured according to Krisch). In all collectives a normal distribution became apparent.

To obtain an idea of the extent of the paraoxon clearance in serum we determined the paraoxonase activity in 30 persons using the method of Krisch. The sera of the same probants were incubated with paraoxon and the decay of paraoxon was determined by gas chromatography. An exponential function has been obtained. We found a linear relationship between paraoxon deterioration and paraoxonase activity (r = 0.9490).

The half-lives under these conditions were between 60.4 and 8.6 min. exhibiting significant differences. Sera with low paraoxonase activity have relatively high half-lives and sera with high activity have a very short half-live of 8-10 min. This clearly shows that human paraoxonase under physiological conditions can hydrolyse considerable amounts of paraoxon in individuals with high paraoxonase activity.

Paraoxonase protects serum cholinesterase considerably during intoxication with paraoxon. We measured the cholinesterase activity before and after incubation with paraoxon and found a similar distribution of the remaining cholinesterase activity as for the paraoxonase activity (Geldmacher-von Mallinckrodt *et al.*, 1976). This shows the important role that paraoxonase plays in poisoning with parathion.

In investigations concerning the specifity of human serum paraoxonase and using the method of Krisch we systematically changed the structure of the substrate and obtained the results shown in figures 8 and 9.

Dicapthoxon and fenitroxon as substrates show normal distributions of the activities, whereas paraoxon-methyl, PO-chlorothion and PO-EPN show polymorphic distributions very similar to para-

PARAOXONASE HYDROLYSES

ETHYL-PARAOXON

PO-CHLOROTHION r=0.90

METHYL–PARAOXON r=0,965

FENITROXON r = 0,579

PO-EPN r= 0,950

DICAPTHOXON r=0,506

FIG. 8.

PARAOXONASE does not HYDROLYSE

PARATHION

ISOPROPYL- PARAOXON

MONO-DESMETHYL-PARAOXON

METHYL- PARATHION

M-NITRO-PARAOXON

DI- DESMETHYL-PARAOXON

CHLOROTHION

O-NITRO-PARAOXON

FIG. 9.

oxon-ethyl (= paraoxon). This is especially interesting because PO-EPN is a phosphonic acid ester and not a phosphoric acid ester.

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Lethal hemorrhages in pregnant mice following one oral dose of T-2 toxin

by H.B. SCHIEFER and C.G. ROUSSEAUX

Toxicology Research Centre, University of Saskatchewan,
Saskatoon, Saskatchewan S7N OWO, Canada

SUMMARY.

Forty-eight hours after oral administration of a single dose (3.0 mg/kg BW) of T-2 toxin dissolved in propylene glycol to mice on days 7, 8, 10, 11 and 12 of pregnancy, 17 % maternal mortality following vaginal hemorrhage was encountered.

At necropsy, it was found that massive hemorrhages had occurred into the reproductive tract. This observation is not in agreement with most published observations, which report failure to induce hemorrhagic disease with pure T-2 toxin.

The results suggest that fatal hemorrhages due to T-2 toxin can occur in mice under certain metabolic conditions.

INTRODUCTION.

T-2 toxin and other trichothecenes have been associated with diseases characterized by immunosuppression, cytotoxicity and hemorrhagic diathesis. However, generalized or severe hemorrhage is not a typical feature of experimental T-2 toxin.

This report describes the occurrence of placental hemorrhage and subsequent death of pregnant mice used in a teratology study in which mice were treated once orally with purified T-2 toxin.

MATERIALS AND METHODS.

Six weeks old female and proven breeder male CD-1 mice were bred for four consecutive days. When vaginal plugs were detected,

the 24 hr period following was considered day 0 of pregnancy. The diet was semisynthetic to eliminate the possibility of exposure to naturally occurring mycotoxins. T-2 toxin, in a propylene glycol vehicle, was administered as one single dose of 3 mg/kg body weight on either day 7, or days 8, 10, 11 or 12 of pregnancy via gavage tube.

RESULTS.

The teratological and reproductive findings are in the process of being reported elsewhere. An overall seventeen percent maternal mortality was seen after the administration of T-2 toxin, peaking at 48 hrs (table I) and death was preceded by vaginal hemorrhage. The blood failed to clot.

TABLE I

Mortality in pregnant dams dosed with 3.0 mg/kg T-2 toxin in response to day of pregnancy

Day of pregnancy dosed with T-2	Number of deaths (hours after administration)			Total	%
	24	48	72		
7	—	2	—	2/23	(9 %)
8	—	2	1	3/21	(10 %)
10	—	1	—	1/18	(5 %)
11	—	4	—	4/26	(15 %)
12	2	8	1	11/38	(30 %)

DISCUSSION.

The finding of hemorrhage with blood that failed to clot after death supports the suggestion that T-2 toxin is capable of producing hemorrhagic disease. Pregnancy indirectly increases the procoagulant activities of blood, thus the placental hemorrhage is difficult to explain. At present, one can only speculate as to the mechanisms involved in the hemorrhagic diathesis observed in the pregnant mice of this study.

Note. — A detailed description is in the process of publication : C.G. Rousseaux, S. Nicholson and H.B. Schiefer : fatal placental hemorrhage in pregnant CD-1 mice following one oral dose of T-2 toxin. *Can. J. Comp. Med.* (submitted for publication 1984).

Clinical and laboratory findings in Iranian fighters with chemical gas poisoning

by M. BALALI

Poison Unit, Emam Reza Hospital, Mashad University, Mashad, Iran

INTRODUCTION.

Although chemical and biological weapons are banned by the 1925 Geneva Protocol and 1927 Convention on Biological weapons, they have been used in South East Asia and in the Iran-Iraq conflict. There are some indications that mycotoxins have been used in Kampuchea, Laos and possibly in Afghanistan (1-5), although a few investigators could not accept it (6-9).

Since September 1983, several Iranian fighters with clinical features of chemical gas poisoning have been admitted to some medical centers in Ahvaz (near the war zone) and Tehran. The first toxicological analysis which was performed at that time in the Tehran University revealed Di-(2-chloroethyl-)sulfide or Yperite. On February 26th, 1984, a heavy chemical bomb attack was made by the Iraqi army on the Iranian fighters in Majnoon Island.

This was reported by all the poisoned fighters, referred to my Unit. Some of the severe poisoned fighters were transferred to some European countries such as Austria and Belgium. Toxicological analyses which were performed later in Tehran and in the Department of Professor A. Heyndrickx (Department of Toxicology, State University of Ghent, Belgium) showed also mycotoxins and a nerve gas called tabun.

Although some works have been done on the toxicological analyses of mustard gas (10-15) and mycotoxins (3, 4, 9, 16), very little has been published on clinical features and biochemical abnormalities in man (17-21). In fact there has been no clinical trial on chemical wargas poisoning published in recent English

llterature. Therefore, the study was designed to estimate the frequency of clinical features, haematological and biochemical abnormalities in patients with chemical wargas poisoning.

METHODS.

In order to study the frequency of symptoms and signs, haematological and biochemical abnormalities in those Iranian fighters poisoned by chemical gas which were referred to the Poison Unit of Mashad University, a questionnaire and special examination chart was designed. Routine Laboratory tests such as blood cell counting, Haemoglobin, Haematocrit, Erythrocyte Sedimentation Rate, Routine Urine analyses were performed for all patients. Other investigations such as arterial blood gases, bleeding and coagulation times, liver and renal function tests, protein electrophoresis and immunoelectrophoresis, chest X-ray, electrocardiogram and electroencephalogram were done whenever clinically indicated.

RESULTS.

Between March 3rd and 15th, 1984, 94 Iranian fighters with chemical gas poisoning were brought to the Unit. Out of them 18 patients were admitted to the ward and the remaining treated as outpatients.

The interval between exposure and admission time was more than 5 days. Toxicological analyses which were already done on most of these patients revealed Yperite.

CLINICAL FINDINGS.

The most common toxic effects were found on eyes and skin. All the in-patients had eye and skin problems.

Conjunctivitis, blurred vision, photophobia and temporary blindness were registered in 94, 80, 72 and 4 % of all patients (table I). Skin reactions including erythema, pigmentation, blisters and severe burning were found in 86, 82, 69 and 12 % of the patients (table II). The scrotum was also injured, erythema, edema, pain and ulceration were seen In 25, 21, 18 and 10 % of the patients (table III).

TABLE I

Frequency of toxic effects of wargas (Yperite) on the eyes of 94 patients

Clinical manifestations	No of patients	%
Conjunctivitis	88	94
Blurred vision	75	80
Photophobia	68	72
Temporary blindness	4	4

TABLE II

Frequency of toxic effects of wargas (Yperite) on the skin of 94 patients

Clinical manifestations	No of patients	%
Erythema	81	86
Pigmentation	77	82
Blister	65	69
Severe burning	11	12

TABLE III

Frequency of toxic effects of wargas (Yperite) on the scrotums of 94 patients

Clinical manifestations	No of patients	%
Erythema	24	25
Edema	20	21
Pain	17	18
Ulceration	9	10

TABLE IV

Frequency of toxic effects of wargas (Yperite) on the respiratory tracts of 94 patients

Clinical manifestations	No of patients	%
Coughing	81	86
Dyspnea	42	45
Wheezing	38	40
Rale	21	22
Chest X-Ray abnormalities	19	20

The irritant effects of the gas on the respiratory tract were as follows : coughing 86 %, dyspnea 45 %, wheezing 40 %, rale 22 % and chest X-ray abnormalities in 20 % of all patients (table IV).

Other symptoms such as headache, abdominal pain, nausea with or without vomiting, vertigo, mental depression, palpitation, loss of hearing were found in less than 10 % of all patients.

One patient had cardiac dysrhythmia with a first degree heart block on the electrocardiogram. Another one not having been in the war zone had an indirect exposure, while he was working in a hospital at Ahraz, having direct contact with the poisoned fighters without adequate personal protection.

LABORATORY FINDINGS.

All laboratory findings were within the normal ranges of methods used, except for mild elevation of transaminase (about 50-70 V/L) in 9 patients (10 %) and also small increases in lactic dehydrogenases in 4 patients. Only two patients had hypo-gammaglobulinemia of 7.2 and 7.7 % normal : 10-18 %) and one had low IgA of < 35 mg/dl (normal 70-440 mg/dl).

DISCUSSION.

Yperite was the only wargas found in the urine of most patients referred to my Unit, but trichothecene mycotoxins and tabun were apparently found in some other patients who were treated in Tehran and the European countries. In addition, the toxicological analyses of the *in vitro* samples — such as the soil of the contaminated area — which were done in the Department of Professor Heyndrickx (Department of Toxicology, State University of Ghent, Belgium) confirmed that at least three chemical wargases were used in the Iran-Iraq conflict.

Regarding mycotoxins, there has been a big debate on the naturally occurring fusarium producing toxin in South East Asia (6-9, 22).

As there has been no systematical survey of the area (Majnoon Island) for toxigenetic fusaria, there might be such an argue. But severe toxic effects on the gastrointestinal tract particularly G.I. haemorrhage and death, which were found in some patients in Tehran and the European countries, are most likely attri-

buted to Mycotoxicosis (4). Toxic effects of trichothecenes have been studied in animals (18, 19, 23-25), perhaps similar effects may occur in humans, as it happened by food contamination (26).

We found no clinical features of an organophosphate intoxication such as tabun poisoning in the patients admitted to the Unit, but it was really too late to find the manifestations, and there was no clinical indication to estimate plasma cholinesterase activity. Less than 10 % of the patients had a history of mild parasympathetic stimulation such as bradycardia, salivation, vomiting and diarrhoea. The patients who had severe nerve gas poisoning possibly died very quickly in the war zone or during transportation to the medical centres.

Poisoned fighters described different smells such as garlic and chocolate, which could be another reason for the use of more than one chemical bomb. Toxic effects of mustard gas have been studied in animals (27, 28). Appart from direct irritation effects on the eyes, skin and respiratory tract, delayed deaths with prominent G.I. effects (diarrhoea), bone marrow depression, lymph node damage, mutagenic activity, carcinogenicity, tumor inhibition and even neurogenic activity were described in experimental toxicology (4).

Perhaps some of these toxic effects have occurred in the patients. In fact, some toxic effects such as bone marrow depression were found by some colleagues in Tehran and the European countries. In fact there have been the biochemical abnormalities in few patients, but they are not specific and common enough to be discussed here. Further study is required to evaluate the clinical and biochemical changes In any of the wargas poisoning.

CONCLUSION.

The frequency of clinical features and biochemical abnormalities in 94 Iranian fighters with chemical wargas poisoning was studied. The most common toxic effects were found on eyes, skin and respiratory tract. The effects were more directly irritant effects rather than systemic intoxication, although general symptoms and biochemical abnormalities were found in less than 10 % of the patients. The clinical and biochemical findings are mostly attributed to mustard gas poisoning. Since mycotoxins and nerve gas (Tabun) were reported to be found in some other pa-

tients of the same chemical bomb attack, mixed poisoning must
be considered.

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Personnel protection against chemical agents : development of antidotal treatment for organophosphorous poisoning

by C.J. BRIGGS and K.J. SIMONS

Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada

SUMMARY.

World events in recent years have resulted in increased concern regarding the use of biological and chemical weapons. Reports of « Yellow Rain » have arisen in South East Asia. Controversy surrounds some of these incidents, but mycotoxins have been implicated in deaths and serious medical conditions where yellow rain has been reported. The source of these mycotoxins is questionable, but their occurrence has led to charges of military use of biological toxins. Poison gas is reported to have been used in the conflict between Iraq and Iran. Many countries possess the ability to manufacture chemicals for use in munitions and both the NATO and Warsaw Pact countries possess stocks of chemical weapons based on organophosphorous compounds, held under a « no first use » interpretation of the Geneva Protocol. These volatile anticholinesterases, soman, sarin, VX and tabun are long-acting, potent nerve agents.

An effective defence policy must include measures for protection against military chemicals. The best approach is physical protection of personnel, using protective clothing and respirators. These are effective against most chemicals potentially used in a military operation, including nerve agents. Equipment and procedures for decontamination are required to supplement personnel protection. Physical protection may be supplemented with chemical defence, using a self administered antidote.

An autoinjector containing atropine as an antimuscarinic cholinergic blocker, with a pyridinium oxime to reactivate inhibited

acetylcholinesterase has been the standard chemical antidote for nerve agents. The effectiveness of antidotes varies with each of the nerve agents. Soman-inhibited cholinesterase is difficult to reactivate, due to rapid « aging » of the phosphonylated enzyme, giving a product which is resistant to conventional oximes. The asymmetric bisquaternary monooxime, HI-6, is an example of the second generation oximes which reactivate soman-inhibited cholinesterase. Pharmacokinetic studies* with HI-6 in dog, rat and mouse have shown that it has 100 % bioavailability when administered intramuscularly, it has a short plasma half life and is able to penetrate the blood/brain barrier. Co-administration with atropine and exposure to soman altered the disposition of HI-6 in mice. HI-6 reactivates soman and sarin-inhibited cholinesterase, but is not effective against the tabun-inhibited enzyme. These new oximes have potential for improving protection against organophosphorous poisoning. Benactyzine has been incorporated into injection for blockade of CNS muscarinic activity. Benzodiazepines may be used to reduce convulsions and prolong survival times in soman-treated rats receiving atropine/oxime therapy. Improved protection is also obtained by pretreatment with pyridostigmine which reversibly inhibits acetylcholinesterase activity up to 40 %. This enhances recovery from sarin exposure and offers some protection against soman. Continued investigation of these therapeutic agents offer the best potential for development of effective treatments for nerve agent poisoning.

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INTRODUCTION.

The first reported strategic use of chemical weapons occurred seventy years ago, early in World War I. There have been confirmed instances of the use of chemicals in military operations since 1918, but there has been general restraint in their deployment even during the Second World War. Only four or five cases are recorded of major conflicts in which casualties due to chemical attack have been substantiated since the First World War.

The early anti-personnel chemical agents have been superseded by compounds which are many times more potent, such as the modern nerve agents. In addition, biological weapons have been

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developed. These have the potential to be much more destructive than any other non-nuclear munitions (1). They may include bacterial, fungal or viral microorganisms, or toxins derived from them.

There is no evidence that microorganisms have been used in any recognized combat, but there have been persistent reports that a mycotoxin product, colloquially known as « Yellow Rain » was the cause of casualties in Southeast Asia. There is considerable controversy surrounding the nature of « Yellow Rain » and the reliability of evidence implicating the product in casualties in Laos, Afghanistan and Kampuchea (2). « Yellow Rain » has been reported to contain high levels of three potent trichothecene mycotoxins which are not indigenous to Southeast Asia. It has been alleged that the presence of these compounds could only have arisen as a result of their utilization in military operations. If confirmed, this would be the first major use of biological agents in military combat.

The United Nations team which investigated the possible use of chemical warfare in the conflict between Iran and Iraq has reported recently that mustard gas and the organophosphorous agent tabun were responsible for many of the casualties. This is the most recent example of a military operation involving chemical warfare, and it further justifies the concerns which have been expressed by many individuals and groups regarding the use of biological and chemical weapons.

The « Geneva Protocol for the Prohibition of the Use in War of Asphyxiating, Poisonous or other Gases, and of Bacteriological Methods of Warfare » dates from 1925 and is still the major political agreement for international regulation of these agents. However, there are many problems associated with interpretation and enforcement of this Protocol. Many countries possess the technology to manufacture agents for chemical warfare. Military analysts have concluded that Iraq has the capability and is probably producing the weapons which were reported by the U.N. team. Iraq acceded to the Geneva Protocol in 1931, but this accession did not prevent probable production and use of sulphur mustards and tabun in the current conflict with Iran. Both NATO and Warsaw Pact countries possess stocks of chemical weapons, held under a « no first use » interpretation of the Geneva Protocol (3). The nerve agents constitute a significant proportion of these reserve munitions. They include sarin, soman, VX and tabun, which are all potent, long-acting anticholinesterases. The United States have not manufactured chemical agents since

1969, but there is pressure for Congress to approve manufacture of second generation binary weapons which would generate nerve agents.

PROTECTION OF PERSONNEL.

The existence of known stocks of chemical weapons and the fact that many countries possess the technology and facilities to produce these materials constitute a potential military problem. Failure of states to adhere to the Geneva Protocol could result in deployment of chemical weapons. An effective defence policy must include measures for protection of personnel against military chemicals. The need for physical protection has been recognized since the first use of chemical agents in World War I. The technology for provision of a safe environment for personnel has advanced, but the principles remain the same. Protective equipment, protective clothing, efficient respirators and development of decontamination techniques are the major approaches to physical protection. The intent is to prevent contact between the agent and the individual to be protected, and in general terms, systems designed for physical protection against chemical agents should be effective also in a biological attack.

Significant advances have been made in design and fabrication of vehicles and operational centres which can be sealed from the external environment, thus excluding any chemical agent. Individual physical protection is provided using protective clothing and respirators. The standard MOPP (Mission Oriented Protective Posture) dress consists of coveralls, mask, gloves and footwear which prevent access of any agent. Such clothing is cumbersome and restrictive and modern research in design of fabrics for this application is directed towards increasing comfort and practical operation in addition to the primary objective of providing protection (4). Development of fabrics with a vapour permeable polytetrafluoroethylene (PTFE) film has enabled liquid-proof cloths to be manufactured which are incorporated into protective clothing. A typical combination consists of three layers, a synthetic non-woven textile or nylon/cotton outer, an adsorbent filled centre layer which is coated with the PTFE film and an inner knit liner to provide comfort. There may be one or two PTFE layers. These are non-wettable but have 0.2 μm pores which permit vapour transmission. This pore size is the same as in a

bacteria proof filter, which would enable the film to protect against spores and bacteria. The nature of the adsorbent and textiles used in these multi-layer fabrics may be modified according to requirements. One approach is incorporation of synergists or reactive chemicals to accelerate the breakdown of the chemical warfare agent, rather than relying on passive adsorption by activated charcoal in the fabric. Advances in polymer chemistry and fabrication techniques make it feasible to produce textiles which contain microencapsulated or covalently bonded reactants or enzymes which will break down nerve agents and other chemical warfare products.

Respirators and decontamination techniques are components of personnel protection procedures. Filter technology and protective seal design has enabled respirators to be made more effective against chemical and biological warfare agents. The ability to decontaminate individuals who have come into contact with chemical or biological warfare agents varies with the nature and extent of the challenge. Speed of removal is the prime factor and emergency removal from skin should be completed in less than three minutes (5). This gives logistical problems for an individual in protective clothing who may still be in a contaminated area. In addition, the products which are effective for breakdown of nerve agents and other chemical warfare materials are too reactive to be used on the skin. Physical removal with absorbent cloths or dusting powders remain the most effective procedures for emergency use. Subsequent washing with water and detergent or dilute bleach is effective for nerve agents. There is a need for improved methods for decontamination of skin, particularly if it is not possible to remove the agent immediately.

CHEMICAL PROTECTION.

The second approach to personnel protection against chemical warfare agents involves the use of chemotherapeutic pharmaceutical products by the individuals exposed to the attack. Standard treatment for poisoning by organophosphorous compounds is termination of exposure and blockage of the effects of excess acetylcholine resulting from inhibited cholinesterase activity. Atropine is used as an antimuscarinic cholinergic blocker and is a component of all regimens for symptomatic treatment of organophosphorous toxicity. Respiratory assistance may be necessary

and a cholinesterase reactivator may be administered to enhance recovery of the enzyme. It is essential that therapy for nerve agent poisoning be initiated rapidly. Military personnel rely on an intramuscular injection of atropine sulphate and a cholinesterase-reactivating oxime as a parenteral antidote for immediate use. This is available in autoinjectors for self-administration.

Pralidoxime [2-PAM, (2-hydroxyimino-methyl) pyridine-1-methyl chloride] and its iodide [PAM] and methanesulphonate (P$_2$S] analogues are N-methylated monopyridinium oximes which have been used widely as cholinesterase reactivators. Toxogonin, [obidoxime, bis-1 [(4-hydroxyiminomethyl) pyridine-1-methyl] ether dichloride,] is a bis-pyridinium di-oxime which is a newer development than the mono-oximes and is more active. Unfortunately, none of these standard oximes has any activity against soman-inhibited cholinesterase (table I). This is in contrast (11) to the second generation oximes, HI-6 and HS-6, which will be discussed later.

TABLE I

Activity of cholinesterase-reactivating oximes (erythrocyte cholinesterase model)

Oxime	Inhibitor			
	Sarin	Tabun	Soman	VX
2-PAM Chloride . .	+	+	0	+
Toxogonin . . .	+ + +	+ +	0	+ + +
HI-6 . . .	+ + + +	0	+ +	+ + + +
HS-6 . . .	+ + +	0	+	+ + +

Several supplemental treatments have been developed to compensate for the deficiencies of the standard injections for chemotherapy of nerve agent toxicity. Pretreatment with pyridostigmine prior to exposure to the agent has been shown to provide improved protection (6). In this case the principle is to partially inhibit (30 %) blood acetylcholinesterase with a reversible carbamate inhibitor. This inhibited enzyme is protected from reaction with nerve agent, and recovery following atropine/oxime therapy is enhanced.

Convulsions are a major adverse effect arising in nerve agent toxicity. Diazepam has been shown to be an effective adjunct to atropine and oxime therapy of organophosphate poisoning, increasing the LD$_{50}$ in rats up to eighty times (7). This combination has been recommended for human use in treatment of nerve agent poisoning (6). Diazepam is an effective drug for pre-

vention of convulsions arising in soman poisoning. It prolongs the survival time induced by atropine and oximes in rats following administration of $5 \times LD_{50}$ of soman (8). An oral dose of up to 15 mg (3×5 mg at fifteen minutes intervals) has been suggested for inclusion in an atropine/oxime/pyridostigmine regimen for immediate treatment of nerve agent exposure in humans (6).

Benactizine is a component of the TAB autoinjector mixture which was issued to both American and Russian armed forces (9). This injector contained toxogonin as a reactivating oxime, atropine as a muscarinic blocker and the benactizine served as a muscarinic blocker with rapid access to the CNS. Benactizine does not possess an optimum spectrum of activity and new approaches to the treatment of nerve agent toxicity have resulted in alternatives to the TAB injector device.

Respiratory failure is a major problem associated with nerve agent poisoning. Recovery from severe poisoning will be enhanced and fatalities will be reduced if forced respiration or resuscitation is available. Procedures and equipment are being developed to prevent asphyxiation arising from obstructed airways. The apnoeic casualty must be treated rapidly and restoration of ventilation must be the first priority. If apnoeia persists, recovery may be possible but the patient will suffer permanent severe adverse effects.

PHARMACEUTICAL CONSIDERATIONS.

There are a number of practical pharmaceutical considerations associated with development of prophylactic pretreatments and self administered antidotes. The routes of dosage for most of these drugs are either oral or parenteral. Oral administration is normally considered to be the most convenient, but this may not be true for an individual in full protective clothing. In addition, atropine reduces fluid secretions, which impairs swallowing and leads to a rise in body temperature through reduced perspiration. The dose of cholinesterase reactivators is high, and tablets can contain one gram of drug. Tablets are particularly difficult to swallow following a dose of atropine. The rate of absorption of drugs following oral administration is not as rapid as by injection, and the latter is preferable for any therapy which must be initiated immediately. Thus, injection is the route of choice for administration of an oxime and atropine mixture in an operational

situation. It has the additional advantage that removal of the res-
pirator is unnecessary. However, formulation of the injection may
present problems of compatibility, stability and patient acceptabil-
ity. Mixed formulations may influence bioavailability of the individ-
ual components. Incorporation of a hydrophobic benzodiazepine
into an atropine/oxime formulation requires a high proportion of
organic solvent which is likely to influence rate of absorption from
an intramuscular site. It may be difficult to ensure that the
mixture remains in solution at lower temperatures. The dose
of oximes produces a high osmolarity which, combined with the
relatively low pH of most oxime solutions, can produce a painful
injection. Many of these factors limit the range of effective mix-
tures which can be prepared for injection. In addition, the varied
biological half lives of the individual components creates prob-
lems of dosage when multiple injections are given, since drugs
such as benzodiazepines tend to persist in the body whilst oximes
are rapidly excreted. However, rational development of mixed for-
mulations can provide convenience and give effective solutions
for incorporation into an autoinjector.

NEW OXIMES.

The resistance to reactivation of soman-inhibited cholinesterase
has led to investigation of many oximes in an attempt to identify
one compound which has a general activity against all nerve
agents, together with acceptable stability and toxicity. The prob-
lems associated with development of an effective antidote for
soman poisoning relate to the fact that the phosphonylated
enzyme is resistant to reactivation after « aging », which
occurs very rapidly. Therapy is complicated by the existence of
central and peripheral acetylcholinesterase, and the problem of
developing oximes which will cross the blood brain barrier in
significant quantities. In the case of soman, it appears that
recovery of poisoned animals is not primarily dependent upon
reactivation of central cholinesterase, and peripheral acetylcho-
linesterase in the diaphragm may be the primary site of action (10).
The mode of toxicity of soman is not fully understood, but it dif-
fers from that of sarin and the other nerve agents.

New approaches to treatment include development and assess-
ment of new oximes. The Hagedorn oximes are a series of asym-
metric bis-pyridinium mono-oximes which have activity as cho-

linesterase reactivators. HI-6 and HS-6 are two of these compounds which have been found to be effective antidotes for soman poisoning, with less inherant toxicity than many of the other compounds in the series (10).

From the standpoint of formulation of a parenteral antidote for nerve agent toxicity, these two compounds have several useful characteristics. Animal studies have indicated that they are more potent than the conventional oximes, which means that a lower
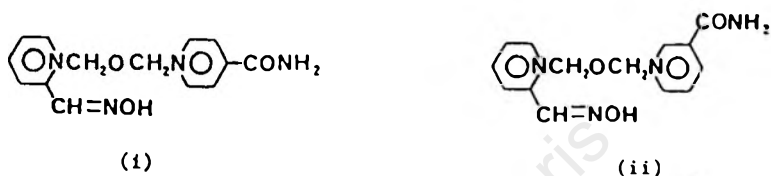
FIG. 1. — Formula of (i) HI-6 and (ii) HS-6.

TABLE II

Stability of cholinesterase-reactivating oximes

Oxime	Stability of aqueous solution
2-PAM Chloride	Stable
Toxogonin	Very stable
HI-6	Low
HS-6	Low

dose can be used. This is advantageous in that it reduces the concentration required in an injector which has a fixed volume. The solutions have a lower osmolarity than the monopyridinium oximes, which would result in less tissue necrosis upon injection. These oximes are highly water soluble and compatible with atropine sulphate. They have a reasonable spectrum of activity with the advantages of effectiveness against soman in addition to sarin and VX. They are not useful oximes for treatment of tabun poisoning. Their disadvantage from a formulation aspect is that both HI-6 and HS-6 have low stability in aqueous solutions when compared with the conventional oximes (11).

BIOAVAILABILITY AND PHARMACOKINETICS OF HI-6.

HI-6 is considered to be one of the most promising therapeutic products in the Hagedorn series (12) and was selected for pharma-

cokinetic studies in dogs (13). Intravenous and intramuscular routes of administration were used. The effects of dilution of solution on the rate and extent of absorption of the intramuscular injection were investigated. Seven pure bred male beagle dogs (9.0 ± 0.4 kg) were subjected to intravenous (250 mg ml^{-1} solution) and intramuscular (250 mg ml^{-1} and 25 mg ml^{-1} solution) aqueous injections of HI-6 on a randomized schedule. HI-6 (Batch #DRES 32) was purified and administered at a dose of 20 mg
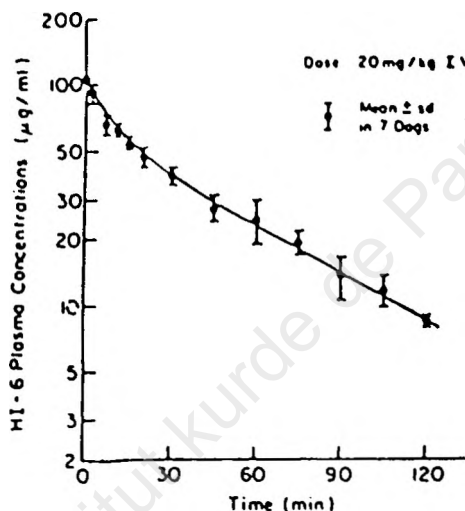
FIG. 2. — Mean ± SD log plasma HI-6 concentration versus time plot for IV injection of HI-6 in dogs.

kg^{-1}. Following intravenous dosage, 3 ml blood samples were withdrawn at 2, 7, 10, 15, 20 and 30 minutes, then every 15 minutes to 2 hours. After intramuscular injections blood samples were drawn at 5 minutes intervals to 30 minutes, then every 15 minutes to 2 hours. Plasma was separated and analysed for HI-6 using a spectrophotometric procedure (13). Overnight urine was collected and assayed for HI-6. Typical HI-6 plasma concentrations versus time plots for IV injection are shown in figure 2.

Mean initial plasma concentration of HI-6 was 93.1 ± 10.8 μg ml^{-1} falling to 8.7 ± 2.4 μg ml^{-1} after two hours. Statistical evaluation of typical results gave a distribution half-life value of 3.9 ± 1.9 minutes with an elimination half-life of 48.2 ± 17.7 minutes. Over sixty percent of the drug was eliminated unchanged in the urine.

Intramuscular injection gave peak plasma concentrations of 45.2 $\pm$ 6.0 and 48.4 $\pm$ 7.3 μg ml^{-1} and occurred at 20.7 $\pm$ 7.3 and 15.7 $\pm$ 3.5 minutes following the 250 mg ml^{-1} and 25 mg ml^{-1} doses respectively. Peak absorption times from the concentrated solution were longer than from the dilute solution, and were more variable. The biological half-life, clearance, apparent volume of distribution, percentage excreted unchanged, and renal clearance calculated for each of the intramuscular concentrations did not differ significantly from those obtained for intravenous injection. Rate and extent of absorption are not affected by concentration. The product is 100 % bioavailable when administered intramuscularly. Its plasma half-life is more than double that of toxogonin [19.9 minutes (14)] which is advantageous, but plasma concentration would still be negligible after four hours. Studies in rats have confirmed that HI-6 has 100 % bioavailability when administered intramuscularly. Concentration of the injection has no effect on rate or extent of absorption from the intramuscular site. Peak plasma concentrations following intramuscular injection were again found to be independent of concentration of injection.

The bisquaternary structure of HI-6 confers high water solubility on the molecule, which inhibits passage from the blood into brain tissue. Ligtenstein (15) has demonstrated that HI-6 can penetrate the blood brain barrier within the first two minutes of intravenous injection, after which passage into the CNS ceases, and the HI-6 redistributes within the brain. The significance of this centrally distributed HI-6 is unclear, particularly in relation to therapy of intoxication by soman. However, Clement has shown that centrally acting HI-6 does reactivate sarin-inhibited cholinesterase in the brain.

Oximes are administered in conjunction with atropine, which has significant pharmacological effects. It was considered that coadministration of HI-6 and atropine could result in altered disposition of the HI-6 in animals. Similarly, feeding is known to affect distribution of many drugs, and particularly the effects of toxic agents. Thirdly, the timing of exposure to soman relative to treatment with atropine and HI-6 is likely to influence disposition of the oxime in the animal. Fasting of mice causes a significant increase in acute toxicity of HI-6 and a significant reduction in ED_{50} value of HI-6 in soman poisoning (16). The pharmacokinetic effects of these different regimens for oxime dosage were studied in male mice (CD-1 ; 25-30 g). HI-6 50 mg/kg

was administered intraperitoneally (IP) to fasted (18 hours) and non-fasted mice. Atropine sulphate (17.4 mg/kg) was administered to one group of non-fasted mice prior to HI-6 dosage. One group of non-fasted mice received 20 mg/kg HI-6.

In studies involving soman, mice were injected IP with atropine sulphate (17.4 mg/kg) and HI-6 (50 mg/kg) 5 minutes prior to dosage (SC) with 100 μg/kg or 287 μg/kg dose of soman. A second dose of HI-6 was administered 4 hours after both soman doses and 24 hours after the high dose soman (Soman $LD_{50} = 130$ μg/kg). Following administration of doses, 5 mice were decapitated at 3, 7, 10, 15, 30, 45 and 60 minutes, exsanguinated and serum separated and frozen. HI-6 concentrations were determined by HPLC.

The serum half-life ($t\frac{1}{2}$) of HI-6 was 5.6 minutes after the 20 mg/kg dose and 7.1 minutes after the 50 mg/kg injection. The latter increased to 11.0 minutes in fasted mice and 11.4 minutes with atropine. After a 100 μg/kg dose of soman the HI-6 $t\frac{1}{2}$ was 12.2 minutes five minutes before soman and 9.4 minutes four hours after nerve agent dosage. The equivalent times with 287 μg/kg soman were 13.9 minutes and 21.6 minutes, reducing to 9.9 minutes in the 24 hour treatment. Half-life values for the 20 and 50 mg/kg doses of HI-6 were not significantly different ($p = 0.05$). Fasting or concommitent administration of atropine prolonged the half-life of HI-6. High dose soman increased the HI-6 half-life four hours after exposure, but this returned to normal control values 24 hours after soman administration. The low dose soman did not increase the biological half-life of the oxime.

It has been suggested that prolongation of HI-6 half-life by soman is due to the hypothermia which is induced by the nerve agent (17). However hypothermia is similar with both soman dose levels after four hours, but only the high concentration of agent produced changes in oxime half-life. It is more likely that the difference is due to distribution changes arising from alterations in the perfusion rate of the kidneys. Fasting decreases the activity of liver microsomal enzymes (8) which could account for the altered pharmacodynamics of HI-6 through changes in *in vivo* metabolism. The implications for use of HI-6 are significant since any modification of half-life of therapeutic drugs following nerve agent poisoning could result in toxicity problems. The extent of prolongation of half-life would depend on the degree of poisoning

and would influence the response to subsequent administration of additional therapeutic drugs.

The H-series of oximes has considerable potential and several of the compounds have significant activity as reactivators of inhibited acetylcholinesterase *in vivo* and *in vitro*. However, there is evidence of interspecific variation in effectiveness (11). The Hagedorn oximes, when co-administered with atropine, are effective against soman toxicity in dogs and rodents. They appear to have less activity in primates. This anomaly requires further investigation. The recent military use of tabun provides a further problem in selecting a second generation oxime as the product of choice at the present time. Conventional oximes such as pralidoxime and toxogonin remain the major candidates for inclusion in current autoinjectors. This selection has been discussed at length by Ligtenstein (11) who favours toxogonin as the product of choice, due to stability, activity, osmolarity and the general advantages of a dioxime.

NOVEL APPROACHES TO NERVE AGENT PROTECTION.

The onset of action of soman is so rapid that it is difficult to determine the site of primary activity. Basic research is required to elucidate the route and mechanism of soman toxicity. Detailed studies on enzymes may lead to improved understanding of this nerve agent and the means to prevent its adverse effects. Development of antibodies to soman (19) will facilitate these studies. Identification of a practical means for inhibition of acetylcholine production through modification of choline acetyl-transferase would provide a practical approach to reducing symptoms of organophosphorous toxicity.

CONCLUSIONS.

The results of the studies on therapy for nerve agent toxicity lead to the conclusion that :

1. Effective treatment of nerve agent poisoning requires combination therapy.

2. Atropine should be a component of any therapeutic regimen for organophosphorous poisoning.

3. No cholinesterase reactivator with acceptable toxicity is effective against all nerve agents.

4. Pretreatment with a reversible inhibitor of cholinesterase, eg. a carbamate such as pyridostigmine, in conjunction with a benzodiazepine improves protection and recovery from nerve agent toxicity.

5. Continued investigation of new therapeutic agents, established antidotes and novel approaches to combination therapy are required for development of an effective broad spectrum treatment for nerve agent toxicity. New oximes have particularly potential for inclusion in these antidotes.

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Problems associated with verification of alleged CBW use in Southeast Asia

by H.B. SCHIEFER and R.G. SUTHERLAND

Toxicology Research Centre, University of Saskatchewan,
Saskatoon, Saskatchewan S7N 0W0, Canada

SUMMARY.

A critical review of the alleged use of CBW agents in SE Asia reveals :

1. *The events have been poorly documented, and insufficient epidemiological data have been collected and presented.*

2. *Basic forensic requirements of collection and handling of specimens have cast doubts on the allegations.*

3. *Analytical difficulties have hampered the establishment of « undeniable proof ».*

4. *Lack of knowledge of natural background levels of the allegedly used toxin or events has cast doubts on the laboratory findings.*

5. *Lack of knowledge of toxic potential and pharmacokinetics of trichothecenes has made scientists and the public suspicious of the allegations.*

6. *The military usefulness of trichothecenes as a CBW agent is questioned.*

The above points are discussed critically in detail, and it is concluded that the major problems are of psychological and political nature, not scientific ones.

Prompt investigation of an allegation of use of CBW agents becomes practical only if there is a political framework (e.g., an International Investigation Organization), which allows for prompt action, access to alleged attack sites, competent collection of

specimens, and epidemiological investigations, and analyses of specimens in qualified laboratories.

A suitable master plan for investigations of alleged use of CBW agents could have been in place for many years, and it is not the lack of contributions by scientists, but the relative immobility of the UN and world opinion which have made it impossible to achieve an acceptable level of verification and control with respect to the prohibited use of CBW agents.

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INTRODUCTION.

Chemical warfare, in all its guises, holds a special significance for Canadians. When the first massive military use of chemical weapons was initiated in 1915 at Ypres, Canadian soldiers were amongst the first victims in the world of this new type of warfare. The event produced a psychological effect on generations of Canadians and, in part at least, accounts for Canada's seeming preoccupation with this issue.

The Prime Minister of Canada focused on the subject at UNSSOD II (1982), and cited verification as probably the single most significant issue in the Arms Control and Disarmament process in the decade of the 1980's.

Before one can verify a violation, one has to have methods to prove that a chemical or biological warfare agent was indeed used. At the present, false allegations cannot be disproven ; true allegations cannot be verified, particularly if the agent in question happens to be a substance which may or may not occur naturally.

Therefore, the debate over the alleged use of mycotoxins as chemical warfare agents, has now to develop into a discussion beyond the straight-forward question of whether mycotoxins were used or not used.

THE EVENTS IN SE ASIA.

Shortly after 1975, it was alleged that mycotoxins had been used as a weapon of terror and a method of warfare.

Mycotoxins are normal metabolites of fungi, and have found widespread use as antibiotics for the treatment of many diseases, but some mycotoxins are very toxic. What is novel about the

use of mycotoxins as CBW-agents is that these toxins might occur naturally in a given country, thus any verification procedure would have to ensure that natural background levels and associated diseases are well known and documented.

The basic script for the proper approach under such novel conditions was actually written already by Sipri in 1971 (1), when it was said that : « ... it follows that an extensive epidemiological investigation of the area in question is a *desideratum* if not a *sine qua non*. Such a study should encompass not only human beings but also animals and insects ». While this was written in the context of bacteriological warfare agents, it applies to biotoxins as well. The neglect of this basic prerequisite has caused much trouble in the evolving discussion of whether the US allegations were based on facts or not. The events in Southeast Asia were the first case ever in which the UN and the world community at large became involved in an attempt to verify charges of CBW use.

The early evidence consisted of oral reports from victims and eye witnesses and were obtained from refugees in Thailand. Still later, reports of CBW use emanated from Kampuchea and from Afghanistan. The symptoms described did not match closely those expected from conventional lethal and incapacitating agents.

If the allegations are true then it means that both the 1925 Geneva Protocol and the 1972 Biological Weapons Convention have been breached. Those are serious charges, and so require a strong case to be made to support them.

DISCUSSION.

A number of points have evolved as the main issues of the debate, and these points will be discussed now.

1. *The events have been poorly documented, and insufficient epidemiological data have been collected and presented.*

While it is understandable that the first reportings of attacks were not treated in a uniform or comprehensive fashion, a major critique is that most of the data submitted to the UN are poorly collected and documented. The most severe critique comes from Evans (2), who charges that interviews have been unevenly, not systematically, collected by personnel untrained in the art of ferreting out information without asking leading questions.

While this may be true in a number of cases, it is nevertheless not impossible to obtain some basic facts and information, from illiterate people, if one overlooks obvious misjudgements, e.g., with respect to the height or type of aircraft carrying out the attack, an error which is easy to understand.

What has become known as the second Canadian report (3) took most of these aspects into account and rendered a solid summary of the events, even though this was done in retrospect and yet was criticized for asking leading questions.

It is worthwhile to remember here that this critical hindsight after the first real test-case of an allegation differs markedly from views expressed a few years earlier. At a US Congress Committee on Foreign Affairs meeting in 1980, Meselson (4) said : « Verbatim transcripts and proceedings could be published, along with evaluations, commentary, and relevant background information. The very existence of such a body or of provisions could have a deterrent effect on potential violators. It could also discourage the making of ill-founded or malicious allegations ». Although the US documentation supplied to the UN can be called vague at times, trying to hide behind the screen of « classified information », by and large the US documentation, and to some extent the Canadian submissions, did precisely what Meselson suggested in 1980.

2. *Non-compliance with basic forensic requirements of collection and handling of specimens have cast doubts on the allegations.*

It is very easy to be critical on this point : as the events in a controversy between two parties unfold, it is not possible to collect evidence in the same thorough manner as is customary in an organized country during peace time. Nevertheless, no matter what one says, there remains a certain degree of doubt when samples have gone through a number of intermediaries and were collected and transported in containers which may release certain chemicals themselves, as the debate over the significance of finding polyethylene glycol shows (5).

3. *Analytical problems have hampered the establishment of undeniable proof.*

The analytical difficulties encountered by the UN Group of Experts have been described in their report (1983), but even within the USA, findings of trichothecenes by one or two laboratories

have come under severe criticism (6), because other laboratories could not confirm the findings or the methodology was questioned. Thus, the problem of very small samples, and most environment samples were either yellow droplets on leaves or yellow material scraped from rocks and other substances, a problem that is common to all forensic analyses, is compounded by the apparent lack of international standards for the analysis of trichothecenes.

4. *Lack of knowledge of natural background levels of the allegedly used toxin or other surrounding or circumstantial events have cast doubts on the laboratory findings.*

When the USA first published their allegations, it was stated that neither trichothecenes nor the fungi capable of producing such mycotoxins occur in Southeast Asia, because trichothecenes are products of fungi in temperate climates. This was promptly attacked by referring to occurrence of trichothecenes in cereal products in India (7). Despite the fact that there have not been any publications of natural occurrence of trichothecenes in food commodities in Thailand and surrounding countries, nor any publications on the occurrence of human or animal diseases with the characteristics of trichothecene mycotoxicosis from this area, the skeptics remain unconvinced. A particularly controversial issue has been the question of whether « Yellow Rain » is not just simply bee excrement (8).

It should be remembered that the probable diverse chemical attacks have become collectively known to the Hmong in Laos as « Tshuaj lom daj », literally, « yellow poison ». This is popularly, in the US and elsewhere, referred to as « Yellow Rain » and the words continue to be the source of much confusion. Bee excrement may indeed have the characteristics of yellow colored spots. But we don't have any evidence that there has been ever any report of disease or death associated with bee feces. Nevertheless, the lack of knowledge of the seasonal occurrence of bee excretions and the hypothetical possibility that bee excretions may contain trichothecenes is obviously an unknown at this point. Closely associated with the question of bee feces is the finding of pollen in « Yellow Rain » samples. Pollen in « Yellow Rain » samples has been described in a number of cases and continues to be debated as to its significance. Again, the lack of background information is very much felt.

5. *Lack of knowledge of toxigenic potential of fungi isolated from SE Asia, and lack of knowledge of pharmacokinetics of trichothecenes has made scientists and the public suspicious.*

Fungi of the *Fusarium* family occur worldwide, and their existence in Southeast Asia was known for at least 30 years (7). Little is known, so far, about the ability of these Fusaria to produce trichothecenes. The first investigations of this question are presented at this Congress at some other place. However, the lack of diseases due to trichothecenes, at least in Thailand, would indicate that trichothecenes are not produced, but the possibility of this potential has to be considered and should be further investigated.

Very little is known about the metabolism and pharmacokinetics of trichothecenes. The few studies that have been done seem to indicate that trichothecenes are excreted rapidly from the body. Under these circumstances, it comes as no surprise that the US Government statements (9) in which it is reported that trichothecenes in tissues of victims were found 1 to 68 days after an attack, have been received by the scientific community with considerable skepticism (10).

6. *The military usefulness of trichothecenes as CBW agents is frequently questioned.*

While it appears to be correct that trichothecenes may not be able to kill as instantly as some of the conventional lethal gases, and while it may be correct that relatively large amounts (when compared with the minute amounts required for conventional CW agents) have to be applied, trichothecenes may play a role as powerful agents for territorial denial and as a terror agent in general. The effects on humans are varied, and can cause severe disease and death, yet at the same time animals and plants are also damaged. Trichothecenes may not be the choice weapon in a highly technically developed country, but in third-world, subtropical or warm countries, the effects could be disastrous.

CONCLUSIONS.

If one tries to summarize what has been said so far, it appears that while not all is well with respect to the scientific handling of the situation none of the problems are unsurmountable.

What appears, however, to be the real problem is the political polarization («them against us») and the inability (or is it just a psychological hurdle?) to comprehend that mycotoxins can indeed cause severe problems.

For most medically trained people mycotoxins are continuing to be an enigma, a topic not taught during undergraduate years, and an area that ranks rather low when compared with infectious or social-attitudinal diseases for instance.

On top of this are the political aspects. Some speakers have already alluded to this problem. The inability of a good number of nations to engage themselves in the investigations, be it by simply denying to accept samples for analysis or to participate in investigations, or by signaling that they don't want to get involved in the debate, or by simply denying an entry permit to a UN-expert team, are examples.

Maybe it is the lack of an existing political framework which is at the heart of all these problems, not the inability or ineptitude of scientists. The concept of a framework for investigation of violations of the CBW-treaties is not new: if one reads through all the documentation on the topic of verification, all the transcripts of the discussions of this theme in Disarmament sessions, one finds out that there have been many excellent proposals in the past, and one gets the impression that a suitable and effective master plan for investigations of alleged use of CBW agents could have been in place for many years already.

For instance, an International Investigation Organization (11) could be created as a standing agency, ready to go into action whenever a complain is lodged with the Secretary-General of the UN. This organization must have, of course, access to alleged attack sites, must be competent in the proper collection of specimens, must be able to carry out epidemiological surveys correctly and efficiently, and must possess, or have access to, qualified laboratories.

One could dream of a truly independent and novel organization of this nature, to be created and set up in a suitable place. To create such a new organization may appear to be a major financial burden in view of the hopefully very limited number of possible violations of the CBW-treaties, but one could put this organization to work by carrying out investigations of major environmental disasters (12). This would keep the members of the organization «on their toes» and alert all the time.

Failing to be able to finance such a new organization, one could try a decentralized approach, in which a skeleton staff in the UN would be able to activate a network of laboratories and experts from countries all over the world, very much in tune with what Dr. Dutt from Singapore suggested yesterday.

Shortly before the events in the Middle East ever occurred, I was much more pessimistic in my assessment of the potential to achieve such a worldwide collaboration. The multinational collaboration which occurred actually in this particular instance, and the fact that this World Congress is taking place, make me much more optimistic. I think we can achieve the goal of a worldwide collaboration in verification of CBW use.

In closing, I would like to express not only my thanks to you for listening patiently, but also to Prof. Heyndrickx, his Organizing Committee and the Government of Belgium for calling us together and making us talk, and hopefully listen, to each other.

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Bacteriological determination of samples collected in gas warfare of Iran

by B. MROZOWSKY

Department of Bacteriology and Virology, University Hospitals,
State University of Ghent, Belgium

In the beginning of April 1984, samples collected in the gas warfare in Iran were handled over to me by the Service of Prof. A. Heyndrickx (Department of Toxicology, State University of Ghent, Belgium).

These samples consisted of :

1. A little quantity of liquid known as Yperite, taken from a bomb on the battlefield in Iran.
2. A few grams of earth, soaked with the same substance, both in a quantity that can make possible each bacteriological examination.

We did not proceed to a direct microscopic examination and we confined ourselves exclusively to the use of bacteriological cultures.

Our interest was directed to the aerobic and anaerobic flora, important in human pathology.

To assure a possible development of each pathogenic bacterium, we used for the first sample, this means liquid from the bomb, the solid mediums of :

a) blood-agar, one of the most useful laboratory mediums, since practically all bacteria grow well on it ;
b) glucose-cystine-agar for the more delicate organisms ;
c) eosine-methylene blue-agar for the eventually possible enterobacteria ;
d) liquid medium of Thioglycollate, used by excellence by the Federal Security Agency for Food and Drug Administration for the sterility testing.

For the earth, soaked by the Yperite, especially to reduce any manipulation that could contaminate the sample, we only used liquid Thioglycollate, just like we did for the first sample.

All the sown mediums were incubated on a temperature of 37°C.

RESULTS.

1. In the cultures of the first sample (liquid from a bomb) controlled each day, during 3 weeks, we did not note any development of microbean flora in any of our culture mediums.

2. The cultures of soaked soil also were sterile during the 16 days. The 17th day we discovered a small turbidity in the Thioglycollate-broth.

By microscopic examination (of the stained preparation) we found the presence of Gram-positive rods. The subculture on the blood-agar-plate and the new Thioglycollate-broth allowed us to determinate a non pathogenic Bacillus Subtilis, a microbe we can easily find anywhere in nature.

Our culture bottles had not been opened from the moment of inoculation until the appearance of the turbidity.

A laboratory contamination then of course is excluded.

The soil did not contain any pathogenic bacteria. The presence of Bacillus Subtilis certifies that the small concentration of Yperite in the soil-sample could not exterminate, or desinfect any bacteria.

The period of 16 days was probably necessary for the adaptation of bacteria to the medium partially sterilized by a low concentration of Yperite.

We did not know this concentration.

To conclude...

Concentrated Yperite seems not to be able as vehiculum for a microbean flora, but in low concentration it cannot sterilize the total bacterial development.

We have not practised any virological examination.

This preliminary publication cannot resolve many problems, but it seems that a collaboration between a Laboratory of Toxicology and a Laboratory of Bacteriology and Virology could be advised.

Autopsy observations
in an Iranian soldier exposed to war gas

by F. D'HALLUIN and H. ROELS

Institute of Pathology and Morbid Anatomy,
State University of Ghent, Belgium

SUMMARY.

The autopsy findings in a 43 years old Iranian soldier, exposed to war gas 15 days before death, are reported. The macroscopical observations can be summarized as follows : the most important lesions are found in the skin and the respiratory tract = extensive burns and defects of the thoracal, shoulder, abdominal and genital region, erythema of the face and neck, oedema of the head, neck, hands and feet, hemorrhagic tracheitis and bronchitis. Other lesions are acute ulceration of the bulbus, generalized hyperemia of the mucosa of the gastrointestinal tract, congestion and oedema of the lung.

Microscopical examination discloses necrotizing bronchitis in recovery phase, only slight leucocytic infiltration around the skin defects, limited bronchopneumonia, marked vasodilatation of the mucosa of the gastrointestinal tract, atrophy of the lymphoid apparatus and sequellae of an earlier tubular necrosis.

CLINICAL HISTORY.

The patient, 43-years old, was admitted into a hospital in Teheran after exposure to mustard gas. Acute respiratory distress syndrome, polymucositis and bullous dermatitis were diagnosed. He was transferred subsequently to the Academic Hospital in Ghent with symptoms of necrotising bronchitis and leucothrombopenia.

He died from septic shock 15 days after the injury.

MACROSCOPICAL FINDINGS.

External examination revealed erythema and oedema of head and neck, and extensive skin lesions consistent with chemical

FIG. 1. — Severe skin lesions with denudation of the dermis.
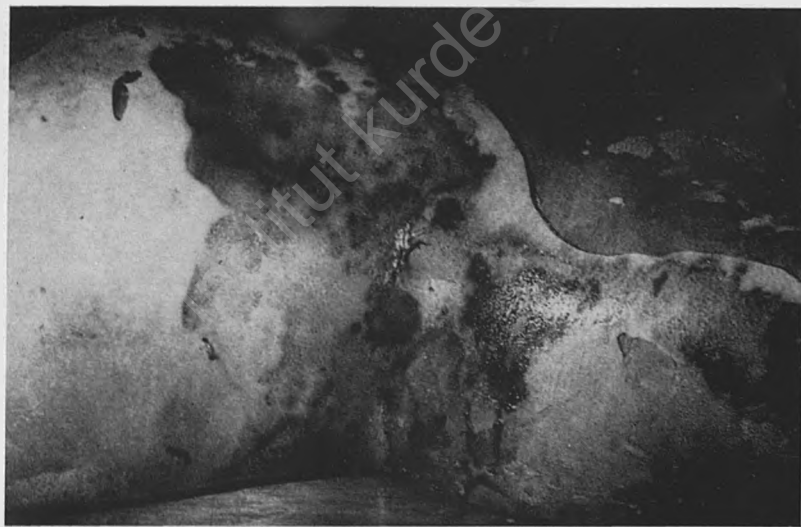
FIG. 2. — Severe skin lesions with denudation of the dermis.

burns on shoulders, thorax, abdomen and genitals. Some skin defects were large, with denudation of the dermis (fig. 1, 2 and 3).

On internal examination the most obvious lesions were seen in the upper respiratory tract. The lungs were partially well expanded and showed a moderate degree of anthracosis.

FIG. 3. — Severe skin lesions with denudation of the dermis.

FIG. 4. — Ulcerated mucosa with formation of membranes.

Larynx and trachea disclosed and ulcerated mucosa with formation of membranes (fig. 4). On section the lungs were oedematous and congested, both lower lobes showed foci of consolidation.

Inspection of the gastrointestinal tract revealed a generalized hyperaemia of the mucosa.

The liver and kidneys were congested, the mucosa of the bladder was hemorrhagic.

The other organs, including the brain, did not show remarkable alterations.

MICROSCOPICAL FINDINGS.

Paraffin-embedded histologic sections from every organ were stained by the haematoxylin-eosin technique.

Microscopical examination of the skin lesions showed large defects of the epidermis, covered by fibrin. There was bacterial overgrowth but no inflammatory reaction (fig. 5).

Between these defects the epidermis was thin and consisted of only 4 layers. Irregular nuclei, hyalin bodies and intraepidermal and subepidermal vesicles were observed (fig. 6).

Microscopically, the most obvious lesions were seen in the upper respiratory tract. The covering epithelium was necrotic in several places and one could see active reserve cells. There was extensive fibrinous exsudation and incorporation of fibrin in the stroma, where reactive fibroblasts with irregular, hyperchromatic nuclei were proliferating (fig. 7). There was a moderate inflammatory reaction. These alterations were seen principally above the level of the small bronchi. Multiple sections of the lungs were made. There was a moderate degree of oedema and congestion of the alveolar capillaries. In the lower lobes we observed foci of bronchopneumonia with numerous neutrophils and a small foreign body granuloma. The microscopical examination of the gastrointestinal tract disclosed only a marked congestion of the vessels.

The hepatocytes contained some iron and fat droplets. We noticed in the kidneys sequels of an acute tubular necrosis. Some tubules were covered by regenerating epithelium and contained protein and pigment casts.

The lymph nodes showed a striking degree of lymphoid depletion. We saw small lymphoid follicles without germinal centers and depletion of lymphocytes in the paracortex (fig. 8).
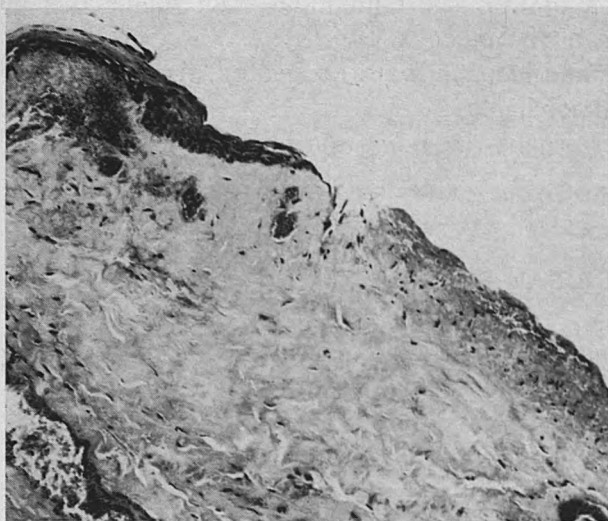
FIG. 5. — Large defects of the epidermis, covered by fibrin, and without inflammatory infiltration.
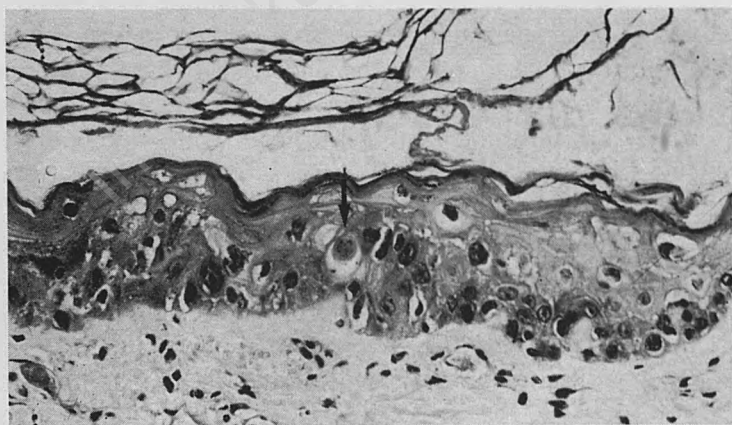
FIG. 6. — Thinned epidermis, irregular nuclei and a hyalin body.
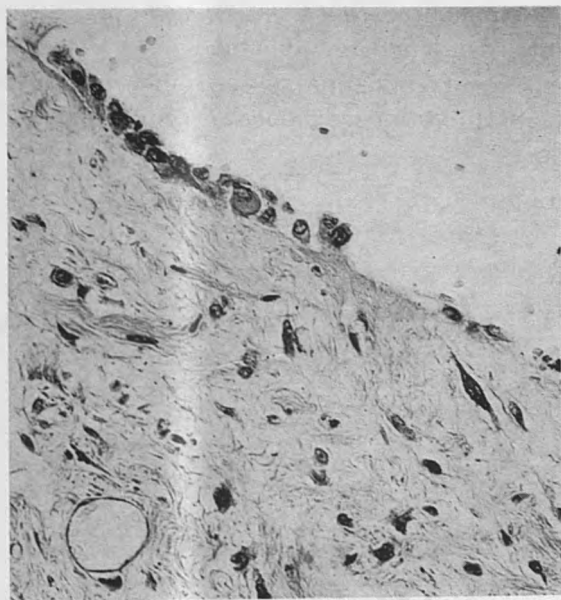
FIG. 7. — Laryinx : covering with reserve cells, reactive fibroblasts in the stroma, reduced inflammatory infiltration.
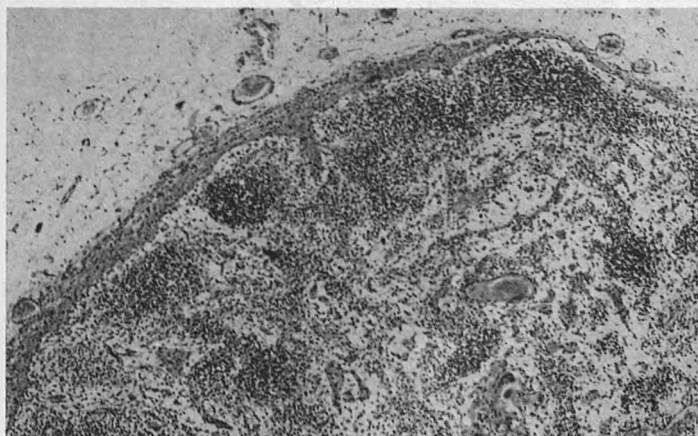
FIG. 8. — Small lymphoid follicles, without germinal centers, depletion of the paracortex, large sinuses.

Immunoperoxydase demonstration of J-chain confirmed this observation. The Malpighi corpuscules of the spleen were also inconspicuous and contained few lymphocytes.

Histopathological examination of the other organs did not disclose remarkable alterations except for congestion and dilatation of the vessels.

CONCLUSION.

The clinical picture and the general histopathological findings, but especially the lesions of skin, respiratory tract and lymphoid tissue are consistent with poisoning by mustard gas.

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Clinical manifestations
of chemical agents on Iranian combatants
during Iran-Iraq conflict

by H. SOHRABPOUR

Labafi-Nejad Medical Center, Tehran, Iran

SUMMARY.

Since the beginning of Khaibar operation, more than 350 patients have been admitted in Labafi-Nejad Medical Center in Tehran.

All these patients were of two major groups :

A. Those who suffered mainly from skin burns, eye irritation, respiratory symptoms and hematological complications.

B. The second group suffered mainly from neuromuscular symptoms. In this report we have tried to explain major medical problems and discuss our experience regarding the treatment as well as the complications of these chemically wounded patients.

INTRODUCTION.

Since the beginning of the imposed war on September 1980, Iraqi invadors have used chemical weapons repeatedly. From the beginning of the Kheibar operation (in March 1984) chemical agents have been used against Islamic forces in a large scale. These savage attacks have led to a number of mortalities and morbidities which have been a new experience for Iranian physicians who are dealing with war wounded patients.

In this report we tried to summarize the clinical manifestations of these chemical agents on our patients and the modalities we adopted to treat them and also the complications that these patients have developed so far.

From the beginning of the Kheibar operation until the time of this report, a total number of 528 chemically wounded patients has been admitted in the Labafi-Nejad hospital.

These patients have been of two major groups :

A. Those who had skin burns as well as ophthalmological symptoms, respiratory and GI problems and hematological complications.

B. Another group who suffered mostly from neuromuscular symptoms.

In view of the fact that more than 90 % of our patients belong to the first group, this report is limited to these patients only.

According to the history taken from these patients the main source of chemical agents has been air bombs and artillery shells.

First all patients noticed a garlic smell and after an interval of 3 to 4 hours an eye irritation and burning sensation ; then lacrymation started. These symptoms were followed by blurred vision and photophobia. On physical examination there were signs of Blephro Kerato conjunctivitis and myosis as well as corneal abrasions. Of all cases only two had corneal ulcers and two had Irritis plus Noso Lacrymal duct obstruction due to severe lid burns.

SKIN LESIONS.

After an interval of 8 to 24 hours the skin lesions appeared. The first symptoms were especially an itching and burning sensation in most areas of the body such as armpits, base of the neck and genital area. Initially they developed erythema and gradually during 2 or 3 days blisters appeared which contained a yellowish fluid. Most of the skin burns were of grades 2 and 3 and covered between 15 to more than 60 % of the body surface. All parts of the skin were affected except soles and palms.

Pigmentation and black skin discoloration was noted in 1/3 of the patients.

RESPIRATORY SYMPTOMS.

There were two major syndroms as far as the respiratory system was concerned.

1. *Upper airway involvement :*

These symptoms developed after 24 to 48 hours and consisted of burning sensation in mouth, nostrils and pharynx dysphonia, cough and sensation of choking. Physical examination including boryngoscopy revealed signs of pharyngitis and borynigitis. We did not notice any ulcerative lesion in the mucosa, and not any of our patients did have hemoptysis.

2. *Lower airway involvement :*

This group of patients was in a more critical state. They had shortness of breath and sensation of tightness in the chest. Cough was more severe and continuous. Physical examination revealed tachypnoea sometimes over 35/minute, as well as tachycardia. Their blood pressure was unstable unless they developed respiratory arrest.

Examination of the chest revealed adequate air entry, no evidence of consolidation, but wheezing and rhochi invariably could be heard and in some cases fine crepitations on lung bases were present.

Cough was productive and sputum was mucoid. As mentioned before, hemoptysis was not a common finding.

Chest X-rays on these patients were essentially normal unless for congestion in hilar areas which could have been secondary to persistant cough. In some cases of fever, repeated chest X-rays showed pulmonary infiltrates most probably due to secondary infections.

In one of our patients who eventually was intubated and managed on mechanical ventilation, multiple patchy infiltrates developed which did not respond to antibiotics. Among these groups of patients mortality was higher, especially when it was complicated with leukopenia and secondary infection. Blood gas analysis on these patients invariably showed severe hypoxemia PO_2 (50 mm Hg) and increased (A-a) PO_2, irresponsive to oxygen therapy, which could be due to increased shunting.

Although some of these cases could be managed conservatively, the serious cases had to be intubated and mechanically ventilated. PEEP was applied in cases in which hypoxemia could not be overcome by usual means.

G.I. SYMPTOMS.

GI symptoms were less frequent compared to other symptoms. Most patients did have nausea, and a few had vomiting.

Dysphagia was not an infrequent symptom, especially in the first 24 to 48 hours.

GI bleeding was rather rare. Only 5 of our patients developed GI hemorrhage. One among them had previous duodenal ulcer and one had gastro-enteritis with bloody diarrhea. Others had massive upper and lower GI bleeding manifested by hematemesis and melena. Unfortunately these patients died. In one of them post-mortem examination showed a large stress ulcer in duodenum as well as bleeding all over the GI tract especially in descending colon and sigmoid.

We did not diagnose any clinical hepatitis nor did we have any significant derangements in liver function tests.

HEMATOLOGICAL FINDINGS.

Hematological findings were among the most serious complications in these patients.

Leukopenia was the first manifestation to appear which happened between 10 days to 2 weeks after exposure.

Thrombocytopenia and anemia followed later if patients survived.

We had cases in which WBC count dropped to less than 1,000 per cubic mm.

Although most of these patients had severe skin burns, we came across cases who had minor skin lesions and yet developed leukopenia.

Bone marrow biopsy revealed hypocellular marrow involving all elements.

Iron stores were normal.

Aside from thrombocytopenia, coagulation profile was normal.

Infective complications occurred in some patients who had severe and large skin lesions.

Skin infections and in some cases pneumonia was the most common type of infection especially when patients developed leukopenia.

Pseudomona and streptococci were among the most common organisms isolated from the patients.

NEURO-PSYCHIATRIC COMPLICATIONS.

These consisted of agitation, insomnia, depression and muscle weakness particularly in lower limbs.

No focal neurological abnormalities could be elicited.

TREATMENT.

Because of the fact that our patients had been taken care of immediately after exposure in the war zone, we treated their problems according to a protocol which is as follows :

Ophthalmopathy :

a) Irrigation with at least one liter of ringer solution.

b) Application of 1 % cyclopentolate QID.

c) Application of 20 % sulphacetamide solution QID.

Corticosteroids were not used until the healing of the corneal epithelium took place completely.

Because of photophobia and irritation at times eyes were kept closed for the first 24 to 48 hours.

Almost all of the patients recovered without any significant complication.

Dermatopathy :

IV solutions were used according to the state of hydration, as well as the serum electrolytes.

Blisters were aspirated but the epiderm was left intact only to slough naturally.

Daily bathing with tap water followed by rinsing the affected areas with normal saline.

Furanit ointment was used for infected skin burns.

Flammazine ointment was applied on necrotic areas, and hydrocortisone ointment only when there was a severe inflammatory process.

Skin left open while using sterile sheets.

High caloric diet was given to all patients.

Complete recovery took place within 15 to 20 days and none of the patients needed skin grafts.

Pneumopathy :

We used vaporizer and moist oxygen in those who had upper airway involvement.

Bromohexine as mucolytic and codeine compounds for cough suppression were utilized.

Antibiotics were not used unless we were convinced that the patient had got pneumonia. In such cases a combination of gentamycin either with cephalotins or carbenecillin were used and the results were satisfactory.

In severe cases of respiratory distress and hypoxemia, endotracheal intubation and mechanical ventilation sometimes with PEEP was performed.

Unfortunately the outcome of this complication is not favorable and almost all of those who lost their lives developed this syndrome as a terminal event.

It is apparent that the chemical tracheo-bronchitis in these patients is very difficult to treat, as it does not correspond well to any type of therapy.

Pulmonary function studies in some of the cases with persistent cough so far, have shown moderate to severe obstructive patterns with decreased FVC, FEVI and FEF.

Bone marrow depression :

As soon as WBC count dropped to less than 2,500, leukocytes, transfusion started, giving one unit of cross matched leukocytes every 12 hours. If leukocytes were not available then whole fresh blood was substituted, two units for each unit of leukocytes.

Reversed isolation and broad spectrum antibiotics were utilized.

CONCLUSION.

The chemical agents used by Iraqi invadors against Islamic forces, have a devastating effect on many organs of the human body. Aside from those already mentioned, according to the literature including the WHO report on Health aspects of Chemical and Biological Weapons, these highly toxic materials may have teratogenic as well as carcinogenic effects.

Respiratory and hematological complications are potentially fatal, and could be long lasting. Although many of the victims of these savage attacks have been discharged, long term follow-up

is required in order to assess the morbidity and even mortality rates.

According to the Geneva Protocol (1925) and also the Convention of 1972, which have been signed by more than 100 countries, production and storage of these weapons are banned but unfortunately superpowers supply their merceneries with this kind of weapons. I believe that not only the users, but also the producers and suppliers of these weapons should be condemned. WHO and other responsible and free minded medical communities should try to do their best to put an end to the use of these weapons which are serious threat not only for human community at present, but also for those to come.

Clinical observations and therapy of injuries with vesicants

by F. COLARDYN and J. DE BERSAQUES

University Hospitals, Ghent, Belgium

We had recently the opportunity to observe and treat five Iranian patients who were the victims of an exposure to vesicants.

The incident was described as more or less four explosions from bombs dropped by airplanes. After the explosion there was a black rain. The contact with this black rain was several hours (2 to 7) and was followed by suffocation, loss of consciousness and later on irritation of the eyes and the throat with irritative cough and dyspnoe. There was also itching of the skin.

The following day the patients were transferred behind the front-line and after 48 hours they were sent to a hospital in Teheran.

At the moment of the exposure they had no special clothing and a gas mask was only used for a few moments and not by all soldiers.

In the hospital in Teheran the patients received intravenous fluid, bronchodilator therapy and antibiotics.

Some received also corticosteroids and all had local therapy for the skin and the eyes. One received blood transfusions.

Ten days after the exposure, the patients were transferred to the University Hospitals of Ghent.

CLINICAL STATUS ON THE ADMISSION.

All patients showed extensive skin lesions which can best be compared with chemical burns. Two of them had an involvement of more than 60 % of the skin surface and in these two patients there was also a sign of Nikolsky. The dermis was denuded and a skin biopsy showed that there was also necrosis of the upper dermis. Less severely damaged areas showed alternating zones

of pigmentation and depigmentation. The other three showed an involvement of 25 to 30 % of the body surface.

The eyes showed signs of irritation but no deep inflammation. All had signs of pulmonary involvement with irritative cough and bronchopneumonia with dyspnoe and low arterial pO_2. Most of the patients had also complaints about pain in the throat.

There was one patient with inflammation of the small and large bowel and one patient complained about polyneuropathy of both legs. On biochemical screening there were signs of deshydration at the admission with a slight renal and liver impairment. Two patients showed leucopenia with neutropenia and all had a lymphocytopenia. Besides the polyneuropathy in one patient there were also signs of neurological involvement with mental clouding in the one patient who died the sixth day.

Multiple cultures were done : the skin showed several potential pathogenic microbes ; on different days haemocultures were also positive with up to three different microbes, some with the same pathogen as on the skin.

Sputum cultures were positive in all patients.

TREATMENT.

The treatment can be divided in two parts :

1. A general treatment as usual in septic patients with signs of deshydration and denutrition (most of the patients had lost 10 or more kg).

2. A treatment with the aim of eliminating the eventual toxic agent(s).

Rationale.

From the clinical signs and the description of the exposure it was evident that the patients had been exposed to vesicants, most probably with Yperite (sulphur mustard gas). Unfortunately it was not sure if that was the only chemical involved in the exposure and among others there was some concern about the presence of Mycotoxins. Therefore besides the therapy of the lesions provoked by Yperite we planned to treat the patients for one or more eventual unknown toxic substances.

Therefore the following therapy was instituted :

1. Rehydration with intravenous fluids, combined with vitamins, high-caloric nutrients and aminoacid solution.
   Antibiotics were instituted as necessary, guided by the results of the cultures.

2. Toxicological therapy :

   a) activated charcoal was given orally, 40 grams every 4 hours, until there was a complete gastrointestinal transit of the charcoal ;

   b) the skin was soaked at least six times a day with a solution of chloramine ;

   c) intravenous acetyl cysteine was given the first week in divided doses ;

   d) three times a week single needle haemoperfusion over coated activated charcoal was performed for 2 or 3 hours during a period of two weeks (Prof. Dr. S. Ringoir).

3. Therapy of the skin lesions : the first day the skin was treated with frequent wet dressings of chloramine solution ; after 4 or 5 days the patients were changed to a treatment with silver-sulfadiazine cream (Flammazine®).

TOXICOLOGICAL RESULTS.

These were done by the laboratory of Prof. Dr. A. Heyndrickx, who will present his results.

EVOLUTION AND RESULTS.

One patient died the sixth day after admission in an overwhelming septic shock and signs of ARDS with stiff lungs. This was the patient with mental clouding on admission who never regained full consciousness and who also had very extensive skin lesions.

The other four patients showed a slow and progressive improvement and the signs of sepsis subsided around the tenth day after admission.

All antibiotic therapy could then be stopped.

The skin lesions also improved very rapidly.

The signs of bronchopulmonary injury also subsided but hoarseness and an irritative cough was still present in one patient at his release of the unit.

The last two patients could be released from the Academic Clinic after 37 days.

We can resume the lesions at the end of their stay as follows :

1. Pigmentation changes of the skin with diffuse but superficial scarring ; one patient also showed some bullae.

2. Signs of bronchial involvement which can best be described as the clinical picture of chronic bronchitis.

3. Slight biochemical alterations, for example an increase of the gammaglobulines.

Report of the specialists appointed by the Secretary-General of the United Nations to investigate allegations by the Islamic Republic of Iran concerning the use of chemical weapons

Comments by FOROUTAN ABBAS

Islamic Revolution Guards Corps. Medical Department.
Bedhari-e.Sepah (Block 6). Ghasr-e-Firoozeh. Piroozi Ave. Tehran - Iran

Despite the fact that the utilization of chemical weapons has continued ever since World War II, detailed scientific reports of these instances by International Organizations are, unfortunately, very scarce. According to the SIPRI 1982 Yearbook, between the years 1974 to 1982, aside from Iran, chemical weapons were deployed in 11 locations throughout the world, but complete and accurate accounts were not made available.

Following the Islamic Republic of Iran's repeated requests to the United Nations Security Council, a group of specialists was finally despatched to the area to investigate Iran's claim of the deployment of chemical weapons by Iraq. During their 6-days stay in Iran, the group investigated the issue from three viewpoints :

1. Munitions.

2. Chemical.

3. Medical.

The structure of the bombs revealed that they were not of the ordinary type ; they were meant to carry a liquid, and upon explosion, expel their contents in the form of a vapour or fine particles over a large area. A sample of the chemical contents inside the bombs at the war zone revealed the presence of at least two substances, mustard gas (a vesicant agent) and

tabun (a nerve gas). For medical purposes, a number of the martyrs and wounded victims of the incident, both at the war fronts and in Tehran, were examined. All clinical symptoms and postmortem examinations conclusively showed the use of a blister-causing agent, as well as a nerve gas, probably of the Organophosphorous type.

Having been a medic for the Pasdaran at the war fronts, I obviously was a direct witness to the bombings and would cooperate with the team of specialists from the UN. Before taking a closer look at the UN's report, it must be noted that it is only a small part of what has taken place in Iran, taking into account only those events which occurred between 13-21 March, 1984 ; that is, during the team's stay in the country.

I will firstly mention the history of chemical weapons deployment in Iran, then consider several points in the UN's report, and finally discuss the chemical bombardments which still take place today, after the condemnation of Iraq by the UN.

Ever since the outset of the war on September 22, 1980, up until the 26th February, 1984. Iraq utilized chemical weapons against Iran in 49 instances. We will first consider the most important of these attacks.

In August 1983, Piranshahr and the surrounding areas were subjected to chemical bombardments 7 times (these attacks occurred a few days after Iranian forces recaptured several important heights overlooking a number of Iranian cities). Two groups of victims were examined : the first group having been exposed to the chemicals at 6.30 p.m. local time on August 8, and the second at 7.00 a.m. on August 9. Of course, a large number of local residents had been exposed also, and were hospitalized in the same area. From the total number of victims, 41 persons who were in a more critical condition were transferred to Tehran. The event was as follows : after the passage of the airplane and the explosion of the bomb, a cloud of dust and smoke covered the area, and small, oil-like particles settled on the ground, objects, clothing, vehicles, etc. A smell of Garlic was widely reported. During the first few hours, no symptoms arose, but gradually after that, the eyes became red and a sensation of burning, lacrimation and photophobia set in. This was followed by severe vomiting which lasted at least 2 hours, and nausea which lasted up to 12 hours. A shortness of breath and coughing was also experienced by some, which remained for several weeks.

Manifestation of dermatological signs began 5-7 hours after contamination in the form of erythema and burning sensations, and the blisters usually developed between the first and third day. Symptoms of the eyes included conjunctivitis, diffuse superficial keratitis and chemosis, resulting in temporary blindness which passed, in most cases, within 7-10 days. Pulmonary symptoms were severe in several victims, three of which had fever, leukocytosis, polynucleosis, and an overall critical condition. One of these had a purpuric rash on the lower leg region.

In any case, all victims achieved relative recovery and were discharged from the hospitals. No military goal was obtained by the Iraqi forces in this chemical bombardment, and all strategic heights and positions in the area are still under Iranian control.

In October 1983, several days after victorious operations by Iranian forces in which another series of strategic heights were captured, Iraq again resorted to chemical bombardment. In addition to military areas, this attack included cities such as Marivan and Sardasht (on October 25) and Baneh (on October 30), as well as surrounding villages. Despite the vastness of the attack, Iranian forces, being prepared this time, were injured much less and the number of casualties was very low. Again, Iranian positions remained unchanged, and no setbacks were inflicted. However, the situation in the cities and villages was another story ; the number of martyred and wounded being greater, because the civilians were not expecting the chemical bombardment. Several of these victims were from Bayanjan village which was bombarded with mustard gas on October 25. After 48-72 hours, 9 of these persons achieved martyrdom in hospitals, the main cause of their deaths being acute pulmonary edema. Several soldiers also died of this cause. Upon interviewing several villagers who were victims of the attack, I was told that they were busy at work outside the village, and when they returned in the afternoon, they witnessed that the people were vomiting and some had died with bloody froth having been expelled from their mouths.

In November and December 1983 and also January 1984, chemical weapons were again deployed against Iran but to a more limited extent, until an important Iranian offensive (the Kheybar operations) was initiated and important military objectives and strategic positions were obtained. Several days later, on February 26, Iraq's widespread and heavy chemical bomb attacks began,

which were unprecedented in the more than four years of war between the two countries.

My colleagues and I were in the postoperative care department of a field hospital on February 26 when in the afternoon, we witnessed a large number of soldiers with severe vomiting being brought to the hospital. Their uniforms and equipment were contaminated, and a sharp odour was distinctly present. Their eyes were extremely red and several victims had hematuria. After decontamination, they were transferred behind the lines.

At approximately 2.00 a.m. in the Shatt-e-Ali region (area number 1 which was investigated by the UN team of specialists on March 14), we were able to locate one of the contamination sites after searching for several hours. From a distance of several kilometers from that area, the smell of mustard gas was distinct. At the site of the bomb's explosion, the soil had become mixed with the mustard liquid and had taken the form of black, muddy soil. Fine black particles in the form of dots (which was mustard gas) could be seen on the surface of the water. The suitable environmental conditions, especially the absence of wind and rain, and suitable temperatures, contributed greatly to the effect of the gas. The entire area was subsequently cleaned by the decontamination teams.

Then we returned to Ahvaz and took the chemical bombardment victims to the Takhti Infirmary for treatment (a sports stadium that had temporarily been placed at the disposal of the Pasdaran). The day after, I contracted severe headache, severe pain in the frontal sinuses, lacrimation, photophobia, rhinorrhea and severe dermal itch. Also, vesicals formed around my waist, and dermatological signs such as papules and rash ensued. (This was probably due to being allergic to mustard gas, because on August 9, 1983, upon touching a sample of soil contaminated with mustard gas from the bombardment of Piranshahr, I developed blisters which quickly healed). From that day until the arrival of the UN team (March 13, 1984) a total of 10 major chemical attacks took place, leaving behind critical victims. (The actual number of attacks was greater, but I will only mention here those cases which caused substantial human injuries).

On February 26, a total of 4 areas were chemically bombarded. Before the attack, conventional bombs were dropped on the area probably to determine wind direction and to precise conditions of the chosen area to be bombarded. The attack was carried out

with approximately 8 jet fighters. After explosion, a cloud of white or yellow smoke would cover the area and various odours such as that of fresh greenery (as in freshly cut grass), garlic, chlorine or rotting were reported.

At 4.00 p.m. the same day, a different type of chemical bomb was deployed which upon explosion, at first emitted a cloud of yellow-coloured smoke, and after that a number of small compartment-like projectiles were shot into the air from inside the bomb. These in turn began spinning, while emitting a smoke from themselves which covered the area, and later fell to the ground. The victims of this attack mainly had eye and respiratory complications and none developed dermal blisters such as those induced by mustard gas, but rather, developed small vesicular skin eruption on the face and neck which began to desquamate in 2-3 days.

A chemical bomb similar in structure to this was described in Sterling Seagrave's book « Yellow Rain » in description of the attack on Alkomeh in Yemen.

The next day, there were at least another 3 instances of chemical bombardment ; the most intensive attack having taken place at 7.00 a.m. in the zone of Shatt-e-Ali where the chemical material in the form of tiny droplets poured down on the soldiers. At least 100 bombs were utilized during these 2 days (not considering the bombs which fell in the water of Hur-al-Hoveizeh), and about 1,000 people were injured, of which about 150 were in critical condition. There were 1,748 persons suffering from the chemicals by the 14th March (when the UN commission arrived). Of course, most of them had received treatment and had recovered enough to continue their duties, but some which required further treatment had been transferred to Tehran. After continuation of chemical attacks, there were other victims which were sent to the hospitals and who were examined by the UN team.

On March 14, the site of the construction Jihad was hit by chemical artillery fire (the construction Jihad is a compendium of non-military volunteers which serves to rebuild war-ruined cities and villages, as well as pave roads and other various agricultural and industrial activities). Several of the victims of this attack have been reviewed in the UN commission's report (Appendix VII, cases 1, 2 and 3).

After penetrating the roof of the structure in which they were situated, the chemical artillery shell directly struck Alireza

Sedighi on the leg, completely crushing his leg and inficting a deep wound, filling the wound with mustard liquid. Aside from him and the afore-mentioned three cases, 46 of the volunteer forces were very severely wounded.

Case 1 : Hamid Reza Rezaii : an engineer in the « Jihad » who aside from dermal blisters, had respiratory problems, corneal abrasion in both eyes and severe chemosis. (His corneal abrasion was seen after staining with fluorescein in the video tape which was shown at the congress).

Case 2 : Mostafa Hezardastan : another engineer in the Jihad who also had severe respiratory complications and fever in addition to large blisters and corneal abrasion. The inferior lobe of his right lung had developed atelectasia. On the 24th March, 1984, in the ICU of the Academic Clinic of Ghent, Belgium, he achieved martyrdom due to septic shock.

Case 3 : Mohsen Shariff : aside from numerous blisters and pulmonary edema, he suffered from confusion and hypotension. He also achieved martyrdom few days later in the ICU of Tehran's Heart Hospital due to respiratory failure.

One case of the Jihad's victims however, for whom the UN team unfortunately did not find enough time to examined him, was Alireza Sedighi ; that is, the same person who was directly hit by the artillery shell.

His general condition was very bad and he was completely disoriented. Approximately 20 hours after contamination, his blood pressure dropped to zero. With treatment, his blood pressure rose back to 12 and he partially regained his awareness, but he had no urine output. With treatment efforts however, his urine output was brought to normal. Nevertheless, his overall condition grew worse each minute and his entire body developed inflammation. His blood pressure was still unsteady and again dropped a few hours later. The patient had severe pulmonary edema and bloody froth would continuously be discharged from his trachea. His lungs showed severe resistance to artificial respiration with an ambu bag. He gradually developed epistaxis, followed by two cardiac arrests, and recovery measures could not revive him the second time. He achieved martyrdom at 2.00 p.m. on March 15, 38 hours after exposure.

Case 6 : Hojjat Dastjani : his major complication was **respiratory** complication, along with cough and hemoptysis. His general condition was gradually improving until on April 3, 26 days after exposure and being near discharge from the hospital, he underwent cough, hemoptysis and cardiac arrest and achieved martyrdom.

Case 7 : Aliyar Eslampanah : I saw this patient about one month after the attack, at Tehran's Labbafi Nejad Medical Center. His WBC's had dropped to 400 and he suffered from severe respiratory difficulty. Fortunately, his blood complications subsided, but recurrence of his respiratory difficulties still persists.

Case 8 : Sohrab Noroozi : this patient was one of the most critical victims. Within 5 days, he developed pulmonary edema, severe respiratory insufficiency and septic shock, and subsequently achieved martyrdom.

Another victim who was wounded along with Sohrab Noroozi by the name of Ali Juma Daryani, also died within a few days in Ahvaz, and underwent a complete autopsy in the presence of one of the UN specialists.

Case 25 : Mohammad Hassan Kokabian : he achieved martyrdom on the 16th March at the Motahary Hospital.

Case 26 : Abdulkarim Reaisi : he achieved martyrdom on the 19th March at the Motahary Hospital.

On the 17th March, that is at a time when the UN team was still in Iran, three chemical attacks utilizing the gas « Tabun » took place ; two at 11.00 a.m. and the third at 5.00 p.m. Cases numbers 32-37 in appendix VII of the UN report are victims of these attacks.

In the treatment of these victims, Atropine and Obidoxim was administered, with very good results. The total number of victims of the March 17 attack was 550 persons, 50 of whom were in critical condition. Fortunately, all of these victims were released from the Takhti Infirmary in an overall satisfactory condition. One of the reasons for the minor clinical symptoms, as stated in the UN report was the Atropine auto-injection by the Pasdaran.

On the 20th March when the United Nations team of specialists left Iran, many other chemical attacks using Tabun gas were effectivated, leaving behind eight martyrs, seven of whom died at the war fronts and one of whom at the Golestan Hospital

in Ahvaz. The cause of death was respiratory arrest. On the whole, the general condition of this group of patients, which numbered around 400, was more severe than those in the tragedy which occurred on the 17th March. But fortunately, all the injured patients were successfully treated and discharged from the infirmary.

All the chemically injured patients since February 26, 1984 up until now number more than 3,500. About 60 of these patients were sent to various countries such as Austria, Sweden, Switzerland, France, England, Belgium, Holland, West-Germany and Japan, in order that the occurrence of this crime may be proven to everyone.

From this number, 7 achieved martyrdom in those countries. The total number of victims which have been martyred from February 26 up until present is 70 persons. According to several reports, a number of the victims, after achieving relative recovery and after dismissal from the hospitals, died suddenly of unknown reasons in their homes.

It should be mentioned that Iraq's chemical bombardments continued after March 20, the more important attacks occurring on the 28th, 29th and 30th March. The 30th March, is in reality the same day that the UN Security Council examined the report of its specialists, which had been despatched to the region, and condemned the usage of chemical weapons. Mohammad Reza Razzaghi, whose autopsy was seen on film at the Ghent Conference, was among the victims of the attack on March 30 (he achieved martyrdom 8 days later).

This shows that this type of condemnation is worthless in the eyes of the perpetrators of the crime. Of course, the UN did not even name Iraq as the perpetrator of these criminal acts. Not only did this move by the UN fail to have the least effect on restricting Iraq's chemical attacks, it even increased them. Iraq learned that even if it utilized a weapon more deadly than chemical bombs, the only reprimand the International Organizations can enact is to recite a mild moral statement.

The attacks continued after March 30, until on April 12, another widespread attack had begun, leaving behind large numbers of martyred and wounded.

The clinical symptoms of this group of victims differed greatly from those of the victims of mustard gas contamination. No blisters were seen, but small vesicles which began to desquamate

after a few days were present, and their eyes were severely affected. Vomiting was very severe and projectile ; muscular weakness and hypotonicity, along with sensations of sleepiness in the first hours, was felt. None of the victims suffered from bone marrow depression, but mainly had severe respiratory complications, several of them dying 5-6 days later due to respiratory failure.

Even after April 12, the attacks continued and it is now clear to us that if more deadly and sophisticated chemical or biological weapons are placed at Iraq's disposal, they will certainly be deployed against us. Must we remain indifferent to the torturous deaths of the people of our country and only suffice with complaining to the United Nations ? Do the people of the world not fear that if no decisive and firm action is taken against these crimes, the path will be paved for these horrible acts to be carried out all over the world ?

The toxicological experiments of Professor Heyndrickx have shown the presence of mycotoxins in the blood of several Iranian victims, and it is well-known that the only countries which possess the technology to obtain this substance are the World Powers, especially the Soviet Union. Also Iranian officials have proof that Western countries, especially the United States, play an important role in supplying Iraq with chemical weapons. Obviously, if international circles continue to regard these crimes lightly, oppressed nations will most definitely resort to retaliatory actions in order to secure their future existence.

At this point, I, as a medical doctor, would like to mention a problem in this regard : the problem of treatment. If this manner of warfare is to continue, it is necessary that the mechanism of the chemical agents' effects on the human body, as well as the method of treatment of their victims, should be taught in the Medical Schools of the world so that at least the Medical Society may be capable of confronting this serious threat to world peace.

Management of trauma in Vietnam and imposed Iranian Iraqi wars

by H. KADIVAR

Baylor University Medical Center, Texas, USA
and Ahvas University, Ahvas, Iran

IMPORTANT RULE IN TRAUMA.

Be calm and organized.

The primary assessment must be rapid, complete, and calmly performed. Do a head-to-toe 60 seconds physical examination. General-thoracic surgeon most experienced in trauma must be in charge as *one physician* is better than two but three are fatal.

CHEST INJURIES.

Chest tubes are diagnostic and therapeutic. Conservative managements is the rule, a small percentage (8 %) requires thoracotomy.

Obtain a stable, intact chest wall, a fully inflated lung, a stable mediastinum and an adequate circulating blood volume. « Wet lung » generally can and should be prevented. Blood gas determination as an indication of the severity of the injury and the effectiveness of therapy.

EARLY THORACOTOMY.

1. Continued intrathoracic hemorrhage (most penetrating wounds of the aorta and the pulmonary artery lead to immediate exsanguination).

2. Persistent, significant air leak.

3. Mediastinal involvement (cardiac tamponade, esophageal and tracheal wounds).

4. Chest wall wound (escharotomy).

5. Thoraco abdominal injuries with an indication for initial thoracotomy and closure of right diaphragm.

6. Rarely for early treatment of *extensive* pulmonary contusions, lobar consolidations and hypoxemia (not responding to positive pressure respirations and high oxygen flow). Massive hemoptysis.

ELECTIVE THORACOTOMY (later complications).

1. Recurrent pneumothorax. The presence of a full-expanded lung is no guarantee that this complication will not occur, it recurred at all echelons of medical treatment (evacuate with *heimlich valve*).

2. Bronchopleural fistula.

3. Clotted hemothorax — decortication is required in only 1 % of chest injury (thanks to single large caliber chest tube properly inserted).

4. Empyema.

5. Pulmonary parenchymal hematoma will resolve without complications, occasionally cause sepsis or hemorrhage.

6. Foreign body — the decision for removal of fragments based on :

A. Size of fragment.

B. Location of the fragment.

C. Possible routes of access to the fragment.

D. Possible complications resulting from retained fragments.

FOREIGN BODY.

Early removal if there is hemorrhage, infection, tamponade or recurrent pericardial effusions.

Late removal in an asymptomatic patient is a matter of overall evaluation of the patient and surgical judgement.

Prevention of embolism or an associated thrombus.

Reduction of the danger of bacterial endocarditis.

Prevention of recurrent pericardial effusion.

SITE OF INJURY.

A wound of entry over the pericardium is by no means a definite sign of wounding of the heart.

A penetrating wound may injure the pericardium, a coronary vessel, a major vessel, or even the myocardium without entering a cardiac chamber. The injuries to the left and right ventricles are about 70 % of cases.

PENETRATING WOUNDS OF HEART.

Approximately 81 % of persons with penetrating cardiac wounds fail to reach the hospital alive, and of those over two-thirds are victims of gunshot.

Cardiac tamponade should be suspected in all patients who do not respond to the usual resuscitation measures with a fragment on X-ray in the location of the heart or great vessels.

A high field pre-hospital mortality undoubtedly accounts, in part at least, for the low incidence (2.8 %) of cardiac wounds among other intrathoracic injuries evaluated for treatment by the first fixed medical facility during Vietnam war.

CAUSES OF DEATH.

Two major causes of death are cardiac tamponade and massive hemorrhage.

Rarer causes of death are myocardial infarction following coronary artery injury and complete heart block from injury to the atrioventricular node or common conduction bundle.

Closed chest cardiac massage is contraindicated in wounds of the heart since either hemorrhage or tamponade is almost certainly the cause of arrest.

In general, it must be assumed that all cardiac wounds are potentially lethal and if there is the slightest doubt that conservative management may not be effective, immediate thoracotomy for inspection of the heart and the control of bleeding should be done.

Pericardiocentesis was used only for diagnosis or to reduce tamponade until thoracotomy is done.

Also remember, if most or all of the blood in the pericardium has clotted, an *uncommon finding*, aspiration may yield nothing.

TECHNIQUE FOR CARDIOGRAPHY.

A general anesthesia is preferred. However, if tamponade is present, the induction of anesthesia may cause cardiac arrest, necessitating immediate thoracotomy and pericardial decompression after endotracheal intubation. Also, if the patient is moribund, anesthesia is not necessary. An incision into the pericardium and the control of bleeding should be executed most expeditiously. The choice of thoracic incision is dependent on the location of wound and associated injuries. Wounds that are presumed to involve the left side of the heart, the posterior wall, the pulmonary conus, or the left pulmonary artery or descending aorta, are best approached through the left sided incision. Wounds involving the right side of the heart, the vena cava, or the ascending aorta are approached through the right thoracotomy (anterolateral, posterolateral, bilateral anterolateral, median sternotomy, thoraco-abdominal incision, anterior midline thoracoabdominal incision (autopsy incision).

IMPORTANT POINTS.

1. Open pericardium widely.

2. Avoid injury to phrenic nerve.

3. Dislocation of the heart was held to minimum.

4. Close small ventricular wound by several over and over 3-0 or 4-0 sutures.

5. Larger wounds may require mattress sutures reinforced with pledgets of teflon felt or pericardium. Sutures in the ventricle are always drawn together with utmost care to avoid tearing.

6. Wounds of the atrium approximate with multitoothed forceps or using vascular clamp then close it with 4-0 silk.

7. For control bleeding from a large wound, tamponade by a foley catheter balloon of 30 cm³ capacity.

8. The pericardium is left widely open for drainage into the pleural space and the dependant part of pericardial sac left open and drained.

9. Tubes are placed on suction.

PENETRATING INJURY OF ESOPHAGUS.

1. Carefully performed esophagogram with gastrografin if time permits.

2. Diagnosis may easily be missed particularly because of associated injuries.

3. Injuries to the intra-abdominal portion may simulate an acute abdomen.

MALTESE TWIST.

With the victim's neck extened, a long knife was thrust into the thorax through the jugular notch and twisted so as to sever the greater vessels at the base of the heart.

TECHNIQUE OF CHEST TUBE INSERTION IN TRAUMA.

1. Chest tube is diagnostic and therapeutic.

2. The chest tube should be single and of a large caliber (36-40F) with multiple extra holes.

3. Troca chest tube is the best and safest, can be guided all the way to the apex.

4. Patient's position sitting if possible.

5. Location 5-6th intercostal space at all for both air and fluid.

6. Do not insert at 2nd or 8 ICS, as recommended in textbook as you will be greeted by massive bleeding or find tube in peritoneal cavity with perforation of spleen, liver and diaphragm.

7. Clamp insertion of chest tube to be avoided except if troca chest tube is not available.

8. Obtain sitting position X-ray of chest immediately and repositioning done if necessary.

9. Constant adequate suction (20-30 cm H_2O) should be applied.

10. Proper management of chest tube is basic thoracic surgeon training and prevents end stage thoracoplasty.

11. Obtain daily sitting position of chest as long as chest tube is in and the day of removal of chest tube and the day after and later on as indicated.

12. Put purse string suture around chest tube for the time of removal. Don't depend on vaseline, as sometimes the chest tubes are needed for several days.

13. Ambulate or transfer patient with Heimlich valve. The valve is of greater benefit in removing the air and is less effective in draining blood. It is mandatory that valve be placed properly and surrounding plastic bag vented.

14. Clamp the chest tube 12-24 hours. Obtain X-ray if lung well expanded then remove chest tube.

AEROMEDICAL EVACUATION.

Preferably, patients should meet the following minimum criteria for routine AE.

1. Stable hematocrit of 35 % and HGB of 11 grams percent.

2. Stable vital signs.

3. No active bleeding.

4. Adequate hydration.

Usually within 24 hours, the patient is placed aboard an aircraft for movement. Depending on the destination hospital, this movement may be made by ground or helicopter transportation. Then patient designation to Pacom or Conus Hospitals. The aircraft utilized for long range, high altitude transfer was C-141. It is pressurized to maintain a cabin altitude of 6,000 to 8,000 feet. Combinations of ambulatory and litter patient transfers are normally arranged for approximately 60 patients per flight with two to three nurses, three to four technicians and a surgeon. Patients may be removed from the Evac system at any part enroute when it is the professional opinion of the evaluation surgeon that patient's safety will be compromised by continued movement.

1. Tracheotomy care — tube should be of proper size and changed prior to placement in the Evac system due to the low (10 %) humidity of aircraft cabin atmosphere, the use of

some humidification devices is recommended. Patient should not be transported with endotracheal tube.

2. Chest tube — chest tubes to be connected to Heimlich valve. Chest X-ray should be taken and interpreted just prior to patient movement. Preferably, the patient should not be evacuated by air within 72 hours after removal of the chest tubes.

3. Nasogastric tubes — leave NG tubes in during Evac, the combination of the basic medical problem, air swallowing due to anxiety and pain and the reduced barometric pressure at altitude could result in difficulties. Abdominal pressure under a cast, pain from distention of hollow viscera and most importantly, vomiting with aspiration.

4. IVC and foley cath usual care.

5. Burn case care — deep burn greater 40 % have a surgeon attendance, good IV and urinary output, functioning airway (tracheotomy), wound coverage via the two sizes of field compresses, NG tube, immobilization of associated injuries.

6. Cranial tongs — traction must be maintained by a close system, preferably with the Colling spring.

7. Stryker frame — portable frames are available for long distance transfers by air.

8. Plaster cast — all circular casts should be bivalved. This allows for the expansion of soft tissue at decreased atmospheric pressures as well as rapid access to a serious wound beneath the cast. Put the date and time of injury, the date of surgery and cast applications and a simple sketch of the bone injury.

9. Vascular injuries — cast should be bivalved and windowed to provide easy emergency access to control hemorrhage. Patient should not be transferred from Vietnam for 14 days after repair. On the cast or dressing should have the repair date, location and type of repair.

10. Medication orders must include routine drugs such as maleria prophylaxis or type and cross match whole blood if the patient needs transfusion. Antibiotic-narcotics and anal-

gesic should have a recorded « stop order » to avoid an undesirable extension of this course of therapy.

11. Physician attendants — selection to the type of care of patient anticipated in flight — attending surgeon is expected to accompany seriously ill patient all the time and all the way to the destination hospital.

EVACUATION TIME.

The average time required for evacuation of a patient from battlefield to a site where definite therapy could be started was :

1. 10 hours in World War I.

2. 3.6 hours in Korean war reduced to

3. *35 minutes* in Vietnam war.

MORTALITY OF MILITARY WOUNDED IN ACTION IN VARIOUS WARS.

World War I (1917-1918)	8 %
World War II (1941-1945)	4.5 %
Korean War (1950-1953)	2.4 %
Vietnam War (1961-1973)	1.8 %
World War III (middle east war — nuclear war). .	?

REASON OF LOWER MORTALITY IN DIFFERENT WARS AND LESSONS LEARNED.

World War I (1917-1918) — debridement of gunshot wounds, use of blood transfusions and intravenous fluids.

World War II (1941-1945) — management of thoracic and vascular injuries.

Korean conflict (1950-1953) — improved management of vascular injuries, liberal use of whole blood, shorter evacuation time, routine use of antibiotics.

Vietnam war (1961-1973) — rapid resuscitation and evacuation, improved management of thoracic and vascular injuries, prompt treatment of haemorrhagic shock with availability of an almost unlimited amount of fresh whole blood on a daily basis, the avail-

ability of *highly qualified surgeons*, modern equipment and advanced surgical techniques.

TISSUE DESTRUCTION CAUSED BY GUNSHOT WOUNDS.

The severity of the injury resulting from a bullet is directly proportioned to the amount of energy it delivers to the tissues. The amount of kinetic energy possessed by a traveling bullet may be calculated by using the formula $KE = \dfrac{MV2}{2\,g}$. Where M is mass of the missile, V is the velocity of the missile and g is the gravitation acceleration. Thus, the kinetic energy is directly proportional to the mass of the bullet multiplied by the square of the velocity. Bullet velocities of less than 1,200 feet per second (FPS) *as low*, those from 1,200 to 2,500 FPS *as medium*, and velocity in access of 2,500 FPS *as high*. Bullet velocity is the most important factor determining the extent of tissue destruction. The most extensive tissue damage is produced when *velocity is high* and *mass is great*. Thus, an M16 rifle with a muzzle velocity of 3,250 FPS will produce a much more severe injury than A .22 caliber rifle with a muzzle velocity of 1,000 FPS even though both utilized bullets of approximately the same size, shape and weight.

On the other hand, A .45 caliber automatic pistol with a muzzle velocity of 860 FPS will produce a much more severe injury than A .22 caliber rifle with a muzzle velocity of 1,000 FPS. Even though the muzzle velocity of the latter is greater than a muzzle velocity of the former, the more severe injury results from the markedly, heavier slug expelled from the 0.45 caliber pistol.

IMPORTANT POINTS.

1. Non-disintegrating bullets that pass completely through the tissues do not deliver all of their energy. Such bullets cause less tissue destruction than those of similar weight and velocity that do not pass completely through.

2. A soft bullet that disintegrates on contact with the tissue imparts all of its energy to the tissue and produces a much more serious local injury. Since fragments of shattered bone serve as *secondary missiles* that transmit all their energy to

the surrounding tissue, the bullet that strikes bone will produce a more serious injury.

3. A bullet that tumbles and strikes the tissues broadside at a velocity comparable to that of a bullet that spins and rolls from a rifled gun barrel will also cause greater tissue injury because more of its energy is imparted to the tissue at the larger area of exposed surface.

4. In close range shotgun blasts, a large mass of pellets is expelled with a greater amount of energy over a relatively wide area.

5. When a bullet strikes soft tissue, « *shock waves* » are immediately transmitted to the tissue involved. These shock waves spread out from the missile tract through tissue at the speed of sound and cause extensive local tissue damage. Tissue damage far from the primary tract may also result. In addition a temporary tract must be wider than the primary missile tract is formed by sudden expansion. The vacuum thus formed sucks foreign debris into the primary tract and may also cause local blood vessel and nerve damage.

6. Since most rifles expel bullets of greater mass at a medium or high velocity, gunshot wounds produced by rifles are considerably more severe and require much more extensive debridement than do gunshot wounds produced by .32, .38 and .45 caliber hand guns which are commonly available to civilians. Exceptions to this generalization are the .357 magnum pistols.

RULES OF WOUND TREATMENT (dress them open).

Skin : save all you can.
Muscle : if in doubt, cut it out. Use 4C criteria for viability.
Tendon : cover them, repaired electively.
Nerve : cover them, preserve length, repaired electively.
Vessels : immediate repair.
Bone : save it, attached or detached.

DEBRIDEMENT.

Debridement is the surgical technique of excising devitalized tissue. The experience in general wars has demonstrated that

proper debridement is the key to surgical treatment of soft tissue wounds and provides the best means of reducing morbidity and mortality. The extent of tissue damage is related to the type of missile, its velocity, the rotational axis, and the nature and extent of secondary missiles acting within the tissues.

Skin. — The elasticity of the skin allows for stretching as a missile passes through, therefore, damage does not usually extend far beyond the traumatized edges. *Excessive* debridement is unnecessary.

Fascia. — Damage to the fascia is related more to loss of substance from a direct effect rather than destruction from lateral energy. The innocuous appearance of the fascia may disguise extensive cavitation beneath. The surfacial plane is a ready avenue for extension of infection after improper debridement. The fascia should be opened widely.

Muscle. — Of the soft tissues in extremities, skeletal muscle is the least able to withstand the shock wave and cavitation caused by dissemination of the lateral energy of a high velocity missile. Devitalized muscle can be recognized by its dark colour, soft consistency, non-contractibility and decreased capillary bleeding of the cut surface.

Artery. — Arterial injuries may be encountered as complete.

Transection, open tears, small holes occluded with thrombus, contusions with internal tears, contusion with aneurysms formation and rarely, local spasm.

The microscopic pathology of the high velocity arterial wound extends several millimeters beyond that which is observed grossly.

Hematomas adjacent to vessels should be explored to rule out vascular injury.

Tendon and nerve fibers withstand lateral energy better than skeletal muscle. Since inspection will readily demonstrate the extent of devitalized tissue, conservatism in debridement is recommended.

Technique :

1. Debridement should be performed with a scalpel and not with electrocautery.

2. Copious irrigation is very effective in flushing out clots, debris and foreign material and exposing hidden vascular injuries.

3. For extremities longitudinal incisions, when the joint creases are traversed, a curve incision to prevent contractures.

4. Repair of tendons or nerves should not be performed, they should be covered with soft tissue when feasible. The epineurium may be tacked to adjacent soft tissue with non-absorbable fine sutures to prevent additional displacement and later identification. Digital and the facial nerve may be repaired primarily when feasible.

5. All wounds in the vicinity of major blood vessels must be explored thoroughly for vascular injury — when arterial injuries have associated unstable fractures, spicules of bone near the repair should be smoothed.

6. Formal arthrotomies should be performed in all wounds involving joints.

7. Hemostasis is imperative.

8. The wound should be dressed with sterile fine mesh gauze *without drains*. For deep wounds, fluffs of gauze should be laid over the fine mesh gauze but the wound *should not be packed*.

9. The initial dressing should not be removed until the time of delayed primary closure or redebridement unless signs of infection are present.

10. Patients having extensive soft tissue wounds of the extremities should have the extremity immobilized in a position of function.

DEBRIDEMENT OF SPECIAL REGIONS.

Face. — Due to the abundant blood supply, less extensive debridement and should be closed primarily if possible without flaps — split thickness skin grafts may be used as coverage in large avulsed wounds.

Neck. — close primarily after debridement.

Chest wall. — Defects in the chest wall may require rotation of a flap of muscle or skin and subcutaneous fat to obtain airtight closure. Exposed costal cartilage should be covered to reduce the possibility of chondritis.

Hand. — Hand wounds should be left open following debridement — excessive debridement should be avoided.

External genitalia. — Because of abundant blood supply, less extensive debridement and primary closure with dependent drainage. Conservatism debridement of testicles and coverage.

Foot. — Properly placed incisions between the metadorsal heads avoiding the weight — bearing areas are recommended. Foot wounds are not to be closed.

WOUND CLOSURE.

Primary repair of combat wound should be limited to the wound of face, oral cavity, neck, scalp and scrotum. Delayed *primary* closure — *4-7 days* after adequate initial debridement. It is realized that some wounds will require further debridement and the closure must be delayed for 4 to 7 days more. If the wound appears clean on examination, no skin edge excision is recommended. Undermining of the skin edges and the creation of new planes of dissection should be avoided if possible. In some situations, coverage of the wound with a split thickness skin graft is desirable in the 4 to 7 days. The wound should be closed with non-absorbable, non-reactive suture.

Exploration of circumstances at the battlefield, diffusion of gas bombs, secure and transportation of poisonous content

by G. FREILINGER

Department of Plastic Surgery, 2. Surgical University Clinic, Vienna, Austria

SUMMARY.

This is a background information on the local circumstances during my 6 days visit in Iran and near the combat zone in the province Kuhzestan.

Diffusion of gas bombs was effected in my presence. Problems of transportation of poisonous contents out of the country follow.

Problems of receiving and treating patients from foreign countries are due to the fact that pre-information about the disease or injurie goes often by diplomatic channels and this specially for countries at war.

This means that the information is not based on medical facts and therefore insufficient and sometimes incorrect. To improve this, a written medical report or in acute cases a direct contact from doctor to doctor or specialist to specialist would be necessary.

The Embassy of the Islamic Republic in Vienna was interested in a better medical cooperation and asked me to visit hospitals in Teheran and maybe field hospitals near the combat zone. This was the reason that I left for Teheran on March 14, 1984.

I had set myself two tasks :

1. To get acquainted with local doctors and specialists in my field for better information and cooperation and to see the condition and standard of hospitals in the country.

2. On request of Prof. Heyndrickx, who kindly came to Vienna and toxicologically tested our patients, try to get a hold of poisonous content inside the bombs and on the ground, where they exploded.

The cooperation from the Iranian doctors and their interest and help during my 6 days visit was perfect. I saw hundreds of victims in different Teheran hospitals with exactly the same signs of intoxication and skin lesions as on our 10 Viennese patients. I exchanged ideas for better treatment and realized that here and there new therapeutic measurements had to be found. There exists little or no information in the literature, especially for a combination of intoxication such as mustard gas and mycotoxin or tabun.

One afternoon was reserved to visit the forensic medicine department in Teheran and the attached mortuary. The corpses of 10 chemically intoxicated soldiers, who died in Teheran, Vienna, or Stockholm were placed here. In another cold-storage room about 50 cadavers were kept, all unidentified victims because of not being registered. To explain this, I have to point out that the Iran army consists of three types of soldiers. The regular, well-paid army, the so-called Pasdaran, which are revolutionary guards, and the Baseey, which are mobilization forces. The two latter are young but also old male people, many times not registered and have only a short military training.

On my urgent request I flew together with the UNO fact-finding delegation to Ahvaz, the capital of the province Khuzistan, about 1.000 km South of Teheran near the Persian Gulf.

Separately from the delegation then I visited field hospitals in this region. I saw again dozens of hospitalized and intoxicated soldiers by, so I was told, tabun, a nerve gas from an attack 2 days previously. The clinical signs of these combatants were dizziness, headache, vomiting, low blood pressure, bradycardia, and in spite of high dosis of Atropine administration, myosis and tonic and clonic convulsions.

In another hospital I took part at an autopsy. The victim was a 26-years old strong man, platoon leader, who was intoxicated

during the same attack as our Viennese patients. Of special interest was the lung, which showed extensive hemorrhage and bronchopneumonia of both lungs as well as abscesses in both lungs.

I could convince myself of the strategic warfare of this particular region. It seems to be a war with a rather stable front-line. The ground is flat and there is spare vegetation.

This means the soldiers are digging themselves a hole in the ground for protection and have to have close contact to the ground. In this environment a most effectful way to attack the enemy and put them out of action is by gas. The combatants I asked agreed that bombs were thrown at them from deepflying airplanes. After explosion, a white to yellow cloud close to the ground was observed, which smelled like depraved vegetables. Obviously the compounds, being heavier than air, spread low on the ground. I was able to take blood and urine examples from one soldier for examination, who was in the neighbourhood but not directly involved in the attack.

Back to Teheran I was able to take part at the diffusion of non-exploded bombs brought here from the battlefield. In a military zone several Iranian experts with protective clothing and gas-masks opened the bomb and extracted the fluid in my presence. The ignition was inscribed in Spanish language, the bomb itself with a weight of 250 kg was inscribed in English text. Then I took the fluid content out of the bomb and carried it to Vienna for further examination. The report on this fluid in Vienna as well as in Prof. Heyndrickx's laboratory in Ghent revealed pure mustard gas. There were no toxic findings in the urine specimen of the soldier.

Ladies and Gentlemen, this was just a short information on my recent experiences in Iran, which might contribute to better understanding of circumstances of the problems of chemical warfare.

First contact with intoxicated victims by chemical warfare

by G. FREILINGER

Department of Plastic Surgery, 2. Surgical University Clinic, Vienna, Austria

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SUMMARY.

After an urgent call for help, I received 10 Islamic combatants in Vienna on March 2, 1984.

On arrival I soon realized that instead of extensive burn-patients, victims severely intoxicated by an unknown agent had arrived. A close description of early findings in these combatants and the organization and distribution to different medical centers in Vienna follows. Twelve days later on invitation of the Islamic Republic I visited hundreds of intoxicated victims in hospitals at Teheran and Khorramshar.

A general survey of these intoxicated soldiers by different chemical agents and the exchange of the introduced therapy in Viennese and Iranian hospitals is presented here.

Plastic and reconstructive surgery deals — among many other problems — with mutilations of war casualties. This has always been a strong stimulus for the development of this speciality. For this reason I have been twice in Vietnam and later on in Afghanistan. Since 1982/83 I received combatants from the Iran-Iraq war with severe facial injuries for reconstructive work. The contact to the embassy of the Islamic Republic in Vienna was therefore established.

Early in March 1984 I was asked by telephone to care for heavily burnt soldiers. Vienna has no big burn unit yet, but it was clear that these victims needed intensive care. Intensive care units are always busy and it is most difficult to occupy

even a single bed. A quick inquiry in several big Viennese hospitals informed me that ten soldiers could be placed at the maximum. On March 2, 1984 Iran Air landed at Vienna airport late in the evening.

May I now inform you about my impression aboard of the Jumbo. In the empty carrier 15 stretchers were placed, keeping the wounded soldiers. All of them were extensively bandaged, several had urine catheters and were put on intravenous fluids for the 5 hours flight. After opening some of the bandages, I faced severely injured soldiers of all ages, some of them very young, but not heavily burnt cases as they had been announced. It looked to me as first and second *degree* corrosions on smaller and larger areas of the body and of the extremities, which on palpating seemed to be extremely painful. The genital region was predominantly afflicted, several soldiers had swollen eyes with medium or severely conjunctival irritations. Some could not open their eyes at all. However, the coughing, the rattling, the growling, the whimpering, and keening and the hoarseness of some of the victims indicated not only pain but respiratory problems and this was quite obvious.

From the protocol it became clear that these casualties were exposed to an attack in Al-Ozair and in the region of the Majnoon - islands on February 25, 1984, which means six days previously.

May I show you some slides of the corroded areas around the body. Please notice the difference to a burn caused by fire or hot fluid or electricity. In these cases I was convinced that the victims were exposed to some other agents, and this has caused liberally spot-wise or in larger areas corrosions of the skin surface. There was no burn scar nor deep necrosis rather than superficial to deep first and second degree errosions.

To my knowledge, there is no single case, which needed skin transplantation later on. On the opposite the lesions healed rather quickly on all kinds of local treatment. I do not want to get into the treatment of these cases, because you will get an extensive report on this following my paper by Doz. Pauser and Doz. Mandl.

I just want to conclude that the casualties we lost were neither on skin problems nor on other visible lesions. It became obvious that several of the victims needed urgent medication, pain relief and treatment.

The selection, who of the 15 casualties could remain in Vienna and who would go on to Stockholm was entirely up to the Iran officials and it took around one hour till this was decided, and ten victims due to Viennese hospitals were embarked.

The ambulances in front of the airplane were ready to bring these soldiers to the outpatient ward of the airport, the registration and visa control was effected by the immigration officer. Depending on the severeness of injuries, the victims were brought then to different units in town. Five cases were brought to my hospital, two of them because of their severe condition were immediately taken to the intensive care unit.

First report on victims of chemical warfare in the Gulf-war treated in Vienna

by H. MANDL and G. FREILINGER

Department of Plastic Surgery, 2nd Surgical Clinic,
University of Vienna, Vienna, Austria

SUMMARY.

On March 2nd, 1984 fifteen Iranians were brought from Teheran to Vienna suffering from burn injuries of different extent. According to our first informations we were expecting some heavily burned patients from Iran whom we were asked to treat in Vienna. But after the first examination of the ten patients considered to stay in Vienna — while five others were flown to Stockholm — it was evident that these burns were not the effects of any well known causes for burns.

But before the question of the origin of those injuries could be tried to solve another problem was more urgent : the hospitalization of the patients. There were ten instead of the expected number of 8 patients to be identified and examined in order to get a general view of the extent of injury. Based on these informations the patients were sent to three different hospitals in Vienna. Then the findings of the first examination of the patients together with the hint of official Iranians that those men were suffering from a gas attack in the Iraqi-Iranian war caused feverish activity. During the next hours and days we had to learn a lot about chemical weapons, their effects — and how little is known about the treatment. The effects of different chemical weapons described in literature were compared to those found in our patients. In this way it was found that the symptoms of our patients were matching very well to those of mustard gas.

Now the next questions arose : 1. How could an evidence be found for an intoxication with mustard gas ? 2. Is there any danger for the caring nurses and physicians by contact with the patients ? 3. What general and specific therapy should be used ?

To 1. In the Department of Toxicology of the University of Vienna there was found evidence of mustard gas in a urine sample of one patient. For confirmation of this finding it was proposed to send samples to another toxicological laboratory in Ghent. So we did. In these samples of blood, urine and faeces Prof. Heyndrickx found not only evidence of mustard gas but also of some different myco-toxins.

To 2. As the question of danger for caring persons could not be denied it was ordered that all contact with the patients or their secretions should be avoided by wearing gloves.

To 3. First the injured skin was treated as second degree burns. Antibiotic prophylaxis was added. When difficulties of gas exchange arose — what happened in 7 of the 10 patients — patients were brought to intensive care units. The number of leucocytes decreased to about 1000 in all cases. Four of the 10 patients died between the 7th and the 36th day after the gas attack. Since the 14th day all patients were treated in the following way : for decontamination the skin was washed with chloramine-solution. Animal charcoal (10-20 g/day) was given together with magnesium sulphate as laxative for acceleration of elimination. Cysteine-solution (4 × 10 ml/day) was infused to decontaminate the blood. In some very bad cases haemoperfusion was added. This combined treatment was successful in six patients. Three of these six patients had been considered to be hopeless cases.

INTRODUCTION.

On March 2nd, 1984 15 Iranians were brought from Teheran to Vienna suffering from burn injuries of different extent. According to our first informations from the Embassy of the Islamic Republic of Iran we were expecting some heavily burnt patients from Iran, whom we were asked to treat in Vienna. But after the first examination of the 10 patients considered to stay in Vienna for treatment — while the remaining 5 were flown to Stockholm, Sweden — it was evident that these burns were not the effects of any well known causes for burns.

This first examination had to be carried out still in the area of the Viennese airport. But before the question of the origin of these injuries could be tried to be solved, another problem stood to the fore : the hospitalization of the patients. Instead

of the expected number of 8 patients, there were **10 men** to be identified and examined in order to get a general view of the extent of injuries. There were difficulties in understanding as none of the patients spoke German or English. So all questions and answers had to be translated. Based on these informations the patients were sent to three different hospitals in Vienna. In the next days and weeks it was my job to coordinate the investigations into the intoxication and the information about the recommended treatment in these hospitals.

INITIAL TREATMENT.

The finding of the first examination and exploration of the Iranian patients together with the hint of official Iranians, that those men were suffering from a gas attack in the Iraqi-Iranian war caused feverish activity. During the next hours and days we had to learn a lot about chemical weapons, their effects — and how little is known about the treatment. In the following period several activities had to be carried out at the same time : the initial treatment of the patients, the attempt to find out something about the circumstances and the causal agent of the intoxication and — last but not least — the information of the public media, as the public interest in this affair was such a big one as we never had been confronted with before. This public interest made it necessary to hold press conferences for the information of TV and press journalists. Who ever had to do with journalists knows what that meant for our hospital in the next days.

Now let us go back to the initial treatment. For the beginning the injured skin was treated like second degree burns. All affected areas were covered with antibiotic ointment. General antibiotic prophylaxis was added. As some of the patients complained about itching of the affected skin areas, calcium and antihistaminica were prescribed. When difficulties of gas exchange arose — what happened in 7 of the 10 men — the patients were brought to intensive care units, where artificial respiration was started when necessary.

FIRST SYMPTOMS.

In order to find out what had happened to those men it was tried to get an anamnesis, which was only possible by help of

an interpreter. The details reported by our patients about the course of events was similar concerning the begin of the attack but differed in details of kind and intensity of the effects. The attacks happened in at least two different areas : in a place named Hurol-Azim near Khorramshar and in the marshlands of Madshnun. The events were dated back 5 days prior to the arrival in Vienna. A number of aeroplanes (6-8) were flying an aircraft attack releasing bombs or shells, which did not explode but dispersed a white to yellowish, evil-smelling gas. When the foggy clouds of gas reached men, the eyes and respiratory tracts began to burn very soon. Some patients reported that differently coloured and/or formed objects had been dropped by the planes. After one to five hours people felt nauseated and had to vomit heavily. Later conjunctivitis appeared followed by aversion to light, which in some cases was lasting for several weeks. Some 6 to 8 hours after exposure or even later blisters began to develop on the body. Some of the men reported that after several hours paresthesia appeared in the face and hands. The men were brought to the next military hospitals, where they could change their cloths and could take a shower. In most men the skin of the urogenital area was damaged heavily so that after 24 to 48 hours it was necessary to catheterize some of them. Later they were brought to Teheran, were dermatological treatment of the affected skin areas was provided. From there they were flown to Austria and Sweden as soon as hospitalization and treatment was promised.

STATUS AT HOSPITALISATION.

When the Iranian patients were examined for the first time, they all showed conjunctivitis of different extent and in some patients connected with sensitivity to light (fig. 1 and 2). The affections of the skin reached from redness of only limited extension to second degree chemical burns of up to 50 % of the body surface (fig. 3 and 4). This skin looked like a heavy sun burn. The superficial layers became dark and separated from the deeper layers (fig. 3). Some patients complained about intensive itching while others did not feel so. The skin of the urogenital area was specially affected in 7 of the 10 patients (fig. 5). Nearly all patients had difficulties and pain at swallowing,

three of them were hoarse or had a croaking voice. In the first days all findings in the blood count were in normal bounds.

TRIAL OF TOXINE EVIDENCE.

Regarding the course of intoxication of the Iranian soldiers Iranian officials spoke about an Iraqi attack with poison gas.
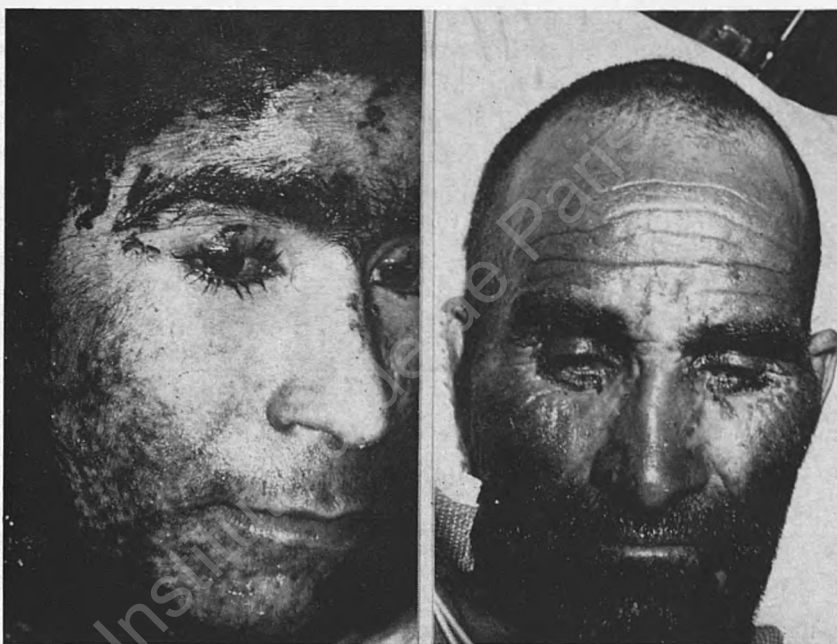
FIG. 1.   FIG. 2.

FIG. 1. — Patient F. with burns in the face and conjunctivitis (7th day).

FIG. 2. — Patient A. showing sensitivity to light (6th day, 1 day prior to exitus).

But nobody of us nor our colleagues had personal experience with that kind of intoxication. In order to find out which poison gas the agent in question could be, it was necessary to compare the effects of different chemical weapons described in literature to those found in our patients (fig. 1-5, fig. 6).

In this way it was found that the symptoms of our patients were matching very close to those of mustard gas. From the moment of this knowledge we tried to find out more about the possibilities of treatment. Contact was taken to several toxicological centers

in Europe. From Prof. Weger in Munich we learned that in animal experiments he had found that a derivate of BAL, the dimercapto propan sulfonate with the registered name « Dimaval » had a positive effect even in mustard gas intoxications. But there were no experiences in humans. In contrast to BAL Di-

FIG. 3.
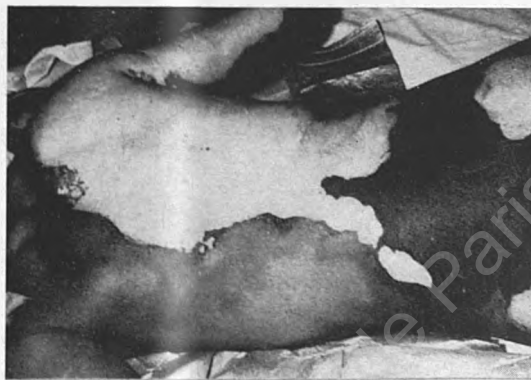
FIG. 4.

FIG. 3. — Patient T. with 50 % burned area (7th day).

FIG. 4. — Patient T. with chemical burns of the dorsum of the hand (7th day).

maval was said to have less side effects. Therefore after some discussion we decided to apply this Dimaval according to the dosage recommendations of the manufacturer : initially 300 mg, followed by 200 mg every two hours during the next 48 hours. Then the dosage was reduced to 100 mg every 6 hours for the next 5 days.

Now the question arose how an evidence could be found for an intoxication with mustard gas. Therefore we sent samples of skin and urine of some of the patients to the Department of Toxicology of the University of Vienna for investigation on chemical warfare agents. The findings of arsenic, phosphorus and sulphur were in normal bounds. But in two cases evidence of

FIG. 5.   FIG. 6.

FIG. 5. — Heavily burned urogenital area (Patient T. on the 10th day).

FIG. 6. — Skin defect resulting from newly developed blisters (Patient T. 10th day).

mustard gas was found in the urine. For confirmation of this finding it was proposed to send more samples to another toxicological lab in Ghent. So we did.

Initially we were suspicious about what we were told by the patients or more exactly by the official translaters of the course of events of the intoxication. Considerations about the possibility of an Iranian fraud were discussed. But two facts eliminated all suspicion on the story we have been told.

First Prof. Heyndrickx in his laboratory in Ghent did not only find evidence of mustard gas in the samples of blood, urine and faeces we had sent to him (thus confirming the first report of our Viennese toxicologists), but he also found evidence of some different mycotoxins, which were thought not to be available to Iran. Furthermore a confirmation of the Iranian allegations was coming from a corner nobody had expected : a speaker

Wiener Zeitung

Golfkrieg: USA wußten vom Einsatz chemischer Waffen durch den Irak

Weltsicherheitsrat erörtert Maßnahmen zur Beendigung des Krieges am Golf

Washington/Teheran — Das Sprecher des State Departments sagte in der Nacht zum Donnerstag in Washington, daß die USA ihre Sorge über diesen Sachverhalt hochrangigen Vertretern der irakischen Regierung gegenüber „mehrfach direkt zum Ausdruck gebracht" hätten. Auf die Frage, warum die USA ihr Wissen erst jetzt preisgäben, sagte sie, man habe bis zuletzt gehofft, auf diplomatischem Weg den Irak vom Einsatz dieser Waffen abzubringen.

Der Sicherheitsrat der Vereinten Nationen ist für den gestrigen Donnerstag zu Beratungen über Maßnahmen zur Beendigung des Golfkrieges einberufen worden. Dabei sollen nach Angaben eines UNO-Sprechers vor allem iranische Anklagen zur Sprache kommen, wonach der Irak chemische Kampfstoffe gegen iranische Streitkräfte eingesetzt habe. UNO-Generalsekretär Javier Perez de Cuellar hat in einer Erklärung am Mittwoch den Einsatz solcher Waffen „kategorisch und energisch" verurteilt.

Zu der geschlossenen Sitzung des Rates hat Perez de Cuellar alle 15 Mitglieder eingeladen, nachdem der Iran eine Untersuchung der Vorwürfe gegen den Irak durch die Vereinten Nationen gefordert hatte. Ein hoher irakischer Regierungsvertreter hat am Donnerstag dementiert, daß der Irak über chemische Waffen verfüge oder diese im Golfkrieg eingesetzt habe. (AFP, AP, Reuter)

Beirut — Heftige Feuergefechte verfeindeter libanesischer Milizen kennzeichneten auch Donnerstag die prespannte Situation Beirut, wo sich die politischen Führer der verschiedenen Bevölkerungen noch auf die Versöhnungsgespräche nächste Woche in der Schweiz vorbereiten.

Radio Beirut meldete, in der unmittelbaren Umgebung des Parlaments eingegen. Sechs Menschen seien dabei verletzt worden. Kämpfe wurden aus den Schufbergen gemeldet. Nördlich von gegenüberstehen sich drusische Milizen und christliche libanesische Kräfte Gefechte.

Der saudiarabische Unterhändler Rafik Hariri bemüht sich weiterhin um die Einheit der Waffenstillstands vom Wochenende. Eine Sitzung der Parlaments auf der eine Kommission zur Untersuchung des jüngsten Zwischenfall.

FIG. 7. — Press report on US knowledge about use of chemical weapons by the Iraq.

of the US-state department announced that the USA had had knowledge about the use of chemical weapons by Iraq since several months (fig. 7).

TREATMENT.

Together with Prof. Heyndrickx, who had been in Vienna for a visite, and with Dr. Pauser, who is working at the intensive care unit II of the Clinic for Anesthesiology, a plan of therapy was elaborated. First all skin areas were carefully decontaminated by washing with a chloramine-solution. For acceleration of elimination animal charcoal was given in a dosage of 10-20 g a day together with magnesium sulfate as laxative. For elimination

by way of the urine the patients had to drink 2-3 liters a day or got this additional amount by infusion. For decontamination of the blood, solutions of cysteine (4 × 10-20 ml 0.5 % solution) were infused. In some very bad cases haemoperfusion over a charcoal filter was added. These cases will be reported by Dr. Pauser in detail.

Vitamine K was given prophylactically for three days. Mustard gas and trichothecens (mycotoxins) both are cytotoxic agents : both are followed by a heavy decrease of the white blood cells, which is connected with a reduction of resistance. This had to be considered in the plan of therapy. Antibiotic prophylaxis had to be continued as long as the number of leucocytes was diminished.

As the question of danger for the caring staff could not be denied, it was ordered that all contact with the patients or their secretions should be avoided by wearing gloves.

COURSE AND RESULTS.

While all patient except one were in the age between 16 and 24 years, one patient was already 45 years (fig. 2). This man seemed to have stayed for a longer period in the contaminated area and therefore was affected more heavily. As symptom for increasing difficulties of gas exchange the pO_2-level was decreasing to 52. He therefore was brought to our intensive care unit, where he soon had to be respirated artificially. Yet it was not possible to save this patient. He died two days later because of not removable troubles in gas exchange. In the X-ray the lungs looked like destroyed. Due to religious reasons this man was not autopsied. For the case of further exitus allowance of autopsy was given later.

The number of leucocytes, which was normal in all patients at the beginning, was decreasing in all men to values between 900 and 2,000. It is remarkable that there was no correlation between decrease of leucocytes and seriousness of illness or extent of affected skin. This decrease happened between the 10th-15th day after exposure and lasted only for a few days.

Difficulties of gas exchange in the lung were found in six other patients necessitating the transfer to an intensive care unit. Three of these patients died on the 12th, 14th, and 36th day after ex-

posure respectively. All died because of therapy resisting bron-
chopneumonia by superinfection.

After the first report of mustard gas and mycotoxine evidence
we sent samples of all patients, who were still alive at that time,
to Prof. Heyndrickx in Ghent. From these 8 patients evidence of
mustard gas was found in 6 cases and of mycotoxins in five cases
(see table I). In two cases only mustard gas and in another two

TABLE I

Toxin evidence and progress of 10 Iranian patients
treated in Vienna for mustard gas and mycotoxin exposure

Patient		Age	Mustard gas	Mycotoxins	Progress
1	F.A.	18	Positive	Positive	Alive
2	A.H.	45	Positive	Not Investment	Exitus (7th day)
3	T.M.	19	Positive	Positive	Alive
4	M.A.	23	Positive	Negative	Alive
5	R.A.	19	Negative	Positive	Alive
6	A.N.	21	Positive	Positive	Exitus (36th day)
7	N.M.A.	22	Not Investment	Not investment	Exitus (12th day)
8	N.M.	16	Positive	Positive	Exitus (14th day)
9	M.J.	18	Positive	Negative	Alive
10	E.A.	24	Negative	Positive	Alive
Total			7/10 positive	6/10 positive	6/10 alive

only mycotoxins were found. In four cases there was evidence
of mustard gas as well as of mycotoxins. The report
of mustard gas ranged from slightly positive to very strong
positive. The values for mycotoxins reached from 0.07 to 0.33 ppm
for Verrucarol, 0.22 to 0.41 for T_2, 0.11 to 0.16 for Nivalenol, and
0.38 to 0.70 ppm for DAS (all values found in blood samples).
In total we had evidence of mustard gas in 7 of 10 men, 2 were
negative and one died before evidence could be found. Regarding
trichothecens 6 of 10 samples were positive, 2 were negative and
2 have died before samples could be taken.

One patient had repeated nose-bleeding, which could be treated
conservatively. Another patient had several attacks of intestinal
haemorrhage without necessitating any surgical procedure. In both
patients there was evidence of mycotoxins.

In some of our patients new blisters appeared in skin areas,
which were free at the beginning (fig. 6). But all affected skin
areas healed at last after up to four weeks without any skin graft-
ing. The skin of the scrotum and penis took the longest to heal.

In summary we have lost four of our ten patients, but on the other hand the combined treatment was successful in six patients. Three of these six patients had been considered to be hopeless cases.

FINAL REMARKS.

By the successful treatment of six out of ten patients suffering from an intoxication with mustard gas and mycotoxins we could not only help these men to survive, but also accomplish an humanitarian duty to give valuable informations about a possible and successful therapy to Iran, where other intoxicated patients will profit from our findings. Furthermore we have gathered valuable experience for the case of a chemical attack in Europe. Following our experience, measures of decontamination as haemoperfusion, cysteine infusions, and charcoal for elimination of toxins from the intestine seem to be successful in the therapy of mustard gas or mycotoxin intoxication. But as in cases of extensive contamination the therapy required highly intensive care, the bounds of capacity of the medical facilities will be reached soon in mass intoxications.

Lethal intoxication by wargases on Iranian soldiers

Therapeutic interventions on survivors of mustard gas and mycotoxin inmersion

by G. PAUSER, A. ALOY, M. CARVANA, W. GRANINGER,
M. HAVEL, W. KOLLER and N. MUTZ

Research Center for Intensive Therapy (Head : Prof. Dr. H. BENZER)
of the Clinic of Anaesthesia and General Intensive Medicine (Head : Prof. Dr. O. MAYRHOFER)
and the 2nd Surgical Clinic (Head : Prof. Dr. E. WOLNER)
and of the 1st Clinic of Internal Medicine (Head : Prof. Dr. E. DEUTSCH)
of Vienna University

SUMMARY.

Ten patients with a reported history of war gas exposition were transferred from Teheran to Vienna by air transport for treatment and rehabilitation. Six of them were admitted to various intensive care units, 3 of these patients (50 %) died. Two survivors — in the toxicologist's view lethally intoxicated — underwent excessive local and systemic treatment regimens. Main pathological findings in both patients were :

1. Large areas of depigmentation and wet lesions of the skin.

2. Lung function disturbances, mainly concerning gas exchange under conditions of unaltered lung mechanics. Angiotensin converting enzyme levels suggested severe disturbances of capillary endothelium cell function.

3. Decreased number of white blood cells in the peripheral blood, due to an almost complete bone marrow depression in punctate material.

4. Repeated attacks of septicemia, due to a high resistent pseudomonas species under conditions of deteriorated immune system variables.

5. Transient liver and kidney failure, probably reflecting the systemic septicotoxic periods.

The multitopic therapeutic approach aimed towards detoxication, stabilization of vital functions and skin preservation and healing by means of extensive nursing procedures :

1. Pretherapeutic blood levels of NIVALENOL = 0.15 ppm, VERRUCAROL = 0.33 ppm and similar values of these and related substances in urine and feces as well as appearant mustard gas amounts in body fluids made prolonged and repeated performance of hemoperfusion via active charcoal reasonable. After this pro-cedure, further concentrations of toxic agents could not be seen.

2. Massive enteral load with charcoal and Magnesium sulfate in order to absorb toxic agents from the gut.

3. Application of N-acetyl-d, 1-homocystein-thiolactone mixed with 1-cystein, vitamins C and K and Piracetame in high doses.

4. Skin therapy was scheduled as packs of charcoal, washing manoeuvres with human milk and application of ointments, mixed together from 5 basic substances in a more times revolving man-ner a day.

5. Vital threatening septic periods induced by a pseudomonas aeruginosa species resistant to any commercial available anti-microbial substance, could be successfully treated by thienamy-cin, a recent developed betalactam antibiotic.

6. Mechanical ventilation, parenteral nutrition, adjuvant thera-peutic drugs and long term monitoring via arterial and pulmonary artery catheters were as well as enteral feeding human milk (1.500 ml/day) and extended passive physicotherapy partly in an air bubbled sand-bed necessary.

Although a control group could not be established in the own department, there are strong suggestions of causability for that therapeutic regimen, considering above cited lethality.

Ten patients with a reported history of war gas exposition were transferred from Teheran to Vienna by air transport for treatment and rehabilitation. Six of them were admitted to various intensive care units, 3 of these patients died. Two survivors admitted to the 2nd ICU of the Clinic of anesthesia and General Intensive Medicine were in the toxicologist's view lethally intoxicated. Following, we would like to cite the conclusion-report, done

by Prof. Heyndrickx, Ghent : « The results of blood, urine and faeces confirm that at least two war gases in combination have been used : mustard gas (Yperite) and mycotoxins (components of the « Yellow Rain »). The amounts found are very high and fatal doses. We know that even in very small quantities, those gases are very toxic for men. There is no scientific doubt that those patients are lethal intoxicated by those chemical war agents ».

The purpose of the paper, however, is to present the pathological findings in those patients and to present a successful therapeutic approach.

Main pathological findings on patients with mustard gas and mycotoxin intoxication are :

1. Depigmentation and wet lesions of the skin.
2. Lung function disturbances.
3. Breakdown of the bone marrow.
4. Deterioration of the immune system, septicemia.
5. Transient liver and kidney failure.

In table I we would like to demonstrate the patient 1, who did not undergo a specific therapy, because we had no information at that time what happened to the patient.

TABLE I

Symptomatic findings in patient 1 after mustard gas and mycotoxin intoxication.
CMV... controlled mechanical ventilation
UV₁ Dräger... a volume generated ventilator by the Company Dräger.

PATIENT 1

1. Skin lesions : 40 %
2. Lungfunction disturbances :

Astrup :		
pH	7.43	7.31
pCO_2	40	49
pO_2	57	56
BE	+ 2.1	— 1.6
FIO_2	0.30	0.5

3 days
CMV — UV₁ Dräger

ACE :	7.26	4.98

3. Bone marrow :
   Punctate : nearly empty
   WBC-count : 3.300   900
4. Immune-system : septicemia

No diagnosis — No specific therapy — Exitus

The patient died within 72 hours under symptomatic therapy only.

Patients 2 and 3 are discussed in detail. At that time we knew about the toxic stuff and tried a more specific therapy, which is presented later in this paper.

Additionally we monitored the angiotensin converting enzyme - levels (ACE). This enzyme is produced by the endothelium of the pulmonary capillaries ; decreased levels occurred within the first

TABLE II

**Data on skin lesions and lung function disturbances.
IMV... intermittent mandatory ventilation**

PATIENT 2 AND 3

1. Skin lesions : 30-40 %
2. Lungfunction-disturbances :

Astrup	Before therapy	During therapy	After therapy
pH	7.44	7.43	7.40
pCO_2	33	31	34
pO_2	58	124	95
BE	— 0.1	— 2.5	— 2.5
FiO_2	0.21	0.30	0.21
Q	0.48	0.32	0.12

IMV-respirator

100 hours in adult respiratory distress syndrome-patients (so called shock-lungs). Higher levels will be seen in any case of proliferation (e.g. sarcoidosis). Normal values are 23 in our laboratory.

One could see that very low levels at the beginning normalized rapidly under the detoxification procedure — hemoperfusion — and controlled mechanical ventilation. Two weeks after discharge from our unit higher levels of ACE occurred, so that a long-term prognosis on lung function disturbances may remain uncertain. With due regard to limitations the increasing levels of ACE may indicate the beginning of proliferation of lung parenchyma.

In order to achieve more information on lung mechanics we evaluated so called volume/pressure diagrams for both patients.

TABLE III

ACE values in patient 2 and 3

HEMOPERFUSION	CMV		HEMOPERFUSION	CMV
7,35			5,77	
5,51			4,28	
4,55			4,98	
6,65			6,12	
7,87			7,70	
9,01			6,82	
5,86			16,01	
8,05			11,47	
10,06			13,03	
11,46			15,40	
6,82			21,87	
13,73			15,31	
13,91			16,1	
15,91			13,03	
15,13			14,52	
13,65			12,07	
15,66			16,80	
16,80			5,25	
17,41			7,0	
			6,47	
28,21			34,42	

2 weeks after discharge

TABLE IV

PATIENT 2 AND 3

2. Lungfunction disturbances

Volume/pressure diagram

ACE 5.86

V/P-diagram abnormal

1. Capillary leak syndrom — Interstitial edema (X-ray)
2. Cuffing of bronchioli
3. Cuffing of bronchial vessels
4. Disturbances of ventilation perfusion-ratio (Astrup)
5. Abnormal deflation limb

ACE 13.65

V/P-diagram normal

1. No interstitial edema
2. Deflation limb normalized
3. No proliferation — compliance normal

As you can see on the left side of figure 1 there is given a volume/pressure diagram (V/P diagram) which was derived in the very early phase of lung disturbances. In contrast on the right side there you can see the diagram in the last, let us call it, the healthy phase. The V/P diagram shows two phases of pressure volume relationship. The inflation limb and the deflation limb ! As you can see on the left side of the figure the inflation limb seems to be a little bit flattened in contrast to the
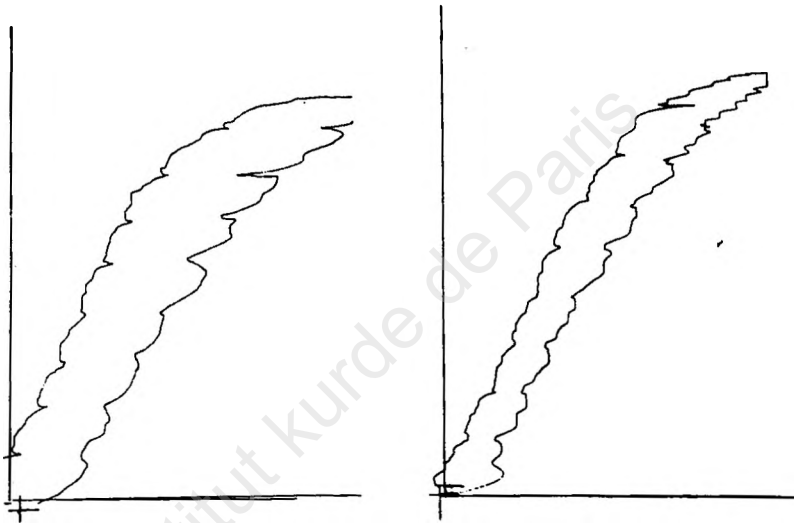
FIG. 1. — Volume/pressure diagram. Left side during early phase of lung disturbances.
Right side normal volume/pressure diagram. Patient 1.

V/P diagram on the right side. This fact could be explained by a slight deterioration of lung mechanics ; but if we focus at the deflation limb there one can find a distinct hysteresis in the curve as can be seen only in newborn infants or canine lungs. In contrast in a healthy lung in adult people you cannot see this mark increased of the so called hysteresis as you can see it on the right side of the figure. This increase of hysteresis on the left side which depicts the very early phase of intoxication, can indicate 2 marked points. First the activity of the surfactant system is not depressed by any intoxication, therefore lung compliance is normal. Second this V/P diagram indicates also that there is a marked reduced diameter of the alveoli. Therefore, a possible explanation of lung disturbances which cor-

relates significantly to our ACE values must be explained by the statements given in figure 2, showing V/P diagrams of patient 2. On the right side there seem to be some similarities of the C/P curves. As you can see on the right a normal function of lung mechanics must be assumed ; but similar to figure 1 the early phase of lung disturbances is expressed by a quite abnormal hysteresis and flattening of the V/P curves.

In this patient, however, the hysteresis is not so impressive as before, because of a little later registration of the curves. In
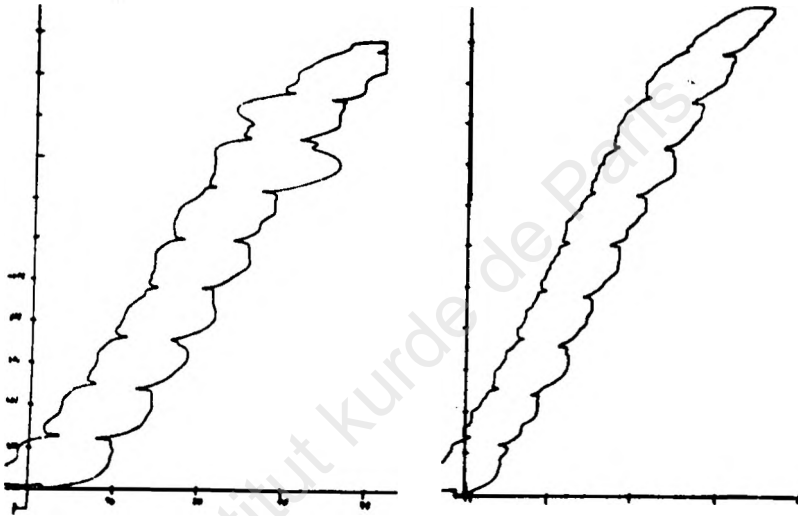
FIG. 2. — V/P curves of patient 2. Left abnormal, right normal.

table V calculated values are given for compliances at any step of the C/P curve. This explanation for lung mechanics was realized by computer program developed on a very simple home computer system at our unit. As you can see, compliances are not different from normal lung function, which will indicate once more that our previous statement is correct, the activity of the surfactant system was not heavily disturbed by the intoxication. Therefore it can also be assumed that visible dysfunctions in gas exchange are due to the statements given previously. Therefore we can say that one of the main parameters for detecting dysfunctions of the endothelium of the pulmonary capillaries and therefore disturbances in gas exchange are the continuous measurements of ACE levels. Furthermore we can con-

TABLE V

Calculated values of compliances derived from the V/P diagram (left column)

Statische Compliance

82.13	ml/cm	H_2O	bei	275 ml
83.78	ml/cm	H_2O	bei	467 ml
86.24	ml/cm	H_2O	bei	673 ml
82.35	ml/cm	H_2O	bei	873 ml
82.77	ml/cm	H_2O	bei	1083 ml
81.66	ml/cm	H_2O	bei	1306 ml
75.88	ml/cm	H_2O	bei	1512 ml
67.07	ml/cm	H_2O	bei	1684 ml
67.07	ml/cm	H_2O	bei	1684 ml
61.18	ml/cm	H_2O	bei	1684 ml
58.78	ml/cm	H_2O	bei	1512 ml
64.50	ml/cm	H_2O	bei	1271 ml
61.60	ml/cm	H_2O	bei	1065 ml
55.67	ml/cm	H_2O	bei	838 ml
51.30	ml/cm	H_2O	bei	838 ml
42.43	ml/cm	H_2O	bei	426 ml
34.80	ml/cm	H_2O	bei	233 ml

Ausdruck	. . . . .	>A<	Speichern	. . . . .	>S<
Noch einmal	. . .	>N<	Ende	. . . . .	>E<

TABLE VI

White blood cell counts in patient 2

White blood cell counts

	2.300 ...... 5.800 ...... 18.000 ...... 7.300
	Discharge

Period of septicemia		
Bars	20	2
Granulo	44	68
Lympho.	20	25
Eosino.	6	—
Monocyt.	10	5

TABLE VII

Parameters reconfirming septicemia

PATIENT 2 AND 3

4. Immune-system : — Septicemia

  1. Body-temperature : — 40.8° C

  2. WBC-count : — 18.000 (I)

  3. Hemodynamics :
    Cardiac index   6.6
    TPR   404

  4. Complete resistent pseudomonas (thienamycin)

  5. Antithrombin III } at any time normal or increased
    fibronectin

clude that mycotoxin intoxication affects the pulmonary endothelium, while mustard gas immersion deteriorates the above mentioned surfactant film covering the alveoli.

Concerning the breakdown of the bone marrow, table VI gives some values of the blood cell counts and differential blood cell counts. It is noteworthy that even under a severe stage of septicemia white blood cells increase up to 18,000 only.

Table VII shows parameters confirming the breakdown of the immune system following a hyperkinetic stage of septicemia.

This vital threatening septic period was induced by a pseudomonas aeruginosa species, which was resistant to any commercial available antimicrobial substance. Only a brandnew chemotherapeuticum — Thienamycin® — was able to cope with this high resistant strain.

As table VIII shows the septic stage never turned into a decompensation. Normal and even increased levels of antithrombin III as well as fibronectin demonstrate a consolidation during the period of septicemia.

TABLE VIII

Levels of antithrombin III and fibronectin

PATIENT 2 AND 3

At III	%	Fibronectin μg (240 ⊥)	
116	116	250	230
121	121	340	—
125	120	240	220
122	106	230	240
136	116	390	240
105	122	280	440
114	135	280	370
109	131	200	180
End of septicemia			
75	81	230	200
68	74	280	—
88	—		

Slight alterations in liver and kidney function as shown in table IX could be due to septicemia and need not to be specific for any intoxication.

Table X shows the highest levels of mustard gas and mycotoxins respectively (different types) before therapy as well as during therapy.

All those toxic stuffs, as shown in table X, disappeared during specific therapy. This therapy is summarized in tables XI and XII.

TABLE IX

Liver and kidney alterations

PATIENT 2 (AND 3)

1. *Liver*

   Bilirubin 2.12

   SGOT 121

   SGPT 147

   LDH 268

   AP 268

   γ-GT 58

2. *Kidney*

   Polyuria — 5000 ml/day

All clearance functions o.k.

TABLE X

Mycotoxin and mustard gas levels

PATIENT 2 AND 3

Mycotoxins and mustardgas

Patient 2 :

Nivalenol .	. .	0.15 ppm	— Blood
Verrucarol	. .	0.33 ppm	— Blood
Verrucarol	. .	0.10 ppm	— Urine
T-2 .	. .	0.18 ppm	— Urine
Verrucarol	. .	0.30 ppm	— Faeces
Yperite .	. .	Positiv	— Urine

Patient 3 :

DAS .	. . .	0.70 ppm	— Blood
DAS .	. . .	0.22 ppm	— Urine
Yperite .	. .	Positiv	— Urine

All those toxic stuffs disappeared during specific therapy.

TABLE XI

Multitopic therapeutic approach – detoxication

1. Hemoperfusion via active charcoal (patient 2 : 12 hours, patient 3 : 29 hours)
2. Charcoal and magnesiumsulfate via the gut and intestinal washouts
3. N-acetyl-D, L-homocystein-thiolactone plus L-cystein, vitamins C and K, piracetame systemically
4. Skin therapy :
   A. Washing manoeuvres with human milk
   B. Ointments (2 % xylocain, lasepton, pantothen, fibrolan, dealyd, vitamin A, betaisadona, canesten, solcoseryl, ultralan, cevitol)
   C. Actisorb-charcoal-pads

TABLE XII

Multitopic therapeutic approach – additional therapy

1. Chemotherapy with thienamycin
2. Parenteral nutrition as well as enteral feeding with human milk
3. Mechanical ventilation with PEEP spontaneous breathing with CPAP
4. Physicotherapy (air bubbled sand bed)
5. Excessive computerized monitoring (BP, PAP, PCWP, CO, fluid balance, blood chemistry A.S.O.)

TABLE XIII

Respiration physiology after discharge in patient 3

VC	100 %
FEV % VC	87 %
TLC	3.01 l
TLC %	101 %
RV	0.8 l
R	3.3 l

Table XIII presents some parameters on respiration physiology, showing normal values evaluated two weeks after discharge.

VC ... vital capacity
FEV ... fraction of expired volume
TLC ... total lung capacity
RV ... residual volume
R ... resistance

Although a control group could not be established in the own department, there are strong suggestions of causability for that therapeutic regimen, considering the above cited lethal intoxication and the fact that mainly those patients survived who could undergo completely the above mentioned therapy.

In summary we would like to state :

1. Only 3 out of 6 ICU patients in Vienna being immersed to mycotoxins and mustard gas survived. Three of them were « lethally » intoxicated.

2. Those 2 survivors out of the 3 were treated by the above mentioned therapeutic approach at the 2nd ICU of the Clinic of Anesthesia and General Intensive Medicine.

3. Treatment has to include detoxication as soon as possible via charcoal-hemoperfusion, charcoal to the gut and a high sophisticated skin therapy more times a day.

4. Lungfunction disturbances are due to a breakdown of the capillary endothelium with all the pathophysiological consequences — ventilatory support, monitoring of ACE and V/P-diagram. There was recovery at the time of discharge.

5. There is no chance in case of complete breakdown of the bone marrow.

6. In case of septicemia high resistant strains are seen. Only very potent antibiotica can help.

7. Any further prognosis is uncertain. Increasing ACE-levels after discharge can be a symptom for an uprising proliferation in lungs.

References available by the author.

Plan of Action

Environmental specimen banking

Hitherto gained experience and possible future prospects

by M. STOEPPLER

Institute of Applied Physical Chemistry,
Nuclear Research Centre (KFA) Jülich, P.O. Box 1913,
D-5170 Jülich, Federal Republic of Germany

SUMMARY.

The world-wide spread of anthropogenic chemicals into the total environment, including man himself, demands scientific and technical programmes for protective and legislative actions in order to arrive at an effective pollution control.

Within these tasks environmental specimen collection and storage (« specimen banking ») is of paramount importance. In the Federal Republic of Germany an interdisciplinary pilot programme on this subject with appropriate scientific, technical and analytical investigations has been supported by the Federal Ministeries of Research and Technology and Interior and coordinated by the Federal Environmental Agency (« Umweltbundesamt ») since 1976. Specimen bank facilities, however, have been inaugurated since 1980 (1). Within this large project up to now 12 institutes from the Research Centres, Federal Agencies and Universities ranging from medical over environmental, biological and geographical to analytical groups took part.

From the view of the Institute which operates the central bank and contributed significantly to all aspects of the German project a survey of the sampling and banking concept, storage techniques and storage conditions for a selection of human and environmental materials and the analytical programme of the pilot phase is given (2, 3). This is followed by a description of the present tasks within the transition phase from the pilot project to the permanent banking state in order to further improve technical and analytical performance and to define and evaluate future sampling

areas. Finally the prospects of a « medium sized » Environmental Specimen Bank for the Federal Republic of Germany from the view of specimen selection and collection, required specimen amounts and available storage capacity are given (4) and the appropriate organizational, technical and analytical efforts are discussed.

INTRODUCTION.

At present, approximately eighty to hundred thousand organic and inorganic chemicals are produced and used world-wide. In addition to those already in circulation every year up to one thousand new chemicals are introduced. The total production of all these substances reaches fairly large quantities. For example the production of organic compounds solely amounted already in 1979 to approximately 300 million tons per year (1) with a production of several million tons world-wide for toxic elements like lead (including organolead compounds for carburetor fuels), nickel, arsenic, cadmium, mercury, etc. After use a large percentage of these compounds, partly in metabolised form — is spread into the environment via the atmosphere, in aqueous emissions or in the form of solids (waste). Regrettably precise informations about the total amounts produced earlier, fields of application and environmental fate (accumulation, metabolisation, decomposition) are only available for a comparatively low number of chemicals. Although a rigid control of the production and possible toxicity of most of the chemicals produced has been started by legislative measures, e.g. the « Chemikaliengesetz » of the Federal Republic of Germany, in force since January 1982 (2), additional means are required for an effective control. Therefore careful and strategically well designed monitoring programmes, including man himself, are important in forming the basis for e.g. governmental actions to protect human beings, animals, soils, plants, lakes, rivers, etc. against harmful compounds.

However, systematic and comprehensive investigations on the toxicological or ecotoxicological behaviour and of discernible effects are practically impossible due to the large number of chemicals. Nevertheless there are strong efforts world-wide to separate, identify and quantify those organic and inorganic compounds that has been identified as hazardous. Such investiga-

tions very often pose the question whether those compounds have been introduced into the environment quite recently or longer ago, and whether the introduction rate is fluctuating, has peaked, or is at a constant rate over a certain time span. Further it would be of significance to know if these compounds are spread over the whole environment or localized in a part of it. Former approaches to answer these questions included careful analyses of these compounds in naturally stored materials from e.g., sediments, tree rings, fossils (museum specimens) bones, teeth and polar ice layers, which, in some cases, could be successfully traced back to the source (3, 4, 5).

These questions, however, could only be expected to be answered reliably and on a broader scale if there would be available specimens from the past which would have been selected from well-defined important regions or compartments. Moreover, those materials should be analytically characterized at the time of sampling and subsequently stored without deterioration and contamination. Informations from analyses of such « fossilized » specimens, including the retrospective analysis of recently identified hazardous compounds, certainly would support decisions, e.g. for governmental regulations. Therefore, since environmental surveillance and protection have become increasingly important during the last decades, the need for feasibility studies for long-term storage of specimens from human and environmental origin and their meaningful selection has been amply discussed and positively answered by numerous experts from various countries within the last decade (6, 7). This was accompanied in the United States of America as well as in the Federal Republic of Germany, Japan and Canada, by pilot environmental banking projects that also include the construction and operation of banking facilities for low temperature (mainly below —80°C) storage of various materials.

PILOT ENVIRONMENTAL SPECIMEN BANK PROJECT FOR THE FEDERAL REPUBLIC OF GERMANY.

The ESB project in the FRG, designed and coordinated by the Umweltbundesamt (Federal Environmental Agency), Berlin and financially supported by the Ministeries of Research and Technology and of the Interior, somewhat differs from the approaches in the USA, Japan and Canada. The other countries prefer a single

institution to be responsible for all technical and analytical aspects. In the FRG, however, the project has been organized at least during its pilot phase as a cooperative task shared by twelve independent research laboratories that range from ecology, biology and toxicology to environmental and analytical chemistry (8, 9). A central storage bank for all materials with appropriate dust-minimized facilities has been constructed in Jülich and inaugurated May 1981. This bank has a total storage capacity of approx. 20 m³ at liquid nitrogen vapour temperatures, i.e. the samples can be stored in the temperature range from approx. —160°C to approx. —190°C. For comparative storage also 2 m³ is available at —80°C. All these details have been described already elsewhere (10, 11). Due to the paramount importance of human materials a particular bank for those materials has been constructed in Münster. This bank has a total storage capacity of approx. 34 m³ at —85°C and has been inaugurated already 1980. For rapid freezing and comparative storage it offers also a capacity of 1.9 m³ at liquid nitrogen vapour temperatures. Special banks for comparative and complementary storage are situated in Kiel, Ahrensburg, Berlin and München, however, with rather low additional storage capacities. All these facilities in the FRG at present offer a total storage capacity around 60 m³ either at liquid nitrogen vapour temperatures or at < —80°C. This must be considered for the discussion of future prospects.

Within the pilot project the feasibility of all steps of specimen banking from sampling to long-term storage had to be investigated.

Thus, besides careful sampling studies a selection of initially fifteen than sixteen different materials ranging from human to environmental samples has been applied for the programme of the pilot phase (see table I).

In order to attain comparable results, all materials to be investigated have been homogenised fresh in one appropriate batch, if not *per se* homogeneous, and subsampled in polyethylene vessels for metal analyses and in glass vessels (borosilicate glass) for analysis of organic compounds. The total volume of the mentioned vessels varied due to the analytical requirements from a few to approx. 100 ml amounting approx. 10.000 subsamples. The selection of the individual specimens has been performed in such a manner that they should be as far as even possible representative for human and environmental

TABLE I

**Specimens selected for comparative low temperature storage
and long-term stability studies (organic compounds and toxic metals)
within the Pilot Specimen Bank project of the Federal Republic of Germany**

Human materials :

Whole blood ; adipose (fatty) tissue ; liver ; urine (recently introduced).

Terrestrical ecosystems and food chain :

Soil *(Parabraunerde ;* sewage sludge (from Hamburg) ; earth worm *(Lumbricus rubellus) ;* carab beetle *(Carabus auratus) ;* grass *(Lolium multiflorum)* wheat ; poplar leaves *(Populus nigra Italica) ;* cow milk.

Aquatic and marine environment :

Carp *(Cyprinus carpio) ;* mussel *(Dreissena polymorpha) ;* brown algae *(Fucus vesiculosus).*

studies as well as from the view of analytical methodology. It was intended to check or to adapt already existing trace analytical procedures for a variety of organic compounds and some toxic metals. The organic chemicals, including groups of compounds as well, and the metals and organometallic compounds to be analyzed during long-term stability investigations are listed in table II.

TABLE II

**Organic and inorganic compounds to be determined in the materials
selected for stability studies within the Pilot Phase
of the Environmental Specimen Bank in the Federal Republic of Germany**

Toxic metals and organometallic compounds :

— Mandatory substances : Hg, methyl-Hg, Cd and Pb.
— Further desirable metals and metalloids : As (including methylated metabolites of As [III] and As [V], Arsenobetaïne etc.), Ni, Co, Cr, Cu, Se, Tl etc.

Organic compounds (pollutants) :

— Halogenated hydrocarbons and polychlorinated biphenyls (PCB).
— Polycyclic aromatic hydrocarbons (PAH).
— Aromatic amines.
— Phenolic compounds.
— Hormones and anabolic steroids.
— Pesticides and insecticides.

Other organic compounds (model compounds for stability investigations) :

— Ascorbic acid.
— Unsaturated fatty acids.

Organic analysis has been performed in different laboratories for various specimens. In the author's laboratory, e.g. polycyclic aromatic hydrocarbons as well as hormones and anabolic steroids have been analyzed by different methodological approaches (11). All metals, however, have been determined consecutively for long term stability in all specimens where feasible in the author's

laboratory using different analytical methods and in some specimens also in other laboratories. This was particularly the case for human samples that where analysed in Münster also for a selection of additional metals by atomic absorption spectroscopy in connection with the bank for human materials (12).

The results obtained so far indicate in general that long-term storage at temperatures below —85°C most probably can prevent deterioration for organic compounds and that due to improved and partly new analytical concepts trend monitoring for various elements and compounds is possibly feasible in the near future from within 5 % alteration of concentration levels (13, 14).

As an example the attained long-term reproducibility for total mercury determinations in eleven out of the sixteen materials is given in table III.

TABLE III

Overall means for consecutive mercury determinations from mid 1981 to fall 1983 in some specimen types of the German ESB, values in μg/kg

Specimen type	Overall mean	RSD (%)	Remarks
Human whole blood	1.31	2.3	
Human liver	59.9	2.2	
Sewage sludge	480	2.1	
Soil (Parabraunerde)	52.0	3.8	Possibly inhomogeneous
Earth worm	21.3	1.9	
Carab beetle	63	2.1	
Grass	4.3	2.3	
Poplar leaves	18.4	2.7	
Carp	72	6.9	Material not particularly homogenised before subsampling
Mussels	11.9	5.0	Possibly not homogeneous
Brown algae	9.9	8.1	for mercury

This is a significant example for the improvement of a routine method and that it is now possible to attain in fairly homogeneous materials average long-term reproducibilities for mercury that are around or even below 3 % even at the μg/kg level. This has been achieved by a new version of cold vapour atomic absorption spectroscopy (15) in combination with an extremely effective and blank minimized digestion procedure using closed quartz tubes (16). The results obtained for a few materials (soil, carp, mussels and brown algae), however, indicate that for the pilot phase not in all cases the desired homogeneity could be achieved. This somewhat limits at present statements for long-term stability also if organic compounds are concerned

so that for future investigations significant improvements of sample homogeneity are needed at least for a few « difficult » specimen types. In a summarizing progress report of the pilot phase, just now appeared, this is advocated as highly desirable for the transition and the final banking phase.

TRANSITION PHASE.

The transition phase, i.e. the intermediate phase between the pilot and the real banking phase — during the pilot phase only one batch of each material has been repeatedly analysed for homogeneity and long-term stability — has begun early 1984. Within this phase final investigations have to be carried out for the identification of a few additional specimen types, monitoring areas and sampling locations. For the latter the present concept comprises for each specimen type sampling in a polluted and a nonpolluted location, whatever this means, because of the fact that the term « polluted » has to be differently defined for different compounds. The author's laboratory, for instance, is presently performing long-term studies on seasonal variations for trace metals (lead, cadmium, mercury, arsenic, etc.) in brown algae from different locations (supposed to be polluted and nonpolluted) from the Baltic Sea and the North Sea in order to clearly define sampling areas to be used within the banking phase. We do expect to finish this study and to start sampling for storage in the Specimen Bank already autumn 1984.

Another task, the improvement of homogenization for the banking phase now is also within realization. A prototype of a homogenization device, able to homogenize all materials of the specimen bank at liquid nitrogen temperatures, will be tested soon. The idea is to bring all environmental materials down to an extremely low temperature, i.e. close to that of liquid nitrogen (—196°C), just after sampling in order to perform homogenization and subsampling at the same temperature level (17). All further manipulations, transport to the storage containers and/ or analytical laboratories, of course, shall be performed under similar conditions. For this purpose the Central Bank operates three special vans equipped with liquid nitrogen vapour deep freezers and can further apply a mobile laboratory for field operations under clean room conditions, if necessary (18).

The transition phase is also characterized by current improvements in methodology, i.e. the increasing application of control materials for metals and organometallic compounds. These materials have been prepared mainly from freeze dried, finely ground and subsequently sieved surplus materials of the Specimen Bank or very similar materials so that at least the main matrix constituents and their contents are completely the same. These control materials shall be used — and have already been used — to check homogeneity of the freshly homogenized materials. In the future they shall be used for retrospective methodological comparative measurements and as a reference for at present not analysed elements as well. The use of control materials in comparative analytical determinations significantly improves precision up to a factor of two in favourable cases (19), which would be of paramount importance for an early detection of pollution trends either for increasing or decreasing values. Accuracy for the contents of the elements to be characterized was and will be further achieved by the continuous use of various physically different analytical methods as has been described recently in detail elsewhere (11, 13, 14, 15, 20). At present control materials are under study from the following specimens : *Human whole blood* (various materials, freshly stored and lyophilized, also from commercial sources (21) ; *liver* (spiked pig liver instead of human liver) ; *urine* (various, also lyophilized materials under study now) ; *soil* ; *sewage sludge* (from Hamburg but somewhat different from the batch stored for the pilot phase) ; *grass* ; *poplar leaves* and *brown algae*.

Further activities of the author's laboratory are in the field of direct metal analysis in liquids, but also in solids, i.e. without digestion prior to determination which significantly reduces blanks and enhances determination limits (15, 22, 23). This is possible now by using new and more reliable instruments for atomic absorption spectroscopy with better background compensation techniques such as Zeeman compensation. The latter constitutes a remarkable step forward if precision and accuracy are concerned. Improved determination methods also make possible the reliable identification and quantification of organometallic species in a number of Specimen Bank materials, which is at present particularly the case for methylmercury and the differentiation between inorganic arsenic and metabolites and higher molecular compounds like arsenobetaines from aquatic and marine sources (24, 25, 26). From this it can be expected that at the end of the

transition phase, i.e. winter 1984 all at present stored or even investigated materials of the Specimen Bank will be characterized for those organometallic compounds as well.

FUTURE PROSPECTS (PERMANENT BANKING PHASE).

Although experience in all aspects of specimen banking will still grow and further optimalisation is to be expected, the feasibility of meaningful specimen banking can be certainly regarded as established in general. This state-of-the-art has been clearly demonstrated during the technical and scientific sessions of the last International Workshop on Specimen Banking and Monitoring as Related to Banking (9) and also at least within the most recent progress reports of the Pilot Projects in the USA and the FRG.

If storage at temperatures below —80°C, preferably in the gas phase over liquid nitrogen (i.e. below —160°C), is performed, it is to be expected that authentic specimens representative for man, the terrestrial and the aquatic environment can be stored without alteration for quite a reasonable number of years. This is most probably valid for organic and inorganic compounds. Additionally, for heavy metals and metalloids of ecotoxicological significance, recent experience suggests that for most if not all specimen types storage of freeze dried samples will be sufficient.

Hence there is unanimous agreement among experts that efficient and comprehensive current and retrospective environmental monitoring has to be based in the near future on the operation of sufficiently capable national specimen banks or specimen bank systems that, of course, depend on the size and the ecological situation of the respective countries as well. Obviously a compromise between scientific needs and desirabilities and budgetary possibilities has to be found.

Based on the already available space for deep frozen storage and the situation in the Federal Republic of Germany from the view of various scientific research programmes a general concept for a « medium sized » specimen bank has been discussed during the recent workshop already mentioned above (27). This appears to satisfy the most important requirements by a well balanced cost-benefit compromise.

The already operational total space of approx. 60 m³ for storage at —80°C (mainly in Münster) and below —160°C (mainly in Jülich) would make possible to store, until the end of this century

more than six metric tons of materials. This estimation, of course, also includes a parallel storage in most cases for security reasons.

Since already from the investigations of the pilot phase a certain framework of specimen types exists, this has been extended from current considerations to cover monitoring and banking requirements from the environmental pollution situation in the FRG. A tentative list of approximately thirty specimen types or groups of specimen types, for this purpose is given in table IV, which still might be due to changes in some cases but in general will certainly show the fairly correct selection tendency for the materials of the specimen bank of the FRG.

TABLE IV

Tentative list of representative specimen types for a « medium sized » Environmental Specimen Bank in the Federal Republic of Germany

Human samples	Terrestrial environment	Food* chain	Limnic environment	Marine environment
Liver	Sewage sludge	Up to 7 various meat and vegetable products	Sediment	Sediment
Adipose tissue	Soil(s) (up to 3)		Fish (2)	Algae
	Earth worm		Mussels	Mussels
Placentae	Grass			Pelagic and benthic fish
Urine	Poplar leaves			Bird and/or bird feathers
Milk	Pine needles Bird and/or bird feathers			

* Food chain products selection is now under discussion in a particular expert group but most probably will be finished end 1984.

From the experience gained during still ongoing investigations (see previous chapter) it appears to be sufficient to collect the mentioned specimen types on average in a biannual sequence and in an average amount (final homogeneous or homogenized material) of life kg, bottled in approximately 230-300 20 ml standard vessels (glass and polyethylene) depending on specific weight, per sampling location for permanent banking. As has been already mentioned, commonly each specimen type should be selected in such a manner that one composite sample is taken at an exposed another at an non-exposed location. The exposed loca-

tion should « fossilise » peaks, etc., the non-exposed concentration profiles or overall trends.

Every year this will amount to approximately 150 kg of stored material for 15 specimen types. If this amount will be approximately doubled for parallel storage, and some additional samples from particular sources (human, environmental) without treatment i.e. for later homogenization, the present storage capacity at least allows reliable storage until the end of this century, even of a selection of appropriate control materials. If necessary some additional specimen types could be included later, without any space problem.

The prospect to use freeze dried and under controlled conditions but at ambient temperature stored additional materials or to store a distinct percentage of the already selected materials in a freeze dried form would further add some more flexibility to the whole system.

Hence it can be concluded that environmental specimen banking at least for the Federal Republic of Germany will reach very soon the stage where it can provide important and unique contributions to environmental protection and management as well as to scientific research.

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The practice of chemical warfare in Kampuchea and Laos

by Pol. Maj. Gen. (retired) Chana SAMUDAVANIJA

Chairman of the Asian Lawyers Legal Inquiry Committee
on Alleged Violations of Human Rights in Kampuchea and Laos,
Bangkok, Thailand

SUMMARY.

From June 4 to 11, 1982 the Asian Lawyers Legal Inquiry Committee comprised of members from Bangladesh, India, Indonesia, Singapore, Thailand and participated by observers from Australia, Japan and New-Zealand held an inquiry in Thailand on alleged violations of human rights in Kampuchea and Laos and found that :

1. *Vietnamese forces had and are using lethal chemical and biological weapons against the people of Kampuchea and Laos.*

2. *The afore mentioned chemical and biological warfare is intended to destroy resistance to regimes established and maintained by Vietnamese military intervention in total disregard for the right to self-determination of the people of Kampuchea and Laos.*

3. *The hostile presence of Vietnamese forces in Kampuchea and Laos and the use of lethal chemical and biological weapons by these forces has resulted in exodus of refugees depriving them of their basic rights to home, country, property and personal security by an invading army :*

In the course of investigation the Committee members visited the refugee camps on the Thai borders and examined the victims. The victims reported that the chemical-biological agents had been delivered by both artilleries and rockets fired from aircrafts. Methods were also adopted for poisoning sources of drinking water.

A variety of toxic agents and carriers were described. Reports of yellow, moist and sticky agent-yellow rain- predominated, but

occasionally granular substances were reported, as well as green, blue-green and black agents.

By investigation it was found that haemorrhaging from the gastro-intestinal tract was the most striking symptom of seriously affected victims : reduced contact produced a blistering rash, especially under the arms and in the genital area.

The young and the aged were especially hard hit and the animals nausea, bleeding from eyes, burning the skin, vomiting, diarrhoea with blood, fainting were common and immediate symptoms. The death rate was high. Survivors took one month to recover to ascertain the above conditions. The members of the Committee examined the victims of the chemical-biological attack, the case histories of some of the victims and doctors who were at that time in charge of the refugee camps reached to the findings mentioned above.

On December 12, 1980 the General Assembly of the United Nations passed its resolution 35/144C to carry out « an impartial investigation to ascertain facts pertaining to reports regarding the alleged use of chemical weapons and to assess the extent of the damage caused by the use of such weapons ».

Following the resolution 35/144C, the Secretary-General of the United Nations appointed the members of the Group of Experts to investigate Reports on the Alleged Use of Chemical Weapons.

The General Assembly, in November 1981 had received the report by the Chairman of the Group, Major General Dr. Esmat A. Ezz, Head of Scientific Research Branch, Egyptian Armed Forces.

According to the General Assembly, the distribution of the report (No. A/36/613) dated November 20, 1981 stated that the Group of Experts found itself unable to reach a final conclusion as to whether or not chemical warfare agents had been used.

The inconclusive nature of the investigation made by the Group of Experts in November 1981 prompted various criticisms and a series of more active interest over the practice of chemical and biological warfare began to take effects.

In Thailand there had already been widespread news and articles circulating in the local newspapers suggesting evidences that the practice of chemical warfare in this part of the world was for real. Much of the news that had been generating in Bangkok was quickly picked up by various international news ser-

vice agencies. In January issue of *Keizai Orai Magazine* published in Tokyo, Japan, Ichiro Totsuka wrote an article entitled, « Soviet Union's Yellow Rain, Menace to Mankind ». A passage in the article ran the following accounts, « A Mig-21 fighter circled over where the assistant village headman was, and dropped four brown rice bags from its fuselage. No explosion was heard. The fighter turned its nose to the north and flew off. During their descent, the bags opened and a yellow mist was released. It was a windless spring morning and the mist silently fell toward the houses of the village. Several minutes thereafter Yong Mang Yang saw villagers beginning to die. When the people came in contact with the « Yellow Rain », fist women and children began vomiting and lost consciousness ; two or three seconds later, men fell into a similar condition. Yong Mang Yang became unable to breath through his nose which started to run and he felt an irritating sensation. His eyes felt as if sand had got in them and he started shedding tears of blood ».

The origin of Ichiro Totsuka came from an account given to Bangkok Post by the Hmong refugee. The story in this English daily newspaper appeared in the 25 October 1981 issue.

The United Nations report no. A/36/613 came out one month later and delivered inconclusive findings as earlier suggested in this paper.

Barry Wain, the correspondent based in Bangkok for the Wall Street Journal, wrote in the newspaper on Wednesday, December 30, 1981. He made the point claiming the United Nations bureaucracy to take 11 months to come to Thailand. Furthermore he wrote that the so called, « Group of Experts » failed to enter Laos and Cambodia. The fact was, as Wain suggested in the article, the team to investigate the matter was originally invited publicly to visit Cambodia by the Khmer Rough government, the recognized Cambodian government by the United Nations.

Blaming on the United Nations bureaucracy, the Wall Street Journal's correspondent found that the United Nations team came too late to gather qualified eyewitnesses reports from the Hmong refugees. The Hmong refugees who actually saw the event of the use of chemical weapons had already left Thailand through resettlements overseas. The situation in 1981, suggesting that the United Nations appointed Group of Experts was not willing to spend a longer period in Thailand and little willingness to take the issue seriously, had been reported widely in the local press. Despite such situations mentioned above, local news in Bangkok kept busy with various

fresh reports of the practice of chemical weapons along the Thai borders with Laos and Kampuchea. The active party which continued the study after the Group of Experts had left Bangkok were the Americans.

Among the Americans in Bangkok, the name of the 51 years old Amos Townsend — a Doctor working with Laotian refugees for a voluntary agency was predominant. It is very interesting to note here that prior to any serious attempt by any party to investigate the use and practice of chemical warfare, Dr. Townsend had already gone far enough to identify various symptoms suspected of being the effects of the chemical agents through blood tests.

Apart from Dr. Townsend, only Canada took the matter to start its own independent inquiries based on international law. Canada was to take the matter with more concern because of what it claimed « the most important work to be the standard instrument concerning the prohibition of the use of chemical weapons ».

By then the issue of the practice of chemical and biological warfare in Kampuchea and Laos had already turned into a hot 'cold war' issue. Soviet Union and the US government were at pain to start denying and convincing the allegations.

On March 22, 1982 the United States Department of State published a report to the Congress from Secretary of State, Alexander M. Haig, Jr., entitled « Chemical Warfare in Southeast Asia and Afghanistan ». The report quoted heavily on medical evidences in Southeast Asia. Evidence has been traced up to 1975 by many sources such as refugees, relief workers and medical personnel. It said, « Victims in Southeast Asia subjected to a direct attack of the yellow powder, mist, smoke, or dust would be seen to begin retching and vomiting within minutes. These effects and those described ... were not pronounced in individuals even 100 meters from the attack zone, indicating a relative dense chemical/carrier combination that was effective in low wind conditions.

Following the victim's exposure to yellow rain, the initial induced vomiting-unlike that caused by the traditional riot-control nausea agent-was protracted over hours to days. It was often accompanied by dizziness, rapid heart-beat and apparently low blood pressure, chest pain, lost of far-field vision, and a feeling of intense heat and burning on the skin, although not described as being most acute in the groin and axillae. Thus, the acute signs and symptoms match some effects of traditional vomiting and blister agents but clearly not all ».

The report to the Congress from the Secretary of State, was in fact the summary of the many cases reported earlier among the Hmong refugee fleeing war from Laos and later by civilians crossing the border into Thailand from Kampuchea.

Then it was on June 21, 1982 that Canada joined the campaign in fighting against the practice of chemical warfare in disclosing the findings of a scientific study showing the chemical agents had been used in Western Kampuchea.

Canada's Foreign Minister Mark McGuigan told at a press conference at Oriental Hotel in Bangkok that the finding of the commissioned Dr. H.B. Schiefer of University of Saskatchewan who came to Thailand and conducted an independent on-site inspection close to the Thai-Kampuchean and Thai-Laos borders.

The most striking evidence was made again on July 22, 1982 when a Vietnamese deserter Corporal Trin Dine Whan appeared at the press conference in Bangkok. Trin Dine Whan formerly of the 210th Artillery Regiment of the 7th Division told that 27 chemical shells were lobbed into Sok Sanh village controlled by the Khmer People's National Liberation Front (KPNLF) with an aim to annihilate the Khmer resistance. The corporal who served in Kampuchea since December 1979 and escaped to Thailand confirmed that the chemical weapons were supplied by the Soviet Union.

During June 4-11, 1982 Asian Lawyers Legal Inquiry Committee on Alleged Violations of Human Rights in Kampuchea and Laos met in Bangkok and started the fact-finding missions to various refugee camps in Thailand. The Committee originally wanted to go to Phnom Penh the occupied capital city under the Vietnamese backed Heng Samrin and to Vientian the capital city of Laos. The Committee was denied entries to both cities. The fighting Khmer Rough resistance groups as well as other fighting forces to liberate Kampuchea were also invited to freely interview on the sites but the Thai military authorities responsible for birder security considered the crossing of the border was too dangerous. Thus the findings of the Committee had to settle for the refugee camps at Khao I Dang and to Nong Khai Refugee Camp for briefing on chemical warfare against the Hmong in Laos by Dr. Amos Townsend, Medical Coordinator for the Nong Khai Refugee Camp. He was joined by Dr. P. Passmore of Australia, a chief administrator of World Vision International, a humanitarian organization responsible for relief operations at Ban Vinai camp

where eyewitnesses and those effected by the chemical agents testified to the Committee.

The Committee went also to the Phanat Nikhom refugee camp where it was allowed to question Vietnamese soldiers who defected to Thailand from the service in Kampuchea. Evidence verbally described through the court room-liked investigation led the Committee to believe that the practice of chemical warfare had been conducted both in Kampuchea and in Laos.

The Committee found out that :

1. Vietnamese forces had and are using lethal chemical and biological weapons against the people of Kampuchea and Laos.

2. The afore mentioned chemical and biological warfare is intended to destroy resistance to regime established and maintained by Vietnamese military intervention in total disregard for the right to self-determination of the people of Kampuchea and Laos.

3. The hostile presence of Vietnamese forces in Kampuchea and Laos — and the use of lethal chemical and biological weapons by these forces — have resulted in exodus of refugees depriving them of their basic rights to home, country, property and personal security by an invading army.

The present situation on the practice of the chemical warfare is not over at this time. There are still day-to-day reports on new discovery of the deployment of these dangerous chemical agents. It must be pointed out that not only they are being deployed in Kampuchea and in Laos, civilians of Thai citizens along the borders with the two countries are also being the target of these chemical agents. Therefore it is very important that instead of letting the issue dies out from the concerned United Nations, there should be more active impartial party to take a renewed interest and there should be international pressure to establish a standard procedure in prohibiting the deployment of these dangerous agents as soon as possible. The chemical warfare is very much alive but it appears that today we tend to let thing easily forgotten.

Chemical disarmament and the Third World

Problems of verification and prevention of proliferation

by Z. BINENFELD and V. VOJVODIC

Laboratory of Organic Chemistry and Biochemistry, Faculty of Science,
University of Zagreb, Zagreb, Yugoslavia
Military Medical Academy, Beograd, Yugoslavia

SUMMARY.

Chemical weapons do not represent a real threat to thé super-powers as they do to the Third World. Therefore the approaches to the problems of verification and proliferation are not identical. This is one of the reasons for the « dead course » in chemical disarmament. This paper reviews these problems.

As a result of the vast resources allocated to military purposes, the technology of the manufacture of means of destruction is developing extremely fast, in striking contrast with the slow process of the negotiation on disarmament, where no concrete results have been achieved. In fact, we are approaching the point where the disarmament negotiations will no longer be able to exert any influence on the arms race and there is a real danger that this race will escape all control, with incalculable consequences for all mankind.

The government representative of superpowers is prepared to declare its readiness and make solemn promises to reduce arms investments if, naturally the opposite side, generally referred to as the aggressive one, would take the first step. In fact, the lack of trust among superpowers, has reached such an extent that each of them seeks the guarantee for its own safety exclusively through the increase in military power from producing newer and newer types of arms. In particular the nuclear and chem-

ical arms race feeds on the continuous input of scientific innovation, and there is a growing belief that the momentum of this arms race is determined by the actions of scientists. Introduction of any new weapon is an irreversible step, and in this scene the role of the scientists in the arms race is of crucial importance. Unfortunately, nothing would have been done to improve the security prospects of smaller countries — the traditional « targets ». A convention is not currently in sight, despite great efforts by the CD's *ad hoc* working group on chemical weapons to address the technical elements of an agreement.

The sense of insecurity smaller countries feel is potentiated not only by the postponement of any serious discussion on chemical disarmament but also by the depreciation of their military usefulness in comparison with other powerful weapons. Smaller countries clearly do have considerable cause for concern with a situation where chemical weapons remain in the hands of the two superpowers and may « fall into » the hands of a possibly growing number of regional military powers. The use of CW against smaller countries could have a strategic implication. Such a danger does not exist or is much smaller for superpowers and those countries which are under their protection. The inevitable conclusion is that smaller countries cannot simply wait for the superpowers to resolve all issues in the field of arms control, especially since the superpowers have their own immediate priorities which have been, and will remain, quite different from those of small countries.

It is promising that at this Congress the problem of chemical disarmament can be approached also from the point of view of small countries. A number of « non-aligned » and other smaller countries which do not belong either to military pacts or to « non-aligned » make a very complex group and they have very similar views regarding disarmament which were expressed on many occasions by their representatives. This group comprises also countries which surely are not be named « smaller », e.g. India, as well as highly developed countries such as Sweden and Finland. The fundamental common characteristic of these countries is that they do not possess their own CW, do not intend to acquire them, and no CW are located on their territories. Consequently, in contrast to countries associated with superpowers through military pacts or otherwise, even in war they cannot use CW, neither CW could be located or delivered from their territory (except in the case of occupation). For these countries CW

represent a greater danger than for *a)* superpowers, with sophisticated antichemical defence, soldiers protected with suitable equipment and in addition possessing other mass destruction weapons, and *b)* countries associated with superpowers, which also have a high level of chemical defence and are « protected » by the doubtful doctrine of deterrence. In most of smaller countries the chemical defence as well as the medical protective measures are so poor that they would be practically unprotected against the consequences of chemical warfare.

Chemical weapons represent a very attractive possibility in so called « local wars » which are without global consequences. The acquirement of such weapons would mean to gain the supremacy over the adversary, and from acquirement to use there is only one step.

Smaller countries are without any restriction interested in banning of chemical weapons and their elimination from military arsenals. Any approach to chemical disarmament which leads to a comprehensive prohibition of manufacture and stockpilling and to the destruction of the existing stockpilles of CW represents for them an acceptable solution. The so called stepwise approach to this problem in which the destruction of supertoxic agents should be the first step in a comprehensive chemical disarmament and in which the destruction of other categories of chemical warfare agents should be postponed for a longer period of time is not acceptable because the potential danger of using CW would remain present.

Many of the World War I agents would be more than adequate against an enemy who did not have gas mask, protective clothing, decontamination equipment and appropriate drugs and medical facilities. In West, where such defensive measures do exist and where military forces are trained to cope with such an environment, chemical weapons incorporating some of the World War I agents still cause considerable concern. The present monopoly of superpowers represents a constant threat to small countries because they and their allies could use CW as well as proliferate them to countries which do not have them.

In this context the binaries represent a special problem. Binary components offer a greater possibility of proliferation with the aim of « terminating on place » or otherwise.

Chemical production facilities previously considered unsafe for the production of nerve agents may now be safe enough to deal with the binary agent precursors, due to the reduced toxicity

hazard of the intermediates. Existing technology — readily available toxic industrial and agricultural chemicals and their production facilities are available or easily developed. Rudimentary means for disseminating could serve as the basis for the development of a chemical offensive capability.

The arms limitation agreements must be founded on reasonable confidence, as is the case with some existing contracts. If there is a decrease in confidence or if there is any doubt concerning the violation of agreements, then only verification measures can restore confidence among States signatories of the agreement. This is particularly true for the countries which possess production facilities and stockpiles of chemical weapons because the arms race, which is most often motivated by acquiring an advantage, or is just justified by the need to not lag behind in the creation of new weapons, is as a rule initiated by these countries. Bearing in mind the specific characteristics of CW, the proposed international verification procedures were the consequence of either political or technical difficulties. On the basis of negotiation it seems, in our opinion, that systematic international verification procedure would be acceptable for majority of nations. There are three fundamental categories of verification :

a) comprehensive (absolute) verification,

b) essential (necessary) verification,

c) limited (insufficient) verification.

a) Comprehensive (absolute) verification presupposes the voluntary acceptance of international inspection and a maximum of openness regarding the obtaining and gathering the necessary data in all stages of the verification procedure.

b) Essential (necessary) verification presupposes a mutually agreed acceptance of international inspection which is in accordance with the conditions stipulated in the Convention. It can be carried out periodically (once or several times in a year) or when the need arises.

c) Limited verification does not include the international verification procedure. Under certain conditions this verification can also encompass off-site inspection.

The problem of verification, as the key problem of chemical disarmament, means for smaller countries : a) controlled destruction

of the existing arsenal of chemical agents and weapons and of manufacture capacities in a period of time as short as possible, and *b)* prevention of proliferation not only of weapons but also of technology and specialists to countries which do not have CW. The crucial problem is what kind of verification can prevent proliferation. Small countries should have the information on the actual situation in chemical armament, including also binaries. They would also like to know where and when the critical chemicals were produced, the location and amounts of stockpiled CW, and the planned rate and manner of distruction of all existing CW.

In conclusion we would like to repeat that superpowers look at CW in the first place as a threat for those not possessing them while small countries consider CW as a direct threat for themselves. This controversy is the main reason for the present « dead course » in chemical disarmament.

Les procédures d'enquête, en cas d'usage allégué d'armes chimiques ou biologiques, sur la base de la résolution 37/98 D

par S. SUR

Professeur à l'Université de Paris X - Nanterre
Co-Directeur du Centre de Droit International de Nanterre (CEDIN)

Le présent texte a pour objet d'exposer très sommairement les principes directeurs des procédures élaborées dans le cadre du groupe d'experts-consultants auprès du Secrétaire Général, établi sur la base de la résolution 37/98 D.

Cette résolution a été adoptée, le 13 décembre 1982, par 86 voix pour, 19 contre et 33 abstentions. Elle n'a donc pas fait l'objet d'un consensus, et les mécanismes d'enquête qu'elle envisage sont l'objet d'une controverse juridique entre gouvernements, qui n'est pas ici notre propos.

La mission assignée au groupe d'experts-consultants par le § 7 de la résolution comporte deux aspects : préparer les procédures d'enquête ; rassembler et organiser systématiquement la littérature scientifique concernant les signes et symptômes associés à l'usage d'armes chimiques ou biologiques ainsi que leur traitement. Seul le premier aspect fait l'objet du rapport du Secrétaire Général publié en 1983 (A/38/435).

La procédure d'enquête suggérée peut être analysée suivant ses principales étapes : son déclenchement, sa conduite, son aboutissement.

I. DECLENCHEMENT DE L'ENQUETE.

1. Il convient d'abord de déterminer qui peut agir, qui peut saisir le Secrétaire Général d'une demande. La résolution 37/98 D dicte elle-même la solution : tout Etat membre peut porter à la con-

naissance du Secrétaire Général des faits de nature à **justifier** l'enquête. Ce peut être *tout* Etat membre, à propos de *n'importe quel* usage, où qu'il se soit produit.

2. Il convient cependant d'éviter les allégations fantaisistes, ou purement propagandistes, qui ne pourraient déboucher sur une procédure sérieuse. Aussi l'Etat membre doit-il apporter des informations suffisamment étayées et précises, sur la base desquelles le Secrétaire Général procède à une évaluation préalable de la demande. Il peut solliciter des renseignements complémentaires, et se faire assister par un ou des experts afin d'apprécier l'utilité de l'enquête. Sur la base de cette évaluation, il décide de ne pas entreprendre une enquête, ou au contraire d'y faire procéder.

II. **CONDUITE DE L'ENQUETE.**

1. Le Secrétaire Général doit d'abord procéder à la désignation d'un groupe d'enquêteurs. Il les choisit sur une liste qui comprend les noms des personnes qualifiées proposées par les Etats membres. Il lui appartient de sélectionner les spécialistes les mieux adaptés en fonction du type d'enquête requis. Le groupe est accompagné par des membres du Secrétariat et des interprètes.

Le Secrétaire Général prend également contact avec des laboratoires choisis sur une liste constituée dans les mêmes conditions. La coopération d'un nombre suffisant d'Etats membres est donc une condition indispensable au bon fonctionnement de la procédure.

2. Ensuite, le déroulement de l'enquête est tributaire des circonstances et du comportement des Etats intéressés. A cet égard, il convient de distinguer trois hypothèses.

a) Une enquête *sur place* est possible. L'Etat où l'usage allégué d'armes chimiques ou biologiques s'est produit acceptant l'enquête sur son territoire et apportant au groupe d'enquêteurs l'assistance nécessaire. Cette situation est naturellement la plus favorable à la conduite d'une investigation efficace. Elle repose sur le consentement du — ou des — Etats intéressés.

b) Une enquête sur place n'est pas possible, parce que l'Etat concerné refuse son concours ou n'est pas en mesure d'apporter

l'assistance nécessaire. Mais l'accès à un *territoire voisin* est possible, et des informations utiles peuvent y être recueillies, telles que témoignages, examens de blessés, de réfugiés, collections d'indices ou d'échantillons variés. L'enquête se déroule alors dans les mêmes conditions que pour la première hypothèse.

c) La troisième est la plus délicate, parce que l'accès au territoire concerné ou à un territoire voisin n'est pas possible. On ne doit pas cependant renoncer à tenter de recueillir toutes les informations pertinentes.

Dans les trois hypothèses, le rapport recommande un ensemble de mesures techniques, portant sur le conditionnement des échantillons bio-médicaux, l'interrogatoire des témoins, les méthodes d'analyse de l'ensemble des éléments de preuve dont le groupe d'experts peut avoir connaissance.

III. **ABOUTISSEMENT DE L'ENQUETE.**

C'est le point essentiel. Le rapport ne traite que de l'aspect technique et laisse de côté toutes les implications juridiques et politiques, qui sont de la responsabilité de l'Organisation des Nations Unies et des Etats membres. L'objet de l'enquête est simplement d'établir, et même d'évaluer des faits.

1. Le groupe d'enquêteurs élabore un rapport qui comporte les principaux éléments suivants : la composition du groupe aux différents stades de l'enquête ; la description des différentes phases de l'enquête et de l'ensemble des éléments recueillis ; les résultats des analyses effectuées en laboratoire ; les conclusions proposées par le groupe, qui doivent permettre d'apprécier la réalité, la nature et l'étendue de l'usage allégué d'armes chimiques ou biologiques.

2. Le groupe n'est pas cependant tenu de se prononcer par « oui » ou « non », mais peut procéder à une évaluation de la probabilité de l'usage. Toute autre formule serait contraire à la rigueur scientifique particulièrement requise dans ce type d'enquête. L'appréciation de la probabilité peut également porter sur la nature des armes utilisées ainsi que sur la quantité des substances impliquées.

3. Le groupe travaille et prépare le rapport en principe de façon collégiale. Des opinions individuelles sont toutefois possibles, et

chaque membre est autorisé à adjoindre au rapport les précisions, compléments ou objections qui lui paraissent devoir s'imposer.

En conclusion, il convient de souligner une nouvelle fois combien la coopération des Etats membres est indispensable aux procédures ainsi proposées. Il est également loisible d'émettre un vœu : qu'une fois pleinement opérationnelles, l'existence même de ces procédures soit suffisamment dissuasive pour qu'elles ne soient jamais utilisées, ce qui serait la meilleure preuve de leur utilité. On rappellera enfin que les travaux des experts consultants se poursuivent et devraient en principe prendre fin en 1984.

Chemical and biological warfare in Cambodia

by S. SANNARD

Information Office of the Cambodian Nationalists - Benelux,
Brussels, Belgium

On behalf of the Nationalist Cambodians and as Representative of Prince Norodom Sihanouk in Belgium, I have the honour to bring to your attention one of our numerous resentments against the Socialist Republic of Vietnam ; so as to say the use by this country of chemical weapons against our people.

Unfortunately for us, the use of illicite weapons by Hanoi constitutes only one aspect of Cambodian tragedy.

Numerous narrations and testimonies of our soldiers, of defectors from Heng Samrin troops and of Vietnamese prisoners, and also the various medical observations recorded among our civilian population as well as among Thai people living at the border with our country are highly significant.

Thousands of Cambodians have died by these illegal methods of war. During 1983, more than 500 of our combatants have suffered by them.

During each of their annual seasonal offensives, Hanoi troops use toxic gases, chemical weapons cf all kinds as well as highly harmful substances spread in water spots and our civilian population established in khmer territory along the border with Thailand is the main victim.

This dramatic situation has been renewed last April during the recent attacks of Vietnamese troops against our bases and our civilian camps.

The variable methods of dispersal — from bombs and rockets to sprays — and the wide range of symptoms experienced by the victims indicate clearly that the attackers were using several different chemicals.

Certain victims died within one week time, others after a few minutes. The most current symptoms are : asphyxia, dehydration,

bleeding, burning, eyes inflammation, fit of coughing, **nausea,** vomiting, lacrimation, etc...

Several experts from different countries such as USA, Canada, U.K., Belgium have reached the conclusion that Soviets developed chemicals and toxins and transferred them to Vietnamese military forces.

In 1979, a team of medical specialists traveled to refugee camps in Thailand conducted extensive interviews of survivors of attacks as well as physicians who had treated and cared for them. The experts identified 3 basic sets of symptoms : burn to the skin, eyes, nose and throat ; spasms and convlusions and heavy bleeding and they concluded that the attacks involved at least 2 and possibly 3 chemical agents including mustard gas and nerve gas.

In December 1980, ad UN commission members concluded that the symptoms in some cases suggested « possible use of some sort of chemical warfare agent ». The group asked to investigate territories where chemical attacks had occurred but the authorities in Kampuchea denied them permission.

On September 1981, the United States announced preliminary test results of a vegetation sample taken from a reported attack site in Kampuchea which confirm the American concerns about chemical warfare in Southeast Asia.

At the end of 1981 and beginning 1982, the State Department presented more findings based on blood and urine samples of survivors of chemical attacks in our country which confirm traces of T2 toxin or its derivatives.

Numerous and varied samples are regularly collected by us and given to Thai armed forces which send them to foreign countries where adequate laboratories such as the one managed by Professor Heyndrickx in Ghent carry out the necessary investigations.

The use of illicite weapons by Vietnam against Cambodian people has given rise to several and regular declarations and severe protests from our leaders and in particular from Prince Norodom Sihanouk, President of Democratic Kampuchea as well as from Mr. Son Sann, Prime Minister of our Coalition Government.

The Council of North Atlantic which met in a ministerial session in Brussels on 8 and 9 December 1983, has condemned in its final statement the use of chemicals in Southeast Asia. Here is a short excerpt of it in French :

« Les Alliés demeurent gravement préoccupés par de fortes présomptions établissant qu'au mépris du droit international, des armes chimiques continuent d'être employées en Asie du Sud-Est, en Afghanistan et que l'URSS est impliquée dans l'utilisation de telles armes. »

Consequently, there is no doubt for us, Nationalist Cambodians, that the Vietnamese aggressor totally rejects the Protocol of Geneva Convention signed in 1925 ; Convention stipulating the prohibition of using chemical products in periods of armed conflicts.

Thank you for your kind attention.

The evidence of chemical and toxin weapon use in Southeast Asia and Afghanistan

by Gary B. CROCKER

Department of State, USA

SUMMARY.

The author is currently attached to the US Mission, Geneva, while conducting research on chemical an biological warfare issues as a participant in the Exceptional Intelligence Analyst Program. This research paper is not an official US Government document or statement of policy. The author is authorized to speak and write on chemical warfare issues by virtue of his ten years of experience in the Department of State prior to June 1, 1983, during which time he was an official spokesman on the US evidence pertaining to chemical weapons use in Southeast Asia and Afghanistan.

The United States Government officially presented evidence on the Vietnamese and Lao military forces use of lethal chemical agents on the Hmong people in December 1979 at hearings before the House Foreign Affairs Committee. Information on chemical attacks in Kampuchea and Afghanistan was presented to the same committee in July 1980. The United Nations established an international investigative team in December 1980. An intensive effort to collect information by the United States and other countries led to the publication of a report by Secretary of State Haig in March 1982 which was based on a classified National Intelligence Estimate. That report stated, with no qualifications, that chemical and toxin weapons had been used in Laos, Kampuchea and Afghanistan in direct violation of two international treaties.

In November 1982, Secretary of State Shulz submitted another report to Congress and the United Nations, again based on a National Intelligence Estimate, which provided further evidence. The United Nations accepted and released the report of the investigative team in December 1982. The final conclusion was « While the Group could not state that these allegations had been proven, nevertheless it could not disregard the circumstantial evidence suggestive of the possible use of some sort of toxic chemical substance in some instances ». During 1983, the United States provided additional evidence to the United Nations, and most recently, a report was submitted to the UN which pointed out that there had been no confirmed reports of chemical attacks in Afghanistan for a year and a half, but regrettably reports of Vietnamese use of chemical weapons continued to be received from Laos and Kampuchea.

The United States government did not originate the charges of chemical weapons use. Victims from all three countries, as well as military and political defectors, told their stories to refugee camp officials, physicians, journalists and UN investigators. Canada conducted an independent investigation and provided several documents to the UN. Thai officials have made statements based on their own investigations and observations, to include attacks on Thai territory which date back to at least 1980. Other governments have conducted investigations which contributed to the US assessments over a period of several years. Private organizations, particularly in the scientific and medical fields, have provided valuable research on specific aspects of the analytical problem of determining what agents have been used. Lastly, newspapers and periodicals from all over the world have been carrying stories about the illegal use of chemicals and toxins in the three countries for years, in most cases before any government statements were made.

Considering all the evidence that has been presented and the international attention that has been focused on this issue, why are we today engaged in a scientific debate over the validity of the charges and the evidence. There are a host of political and military reasons, some obvious and some not so obvious, that have caused governments and individuals to either be cautious in accepting the evidence or to actively work to disprove the evidence. It is not the purpose of this paper to examine the motivations of governments and individuals. Rather, it is to review the evidence, much of which has been forgotten or tossed

aside because of the preoccupation with what is popularly known as « Yellow Rain ».

The term « Yellow Rain » originated from descriptions by Hmong refugees of a wet, sticky substance which was sprayed from airplanes and dropped on their villages like rain. The substance was reported to have caused many deaths, illness, skin disorders as well as killing livestock and crops. People who consumed contamined water or food died or became seriously ill. The symptoms reported by the Hmong, and later the Kampuchean resistance, puzzled chemical warfare experts because they did not accord with symptoms produced by known chemical warfare agents. Samples of vegetation and other materials like bark and water were tested, but found to contain no known chemical warfare agents. In the fall of 1981, Dr. Chester Mirocha at the University of Minnesota made a breakthrough for US government analysts when he discovered high levels of mycotoxins in a vegetation sample from Kampuchea. Since that time, many samples of vegetation, water, blood, urine, tissues and even the yellow material itself have been analyzed and found to contain toxins. In one case the US found the same toxin on a captured Soviet gas mask from Afghanistan. All of the sample results are available to the public for review. In part, the sample results led to the US conclusion that toxins were being used by the Vietnamese in Laos and Kampuchea. However, it should be remembered that the US Government was convinced that a variety of chemical warfare agents were being used in all three countries before the use of toxins was confirmed by sample analysis. Moreover, in the absence of a successful sample collection program for Afghanistan (compared to Southeast Asia), the US government was confident in the assessment that toxins were used by the Soviets on the Afghan resistance.

The large body of multi-source information which compliments the evidence obtained from sample analysis, continues to be ignored or cast aside in the current debate about the origin of « Yellow Rain ». That body of information, if carefully examined, contains the answers to many of the questions about the type of agents that have been used in all three countries. Comparative analysis of the data from three countries, plus information acquired from attacks on Thai territory, will cast *considerable* doubt on alternate hypothesis that contradict the US Government conclusions. Moreover, the historical record of evidence, which has yet to be fully analyzed, shows that toxins have

not only been disseminated as a wet spray (yellow rain), but by other means. Furthermore, other lethal and non-lethal agents have been used which cause symptoms ranging from short periods of discomfort to instant death.

Sensitive intelligence information played an important role in convincing US officials that chemical and toxin weapons were being used and that the Soviet Union produced and provided those weapons used in Southeast Asia. Much of the information has been used in the official public documents, but the specific sources of information were protected. However, there is sufficient information available in the public domain to make a very convincing assessment if researchers take the time to correlate the data. What appears to be missing is dramatic evidence that can be used on television news. No one has been able to film a victim being hit with chemical agent, although several films are available of helicopters dropping chemicals and victims were filmed afterwards. Nor has anyone been able to bring out a shell or bomb filled with chemical agent. In the absence of such dramatic, and apparently « convincing » evidence, it is necessary to obtain proof the hard way : painstaking analysis of thousands of pieces of data. Unfortunately, this does not capture the public's attention and critics can focus on specific aspects while ignoring a large percentage of the evidence.

It is not possible here to describe all the evidence or even list all of the organizations and individuals involved in the investigation, but the evidence can be reduced to it's essential elements for purposes of comparative analysis.

CHEMICAL WEAPON DELIVERY SYSTEMS.

A wide variety of methods for disseminating chemical and toxin agents has been reported by refugees, defectors, journalists and in a few cases Thai government and civilian observers. Although some methods may seem unorthodox or even bizarre, all are technically feasible and most of the delivery systems are known to be part of the Soviet chemical warfare arsenal. Some new techniques for delivering chemical agent were discovered during the course of the investigation.

Laos.

Captured US L-19 single wing light aircraft was used by the Lao forces from the mid to late 70's to deliver rockets. A Lao

pilot described these operations in detail (March 1982 Haig Report).

Captured US T28/T41 trainer aircraft and Soviet AN-2 biplanes used in the Soviet Union for crop dusting. After 1978 Soviet MIG-17 and MIG-21 jet aircraft were reported firing rockets and dropping bombs. More frequent use of helicopters was reported after 1979 using spray tanks, rockets and cannisters.

There are only a few reports of artillery being used. One report described Soviet advisors using a hand held weapon and another described the use of a B-40 rocket.

There are a few reports of poisons being put in food and water.

Yellow rain, the wet, sticky substance was associated with light aircraft and helicopters, but in some instances the victims did not see an aircraft. There are as many reports of yellow powder, caused by bomb which exploded with a dull thud, creating a dirty yellow cloud. Some death was associated with this substance, but more often people who touched the powder or were in the vicinity of the cloud broke out in small hard blisters. Holes in leaves were associated with this type of attack. Since 1976, the Hmong have claimed that the red cloud was the most lethal. Hmong have also described green and white clouds. In some cases combinations of yellow, red, green, black, grey, or white were reported.

Kampuchea.

Light fixed wing aircraft and helicopters have been associated with the spraying of both yellow and white material in dry or wet form. Soviet jet fighter aircraft has occasionally been observed in chemical attacks with bombs and rockets. Several times large transports were associated with chemical sprays. Most commonly toxic powders were sprayed along trails and the Thai-Kampuchean border where resistance forces operated. Thai observers reported seeing this powder.

Vietnamese forces use much more artillery in Kampuchea than they do in Laos because of the nature of the fighting. Chemical rounds have been reported fired from 60 mm and 120 mm mortars, 105 mm and 107 mm artillery, grenade launchers and hand held weapons described as RPGs, DH-10 as well as B-40 rockets. Chemical grenades have been frequently reported. Mines, or other devices, which are triggered by trip wires have been used to disseminate some form of aeresol in jungle areas.

The sprayed material is almost always white or yellow. There are some cases, particularly with artillery, where the gas is colourless. In some cases there are reports of a yellow-black combination.

The use of poisons is reported much more frequently in Kampuchea than Afghanistan and Laos. Vietnamese carry kits to poison water holes. Several times the Thai have caught Vietnamese agents poisoning wells in refugee camps. In one case the poison was cyanide.

Poison bullets have also been reported.

Afghanistan.

Helicopters, which are widely used in Afghanistan, are the most predominant method for delivering chemical munitions. Eyewitnesses have described spray bars, rockets, bombs and various types of cannisters.

Jet fighter aircraft, sometimes working with helicopters, has used bombs and rockets. A common description from several areas of the country is a combination of yellow and black smoke. In some cases the agent was described as colourless.

IL-28 bombers were identified in 1979, before the Soviet invasion, dropping chemical bombs.

Chemical artillery shells have often been reported and associated with a black smoke.

A special Soviet vehicle with a hose for pumping gas into tunnels, caves and other inclosures has repeatedly been described by resistance fighters and refugees.

Chemical grenades and poison bullets have been frequently described.

A Dutch journalist filmed a helicopter dropping a bomb which exploded with a dull thud, giving off a dirty yellow cloud. The bomb cannister was also filmed on the ground. A black cloud could be seen in the background.

A film taken by the Afghan resistance shows a helicopter dropping cannisters which disperse a white cloud which slowly settles in a valley.

Eyewitnesses have reported that napalm is sometimes dropped on an area after a chemical attack. Other types of incindiary devices have frequently been reported, and in some cases samples have been brought out for analysis.

Refugees and resistance sources have described the use of poisons on food and in water supplies.

There are more reports of blue or blue-green clouds from Afghanistan than from Southeast Asia. Descriptions of dirty yellow clouds, yellow powder, and yellow granular material span the period from 1979 through 1982.

THE CATEGORIES OF AGENTS.

The evidence from all three countries indicates that irritants, incapacitants and a variety of lethal agents have all been used, sometimes in combination. There has been little scientific interest in the allegations that irritants have been used, even though identification has not been made through sample analysis. The symptomology is well known and the use of irritants apparently causes no undue alarm because it is used by police forces. Incapacitants are another matter. They are not available to western military forces and there use is considered quite unpredictable. Use of incapacitants to render resistance forces and villagers unconscious has been reported by victims, defectors and journalists to occur in all three countries, but especially in Afghanistan in 1980 when more than a million refugees fled to Pakistan. There should be more visable concern that the Soviet Union has a BZ type agent in their inventory that wouldn't be detectable by NATO forces on the battlefield.

In the lethal category, the human source material indicates the following type of agents :

— A colourless, odourless agent that kills instantly without any visable physical symptoms. Afghan/Kamp.
— A wet, sticky, yellow substance that causes massive bleeding and death if exposed to the skin. Blisters, prolonged illness, vomiting, some bleeding and chest pains result from contact with the dry powder formed from the wet material. In some cases eating food contaminated with the substance causes the same symptoms or death. Laos/Afghan/Kamp.
— A dirty, yellow cloud caused from a bursting cannister or bomb contains an agent which causes small hard blisters and prolonged illness. Fewer deaths are reported from this agent. Afghan/Kamp/Laos. A black cloud is associated with a yellow cloud in Afghanistan and Kampuchea, with severe symptoms or death reported if the victim is heavily exposed to the agent.

— A lethal white powder that is sprayed or delivered by cannister. Exposure causes serious illness. Afghan/Kamp/Laos.

— An agent associated with a red cloud that causes rapid death. Laos only in the 70s.

— A lethal colourless agent, but with a rotten fruit smell or other similiar odour. Those directly exposed have convlusions and some bleeding before death. Light exposure causes illness. Afghan/Kampuchea/Laos.

— A yellow or blue gas pumped from a vehicle through a hose into inclosures such as caves, tunnels and underground waterways that makes the water bubble. Severe illness and sometimes death. Afghan/Kampuchea. Skin peels off and is very soft after death. Afghan only.

THE IMPORTANCE OF HUMAN TESTIMONY.

Critics of the US evidence often refer to the large body of human testimony as nothing more than unsubstantiated « refugee reports ». These « refugees » have been described as ignorant peasants, mystic tribal peoples or biased members of a resistance force. The H'Mong are tagged as « former members of the CIA secret army ». Kampucheans have been stigmatized by Pol Pot's bloody regime and Afghans are thought of as fierce fighters who all exaggerate and lie, being motivated by extreme anti-Soviet feelings and religious beliefs. A careful examination of the recorded testimony that appears in US State Department documents, the UN report and hundreds of newspaper articles filed by journalists from many countries reveals a much different character profile of the « refugees ».

Laos.

Refugees from Laos who have described chemical attacks for eight years are both H'Mong and Lao ; men, women and children ; professionals and farmers ; soldiers and civilians. Few can be characterized as former members of « CIA's Army ». Furthermore, concerned physicians, diplomats and journalist published the plight of the Hmong, not a well-organized Hmong propaganda network (which doesn't exist). The earliest reporting on CW use in Laos was based on interviews with two French doctors in 1977 who were treating the Hmong. At least three former Lao pilots provided detailed information on how chemical

weapons were stored, moved and used. The former Lao Health Minister, who defected to China, charged that the Vietnamese had killed thousands with chemical weapons. Refugee testimony was questionable, even to US intelligence analysts, in the period 1975-1979, but by the end of 1979 there was sufficient supporting evidence to cross-check the eyewitness accounts of chemical weapons attacks. Physicians from a number of countries published reports that dispelled theories that the Hmong were only suffering from natural diseases.

Kampuchea.

Testimony on chemical attacks in Kampuchea came not only from the Democratic Kampuchean faction (Pol Pot's organization), but also from the non-communist groups resisting the Vietnamese forces. However, US analysts remained suspicious of public charges made by the Democratic Kampuchean spokesmen from 1978-1980, until confirmation was obtained from independent sources. Vietnamese defectors described receiving Soviet supplied chemical weapons, not only in Kampuchea, but on the Chinese border in 1979. Several of the Vietnamese used the weapons or saw them used. Like Laos, the victims were men, women and children. Unlike Laos, journalists, physicians, nurses and nongovernmental organizations had access to Kampuchea, thus being able to examine and interview victims shortly after an attack. Moreover, intelligence could be more easily be collected because of the proximity of the fighting to the Thai border. It was not that difficult to determine when and where an artillery or air attack had taken place. In reaction to intelligence information, physical and biomedical samples could be collected shortly after chemical weapons were used. In a few cases bodies of gas victims were examined. Western journalists were present in Kampuchean field hospitals when blood was drawn for shipment to the US for chemical analysis. Therefore, Kampuchea provided more opportunities for rapid collection and analysis of samples than was the case in Laos where it took weeks for victims to reach Thailand. Moreover, the Thai military could more closely observe the fighting in Kampuchea and were able to conduct their own investigation of chemical attacks in coordination with other governments and private organizations. In some cases Thai military and civilian observers reporting seeing chemical attacks or residues (usually in powder form) that were spread near the border along major trails.

Thailand.

Thai military and civilian observers, and in some cases victims, can in no way be characterized as refugees, members of a resistance or any of the other deragatory labels put on the victims from Laos and Kampuchea. Since at least 1980, Thai military officials have reported chemical attacks across the border in Thailand. A Vietnamese defector stated that toxic powders spread along trails caused prolonged illness and were used as an area denial weapon. Sometimes these powders blew into Thailand and in several cases Thai civilians were affected. The most well-known incident occurred on February 19, 1982, when planes were reported by Thai villagers to have sprayed a yellow material over three villages. A detailed account of this event appears in the UN Report. A Canadian medical team was on hand to conduct a thorough medical survey. Samples of the yellow material were found to contain low levels of mycotoxins (US State Department Report, November 1982). In another case, the Thai captured a Vietnamese agent in the act of poisoning water wells in a refugee camp with cyanide. Thai military officials have stated publicly on numerous occasions that their investigations show conclusively that chemical weapons have been used in Laos and Kampuchea.

Afghanistan.

The background profile of the victims and observers of CW use in Afghanistan is even more varied than for Laos and Kampuchea. A sampling of the human testimony is contained in the two State Department reports and the UN report, but there are many more accounts that have never been published. In general, there is testimony from men, women and children from the different tribal areas who speak several languages and adhear to diverse political and cultural beliefs. Refugees span the spectrum of Afghan society : political, military, professional, farmer, peasant and business. Not all eyewitness accounts come from members of the resistance. Investigators have had to work hard to gain information about chemical attacks because the issue has not been a major feature of the public appeal for assistance against the Soviet occupation of their country. It has not been uncommon for Afghans to question why so much emphasis has been put on chemical weapons. In their view, conventional weapons, and in particular the heavy use of incendiary weapons like napalm, are all being used in a like manner to subjegate the

population to Soviet control. However, some members of the resistance have gone back into Afghanistan to collect information and samples, and in several cases films were made of chemical attacks. A captured gas mask and a sample of contaminated wheat was analyzed in the US and found to contain the same toxin identified on samples from Laos and Kampuchea.

Defectors, Afghan and Soviet, have provided some of the most valuable information on the types of chemical agent that have been used by Soviet forces. Like Kampuchea, the US has good operational intelligence on military activities and can confirm or refute human source reporting by cross-checking the details of a victims story. Physicians and journalists, some with access to Afghanistan, have provided valuable information on specific attacks and medical symptoms. Dr. B.A. Zikria, Professor of surgery at Columbia University College of Physicians and Surgeons and a pulmonary specialist, has examined victims inside Afghanistan and in Pakistan. Journalists from a number of countries have filed reports on specific attacks, in some cases having observed planes or helicopters disseminating chemical and incendiary weapons. A Dutch journalist, Bernd De Bruin, filmed a helicopter dropping a cannister which caused a yellowish cloud in 1980. He also filmed victims and was himself affected by the toxic agent for several months. In sum, the human testimony from Afghanistan comes from such a variety of sources that a confident assessment can be made that the Soviet forces have used a range of chemical agents on the Afghan resistance and civilian population since the summer of 1979.

Other countries.

Human testimony has not been collected only in the border areas of Laos, Kampuchea and Afghanistan. Sources in the US, Europe and Asia have provided first-hand or supporting testimony. Cuban, Soviet, Afghan, Vietnamese, Chinese and Middle Eastern sources have been debriefed in other regions on their knowledge of chemical weapons use or their familiarity with Soviet training on chemical weapons. For example, the US Government concluded on the basis of information from Cuban, Soviet, Afghan, Vietnamese, European and Chinese sources that the Soviets have developed a chemical mine which contains toxin in aeresol form. Such a mine has been used in Kampuchea, and in one case, blood drawn from a victim exposed to the agent from a mine was found to contain toxin.

DEFECTOR REPORTING.

The testimony of defectors tends to be overlooked and does not receive the same attention as the refugee reports. US analysts found striking similarities when comparing the information provided by defectors as well as the correlation between the defector reports and the other evidence that was available.

The US Department of Defense Medical Team that interviewed Hmong victims in 1979 concluded that three classes of agents had been used :

— irritant or riot control agents,

— nerve gas,

— an unknown agent or combination of agents that caused a variety of unfamiliar symptoms that could not be associated with known agents.

In 1979, toxins had not been identified as the cause of the unusual symptoms. However, the identification of three classes of agents was a perceptive assessment in light of the data available today. Moreover, the wide-range of medical signs and symptoms is now known to result from the level and route of exposure. Defectors added information that could not be obtained by interviews with victims, medical exams and sample analysis.

Vietnamese soldiers who were familiar with Soviet supplied chemical weapons, and used them in some cases, described agents that irritate, incapacitate (cause unconsciousness), create illness and skin disorders, or that kill in several different ways. A Vietnamese private told of Soviet advisors firing a chemical agent from a hand held weapon (DH-10) which produced a greyish, white and green cloud that killed quickly. This event occurred in Kampuchea, but an account from Laos described a Soviet advisor firing the same type of weapon, with the same lethal result. Another Vietnamese soldier used a shoulder fired weapon to spread a gas that rendered Kampuchean soldiers unconscious so they could be captured. Yet another described black smoke from artillery shells which produced itchy skin, weakness, skin lesions and decaying skin, often resulting in death from internal bleeding.

Afghan and Soviet military defectors describe classes of agents similar to the Vietnamese. In some cases the descriptions of the

delivery systems, colour of smoke, associated odour and the **medical signs** and symptoms are too similar to be regarded as coincidence.

— A former Afghan helicopter pilot told of dropping cannisters of toxic smoke, tear gas and a anti-respiratory gas that makes the skin very soft after death.

— An Afghan officer in 1981 said the Soviets supplied four agents : one was an incapacitant and the others were lethal, in some cases turning the skin a dark colour after death.

— A resistance leader, familiar with Soviet chemical weapons by virtue of this training in the army, said that besides irritants and hallucinogenic gas, a lethal substance in the form of a white powder was delivered by helicopter.

— Yriy Povarnitsyn, a Soviet CW specialist who defected, described clean-up operations in Afghanistan after chemical attacks, including his finding of internal bleeding in Afghans when he performed autopsies in the field.

— An Afghan pathologist described how he collected soil samples after a Soviet chemical attack to determine the level of contamination.

— One Afghan defector identified where the Soviet forces stored phosgene, diphosgene and the nerve agents sarin and soman. He also listed where and when the specific agents had been used. (This information matched eyewitness accounts of chemical attacks and intelligence information on Soviet operations).

— A Soviet soldier who defected to the Afghans described how containers of lethal chemicals were dropped from helicopters.

— Anatoly Zakharov, a Soviet soldier interviewed by a British journalist in 1982, revealed that at his base the Soviets had a « 100 % » lethal agent that was delivered by rockets. This agent caused the skin to become very soft after death. He also said that Soviet forces at this base had stores of « picric acid » and an incapacitating agent, which were sometimes used together.

— An Afghan airport official who left the country discovered that cannisters stored at the Qandahar Airfield contained three agents : incapacitants, blistering and one that suffocates. One gave off a yellow smoke.

— An Afghan physician who escaped to Pakistan had treated 15 Afghan victims of a chemical attack brought to Kabul by

Afghan government forces. They all had red lesions on their skin.

— A former Afghan officer listed four agents provided to the Afghan military : irritants, incapacitants, a blister agent and a lethal agent that caused severe symptoms before death.

— Brig. General Watay, Chief of the Chemical Department of the 99th Afghan Rocket Regiment, said that the Soviets started using chemical weapons in April 1979. In an interview with a writer from *Jane's Weapons of the World*, he claimed that the Soviets were testing new gases in Afghanistan that were unknown in the West. According to his account, the Soviets use phosphorous and sulphur in liquid and gas form which causes suffocation, burns, nervous disorders and irritation of the skin.

Suffice it to say that US analysts, who had access to the circumstances surrounding the debriefing of these defectors and the ability to cross-check their stories, became convinced that the Soviets had brought a variety of chemical agents into Afghanistan and had used them on a selective basis from 1979 thru 1982. It is understandable that people who do not have access to the official debriefings of defectors, or cannot reinterview them, will be skeptical about the source's credibility. In some cases, however, defectors were not reluctant to be named and they have been reinterviewed by non-US reporters and officials. Regardless, this brief description of defector information was included to point out that human testimony used by the US came from not only « refugees », but individuals who had first-hand information on when and how chemical weapons were employed in all three countries.

THE ROLE OF JOURNALISTS AND WRITERS.

Journalists and writers are singled out in this discussion of human testimony because they have not only reported the stories about chemical warfare, they have also become sources of first-hand information. They deserve much of the credit for bringing the chemical weapons use issue to the attention of governments and the public as well as keeping the issue alive in the face of repeated attempts to downplay it's importance of shelf it altogether. There is yet another reason to single the journalists and writers from many countries who have often risked

their lives to obtain information from denied areas. Very few of these individuals could be characterized as being for the development of chemical weapons in the US, nor in many cases could they be identified with the policies of the US administration in power at the time they were writing. The majority of the press articles on CW use in Southeast Asia and Afghanistan criticized the US Government's handling of the issue. However, there has been a universal condemnation of the Soviet Union and the Vietnamese for using chemical and toxin weapons by journalists from all over the world in papers and magazines that span the political spectrum from left to right. Therefore, it is reasonable to assume that many of these writers have expressed views based on their own assessment and evidence, independent of the US Government.

Sterling Seagraves, author of the book *Yellow Rain : A Journey Through the Terror of Chemical Warfare,* devoted as much time in his book to the ineptness of the US Army Chemical Corps and the need to stop the production of chemical weapons as he did on his investigation of chemical warfare in Southeast Asia and Afghanistan. Nevertheless, Seagraves was one of the first to recognize that toxins were what had been causing the mysterious symptoms in Laos. His interviews in Pakistan and Thailand also revealed the striking resemblance of the descriptions of delivery systems and medical symptoms by people from two separate regions in the same time frame.

Jane Hamilton-Merrit spent many years working with the Hmong and urged the US Government to conduct an investigation. Her article in *Reader's Digest,* October 1980, « Gas Warfare in Laos : Communism's Drive to Annihilate a People », described her personal experiences in interviewing gas victims. There is a striking comparison between the symptoms of her friend, Nhia Yang Vang, who had changed from a vigorous man of 40 years to a « skeleton » with recurrent chest pains, difficulty in breathing and loss of appetite to the symptoms of a virile, middle-aged Afghan leader examined by Dr. Zikria in Pakistan who looked like a withered, old man in a film taken of the examination.

Dutch journalist, Bernd De Bruin, filmed a Soviet helicopter dropping a cannister which burst into a yellow cloud during his trek with the Afghan resistance in August 1980. He took pictures of victims of the attack as well as skin lesions on men and children who walked through a yellowish powder on the ground. Blisters broke out on his hands from exposure to the yellow

cloud and he was ill for about six months according to German physicians who examined him later. His story was published in the European magazine *Newsnet*. One month before De Bruin saw the chemical attack, nineteen Hmong described how their village was hit with an exploding bomb that produced a yellow cloud of powder. Some died in hours, some the next day, while others became ill and had blisters on their skin. Complete medical exams, including X-rays and blood analysis, ruled out known natural diseases like TB to explain the damage to the lungs. Their blisters were small and hard like those on De Bruin's hands. Like De Bruin, they remained ill for a long period. Likewise, De Bruin's picture of the bomb and his description of a dull thud, rather than a loud explosion, was strikingly similar to the Hmong account. De Bruin's account was lightly dismissed by UN investigators, but not by US and European analysts who carefully compared the data from Laos, Kampuchea and Afghanistan, finding many such striking similarities.

Robert L. Bartley and William P. Kucewicz of the Wall Street Journal, a newspaper that has consistently kept the evidence on chemical weapons use before the public, wrote a lengthy assessment in the Spring 1983 issue of *Foreign Affairs*, titled « 'Yellow Rain' and the Future of Arms Agreements ».

Lucio Lami, an Italian journalist, has written a series of articles in Milan's « il Giornale nuovo » based on his interviews in Thailand with CW victims and defectors. The articles include the lengthy transcripts of the interviews, providing considerable detail, particularly from Vietnamese Captain Nguyen Quan who describes the Soviet supported chemical warfare organization and agents used in Laos and' Kampuchea. His articles also severely criticize US and European governments and the press for not pressing the issue.

Many more journalists from the UK, Australia, Japan, France and other countries have traveled into the denied areas and reported on chemical warfare. They have also brought out samples of contaminated material for analysis in a number of countries. Of course there are journalists who seriously sought evidence and couldn't find any, which is understandable when one examines the timing and location of chemical attacks. There are also journalists and writers who have worked hard to disprove the US evidence. Some of these writers have claimed that the CIA has fabricated the whole issue, but that notion has not gained many followers.

Government officials have also written articles supporting the US evidence. Republican Congressman Jim Leach urged the US Government to intensify it's investigation and personally involved himself in the investigation. Likewise, Democratic Congressman Stephen Solarz has written articles defending the US evidence. Other Congressmen, Senators and Governors have written articles or submitted testimony in the Congressional Record. European parliamentarians and government officials, including heads of state and Foreign Ministers have condemned the use of chemical weapons. NATO issued a communique on behalf of all countries expressing concern that chemical weapons continue to be used. Lastly, The Law Asia Society, made up of lawyers and jurists from twelve Asian countries, conducted an investigation in Thailand and issued the following statement : « On the issue of alleged use of chemical weapons, the consensus of the inquiry committee was that circumstances existed which would be sufficient to convince a reasonable person that there had been widespread, but selective employment of some form of chemical weapons in Laos and Kampuchea.

THE ROLE OF PHYSICIANS.

During a heated debate on the medical evidence, a professor remarked that medicine is not a « hard science » and doctor's clinical observations could not be used as proof that chemical weapons had been used. Perhaps the physicians that have made such a valuable contribution to the investigation would concede that some of the signs and symptoms are puzzling, but they would also state that they are capable of positively identifying certain diseases and can accurately describe the symptoms they observe. Thus, physicians from several countries with different specialties have been able to state with confidence what diseases the Hmong, Kampucheans and Afghans *were not* suffering from and they can limit the range of possibilities to be examined.

— Two French doctors working in Laos, with considerable experience on the local diseases, were able to state in 1978 that it appeared that chemical weapons were being used on the Hmong.

— The US Department of Defense Medical Team, headed by Col Charles Lewis, Chief of Dermatology at Brooke Army

Medical Center, Fort Sam Houston, Texas, left Washington DC in 1979 with some skepticism and returned convinced that only several types of chemical weapons would explain the medical symptoms they observed in the refugee camps in Thailand.

-— Dr. Amos Townsend of the International Rescue Committee, spent years examining the Hmong and Kampuchean CW victims. He also collected physical and biomedical samples, including control samples, to assist the US and other countries in identifying the agents being used. Several nurses he worked with in the field hospitals eventually were convinced that chemical weapons caused the unusual symptoms they had treated over a period of years. The UN report contains their written testimony.

— Dr. Bernard Wagner, a professor of medical pathology at Colombia University, conducted a fact-finding visit to Thailand in 1983 and concluded that toxins were being used as weapons.

-— Dr. B.A. Zikria, a professor of surgery at Colombia University College of Physicians and Surgeons, found that Afghans who had escaped to Pakistan were suffering from a variety of symptoms he could not explain as a pulmonary specialist on smoke inhalation. His clinical examination was recorded on film and he testified before the US Congress that he was convinced that chemical weapons which cause severe deterioration of the body have been used on the Afghans.

— Dr. Richard Harruff examined Hmong refugees in 1980 and concluded there was a low incidence of tuberculosis, dispelling yet another alternate explanation that was offered for the unusual lung problems found in refugees who claimed to have been in a chemical attack. In his article « Chemical and Biological Warfare in Asia », *The Journal of the American Medical Association*, July 1983, Harruff stated that his conclusions had been substantiated by other investigators, but he was amazed at the lack of interest shown by the medical and scientific community on the plight of the Hmong people.

Many other individuals in the medical profession have made significant contributions. Dr. Stafford Bourke and Dr. Bird, at their own initiative, conducted examinations of alleged gas victims at

their World Vision hospital in Ban Vinai Refugee Camp with an efficiency difficult to achieve given the facilities and number of patients. Their testimony appears in the UN Report. Likewise, the Canadian Epidemiological Survey, submitted to the UN, provided a useful guideline for future medical surveys in Thailand and elsewhere. Much work needs to be done by qualified medical personnel to correlate the signs and symptoms of victims from the three countries in order to distinguish victims of natural disease from those exposed to chemical weapons. Perhaps the recent experience of Swedish, Austrian, Swiss and British physicians in treating the Iranian chemical warfare victims will stir more interest in this problem. The budget for the US Army program on medical intelligence was almost cut years ago until it was pointed out to the Congress what a valuable service these medical specialists on chemical warfare provide.

THE ROLE OF THE SCIENTISTS.

Scientists from many disciplines have tackled what is the most difficult task in the investigation : the attempt to discover what specific agents have or have not been used. Many scientists have established a demanding set of criteria to be used in determining whether specific chemical agents were employed at a given time. Some of the specialists will not be convinced unless they personally can collect contaminated samples and analyze them in their own laboratories. While the discovery of toxins in environmental and biological samples has gone relatively un-challenged, scientists have questioned the origin and credibility of the samples as well as the cause for the presence of toxins. Moreover, there has been a consistent call for more tests of control samples to determine the natural occurrence of specific toxins in the environment where they were collected.

Whether it is possible to establish absolute scientific proof that specific chemical weapons have been used in areas denied to investigators is a debatable subject. Even when munitions were taken from the Iranian battlefield by the UN team and the agents used identified as mustard and nerve gas, there was not absolute proof that the Iraqi forces had fired the shells or dropped the bombs. Therefore, producing a munition filled with an agent will not meet the scientific requirements to identify the quilty party, or connect the munition to the symptoms of the al-

leged victims. Therefore, we are faced with a situation where the scientific information (sample analysis) must be correlated with evidence provided by eyewitnesses, defectors, physicians, intelligence sources and historical records on chemical warfare. This was the approach taken by the US Government that led to the charges that chemical weapons had been used in Southeast Asia and Afghanistan. Scientists, inside and out of the government, have worked for years to positively identify the agents that have been used.

— Dr. Chester Mirocha, University of Minnesota, developed new techniques with the gas chromatograph/mass spectrometer and used a full mass spectral scan to compare sample findings with known standards. Mirocha concluded, after completing his assessment of samples furnished by the US Government, that the high levels of different toxins found on vegetation and in biomedical material could not have occurred as a result of natural contamination. Details of the experimental procedures used were presented at the Society of Toxicology meeting in Louisville, Kentucky, on August 16, 1982 ; at an International Mycotoxin Symposium in Vienna, on September 1, 1982 ; and at the Association of Analytical Chemistry Meeting in Washington DC, on October 28, 1982. Dr. Mirocha found trichothecene toxins in samples from Laos, Kampuchea, Thailand and Afghanistan.

— Dr. Sharon Watson, US Army (Now Defense Department) Medical Intelligence and Information Agency, Ft. Dietrick, Maryland, was responsible for preparing positive, control and negative samples before they were sent to laboratories for analysis. She also wrote scientific research papers on trichothecene toxins which were invaluable in this field that few, even in the scientific community, understood.

— William Sarver, US Army Chemical Systems Laboratory, oversaw the testing of samples for traditional agents and the building of a GC/MS analytical capability within the Army. Results of tests in his laboratory were checked by the Food and Drug Administration and private laboratories.

— Dr. Joseph Rosen, Rutgers University, analyzed samples from Southeast Asia for the US Government as well as for ABC TV News. He not only found levels of trichothecene toxins comparable with what Dr. Mirocha found, but he also found a synthetic substance, polyethylene glycol,

which he can demonstrate was in the sample material, not a contaminant from the container as some have suggested. Rutgers has published his paper, written with Robert Rosen, « Presence of Four Fusarium Mycotoxins and Synthetic Material in 'Yellow Rain' », 1982.

— Surveys of the toxigenic fungi and mycotoxins naturally present in Southeast Asia conducted by the Mahidol University in Bangkok and the Massachusetts Institute of Technology did not reveal the presence of trichothecenes identified in the samples analyzed by Mirocha and Rosen (T-2, nivalenol, deoxynivalenol, or diacetoxyscirpenol), although other mycotoxins such as aflatoxin were present.

— Dr. Bruno Schiefer, Toxicology Group, University of Saskatchewan, Canada, concluded after a field trip to Thailand that « The events that are reported to take place at the time of alleged chemical warfare attacks cannot be explained on the basis of naturally occurring diseases ». His report, « Study of the Possible Use of Chemical Warfare Agents in Southeast Asia », was submitted by Canada to the UN.

— Dr. Paul Nelson, one of the world's leading authorities on the fusarium fungus, a plant pathologists from Penn. State University, catalogued more than 6,000 isolates of fusarium, 300 of them toxin producers, and says that he has never encountered references to a toxin-producing fusarium fungus in Southeast Asia.

— Dr. A. Heyndrickx, Director of the Toxicology Laboratory, University of Gent, Belgium, traveled to Thailand to collect his own samples and using the GC/MS system, concluded that the trichothecenes were being used as warfare agents in Southeast Asia.

— Dr. Fonnum of the Norwegian Defense Research Institute has studied methods for using toxins as warfare agents, countering some of the unfounded statements that tons of toxin filled munitions would have to be dropped on a small area to create lethal dose rates or the symptoms described by victims.

— Members of the National Academy of Sciences, the National Academy of Engineering and the Institute of Medicine prepared a comprehensive research document, « Protection Against Trichothecene Mycotoxins », National Academy

Press, 1983, which provides an extensive survey of the literature and compilation of data.

— French scientists have also found trichothecenes in samples from Southeast Asia and the French Embassy in Bangkok announced in 1983 that positive traces of toxin had been found in a number of samples collected by the French. Thai chemical warfare specialists have supported findings by other countries, that toxins have been used as weapons, through the analysis performed in Thai laboratories.

THE SCIENTIFIC DEBATE ON YELLOW RAIN.

A number of prominent scientists have consistently leveled their criticism on the US Government's investigation, even though scientists from the private sectors of the US and other countries have reached the same conclusions about toxins being used as weapons. Criticism has been narrowly focused on the toxin issue, with very little discussion devoted to other agents and the evidence beyond that obtained from sample analysis. Political, rather than scientific writers, have charged that the whole issue of chemical weapons use has been fabricated by the United States to further it's own interests. Scientific critics recognize that trichothecene toxins have shown up in Southeast Asia and that there are serious, unexplained health problems evident in the Hmong and Kampuchean refugee population. Some of these scientists are even willing to concede that chemical weapons, other than toxins, have been used. Another characteristic of the debate is that it remains fixed on Southeast Asia, with little attention paid to the evidence from Afghanistan.

Two early hypotheses did not stand up to scientific scrutiny. Initially, Vietnamese forces were said to be using only captured US riot control agents (CS gas) and the Soviets, it was held, were only using non-lethal irritants like tear gas, CS and adamsite on the Afghan resistance. Unless one is prepared to believe that most of the human testimony and medical information is false, it is difficult to equate these two points of view with the evidence.

The Soviet Academy of Sciences, in a document submitted to the United Nations, concluded that the US use of defoliants in Vietnam changed the ecology, allowing elephant grass to flourish and produce fusarium spores that were borne by the winds to Laos and Kampuchea, eventually producing the trichothecene

toxins. The UN investigative group found this explanation implausible because too many unlikely events would have to occur to support this explanation. The Soviet document did not offer an explanation on how the wind would selectively deliver the spores to some areas of Laos and Kampuchea, and not others, nor did it say why the spores didn't go into Thailand. What is important is that the Soviet Academy of Sciences recognized that trichothecene toxins had shown up in Laos and Kampuchea since the fighting there had intensified. According to biologists, the Soviets were even wrong in their opening assumption that US use of defoliants had caused elephant grass to flourish.

The most well-known criticism of the US evidence on toxin use was put forth in an article in *Science* in late 1983. Professor Matthew Meselson, a biochemist from Harvard University ; Julian Perry Robinson of Sussex University ; and Peter Ashton of the Arnold Aboretum presented their conclusion that the presence of trichothecene toxins in Southeast Asia is a natural occurrence. Their investigation shows that swarms of bees in Southeast Asia are known to cause a « Yellow Rain » that matches the descriptions provided by so many Hmong refugees of chemical weapons attacks. These particular bees ingest several types of pollen during their flights in the spring and clear their system of the digested pollen in small, liquid drops that form small yellow spots on vegetation. This would explain, according to the hypothesis developed by the three authors, why several types of pollen have been found in spots on vegetation thought to have been the result of chemical warfare sprays. It is difficult to characterize this hypothesis much further because of the limited amount of writing or documentation available on the details of the assessment. Therefore, questions are posed to those scientists who hold that this hypothesis can withstand scientific scrutiny.

1. What is the relationship between the « Yellow Rain » caused by the bees and the medical problems experienced by the Hmong and the Kampucheans ? Does the hypothesis include the judgment that the bee droppings, which include pollen, provide a good medium for the growth of trichothecene producing fusarium ? If so, how long does it take for the toxins to be produced ? Could food sources, or water, have been contaminated in this manner ? Several toxicologists have already written replies in scientific journals to those that have suggested this as a cause for the presence of tricothecenes. Dr. Mirocha, Dr. Rosen,

Dr. Schiefer and others question the scientific basis of this hypothesis.

2. If question one does not address the hypothesis properly, then are we to understand that the « Yellow Rain » of the bees has been mistaken as chemical warfare and the toxins have gotten into the food chain or the water in some other way ? Or, are the medical problems mistakenly confused as being from chemical weapons actually the result of diseases, known or unknown ?

3. If there is a natural explanation for the presence of trichothecene toxins on vegetation, in water and in the bodies of the Hmong and the Kampucheans, then why haven't the Lao and the Vietnamese reported by now that their troops and civilians suffer from the same mysterious symptoms ? It is understandable that neither government has the capability to detect toxins at the levels found by toxicologists like Dr. Mirocha, but certainly with 40,000 or so Vietnamese troops in Laos and over 150,000 in Kampuchea, all operating in the areas where chemical attacks have allegedly taken place, one would think that these troops would be affected in the same way as the local inhabitants and the resistance forces. We certainly can't be dealing with a disease that only attacks those resisting the communist regimes in power. Some Lao villagers have reported being subjected to chemical attacks by helicopter, causing the local Lao military to be outraged at the Vietnamese, but there has been no documentation provided by the Lao or Vietnamese Government that populations under their control have experienced medical problems similar to those of the Hmong and Kampucheans. The idea may occur to them in the future, but it would be too late of achieve any credibility.

4. When does the « Yellow Rain » caused by bees occur ? A previous hypothesis of Professor Meselson, which preceded his study of Southeast Asia bees, was that the US had only found toxins in samples collected in the spring months. While that is true, with a few exceptions, that period of several months is the dry season when the fighting has been the heaviest. Collection efforts have been intensified in that period, particularly in Kampuchea, because fighting has been close to the Thai border. It should be noted that positive samples were collected from attacks in the spring, particularly March, but there were no

reports of « Yellow Rain » or anything else that could have been mistakenly caused by bees. Victims reported artillery shelling or mines as the cause of the gas. There is also a question about the 1977 Chinese study that has been repeatedly cited as conclusive proof that bees cause « yellow Rain ». The Chinese report states that the event occurred in « September » and their is no mention of illness associated with the bee rain.

5. Could the « Yellow Rain » from bees start as early as February ? There are a good number of reports of yellow material being spread on villages and crops at the beginning of the year in Laos, and in one well-studied case, Thailand. Does the bee hypothesis extend to the February 19 incident in Thailand that was studied by the Canadian team, in addition to the US, French, UK, Australia, the UN and others ? The UN report documents the testimony of eyewitnesses and includes the Canadian assessment. There was considerable agreement that the yellow material spread over the village caused the increased incidence of illness, within twenty-four hours. Could toxins have grown on the bee droppings that fast, assuming bees can't carry the toxins in their body and excrete them with the pollen ? Eyewitnesses said the yellow material came from an airplane. These were Thai villagers, not members of a suspect resistance. Were they lying too or had they been confused like the Hmong and the Kampucheans ? Low levels of trichothecene toxins were in the yellow material, several laboratories confirmed the findings, but pollen was also in the samples. Why the Vietnamese sprayed this material on a Thai village is not known. It may have been an accident or the Vietnamese might have been sending a message to the Thai. One view, which may explain the presence of pollen, is that the Vietnamese were trying to discredit the US evidence by dropping a relatively harmless yellow material where investigators could readily collect samples. They might have been successful if the Canadian medical team had not been in the area.

6. Dr. Mirocha has written several times that there was no pollen in the samples in which he found the high levels of toxin. Furthermore, the vegetation samples in which he found trichothecenes were covered with small yellow spots that do not look anything like the larger spots of bee excrement. Therefore, microscopic comparisons of alleged « Yellow Rain » samples (not provided by Dr. Mirocha or anyone else who has found toxins in vegetation samples) with authenticated bee excrement can only

be used to demonstrate that some samples from Southeast Asia are not related to chemical warfare. An article by Alaastair Haye in the *New Scientist,* March 22, 1984, describes the US evidence as weak and provides an example of the microscopic photo comparison. In other samples, which did not have toxin, laboratories have found several types of pollen. UK and Australian scientists have been cited as having found only pollen. In some cases the US Army found only pollen. Would those who contend that « Yellow Rain » is a natural occurrence, and that there is no proof that toxins have been used as weapons, agree or disagree with the proposition that some samples, but not all, have been covered with bee excrement ? It is not inconceivable that persons collecting samples of vegetation in areas where chemical attacks have occurred, or have been reported to occur, have collected material covered with spots caused by bees or have collected authentic chemical warfare samples that also had bee droppings on them. In other words, Professor Meselson may have found the answer to a puzzling question : why have some laboratories found several types of pollen in a small yellow spot ? It is even possible that in some cases the Hmong mistook yellow rain caused by bees for a chemical attack, but more information is needed on the Hmong's familiarity with the natural activities of bees. The Hmong are known to harvest honey and it is difficult to explain why they would not be familiar with the seasonal habits of bees.

Given the many questions left unanswered by this alternate hypothesis and the lack of documentation and research, it seems reasonable to conclude that a very limited scientific finding about bees has been stretched too far in an attempt to prove that the evidence on chemical weapons use is not credible. Scientists have a legitimate right to debate scientific findings and question hypotheses, but we are not dealing with Einsteins theory of relativity here. People from three countries, who are ruled by governments that do not allow international investigators access to the areas of alleged chemical weapons attacks, have asked the international community to stop the use of these inhumane weapons before more of their people are killed or injured. Alaastair Haye is absolutely correct in his observation (article cited above) that « An independent investigation, under UN auspices, of the areas where yellow rain is alleged to have been used in Laos and Kampuchea might have helped to resolve the Issue ». In the absence of that ideal, government and non-

government analyst have to work with the available information. Rarely does a criminal investigator see the crime. These cases are more akin to forensic inquiry than to scientific experiments in the laboratory. The international effort to stop the use of chemical weapons is being impeded by the challenge to the credibility of the specific evidence on toxin use in Southeast Asia. While it may not be the intent of the scientific critics to discredit all evidence from the three countries, that has been the end result in terms of public perception. Therefore, the scientific critics have a moral as well as scientific obligation to fully document their case.

The US Government knew full well that it accepted serious international responsibilities when it took the Hmong, Kampucheans and Afghans on as defendents and formally charged the Vietnamese and the Soviets with violations of international treaties. That is why it took several years to assess the evidence and compile a convincing case that could be used in public. Rather than move too quickly, as some have charged, the US did not formally present evidence on Laos for five years after the first report of a chemical attack, and then in guarded language. It took two years of study to arrive at convincing evidence on Kampuchea, and one year on Afghanistan. Secretary Haig first announced that mycotoxins had been used on September 1981. There was good reason for being confident that the identification of the agent that had alluded experts for so long was actually a combination of toxins. By the time of the announcement, US officials had stated at several congressional hearings that there was no doubt that chemical weapons had been used in Laos, Kampuchea and Afghanistan.

Before Secretary Haig's announcement, US analysts had discovered that the Soviets had been developing mycotoxins as weapons for many years, a development that had not taken place in the Western countries. By 1981, US intelligence collection means were established to collect on military activities in the two regions, allowing a capability to determine when some chemical attacks took place. Trained teams could be directed to attack sites to collect samples and gather first-hand information from eyewitnesses. The first sample, in which Dr. Mirocha found high levels of trichothecene toxins, was a leaf and stem collected by a team who also brought back other samples of vegetation an water. In addition, they examined the bodies of the

dead Kampuchean soldiers and reported rapid necrosis of the inner lining of the gut, a particular symptom of trichothecene poisoning. Six months after the Secretary's announcement, the US Government presented a document to the Congress and the United Nations which was based on a National Intelligence Estimate. By that time, many more samples had been analyzed and other new information confirmed the previous conclusions that chemical weapons had been used in all three countries. US policy was, and still is, that information on chemical weapons use should be released to the UN and the public as soon as possible, because the principal objective of the US investigation has been to stop the chemical attacks and do everything possible to prevent these weapons from being used again in any region of the world. Doubt about the sincerity of the US charges and the credibility of the evidence, which has stemmed from the on-going scientific debate about « Yellow Rain », has weakened international attempts to stop this violation of international law.

The World Congress on Chemical Warfare to be held in Ghent, Belgium, from May 21-23, will once again bring together concerned experts from many countries to discuss the evidence and propose new methods for establishing the scientific « truth ». Under the auspices of the UN Secretary General, Egyptian General Esmat Ezz, who headed the UN investigation on Southeast Asia and Afghanistan, work has been done on new investigative procedures for future cases of chemical weapons use. These procedures were bypassed when the UN decided to investigate the lastest case of chemical weapons use by Iraqi forces on the Iranians. The work done by this UN group, some of which has already been published in UN document A/38/435, 19 October 1983, offers good recommendations for attendees of the World Congress to discuss. Proposals on how to conduct better investigations in the future is an important discussion topic, but the World Congress is faced with an immediate problem of how to resolve the scientific debate on what has already taken place. This moral and legal issue, which involves the plight of thousands of people, cannot wait indefinitely for the scientific side to make up it's mind about the facts. It is incumbent upon those who continue to challenge the evidence with short articles in newspapers and periodicals to compile their case scientifically so that other scientists, chemical warfare experts, intelligence organizations, governments, congress and parliamentary bodies and the public can

study the challenge and offer rebuttals to tangible, documented evidence.

THE FLOW OF EVIDENCE CONTINUES.

The US Government is confident in it's assessment and conclusions, but dissatisfied that agents other than toxins have not been collected and analyzed to determine their chemical properties. Besides stopping the use of chemical weapons, it is incumbent upon western nations to learn as much as possible about the chemical weapons in the inventories of potential adversaries. Moreover, the more we can learn about the chemical weapons being used, the more quickly we can develop antidotes to help the victims. Reports of chemical attacks in Laos, Kampuchea and Afghanistan continue to be received and analyzed. A US team of specialists in Thailand, working with experts and officials from other countries, ensures that information and samples are passed in a timely fashion to analysts. Developments in Afghanistan are also monitored, even though reports of chemical attacks have significantly decreased. New claims of chemical warfare in the recent Soviet offensive in the Pansjir Valley cannot be ignored, although they must be carefully checked. The use of chemical agents by Iraq adds another case to be investigated thoroughly. Alleged Ethiopian use of chemical weapons on the Erritrean Resistance was never properly investigated and few facts are available to determine whether there was any truth to the claims.

Hopefully, there will not be any more cases to investigate. Prudence dictates that the international community be better prepared to quickly investigate, using proper procedures, when the warning of new chemical attacks is first sounded. Otherwise, we will again face the horrible prospect that innocent and unprotected people will be killed and injured over an extended period of time by these weapons that mankind sought to ban from the battlefield in 1925.

Observations, impressions, pitfalls and recommendations from field CBW research among refugees in Southeast Asia

by A. CROSSLAND and A. TOWNSEND

International Rescue Committee, Bangkok, Thailand

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SUMMARY.

The evolution of the authors' involvement in this form of field research is briefly described. The general function of this endeavour within a broad investigation into allegations of CBW use in Laos, Cambodia and Vietnam is explained along with the complex role that Thailand plays because of its geographic location. The setting of the Refugees, Voluntary Agencies, International Organizations, and the various organs of the Royal Thai Government is outlined as they interact on CBW Field Research in SEA.

Sample collection and handling within Thailand are briefly described as are the comparisons between countries of origin apropos histories, physical examinations and time lag/topographical differences.

Impressions gained from medical histories from different geographic areas are compared with histories plus clinical observations and impressions developed when signs and current symptoms are studied. Trends in mortality and morbidity over the past 8 years are loosely given as impressions and studied speculations after the fact.

Impressions of non-human effects as obtained by history are outlined and broad impact described.

Treatment modalities seen in use or described are presented. Defensive measures taken for personnel protection are briefly described.

Impressions developed about the usefulness of Mycotoxins (Trichothecenes) as a military weapon for broad population control as well as for tactical situations are explained. The « suitability »

of this weapon for different geographic and socio-political popula-
tions is discussed. Chemical warfare and the public attitude are
reviewed.

Pitfalls found most difficult to overcome by the authors in their
SEA research effort. Implications for the decades to come are
considered.

Recommendations for useful research in CBW in general, Tricho-
thecenes in particular and the « wedding » of all forms of warfare.

Comments on the future of International Law, CBW use control,
history and the general character of the human animal responses
throughout his known history on this planet are given.

As early as 1977 journalist reports in various Washington publi-
cations made Dr. Townsend aware of CBW use in Laos. He noted
the general lack of USG responses and interpreted that as indicat-
ing that USG knew that the allegations were without foundations
or that they had foundations but, for various reasons of their own,
chose not to disclose their findings publicly at that time. It was
not until the Fall of 1980, when two members of the US Embassy
approached the medical staffs of Nong Khai and Ban Vinai
Refugee Camps near the border of Laos that he realized that
as yet much basic field information and specimen gather-
ing had not been done. Feeling strong moral obligations to
Hmong as former functional Allies of the US, to this apparent
significant gap in scientific knowledge of potential civilian and
military importance to much of the world and to his own country
and its friends, Dr. Townsend agreed readily to assist
his government's efforts in whatever manner he could within
the limitations of his prior commitment to provide medical help
for refugees under the care of his organization, the International
Rescue Committee, and his own rather out-of-date knowledge of
modern CBW (Dr. Townsend had worked briefly on personnel pro-
tective measures in Biological Warfare at Camp Detrick 30 years
ago and prior to his own medical training). His main involvement
in this SEA-CBW field research began in April 1981, a part-time
effort which became full-time in January 1982.

Ms. Crossland, a British Midwife with additional training in psy-
chosocial aspects of medical care, began her interest in the Hmong
allegations of lethal CBW use against them in Laos while serving
as a Public Health Nurse in Ban Vinai Camp in 1981. She joined

Dr. Townsend full-time in September 1982 stimulated by her concern for the Hmong and at least partially by her own family's traditional involvement within the British Royal Navy. Both Ms. Crossland and Dr. Townsend readily admit further stimulation for their involvement has arisen out of the profound apathy, disinterest and fear displayed by most Voluntary Agencies and International Organizations as well as many of their staffs working with the various refugee groups entering Thailand after 1975. Both authors have sought primarily to pull as much of the shroud of ignorance from a confusing subject in a complex part of the world as their own limited abilities would permit. Both have readily recognized their extreme dependence upon Refugee and Resistance sources for much of their information and many of their specimens as well as upon various governments and universities for sophisticated laboratory studies and alternate forms of information gathering and analysis. Both have endeavoured to raise the consciousness level of this subject as a topic for world concern, rather than purely a local SEA phenomenon, with the hope that eventually greater expertise would enter this arena of sensitive scientific investigation from our own governments as well as many others.

Although an ancient Kingdom that has only recently in time stepped out of a feudal past, Thailand contains within its population elements of almost every other Asian country and ethnic group, particularly those of its immediate neighbourhood. The Thai people are politically still in a state of social and political transition and thus both they and their various government organs find the intermittent presence of refugee groups from countries that have been ancient enemies more than a little unsettling. Indeed the RTG in Bangkok is not always followed with the same diligence in all outlying provinces. Both deep philosophic differences as well as personal ambitions tend to keep both its internal functions as well as its relations with all foreign groups somewhat in a state of flux. Direct confrontation is usually avoided as much as possible as long as the ultimate outcome is still in doubt. Survival with the smallest cost in trauma is most often sought.

In this turbulent political scene have stepped desperate refugee groups of many different ethnic and political backgrounds, Voluntary Agencies with constantly rotating international staffs reflecting widely variable religious and/or political persuasions as much as upon the service for which they were originally formed, and the

many Foreign Embassies, each to different degrees bent on gathering « useful » information for their home countries and on maintaining functionally « good relations » with the host Royal Thai Government and the people of Thailand. In this setting, perhaps with more bravado than sense, we have sought to carry out our work as private citizens, sort of quasi-scientific « amici curiae », if you will, attempting to find at least embers of truth which might be related to the clouds of smoke that most people sought only to get out of their eyes, patently avoiding any real search for a possible source.

It is our understanding that few samples had been collected prior to April 1981 and that, until late 1980, none were conscientiously sought. At that time it was made clear to some of the Lao/Hmong Refugee leaders that specimens would enhance the value of their reports considerably. Though April-June 1981, eight environmental samples passed through Dr. Townsend's hands to the US Embassy, Bangkok, and were sent from there to Washington, DC, for dissemination to government and non-government laboratories along with false positive and negative controls. These samples were all Laotian in origin and came wrapped in paper, plastic, and/or aluminum foil and were sometimes placed inside glass bottles. On only one occasion was one of the samples opened and its content noted to be a black carbon-like powder, originally reported to be from a « blue rain ». Later environmental samples were placed in special containers with absorbent carbon. A special effort was made to avoid plastic bags which, we were told, interfered with the laboratory analyses of the samples. Reports of CBW use and environmental samples appeared from all four seasons within Laos. Later reports of use within Cambodia showed a somewhat different pattern coincideing largely within the Dry Season from November to June. This was also the time of year when most military activity was initiated by the PAVN in that country against various Khmer Resistance forces (79-84). What few samples came out of Cambodia tended to be of more recent origin since most of them were collected within 50 km of the Thai border of the West where the terrain was relatively flat and multiple trails existed through jungle and broken country.

The situation in Cambodia contrasted sharply with that found in Laos where the terrain is vastly different and where the Hmong populations inhabited the mountains usually a long way from the Thai-Lao border, most of which is the Mekong River. The Lao ter-

rain with mountains up to 2,500-3,000 meters is often precipitous and irregular in formation as well as frequently jungle covered. It is guerilla country. Overland movement on foot is formidable even to one in good health and excellent physical condition. Thus both alleged victims and environmental samples have gone through a form of « selective process » by the time they crossed the Mekong River into Thailand. A far greater time-lag exists within Laos than has been seen in Cambodia. As one might expect, this affects the type of person seen when they finally reach the Thai border. Most of the people who complained of chemical contamination in Cambodia after 1980 were usually fairly well fed young men and women in otherwise good physical condition. Usually, they were active in one of the Khmer Resistance groups within the Khmer Coalition. The modes of chemical dispersion most often described in Cambodia were by artillery and mortar shells, rockets, « boody traps », direct water contamination (especially important during the Dry Season), ground areaspraying by either back-pack or aircraft, and mines. The plethora of different modes has been especially impressive. The use of these different modes seemed to depend upon the season and whether the PAVN was on the offensive or drawn up in a more defensive alignment as seen most often during the monsoon seasons. These past nine months have seen much more use of the non-lethal incapacitants by PAVN, especially close to the Thai border. Exactly what is going on relative to CBW use within the interior of Cambodia is at present uncertain. Khmer Resistance has become more aggressive there during this Dry Season while the PAVN has been attacking border enclaves and even entering Thailand on occasion. It is our understanding that Soviet and East European « Advisors » to PAVN are now restricted to travel by aircraft in the rural areas of Cambodia.

Our impression of CBW use within Laos over the past 9 + years has been gained from many sources, literally several hundreds of people Hmong, Lao, Thai, VN + other Refugee worker, singly and in groups, primitive and highly sophisticated, of all ages and both sexes, with and without military experience, both apolitical and some highly political. The pattern has changed during this period and we shall describe our impressions of these changes and their effects giving possible reasons for some of them. Since we have never knowingly been present in any county in an area where an obvious CBW attack was taking place, it should be fully understood that these reports are of « alleged uses

of « CBW » and they represent our own impressions and deductions which of necessity have had to change somewhat with time. We have not been privy to the full range of information gathered by many other sources and techniques and realize that we might have to alter our impressions more, or underscore them further, if we were so informed.

From the earliest known allegation of some form of CBW use with lethal potential as described in the January 1974 issue of the National Geographic Magazine (US), we have been piecing together reports that suggest that by 1975 with the failure of the coalition government in Laos, the Pather Lao and PAVN forces had begun a frequent and effective use of chemicals against the remnants of the Hmong and Lowland Lao forces, especially the former. In the earliest times when large groups of Hmong were fighting in a more traditional style reports of chemical contamination involved rockets and artillery-mortar shelling. Some reports involved the use of aircraft and gradually this became the predominant mode of deaths due to chemical contamination and, since the Hmong most often function as a family unit, these deaths involved people of all ages, and symptoms of a wide variety. One group of 8,000 Protestant Hmong reported their losses from 1975 to 1977 in a July 1977 letter to Thai-American friends and mentioned that a group of 10,000 Catholic Hmong nearby were « suffering similarly ». The English translation of this report which Dr. Townsend saw for the first time in July 1982, described the losses of people by disease and conventional weapons in contrast to the losses by chemical contamination. Unfortunately, this information was not made known to any embassy or news media at the time it was first received in 1977. That report and others from physicians working with newly arrived Lao-Hmong refugees in Thailand also indicated that many groups of Hmong were existing in a highly transient state with little medicine and no adequate supplies of food. Photographs of some of their people, especially their young, resembled those taken of Khmer newly arrived in Thailand, 1979-1980. This combined role of malnutrition and chemicals is a potentially significant one which has often been overlooked as has the combined role of « mild disease of childhood » and malnutrition, as we saw it among the Khmer Refugees, 1979-1980.

Since 1981 there seems to have been a gradual diminution of mortality and morbidity, especially the former, resulting from CBW

used against the Hmong coming out of Laos. Use has persisted although the rather wide variety of aircraft involved seems to have been reduced, possibly as experimental conclusions have been reached. It should also be recognized that thousands more of the anti-Pathet Lao Hmong have either left Laos by now or have been driven into total submission and come down from their traditional mountain top villages to more controllable valley sites. In brief, the chemicals seem to have been used as a « social broom » to effect the desired changes in the political-social structure of Laos. Untold numbers have died but, being « untold » and only an estimate, they are as easily forgotten as are those of Cambodia and other countries of times past.

The short term signs and symptoms that have been reported to us by the Hmong, Khmer and Lowland Lao include : headache, visual disturbances, nausea — sometimes with vomiting and diarrhea and sometimes with blood, anorexia, fatigue, weakness, vertigo, decreased thought control, decreased memory, auditory disturbances, burning sensations up to small clustered blisters in the skin, cough — sometimes with blood, nasal congestion, chest and abdominal pain, tachycardia, cardiac arrhythmia, unconsciousness and death. The long term effects noted by history and/or observation include : intermittent weakness, anorexia, reduced memory and thought concentration ability, intermittent diarrhea, relative impotence, increased fatigue, cough and dyspnea, increased susceptibility to infectious diseases and tumors(?), foetal abnormalities(?), spontaneous abortions(?).

Animal signs described most often include : anorexia, vomiting, diarrhea, erratic behaviour, seizures, unconsciousness, bleeding, and sudden death. Although a few wild birds, squirrels and fish have been described as having been killed by chemicals, most reports indicated that primarily domestic pigs and chickens were the most affected while cows, buffalo, horses, goats, ducks, dogs and cats were less so. The reported lethality would indicate, if accurate, that the domestic animals suffered even more lethality than did the humans in the same area. Animals under cover seemed to fare better, as did the humans in a similar locations, depending in all cases on what contaminated food or water was taken in over the next 24-48 hours.

There were some plant effects described in a few cases, most often affecting the cassava or tapioca plants, but these descriptions were all much more vague than those pertaining to humans

and domestic animals. There has been some mention of root damage as well as of leaf death. Most often only a few leaf holes were noted. There have been very few reports of strictly « defoliation episodes ».

In Laos, it was the normal response of affected Hmong (when they finally decided to flee) to wait until they felt well enough to travel which might be 2-8 weeks after an episode of alleged chemical use before they would make their way to the Mekong River. That trip was often via jungle trails, replete with ambushes, mines and all the hazards of nature while they tried to live off jungle fare enroute. The trip itself might take from 1-3 weeks depending on the distance and route. Although the dead were never brought out and those initially made « very ill » rarely even attempted the trip, many made only lightly to moderately ill by the chemicals were severely ill by the time that they reached the Mekong. Further loss by ambush, assault and outrigh murder at the hands of a boat renter, if used, and the lack of water and River knowledge was often experienced.

The Khmer stood a better chance of survival for their groups used much sophisticated therapy than the Hmong who depended mostly upon opium, sometimes accidently in lethal amounts with the very young, and various local herbs. If there were enough fairly healthy surviving Khmer, they would bring the victims to primitive field Aid stations, begin dextrose and saline IV therapy, and employ various drugs to combat the different symptoms noted. It was some of these patients that Dr. Townsend first saw 4 $\frac{1}{2}$ weeks post exposure in Cambodia in October 1981. At that time their most significant symptoms were : fatigue, weakness, anorexia, anxiety, intermittent nausea, jaundice with fever, mental confusion, relative impotence and sudden death. Two of these nine patients were found to have trace amounts of mycotoxins in their blood although I do not believe that the two included the man with jaundice who was the most ill. Two of the nine were people with medical training who supposedly had been contaminated by handling the people made ill via contamination, water to skin. Some Hmong and Khmer complained of notable symptoms 3-5 years after exposure. This needs to be studied.

Initially few defense measures were used. However, with time both Lao/Hmong and Khmer learned the value of staying cover if an attack was recognized soon enough, of breathing through a

cloth moistened with water or urine in lieu of a gas mask, of not running about hyperventilating in a cloud of chemicals, and of attempting to wash chemicals off their skin as soon as possible. Some of the Khmer Resistance were also testing the use of various mixtures of inhalants similar to those found on some PAVN bodies, i.e., mixes of ether, chloroform and ethanol.

When we first began to study the reports and alleged victims more consistently, there was a great frustration because few cases seemed to fit well any of the known chemical agents of WWI and WWII. Indeed, as we look back on the hundreds of people that we have talked with and examined, only one, a Lowland Lao Resistance fighter of 50 years of age stands out as a « probable chemical victim » on the basis of physical examination alone, and we were very hesitant to believe him primarily because of his description of the weapon, some sort of hand thrown grenade, although he showed peculiar blistering, edema, dyspnea, and weakness as well as other appropriate symptoms... and was very insistent that he had been « gassed ». Mycotoxins were found in his blood. He had only recently been exposed and then only a few kilometers from the Mekong River.

Although at the beginning we knew very little about mycotoxins in general and even less about Trichothecenes, the more that we interviewed and examined alleged victims, the more that we become impressed with the use of a group of natural toxins that seemed to display such a wide variety of symptoms and signs with variable onset in timing and duration, to mimic so many indigenous diseases of variable degrees of severity, and to pose such a challenge to clinicians in the field as well as ultimately to military commanders upon whom rest both defensive measures and appropriate counter measures. Without a rapid means of identification and absolute diagnosis, the commanders are placed in a more tenuous position than the clinicians. Lethality as reported has varied from zero to about 50 % and we are convinced that more than one « mix » of mycotoxin agents exists although there obviously are many other variables such as victim status of health, weather, delivery system used, accuracy of delivery, strength of the agent(s) used, as well as the type(s) of agent(s) used in their mix. Whereas, at first glance, one might think that mycotoxins would be useful only in a tropical setting and against less sophisticated populations, the destructiveness of any war with its drastic reduction in levels of sanitation and rapid

lowering of standards of life tend to make mycotoxins, although far less lethal than nerve gases, a potentially useful adjunct to other military weapons. It is our impression that mycotoxins have been adapted to many types of military weapons and are fully integrated into a wide variety of armaments from the multi-engined transport and fighter-bomber aircraft to the weapons carried and fired by the single soldiers for use in closed-in urban as well as in rural tactical situations with proximities under 100 meters. As moderate to low order lethal agents, mycotoxins would seem to fill a potential military role between the riot-control and incapacitant chemicals on the one hand and the various nerve gases, a virtual chemical counterpart to the nuclear weapons, on the one extreme.

The several pitfalls throughout this « field research' included especially the initial skepticism that we faced on all sides, especially our own. Recognizing that refugee status gave humans a degree of social acceptability only slightly above that of abject slavery, we had to constantly struggle to control our own deep-seated sympathies for all refugees, regardless of their levels of sophistication and political philosophies, in order to ascertain the clearest elements of truth possible in spite of the cultural and other barriers. We were shocked and greatly disappointed by attitudes that reflected, instead of a healthy skepticism and an earnest scientific and social curiousity, rather a deep seated fear of what might take place in their lives as a result of any positive find of the use of lethal chemical weapons. This ultimate concern for the personal, corporate, national, and international « status quo », or what might be perceived as that, began to worry us far more than the presence or absence of any particular new chemical weapon. It still does.

We consider that a strong and healthy skepticism is mandatory in any bona fide scientific endeavour. However, when that skepticism is used as a tool to impede or purposely discourage any examination of any allegations that are brought by small groups without any representation in an international forum, it is not a healthy skepticism. Indeed, it suggests societies that are in themselves intellectually and morally « ill », regardless of their standards of living, or the ulterior motivations of the few who may be manipulated by those that can not survive in an open and concerned world forum. Skepticism should insist upon increasingly more complete and accurate work rather than on no

work at all, or, as we have encountered on more than one occasion, the demand that it must be done only « first class » with a virtually open-ended budget-or not at all.

Another pitfall that has received little attention in the news media is the apathy of the world press generally on the one hand and the almost total inability of such primitive peoples as the Hmong to mobilize any world social concern for their benefit. We have had to seek out most of these individuals to ascertain what experiences they had encountered. Rarely did they ever contact us initially with a report of chemical contamination. Never did they evidence the indignant outrage that would be so commonly expected of European, American and Middle-Eastern people. Even pirate rapine and plunder on the high seas draws more press attention than allegations of the chemical death of unsophisticated people in a far-off jungle.

Yet another early pitfall in the evaluations of these allegations was the scattered presence, and almost total lack of communication between, medical and other workers among the Lao Refugees inside Thailand between 1975 and 1980. Thoughts and impressions were largely kept to one's self, particularly in view of the unusual descriptions given, the inadequate laboratory facilities, and the general lack of experience in military related affairs among most Refugee workers. Even groups that had on their own come to the conclusion that someone was doing something with chemicals of an unknown type to the Hmong in Laos' would not speak out for a number of personal and/or corporate reasons.

It is recommended that several nations and well endowed private institutions on different continents study these allegations thoroughly within Thailand and among Refugees as much as possible on their own before comparing notes and conclusions. Backgrounds of national mycotoxin levels in basic and oft used indigenous foodstuffs should be as known to the medical professions in their respective countries as it now seems to be to the Veterinary professions of many countries. Indeed, it should be known to the consumers as well! It is also strongly urged that thorough studies be made and openly published concerning the various characteristics of mycotoxins when produced under a variety of substrates and conditions and when coupled with potentiating additives. Long range effects of mycotoxins found on all continents should be diligently studied by a variety of disciplines. The particular potential for such a study

would seem to presently exist in any country that has given haven to the Hmong.

Simultaneous with the more « civilian oriented » type of research on the natural mycotoxin levels in locally consumed as well as internationally commercial foods, it is recommended that all potential military applications be investigated by those countries having the capability of so doing. Whereas it would be politically and philosophically more expedient to separate the defensive from the offensive studies, both pragmatism and the basic economics of such work would seem to suggest that they are too interrelated to allow for such costly indulgences. Where countries can cooperate with others, more rapid and theoretically more accurate results should be obtained. We assume that many of these recommendations have already been taking place since they are not unique to us. It is hoped that, as much as possible, the resultant information would be made available to the world public.

Idealistically it is our fondest hope that the various elements of mankind would put to rest the ultimate use of all forms of massive weapons of war, let alone chemical and biological weapons. However, both man's history over the last 6,000 years and our perceptions of his nature and character world-wide is such that at least for a few more generations we doubt that any form of « CBW disarmament agreement » would succeed save that built on the mutual knowledge of each other's possession of such weapons and the ability for their dissemination. They rarely use such weapons whenever the ability to reply in kind is known to exist. This is, of course, the same sort of « mutual respect with fear » that is presently relied upon so uneasily in the case of nuclear weapons. Even when national populations indicate their desire to avoid the use of such weapons, governments, when severely threatened, will tend to use whatever weapons they deem necessary for their survival. We have witnessed such recently in a sad war between erstwhile Muslim brothers. Indeed, they remarkably resemble their Christian cousins in the lengths to which they will go in order to maintain control of their respective peoples. In general, of course, the more dictatorial any government might be the more risks it can take day to day that can jeopardize not only itself but also its people and civilizations for generations to come.

It has been the sincere hope that the UN would assume a more vigorous and responsible role in studying these allegations, par-

ticularly for the purpose of protecting the small nations and ethnic groups who have poor if any representation in international organizations. Thus far, however, the various field agencies of the UN in Thailand have distinguished themselves primarily by the energetic avoidance of the pursuit of such challenging allegations that have gone on for ten years in Laos and four years in Cambodia. It is our understanding that this same level of enthusiams for the subject in Pakistan/Afghanistan based UN field agencies is held. In spite of these generally apathetic UN and other 10 responses, however, we insist on maintaining hope, recognizing that it is apt to be built more out of the fabric of fear, than of logic, for the next several generations.

Comparison of the toxicological investigations in man in Southeast Asia, Afghanistan and Iran, concerning gas warfare

by A. HEYNDRICKX and B. HEYNDRICKX

Department of Toxicology, State University of Ghent,
Hospitaalstraat 13, 9000 Ghent, Belgium

SUMMARY.

Since af few years the Department of Toxicology at the State University of Ghent is engaged in setting up detection methods of environmental and biological samples of man, concerning chemical and biological warfare.

The different results obtained are discussed and the figures found evaluated.

Discussion is also given about the different analytical procedures that can be used and the clinical toxicological evaluation in man. The toxicological values obtained from soldiers in treatment are also compared and the origin of the poisoning discussed.

The background levels of those contaminants in man are also studied and the war gases used determined.

INTRODUCTION.

Our interest was first aroused by foreign students participating at the Foreign Students' Programs at the State University of Ghent. They were telling us about the problems in their respective countries, and asking if we could help them in finding answers to their questions. First of all, students from Thailand brought over samples from the Far East, in casu Vietnam and Cambodia. Those were the samples we analysed first.

Thailand, Vietnam, Cambodia.

The samples we were getting from Cambodia were environmental ones. We could differentiate those samples in two groups :

A. Those containing only pollen.

B. Those containing mycotoxins and no pollen.

The mycotoxins we found were T_2, HT_2, etc., as later discussed. There was no doubt that a new kind of gas warfare had been used in casu mycotoxins (Yellow Rain).

Afghanistan.

As no samples were received from Afghanistan, we did not make any analysis. But we received very interesting medical information from physicians working in Afghan villages. There is no doubt that at least mycotoxins and mustard gas are used.

Iran.

In the spring of 1984, wounded Iranian soldiers were brought over to Europe for emergency treatment.

By those patients, after analysing blood, urine and faeces, we came to the conclusion of the presence of a mixture of at least three gases : mustard gas or Yperite, mycotoxins or Yellow Rain and a third unknown gas. This third gas could be suspected to be a neurotoxic one, but at that time we did not have any background information. Later, after the publication of the Report of the United Nations (8), it became clear that they had been using Tabun, which was correlated to the lower plasma acetyl-cholinesterase activity we found in the Iranian patients earlier. Some unbelief from military people followed our announcement of the use of a mixture of three or more gases. I found the solution to this problem by going over to the battlefield in the desert (Majnoun Islands). Soldiers told me that a great number of bombs and shells with different contents were dropped at the same time. If there is a white dust and chocolate taste, they know, having no time to inject themselves, that they will die very smoothly and quickly. There will be no symptoms on the corpses, we know Tabun has been used.

If the dust is black and there is a garlic taste, the symptoms only appear two or three hours later, the chemical used is Yperite.

If the dust is greyish blueish, they know there will be no symptoms immediately, but they will get sick much later : this must be Yellow Rain.

FIG. 1.
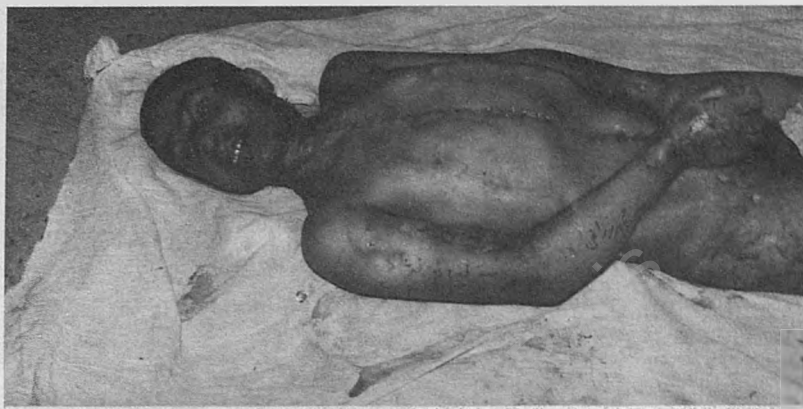
FIG. 2.

FIG. 1. — Lesions caused by a synergetic mixture
much more toxic than the separate compounds themselves
(post-mortem 3 days after death).

FIG. 2. — Symptoms on a corpse (post-mortem 3 days after death).

FIG. 3. — The desert region near the Majnoun Islands,
where I had been invited to by the Iranian Officials
(orange NBC clothing : Heyndrickx A. ; green NBC clothing : Iranian soldiers).

FIG. 4. — Bomb found in Iran same as identified by the United Nations team (8).
Identity : « *Para tiempo de armade — Inferiores a 6 segundos — quitar el tornillo
visor rojo peligro — Esp. MU 09 — Lot 83.01* ».

FIG. 5. — The opening of a mustard gas shell and sampling. Battlefield region, Iran
(orange NBC clothing : Heyndrickx A. ; green NBC clothing : Iranian soldiers).

FIG. 6.

FIG. 7.

FIG. 6. — Dead body, black and swollen (post-mortem 1 day after death).

FIG. 7. — Dead body, black and swollen (post-mortem 3 days after death). Many of the dying get in an edematous state hours before death. Ten minutes before dying a dirty fluid under pressure comes out of the nose. Two or three days after death has occurred, the bodies turn completely red, similar to a Carbonmonoxide (CO) poisoning. They contain a very high amount of methemoglobin.

FIG. 8.

FIG. 9.

FIG. 10.

FIG. 8. — Penis, scrotum of patient and scars, after mustard gas contamination
(4 weeks after attack and treatment in hospital, Teheran).

FIG. 9. — Scrotum : swollen (4 weeks after attack and treatment in hospital, Teheran).

FIG. 10. — Swollen dead, with pus running down from nose and ears
(post-mortem 3 days after death).
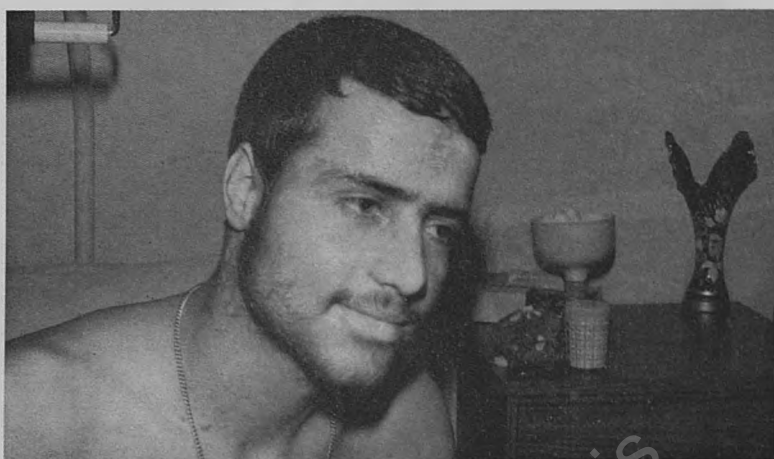
FIG. 11.

FIG. 12.

FIG. 13.

FIG. 11. — Four weeks after attack and treatment in hospital, Teheran.

FIG. 12. — Patients in Teheran, victims from the war gases attack on April 12th 1984.
Scars, swollen scrotum, penis, elbow region, armpit region.

FIG. 13. — Six weeks after attack. Treatment in hospital, Teheran (wounds infected).

What possible protection can be used in those cases ?

Unfortunately, there are practically none : because of the heath in the desert, suits and masks are unappropriate to wear all the time ; and there is not enough time to put them on after the explosions.

CONCLUSION.

Similar looks of the victims in Cambodia. Afghanistan and Iran are evident. We cannot state that the same gases have been used, no analysis having been made by us on Afghan samples. The post-mortem signs as observed in the victims are conclusive.

There is no doubt that there is a parallelism between the soldiers from Iran treated in Europe and the dead people we saw in the morgue in Teheran. There is no doubt that chemical and microbiological war gases are used in the Iran-Irak conflict and that Chemical and Biological Warfare is used today by Irak in Iran.

Perhaps these warfare techniques are going on since many years, we remember the problems in Yemen some years ago ; there have been reports in Laos ; we know that chemical agents are stocked in Ethiopia.

Some symptoms we have seen on patients in Iran cannot be related to the gases mentioned above, a fourth gas or mixture of gases is therefore suggested.

It is absolutely necessary to have a complete ban on those terrible weapons, with the possibility of international control and of their basic materials needed for production, in order to protect man against those terrible weapons, of which we have already a proliferation in the developing countries.

Having seen myself in life a great amount of corpses of political prisoners in World War II in German concentration camps, as well as in the field of toxicology, I can testify that the post-mortem signs shown here are completely different (fig. 7).

The corpses are black colored, mouth and nose are bleeding and pus mingled with a bloody liquid is running out of mouth and nostrils, eyes and ears. Skin peels off very soon after death and sometimes even already before.

Some people in Afghanistan say that those who touch the deads are contaminated and afterwards many die too.

One could assume microbiological warfare but we excluded it (9). We believe the contamination is due to the state of septicemia of the deceased. The symptoms being the same as the deceased in Iran we can assume the same war gases : mustard gas, yellow rain, tabun as a mixture ; or mustard gas alone or mixed with other gases. A fourth gas, or mixture of gases, we could not identify in the Iranian patients treated in Europe ; these symptoms are clinically completely different.

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Various aspects of the Afghan question

by A. NAIM

103 D Greencroft Garden. London NW 6 3PF, United Kingdom

Allow me to convey my thanks for granting the opportunity to speak at this distinguished gathering about various aspects of the problem of my country, Afghanistan, which was ruthlessly invaded by the Red Army. Our nation during the course of its long history, had always struggled for the preservation of its freedom and territorial integrity, its religious values and national self-determination at the cost of its blood ; and unless a political solution, based on international justice and self-determination is found, our people will be resolute in regaining their dignity and their place amongst the free nations of the world. At this very moment as I am standing here, despite the shameful atrocities of the Russians and their local subordinates, the Afghan freedom fighters are continuing their struggle with the utmost determination and devotion, and their morale is still very high.

The independence of Afghanistan has been threatened since April 1978, when for the first time a Communist regime backed by Soviet intrigues usurped power in Kabul, and in December 1979, with the invasion of the Red Army and the installing of Babrak Karmal, Russia formally occupied our country.

This intervention and subsequent invasion was not only contrary to all international norms and practices, it was also contrary to the previous understanding and agreements on which the relations of Afghanistan and the USSR were based. The cornerstone of those relations had been based on reciprocity and peaceful co-existence between two neighbouring countries which had different political, economic and social systems. To us, reciprocity means non-interference in each other's internal affairs and mutual respect for the accepted traditions and values.

Soviet authorities in the past at different levels, repeatedly assured their Afghan counterparts that they would always adhere

to those principles. Likewise, from time to time, the Afghan authorities reminded their Soviet counterparts of consequences of their intervention in Afghanistan. Peaceful co-existence between free Afghanistan and Soviet Russia was regarded as an example between two neighbouring countries.

Lack of appreciation on the part of the West of non-alignment in Afghanistan Foreign Policy ; also its lack of interest in building an economic infrastructure where Afghanistan needs were greater than those of her neighbours ; similarly, the inbalance of the military equation in the region by the expansion of Western military pacts, which was, in the final analysis, a total failure ; as well as the attachment of political strings by the West to the giving of military aid : it was these policies which paved the way for further cooperation between Afghanistan and the USSR. However, the regimes and governments of free Afghanistan, as I mentioned before, in their dealings with Russia had *never accepted any political conditions.*

Whilst on one hand the Russians assuring the Afghans of their friendship, non-interference and continuation of peaceful co-existence, secretly they were supporting materially and ideologically those who were opposed to the national interests of Afghanistan. With a Soviet inspired coup, the so-called PDPA usurped power in Kabul. Thus with this intervention and invasion of Afghanistan, the Afghan question, the subject of this talk, arose.

Unfortunately the concept of the Afghan question is distorted by various interests. They do not wish to correct this concept in order to use Afghanistan as a bargaining point for their own benefit. The communist *Khalqi* and *Parchami* regime in Kabul portray the Afghan resistance as so-called terrorists and bandits, aided by the forces of « imperialism and reaction ». But they were not able, even with the might of the Red Army, to eradicate those so-called terrorists and bandits.

Not only have they not been eradicated — on the contrary, their numbers have increased.

The Western media likewise sketch the Afghan question according to their own shades and colours. Some applaud the courage of the Afghan freedom fighters, certainly not for their fighting for Afghanistan's national interest and religious values, but in order to halt and humiliate the Russians, the prolongation of which, regardless of Afghanistan's future status, they believe is in their own interest. Others picture the situation as

an unequal opposition by those who want to preserve their old way of life and resist all attempts of progress and modernization. Thus they show the image of the Afghan resistance as a romantic and retrogressive step rather than nationalistic and progressive movement. Some are of the opinion that the present state of affairs in Afghanistan does not represent the defeat of the Russians, but rather as « stalemate ». They think that finally the tide of events will turn towards Soviet's advantage, and suggest a « Finlandisation » formula for the solution of the Afghan question. There are however others who doubt the validity of an alternative regime if the Russians withdrew. They argue that Soviet withdrawal would consign Afghanistan « once again into medieval oblivion ».

The true nature of the present Afghan question is the result of the declared opposition of the entire Afghan nation, with the exception of a surrendered minority to the circumstances created by the Soviet intervention in our country's internal affairs along with the coup d'état of April 1978 and the subsequent Russian invasion. Therefore, it is a purely political question rather than a problem of refugees, etc. In fact, those problems are often as a result of the actual question. This intervention and invasion plunged the Afghan nation into an abyss which resulted in the loss of both life and material resources. Since then, as you are aware, Afghanistan has become not only the scene of bloodshed, the victims of which are innocent men, women and children, but all efforts are made to destroy the traditional and spiritual values of the Afghan nation. The Russian occupation of Afghanistan has been condemned in the UN, in Islamic conferences, in the non-aligned gatherings, in many other international forums and by the world public opinion. The Soviet adventure in Afghanistan not only tarnished her image, especially in the Third World where she was regarded as a force for « peace and progress » but these actions in fact revealed her true imperialist intentions. The resistance by the people of Afghanistan on the other hand undoubtedly gave support to the struggle and uprising of the Polish workers. If there had not been any resistance in Afghanistan, surely the events of Hungary in 1956 and Czechoslovakia in 1968 would have been repeated in Poland.

Although the Communist regimes of Taraki, Amin and Karmal supposedly regard themselves as non-aligned, independent and democratic, in reality this is pure imagination. Like all the slogans

of Communist ideology, they are nothing but a series of lies and deceits.

The failure of the Russians to complete the occupation of Afghanistan, the continuation of resistance and its worldwide condemnation, forced them and their installed regime in Kabul to seek a political solution to the Afghan question. However, the very concept of a solution remains vague and all parties concerned are trying to interpret and use this concept to their own advantage.

By solving the Afghan question politically, the Soviets seek to preserve the status-quo in Afghanistan through an international agreement and guarantee. After achieving that, in their opinion, they may « withdraw », but their military, political, economic, cultural and administrative establishments would be unaltered. In line with this concept the Russians encourage the Kabul regime to participate in the indirect talks in Geneva with the Pakistan government under the auspices of the representative of UN Secretary General, of course without any genuine Afghan representation. As long as the status-quo in Afghanistan remains, any suggestion of a political solution is out of the question and any agreement to resolve the aftermath of Russian intervention merely postpones the possibility of a realistic and genuine political solution. Any international agreement and guarantee without consent and consultation of the elected representatives of the Afghan people would be both unacceptable and irrational.

Likewise the solution, proposed by the EEC, to create arrangements to guarantee Afghanistan's neutrality along the basis of the 1955 Austria treaty (often described as « neutralisation » in the Western media) between the USSR and Afghanistan's neighbours is unrealistic and would limit the sovereignty of our country, neutralising her legitimate national interests, as a result of which the Afghanistan of the future will be at the mercy of others leaving her without any freedom of action. The policy of neutrality and non-alignment pursued by regimes and governments of free Afghanistan prior to April 1978 was an active and positive policy based on our free national judgment and was adopted in the interest of world peace and the reduction of international tensions. This policy could not and should not be regarded as synonymous with the passive neutrality imposed and guaranteed by certain powers on certain countries of Northern and Central Europe. Any suggestion based on treaty of neutralization would prevent the prospects of a just and lasting political solution. If invasion of a small country by a super-power is followed by « neutralizing »

the victim, then what is the destiny of all small nations of the world to be ?

Afghans are a peace-loving nation and always have been. Unless our dignity is threatened, we never provoke war, nor do we even refrain from any kind of sacrifice for the restoration of that dignity. As a developing country, Afghanistan has no other desire or design than peace, security and stability, which are the prerequisites of economic and social development. Whenever there is the possibility of a peaceful means of the restoration of that dignity, we always welcome it. Furthermore the concept of negotiation with the Soviets, provided they are sincere, has never been rejected by Afghanistan. But what is not negotiable are the basic principles of our nation. They are national independence, national identity, national sovereignty, territorial integrity and of course, our Islamic values. If Russia is willing to negotiate along these lines, then she should start a clean page, write a new chapter and assist in improving the situation.

The realistic approach in finding ways to a political solution is through the United Nations. The resolutions of the Security Council and the General Assembly on Afghanistan are at the disposal of this organization. If Russia attaches any importance to her commitments to the UN Charter as a founder signatory, and to her responsibility as a permanent member of the Security Council, then she should respect the UN resolutions.

The present procedure, the indirect talks in Geneva, however, are contrary to the internationally accepted approach in the resolving of disputes :

1. It ignores the parties truly concerned and directly involved.

2. Their main emphasis is on the problems of refugees and other human factors, and does not deal with the core of the matter.

Since the nature of the Afghan question, as mentioned earlier in this speech, is purely political and the refugee problems and other human factors are related to the aftermath, any attempt to resolve the aftermath without serious consideration of the core of the conflict is fruitless, unjust, irrational, and cannot possibly produce positive results.

The two confronting sides of the Afghan conflict, Russia and the entire people of Afghanistan are not represented in the indirect talks in Geneva.

The Karmal regime has neither the authority nor the ability to represent the Afghan nation.

Without participation of our people through their elected representatives, any solution imposed and guaranteed by outside interest is doomed to failure. The people of Afghanistan did not create the present conflict and they are under no obligation to take the first step towards a political solution. If USSR wants a political solution, it is up to her to take the first step, by totally and unconditionally withdrawing her troops ; and she should pledge non-interference in Afghanistan's internal affairs, cease to support the Karmal or any similar regime in Kabul, and respect the right of the Afghan nation to determine its political, economic, social and cultural destiny.

If a political solution to the Afghan question through the United Nations is desired, then the present procedure should be altered. The international dimension of the Afghan question requires a special international conference under the auspices of the UN. As has been suggested by Mr. A.R. Pazhwak, an experienced, former Afghan diplomat and once President of the UN General Assembly, the participants at such a conference should be the representatives of the Afghan people, permanent members of the UN Security Council, Afghanistan's neighbouring countries and a number of member nations of the UN from all continents with special regard to non-aligned and Islamic countries, under the good office of the UN Secretary General.

If Russia insists on intensifying her adventure in Afghanistan, then the Afghan freedom fighters, who consist of the entire nation, have no other choice but to fight to the last bullet and last drop of their blood. Under such circumstances, Soviets must not forget their lesson of history, that the outcome of the expansion of empires does, in the final analysis, tear them apart internally and we believe that the Soviet Empire is no exception.

SUMMARY

This issue of the Proceedings of the First World Congress : « New Compounds in Biological and Chemical Warfare : Toxicological Evaluation », May 21st-23rd 1984, Ghent, Belgium, is entirely devoted to the fifty-two papers and communications as presented to the 324 registered delegates from 31 countries.

The following main conclusion is made :

1. From the communication of Prof. C.J. Mirocha it is clear that trichothecenes were found in biological samples of man and the environment in Cambodia in concentrations that never have been detected before ; they cannot be of natural origin. The autopsy material shows death has occurred through mycotoxins used in microbiological warfare.

2. By the paper of Prof. J.D. Rosen it is demonstrated that polyethylene glycol, a man made substance, is present in mycotoxin samples originating from Cambodia : this substance has never been found in environmental samples in nature.

3. From different papers and communications it is proven that mycotoxins used in chemical warfare metabolize quite fast in biological samples of man, and that after three weeks no mycotoxins can be found, after a second analysis of the same sample. It is furthermore scientifically demonstrated that mycotoxins in biological samples of man (urine, blood and faeces) deteriorate by standing ; no mycotoxins can be found either by gas chromatography or mass spectrometry.

4. The outstanding research work of Dr. H. Cohen makes it clear that the very high amounts of trichothecenes found in environmental samples cannot be of any normal origin in nature. The theory of Prof. J. Meselson saying that these trichothecenes come from bee faeces etc. is scientifically impossible.

5. It was also stated that Yperite or mustard gas can be found in biological samples of man after a chemical attack, even 3 weeks

after the intoxication. Mustard gas does not metabolize as fast as it is thought.

Yperite in environmental samples can also be detected several weeks after the bombing.

This World Congress, the first of its kind, shows that much research has to be done, and that many theories have to be revised : the more sensitive our techniques are, the more we will be able to describe the mechanism of action, metabolization and deterioration of the compounds.

Years ago this was impossible : gas chromatography and mass spectrometry did not exist, toxicologists had to rely on more simple techniques than nowadays. Environmental and biological samples of man are much more complicated to study than *in vitro* tests.

These studies in human samples have opened new theories what the toxicity of those compounds is concerned, and at the same time new treatments and desintoxication possibilities are described.

Prof. A. HEYNDRICKX,
Editor.

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* The italics author held the talk at the Congress.

AUTHOR INDEX *

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