THE INTERNATIONAL ASSOCIATION OF FORENSIC TOXICOLOGISTS
23RD EUROPEAN INTERNATIONAL MEETING
TERRORISM : ANALYSIS AND DETECTION OF EXPLOSIVES

SECOND WORLD CONGRESS
NEW COMPOUNDS IN BIOLOGICAL AND CHEMICAL WARFARE : TOXICOLOGICAL EVALUATION
INDUSTRIAL CHEMICAL DISASTERS
CIVIL PROTECTION AND TREATMENT

GHENT, AUGUST 24-27, 1986

PROCEEDINGS

Edited by : Dr. B. HEYNDRICKX, M.D.
Department of Toxicology
State University of Ghent
Belgium

Institut kurde de Paris

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P R O C E E D I N G S

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Sponsored by the National Ministry of Education, the Flanders Government, the Faculty of Pharmaceutical Sciences, State University of Ghent, and the National Science Foundation, Belgium

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Conseil de plus en plus impératif : ne touchez pas aux fûts du *Herald* échoués sur les plages...

Dimanche soir, un des trois fûts échoués sur la plage de Knokke et contenant une matière toxique, en provenance du *Herald of Free Enterprise*, a explosé dans les mains de quatre membres de la Protection civile.

Le transfert immédiat des blessés vers les cliniques de Blankenberge et de Bruges, ainsi qu'un traitement adéquat après consultation du toxicologue de renommée internationale, le professeur en médecine Aubin Heyndrickx, de l'université de Gand, leur a problablement sauvé la vie.

Le professeur Heyndrickx, qui nous a expliqué les réactions du produit toxique concerné, ainsi que les mesures à prendre en cas de contamination, n'est pas rassuré, malgré les indications des lettres, quant au contenu des fûts, lesquels sont soit toujours à bord du ferry chaviré, soit ballottés entre deux eaux à proximité des plages.

« Le papier est complaisant », remarque-t-il... Après l'incident, le procureur Valcke, du parquet de Bruges, a lancé un appel urgent aux bourgmestres des villes balnéaires et aux services de tourisme. Son contenu peut être résumé en quelques mots : « Que personne ne touche ou ne s'approche d'un fût échoué ! »

Un produit chimique dont tout le monde se méfie

On n'a su que lundi, tard dans la soirée, qu'un fût contenant du toluène diisocyanate avait explosé dans les mains des quatre membres de la Protection civile qui l'avaient ramené de la plage de Knokke vers la base navale de Zeebrugge. Une personne a été touchée au pied par un éclat; tous les quatre ont été éclaboussés par le produit chimique que le professeur en médecine Heyndrickx a décrit comme appartenant à la famille de celui qui fit exploser l'usine de l'Union Carbide à Bhopal (Inde), les gaz ayant par après causé la mort de centaines de personnes.

Un traitement adéquat à la clinique Fabiola de Blankenberge a sauvé la vie à Marc Borio, à Francis Vandebossche et à Yvan Laprudence. Tous trois ont pu sortir de clinique mardi. Daniel Desmet, soigné à l'hôpital Saint-Jean de Bruges, pourra probablement rejoindre ses camarades aujourd'hui.

Défense de toucher et d'approcher

Mais qu'adviendrait-il si des enfants ou des promeneurs entraient en contact avec un tel produit échoué sur une plage ? Et que faudrait-il faire en cas de contamination ?

C'est ce que nous avons demandé au professeur Aubin Heyndrickx qui fut dans le passé appelé plusieurs fois en consultation à Bhopal et qui a été chargé dernièrement du traitement des victimes de la guerre chimique entre l'Iran et l'Irak.

« Le toluène diisocyanate produit une violente réaction exothermique au contact de l'eau de mer : des gaz se forment à des températures très élevées, transformant ces fûts en des bombes potentielles. Dès lors, l'explosion de dimanche n'a rien eu d'étonnant pour moi car j'avais déjà averti les services concernés de cette éventualité. Malheureusement, il faut toujours qu'il ait d'abord un accident avant que l'on prenne un avertissement au sérieux ! »

Toujours d'après le savant gantois, cet incident est de nature à prouver aussi qu'il se pose de sérieuses questions quant au contenu des autres fûts.

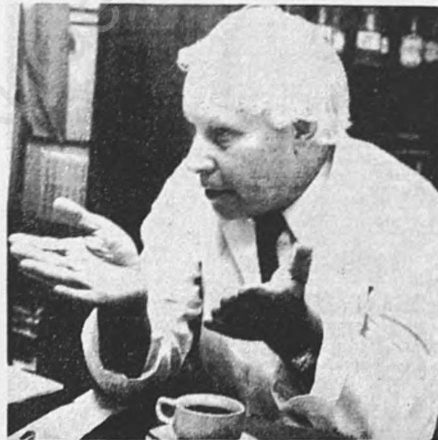
« Nous avons une idée générale de ce contenu, mais quels déchets de produits chimiques se trouvent dans les bouteilles entassées dans les seaux noirs en plastique localisés à bord du ferry ? Tout ce que nous savons, c'est qu'il s'agit de produits très toxiques, sans heureusement être de provenance nucléaire. »

Ne pas laver les yeux

Alors, défense de toucher ou d'approcher, mais si malheureusement le produit vous atteint malgré tout ?

« Le traitement est le même que pour celui que nous avons utilisé pour les victimes de l'ypérite : laver abondamment avec une eau savonnée, puis rincer à l'eau claire pendant un quart d'heure — mais pas plus — et recouvrir les blessures chimiques avec un baume spécial — de la flamarine. Ne pas laver les yeux, simplement les rincer. Si jamais vous inhalez le gaz, qui heureusement se disperse vite à l'air libre, un transfert immédiat à la clinique la plus proche s'impose. »

Un autre conseil : placez-vous toujours dos au vent afin de ne pas inhaler les gaz. En outre, enlevez tout de suite les vêtements éclaboussés et lavez-les ou brûlez-les.



Le professeur Heyndrickx : « J'avais prévenu... »

« Ces produits sont une saleté dont le transport à bord de ferry pour passagers ne devrait pas être autorisé » conclut le professeur en médecine Heyndrickx, qui insiste encore pour que personne ne s'approche de ces fûts devenus des bombes.

Redressement après Pâques

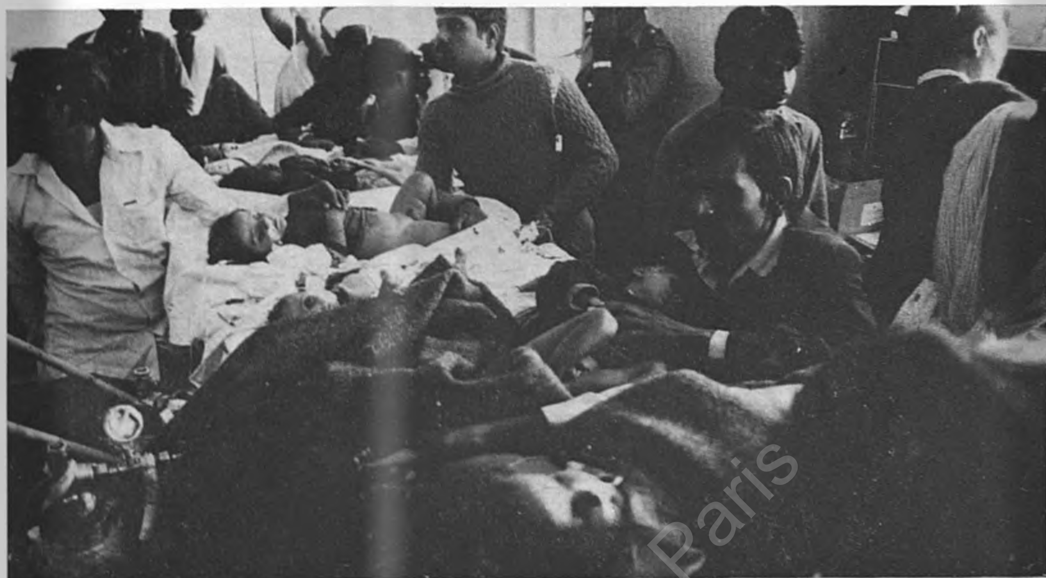
Autour et sur l'épave du *Herald*, les renfloueurs ont recommencé les travaux préparatoires au redressement. A cause d'un épais brouillard qui a empêché l'approche des bigues taklift 4 et 6 près du navire, leurs prévisions d'hier — peut-être trop optimistes — ne se réaliseront pas. Le redressement ne pourra se faire avant le début de la semaine prochaine. Le renflouement proprement dit du navire ne pourra se faire avant début mai !

Mardi soir, alors que certaines sources faisaient encore état de la présence d'une trentaine de corps de victimes dans les cales du ferry, corps qui n'ont toujours pas pu être récupérés, cent septante des cent septante-quatre cadavres extraits du *Herald* ont pu être identifiés et cent cinquante-sept corps ont déjà été rapatriés vers la Grande-Bretagne.

EDDY SURMONT.

Une mine au large

Mardi, seul le remorqueur de la force navale *Ekster* croisait encore aux abords du ferry, le chasseur de mines *Crocus* ayant en effet été appelé, le matin, pour procéder à l'enlèvement d'une mine datant de la Seconde Guerre mondiale, trouvée au large d'Ostende, à un demi-mille (environ 1 km) devant l'entrée du port. Les plongeurs démineurs ont fait exploser la mine, au large, dans le courant de l'après-midi.



Hours after the tragedy, parents at crowded Hamidia Hospital watch helplessly as their children struggle to breathe

RAGHU RAI—MAGNUM

Environment

TIME DECEMBER 17, 1984

COVER STORIES

India's Night of Death

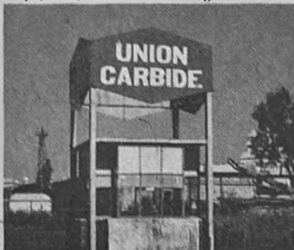
More than 2,500 people are killed in the worst industrial disaster ever

The first sign that something was wrong came at 11 p.m. A worker at the Union Carbide pesticide plant on the outskirts of Bhopal (pop. 672,000), an industrial city 466 miles south of New Delhi, noticed that pressure was building up in a tank containing 45 tons of methyl isocyanate, a deadly chemical used to make pesticides. At 56 minutes past midnight, the substance began escaping into the air from a faulty valve. For almost an hour the gas formed a vast, dense fog of death that drifted toward Bhopal.

The vapor passed first over the shanty-towns of Jaiprakash and Chhola, just outside the walls of the plant, leaving hundreds dead as they slept. The gas quickly enveloped the city's railway station, where beggars were huddled against the chill. In minutes, a score had died and 200 others were gravely ill. Through temples and shops, over streets and lakes, across a 25-sq.-mi. quadrant of the city, the cloud continued to spread, noiselessly and lethally. The night air was fairly cool (about 60° F), the wind was almost calm, and a heavy mist clung to the earth; those conditions prevented the gas from dissipating, as it would have done during the day.

A few hundred yards from the chemical plant, M.A. Khan, a farmer, was lying in bed when he heard several thumps at a nearby dairy farm and sensed that his own cows were milling about restlessly. He arose and went outside. Two cows were dead on the ground. A third gave out a loud groan and collapsed as Khan watched. Then the farmer's eyes began to smart painfully. He ran into the darkness. The day after, at Bhopal's Hamidia Hospital, his eyes shut tightly and tears streaming down his cheeks, Khan described his fear: "I thought it was a plague."

Empty, the plant awaits investigators



ZLOTNIK—SIPA/SPECIAL FEATURES

Others thought it was a nuclear bomb or an earthquake or the end of the world. As word of the cloud of poison began to spread, hundreds, then thousands, took to the road in flight from the fumes. In cars and rickshaws, on foot and bicycles, residents moved as fast as they could. As in some eerie science-fiction nightmare, hundreds of people blinded by the gas groped vainly toward uncontaminated air or stumbled into one another in the darkness. Others simply collapsed by the side of the road in the crush. At least 37 people who had inhaled the fumes died hours later from the effects, having reached what they thought was safety.

By week's end more than 2,500 people were dead in the worst industrial disaster the world has known. At least 1,000 more were expected to die from the fumes in the next two weeks; some 3,000 remained critically ill. In all, 150,000 people were treat-

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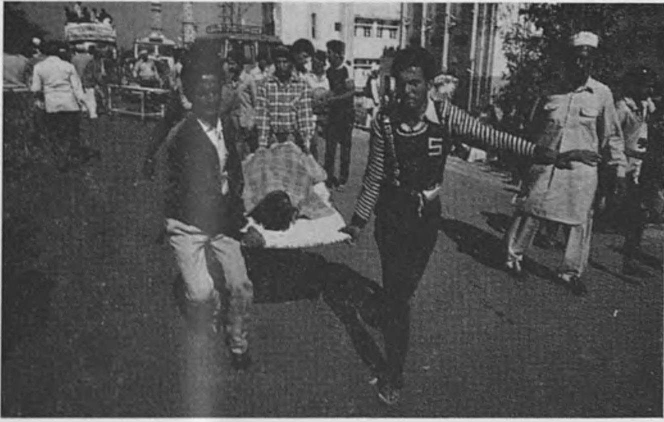

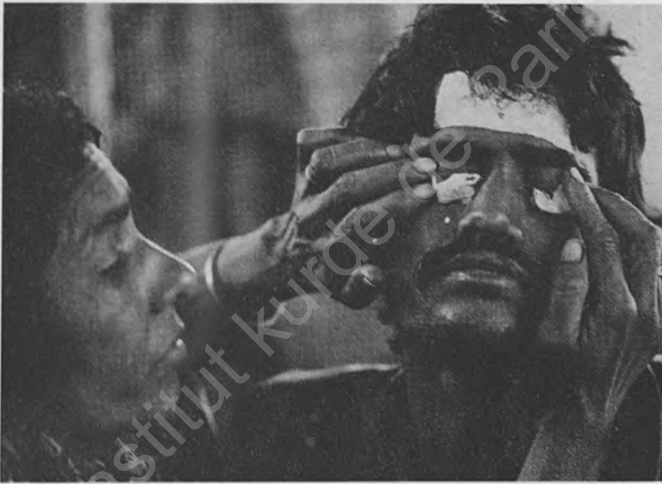

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Illustrated weekly of India
December 30, 1984

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SOIR

TELE week-end
Pages 40 à 43

0 Bruxelles

100 ANS
Libérer le ciel européen en faisant payer le billet ... au prix du train ?

101e année, n° 87
Edition • • •
Sam. 11 et dim. 12-4-1987

M. Gorbatchev décide de détruire l'arsenal chimique de l'U.R.S.S.

Succès populaire dès le début pour M. Gorbatchev qui entamait jeudi une visite de quatre jours en Tchécoslovaquie, pays dont la population espère sans doute, à la faveur de ce qui se passe en U.R.S.S., connaître les vertus d'un nouveau printemps. Le numéro un du Kremlin a-t-il donc dit M. Gorbatchev à Prague. *On sait*, a-t-il ajouté, *que les pays du Pacte de Varsovie n'ont jamais produit ces armes et ne les ont jamais eues sur leur sol. L'Union soviétique n'a pas d'armes chimiques en dehors de son territoire. En ce qui concerne les stocks d'armes chimiques, j'ai*cluait les autres gammes d'armes nucléaires dans lesquelles Moscou avait la supériorité.

Certes, M. Gorbatchev a parlé plus précisément des missiles d'une portée comprise entre 500 et 1.000 km, ce qui laisse dans l'ombre des armes d'un rayon d'action inférieur et qui peuvent parfaitement atteindre les défenses occidentales, l'Allemagne fédérale particulièrement.

CHRISTIAN-GUY SMAL.

Suite en troisième page.

EDITORIAL

by A. HEYNDRICKX

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

Honourable Vice-Prime Minister,

Honourable Past Rector,

Ladies and Gentlemen,

For the second time at the State University of Ghent in Belgium, we are having the World Congress on Chemical and Biological Warfare. We have also as a subject the Industrial Disasters. As toxicologists we are interested in the Environment, we have to deal with it every day.

Since the First World Congress, chemical warfare in the Gulf Region is still going on. We had the opportunity to treat all over Europe in University Clinics many patients who were suffering from those attacks. It is a pleasure that many of the scientists who were treating them are here today to discuss with you their experiences and how we could improve the decontamination and also the suffering of men. Since then also we have had chemical disasters as in Bhopal (India), by Union Carbide, and we can also evaluate what was going on in air, water and soil pollution and how we should treat them. This year, it is 10 years ago that in Seveso, Hofmann-La Roche, the company Icmesa, caused the so dangerous intoxication by dioxines for people living around the factory where we had also to evacuate many in order to prevent a further subacute intoxication or even a chronic one.

It means that today more and more society has to deal not only in the Western industrialized World, with those possibilities. We have to see with the technology we have today how we can im-

prove it in order to avoid such catastrophies. At the same time the responsibilities for the governments to have disaster plans, if something would occur today that we could prevent such disasters, and at the same time take the responsibilities for citizens so that we can protect them and that we can avoid such catastrophies.

Technology has changed the last decades going to massive production of some intermediates which can be so toxic and even more dangerous than chemical warfare agents today. Many of those chemicals we need, we can not avoid them because we have to produce compounds that society today expects. It means that the responsible engineers, chemists, pharmacists, physicians, all of them evaluate the dangers, see what we can accept and apply a technology that could protect us from such disasters. It means also that in developing countries, before we can start up such factories, we have to rely on specialists who can control in the same manner as we do in the Western industrialized world using the same standards of production, maintenance following, and engineering. If that is not the case and if we don't have the necessary specialists, such factories should not be installed and working.

In these fields of toxicology and pathology where we have new intoxications to detect and to treat, where for many chemicals we don't even have antidotes, everyone understands that we have to be much more careful than in the past. I agree that always there will be a calculated risk, that we can not avoid and at the same time we can not stop the industry from going ahead, but all those parameters have to come together and have to be evaluated. This 2nd World Congress where we were adding those chapters, brings together specialists from all parts of the world. I hope that their knowledge and fruitful discussions, we will have during those three days, will be useful for all governments and at the same time for industry. Today public health is the most important parameter for the future. If those compounds don't bring anything forward, what's the use of it and what's the use to produce them. So I hope that in this field we will be able in The International Association of Forensic Toxicologists and in other scientific societies to solve those problems.

It is a pleasure that the 23rd Annual Meeting of the International Association of Forensic Toxicologists meets for the 3rd time in Ghent again. This society, started in London by Dr. Curry, also

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Dr. Honoris Causa of our University this year, has more than one thousand members all over the world, seeing each other regularly. It is a pleasure that they are with us again as they were also by the first centennial celebration of the Department of Toxicology here in Ghent in 1976. They accepted the invitation to come again here as we have done many times in the friendly atmosphere, helping each other in this very difficult field of toxicology.

In the section of Terrorism, TIAFT members will discuss the new techniques of evaluating explosives, the residual analysis and detection, field of great concern in the Western World and the mediterranian region. Air-port safety and control are at risk, forensic toxicology plays a big part in it.

To our present President Dr. McLinden and also to our past chairman Prof. Brandenberger and all of you who are in Ghent, welcome.

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Allocution
by His Excellency Mr. G. VERHOFSTADT

Vice-Prime Minister, Minister of Science Policy

It is with his warmest congratulations that the Minister of Science Policy of Belgium — who I have the honor to represent — wellcomes the participants at the Second World Congress on « Compounds in Biological and Chemical Warfare : Toxicological Evaluation. Industrial Chemical Disasters », organized these coming days at the State University of Ghent.

During this century, a fast scientific and technological evolution, especially in biology and chemistry, has produced large benefits for mankind : everday's life and work became much easier by the introduction of many new products and processes.

Moreover due to the progress in medicine and bio-sciences, many illnesses which ravaged before entire communities, cities and even countries, are today eliminated — at least in the industrial Western World, I would like to say unfortunately only in the Industrial World.

However this progress of sciences led also to the development and often to the introduction on the battlefield of new, ever more devastating weapons. Our country was the testing ground of this new generating of so called C-weapons. Indeed as it is well known, during the First World War, chemical weapons were used massively on the battlefields of the Flanders, only a few miles away from this city of Ghent.

This is, among others, one of the reasons why the Belgium community and its scientists are so sensitive to the consequences of the use of chemical and biological weapons, which potentially can be as destructive as nuclear weapons.

Apart its military use, the rapid development of the biological and chemical sciences and its ever growing applications introduces the risk of industrial accidents, such as the Bhopal disaster.

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Consequently, several important challenges are raised for the engineers and scientists working in these fields : improving strongly the security of the processes applied in the different production cycles, detecting as fast as possible any threat of disaster and developing optimal assistance to potential victims.

Finally, I would like to express the great concern of the Belgian government and particularly of the Minister of Science Policy about the drug problem — maybe the greatest element of destabilisation of our Western civilization.

Drug traffic, the control of it and the treatment of the intoxicated, mostly young people, is a difficult, but very important task of all our governments, and for the scientific community, which has to contribute in this modern war by providing to the authorities powerful techniques to detect narcotics and to treat its victims.

In these fields, the members of the International Association of Forensic Toxicologists have provided outstanding contributions and I want to express in name of the Minister of Science Policy to this outstanding Association and its president, Prof. McLinden, my sincere congratulations.

***

Dear participants, I am convinced that due to the excellent work and dedication of the Scientific Organizing Committee and the enthusiasm of its Cairman, Prof. Aubin Heyndrickx, the present meeting will be very fruitful to you, not only scientifically but also socially.

Thank you.

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Allocution by Mr. R. WYNAKKER

Alderman of the City of Ghent

Honourable Chairman,

Ladies and Gentlemen,

In behalf of the Mayor of the city and the Aldermen, I am extending to you our warmest welcome to come to the old city of Ghent, here in the premises of the city with such a historical past.

To Professor Heyndrickx and his staff who was organizing the 2nd World Congress on chemical and micro-biological warfare, and also the International Association of Forensic Toxicologists European meeting under the direction of Prof. McLinden ; the city of Ghent is very happy that you are here for the 2nd time on the World Congress and the 23rd time for the European Meeting of the International Association of Forensic Toxicologists. The last time you were here for the TIAFT Meeting it was to celebrate, also in this city, the 100 years of existance of the Department of Toxicology of our State University. Since then many things have happened in the world, in the Gulf Region and other parts, where the knowledge of scientists to make the truth known and at the same time through international efforts to prevent peace, one of the biggest achievements of mankind, the State University of Ghent participates in it. All of you have very big responsibilities in your own countries as experts, witnesses, international scientists, who are working all together in the field of toxicology, and at the same time do something for our society. Many chemical agents are used today in chemical warfare, many toxic compounds are made in industry where we have the examples of Bhopal in India, Icmesa the Seveso case in Italy, and in other parts of the world. It means that all of you have very important tasks in order to prevent what is happening, and at the same time take care of the population and see

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how we can protect them. It is an honour for the city of Ghent. The International Association of Forensic Toxicologists has not only the responsibility about those industrial chemicals, at the same time what worries us so much is preventing drug addiction. Many of the young people today are faced with heroine, cocaine, crack, with euthanasia. Many of those formulations did not exist years ago. But at the moment mainly our youth, the generation of tomorrow, has to face those big problems.

I hope that you will have the possibility to visit the historical land marks in the city. The Treaty of Ghent was signed here between the United States and England. Many treaties were signed here in this city hall during the Middle Ages, changing at that time the world. We hope that the efforts that all of you are making will bring us to more security in the world, giving guidance to our governments and the politicians, who have to take the responsibility in their respective country.

In the section of Terrorism, TIAFT members will discuss the problems of detection and analysis of explosives, air-port safety and control. A very important subject in safety and safeguarding our freedom. Toxicology is one of the most important fields in science today. If something is not toxic it can not be dangerous. But if some of those compounds are threatening human life and nature, then it is impossible to accept it. It is not easy to evaluate those risks because science can not always see in the future many years ahead to evaluate the possible chronic toxicity. Toxicology, also the field in medicine, in pharmacy and science, where we have to rely on in public health, and also transfer it to developing countries. Tropical diseases can not be cured without chemicals. Some of them still today are very toxic but they are the only ones that we have. It means that toxicology, one of the most important sciences today is not only of a big scientific value, but has a direct input in daily life. The city of Ghent welcomes you all, I am wishing to you a very fruitful World Congress and a very friendly TIAFT meeting.

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Address by Prof. Dr. H. Brandenberger

Past President of TIAFT

Your Excellencies from the honorary committee and Ambassadors,

Dear Rector of the State University of Ghent,

Dear Prof. Heyndrickx,

Honored members of the congress and guests.

Since the President of TIAFT, the honorable Dr. V. McLinden from Australia, has been taken ill, I will stand in for him with a few words of appreciation.

We all are grateful to the Rector and to the officials of the University of Ghent for receiving us again in this city with its long history, and in this University with its long list of impressive scientific accomplishments. We all have to thank Prof. Heyndrickx and his staff from the Laboratory of Toxicology for planning and organizing this congress. It is a joint venture, a get-together of forensic toxicologists (the members of TIAFT) with other scientists closely involved in the investigation of compounds used in biological and chemical warfare, in industry, as well as in the manufacture of explosives.

We toxicologists know from our contact with the drug scene that banning a compound does not exclude its application. We also know from our experience with pharmaceuticals, pesticides, solvents and metallic poisons that incorrect handling, human errors and human negligence are frequent and that they can cause great damage. Our distinguished TIAFT member Prof. Heyndrickx has always been a toxicologist with a broad outlook and with foresight. He has successively added to his field of research and to the applied investigations carried out by his laboratory many additional problems of national and international impor-

tance. When he organized, two years ago, the first world congress concerned with compounds in chemical and biological warfare, many toxicologists and TIAFT members attended the reunion. This week, we meet together officially, side by side. We all hope that the personal contact between scientists from different disciplines will be mutually inspiring and profitable.

Chemists have helped to create dangerous poisons and weap-one. We chemists and our colleagues from the related fields must work together to keep them under control.

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PART I

THE INTERNATIONAL ASSOCIATION OF FORENSIC TOXICOLOGISTS

23RD EUROPEAN INTERNATIONAL MEETING

TERRORISM : ANALYSIS AND DETECTION OF EXPLOSIVES

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General Topics

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Forensic toxicology
Western Australian experiences

by V.J. McLINDEN

Forensic Science Laboratory, Government Chemical Laboratories
125 Hay Street, Perth, Western Australia, 6000

In discussing forensic toxicology in Perth, Western Australia, it is proposed to raise queries for discussion on a number of general issues which have not received much attention at TIAFT meetings in recent years, such as,

— the scope of the toxicologist's report,

— the extent of investigation in positive and negative cases what constitutes the positive identification of a drug,

— relationship with forensic pathologists,

— specialist versus general laboratories etc.

In December 1985 the Forensic Science Laboratory in Perth completed its first ten years of existence in its own right. It is one of a complex of seven laboratories which constitute the Government Chemical Laboratories, sharing the same administration. Our laboratory could not be described as typical of an Australian forensic science laboratory because they all vary from State to State, both in the scope of their activities and their manner of administration. There is one specialist forensic toxicology laboratory in Australia whilst the others are contained in forensic science laboratories which are generally contained within a complex of other chemical laboratories.

Whilst specialist forensic science laboratories are the norm in some countries and we look enviously at, for example, the system in the UK with its central facilities for providing quality assurance programmes, staff training, information systems etc., there are certainly some advantages in being part of a chemical laboratory complex where we are able to call on expertise and equipment

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available under the same roof. We do, for example, make use of our Food and Industrial Hygiene Laboratory for assistance with analysis and advice concerning pesticides and herbicides when we have an overdose case on hand. This obviates the need to prepare our own GC columns and standards and tie up a gas chromatograph which normally is used for drug work. Similarly we call on the Water Science, Mineral Science and Agricultural Chemistry laboratories for access to atomic absorption, ICP equipment and so on as required. Probably the biggest disadvantage in being in such a complex is the sense of not being identified and the difficulty, when it comes to funding, of convincing the authorities of the priority of forensic toxicology within a Mines Department.

ISOLATION.

Perth is the world's most isolated capital city, its closest capital city being 2 200 kilometres away and is in the world's largest state. Our Forensic Science Laboratory serves a population of 1.5 million, about one-tenth of Australia's population, spread over an area of 2.5 million km^2. This isolation brings with it problems of delays in supply and service of equipment. Suppliers tend to carry very little equipment, spare parts or chemicals and generally draw on the supplies in the head offices in Melbourne or Sydney. Even there the reserves are held to a minimal level, particularly in the present climate which exists in Australia of very high interest rates. Our biggest traumas have been with the GC-MS when on several occasions it has been out of action for several weeks, due in part to delays in supplying parts from the USA. With no back-up GC-MS a huge backlog of work has accumulated over such periods.

In addition to the above problem, there is very little scientific instrumentation manufactured in Australia so that there is the inconvenience in the long delivery time after ordering new equipment, and prices for such equipment, for some inexplicable reason, are much higher in Australia than those in America even after taking into account shipping costs, exchange rates etc. Such problems would be far less severe in the more populated countries but these are the costs we pay for the considerable benefits of isolation.

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ORGANISATION.

The Forensic Science Laboratory in Perth has a staff of 16 including 12 chemists and four technical support staff and is concerned with the following activities :

Toxicology
Alcohol/drugs < drivers / fatalities
Illicit Drugs
Physical Evidence
Breathanalyzer
Prison Drug Offences
Doping Control in Sport

We are the only forensic science laboratory in Australia which is involved in doping control work but we find that our experiences in this field complement our toxicological work particularly in relation to the analysis of low dose drugs.

The forensic pathologists, as in most States in Australia, are separated from the toxicologists both geographically, departmentally and in some cases in our ideas on approaching toxicological problems. All of the forensic pathologists operating in Perth have originated from, graduated and practised in the UK. Their approaches, however, vary widely from a collaborative to an attempted master/servant approach and this is reflected in the type and quality of the exhibits received and the provision of relevant background details surrounding cases.

In comparing forensic science in Australia with the UK for example, the major difference is that the forensic services are the responsibility of each State Government. There is no uniformity in the approach to toxicological problems and consequently standards are uneven across the nation. Annual meetings of forensic toxicologists, which went some way towards overcoming these difficulties, went into recess several years ago when economic restrictions on travel highlighted the low priority held by the forensic sciences in the eyes of the Directors of the various State Laboratories.

PROPOSED NATIONAL FORENSIC RESEARCH INSTITUTE.

Over a period of more than ten years there have been a number of inquiries at federal level into the need for a centralized forensic science research centre. Each of the inquiries has found that the

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need exists and that forensic science laboratories in each State need increased funding. Despite the recommendations of these inquiries changes of Government, economic difficulties but more realistically the low priority of the forensic sciences in the eyes of Governments, have resulted in no progress in this area. It seems that nothing short of a crisis, which did happen in one State and cost that Government severely, is likely to lift the level of funding for the forensic sciences to a level adequate to cater for the community's needs.

In the meantime we endeavour to carry out our own quality assurance programmes. From time to time conscientious toxicologists in one State or another have offered their services by preparing and distributing samples in relation to collaborative studies. Unfortunately the response from the participating laboratories has not always matched the enthusiasm of the organizing laboratory. The answer to this indifference or lack of sense of priority in this matter seems to lie in the certification of laboratories by an approved body, certification being dependant on participation in the programmes organized by the approving body.

My desire to see TIAFT in the long term become involved in the organization of quality assurance programmes is undoubtedly influenced by my experience with the set up of unco-ordinated forensic laboratories, as they exist in Australia.

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TRAINING.

Formal training programmes for forensic toxicologists in Australia are virtually non-existent. Most toxicologists become so through experience and working in the field. Generally chemists have come to our laboratory with a science degree, maybe with honours, but with little or no experience and no training biased towards the forensic sciences. It is envisaged that the recruits of the future will probably have PhDs but still with no training in forensic science since there are no degree or post-graduate courses in forensic science in Australia.

With the enormous increases in all aspects of forensic science work in recent years and the ceilings placed on staff, we simply cannot afford the luxury of a long apprenticeship in the laboratory before the new recruit finds himself confronted with case work. Whilst every endeavour is made to shield these inexpe-

rienced chemists from cases likely to involve Court appearances, he will find himself involved, sooner than would be desired under ideal conditions.

DRUG TRENDS.

In 1985 the Laboratory handled about 440 cases of sudden death which were the result of police investigation. Not only has this number gradually increased over the years but so too has the percentage of positive cases, and the contribution from multiple drug cases. Table I shows the drugs or poisons encountered in 1985, relating to incidence only and not to toxicological significance.

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TABLE I

Drugs / poisons detected in toxicology cases 1985

Drug or poison	*No. of identifications*
Carbon monoxide	55
Morphine	41
Codeine	37
Oxazepam	24
Paracetamol	21
Diazepam	18
Desmethyldiazepam	16
Propoxyphene	12
Benzodiazepine metabolite*	12
Phenytoin	12
Carboxy THC	11
Amitriptyline	10
Nortriptyline	8
Trichloroethanol	8
Pentobarbitone	7
Salicylates	6
Carbamazepine	6
Verapamil	6
Doxepin	5
Lignocaine	5
Chlorbromazine	4
Dextromoramide	4
Various**	91

* Parent drug not identified.

** Amylobarbitone, chlorpheniramine, cyanide, flunitrazepam, lead, pethidine, phenobarbitone, propranolol, quinine, temazepam, thioridazine, trimethoprim (three each).
Benztropine, chloroquine, desipramine, diphenylpyraline, flurazepam, imipramine, metoprolol, nitrazepam, oxycodone, promethazine, quinalbarbitone, toluene, xylene (two each).
Acetone, chlordiazepoxide, dichlorvos, diclofenac, diflunisal, diphenhydramine, diethylpropion, dihydrocodeine, ephedrine, ethylfenthion, freon 11, haloperidol, metasystox, methadone, mianserin, norpethidine, orphenadrine, paraquat, procainamide, prochlorperazine, propane, pyrimethamine, quinidine, ritalinic acid, salbutamol, strychnine, thiopentone, trichlorphan, valproic acid (one each).

In each of the last ten years carbon monoxide has been the most frequent means of suicide in WA. In recent years, in line with world wide trends, heroin deaths have worked their way towards the top of the list. The increase in heroin deaths corresponds with the large increases in heroin drug seizures also examined by the Laboratory. The total number of different drugs or poisons encountered in 1985 was 75. Table II shows those encountered over the last ten years.

TABLE II

Number of different drugs / poisons detected in 1976-1985

Year	Number
1976	31
1977	51
1978	56
1979	63
1980	76
1981	60
1982	99
1983	78
1984	82
1985	75

Over this period there has been a total of about 130 drugs and 30 non-drug poisons encountered. From the list of these drugs and poisons a « feel » for the « common drugs » to be screened for on a routine basis is obtained. This becomes relevant in answering the query which confronts every toxicologist at some time or another of how far should be screen for drugs and poisons be extended in an « unknown » case. Using the above information we can report with assurance that no « common drugs » were detected on the understanding that « common drugs » refers to those which have been encountered previously in the last ten years or drugs closely related to them and should any of these drugs have been present then they would have been detected and reported.

Unless there is some additional information supplied by the pathologist which indicates the presence of a drug which requires a target analysis beyond the normal screening, then no further work is carried out. The understanding is that the onus is on the pathologist and the investigating officer to provide the additional information if additional work is required.

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TABLE III

Extract from a multiple drug case report

Exhibit : Blood (Lab No. 85G56)

Marks :

Result of examination :

Alcohol	0.107 percent
Trichloroethanol	Not detected
	mg/l
Amylobarbitone	33
Pentobarbitone	15
Carbamazepine	7
Codeine	0.8
Morphine	0.03
Dextropropoxyphene	< 0.1
Diethylpropion	< 0.1
Diazepam	0.5
Desmethyl diazepam	0.2
Oxazepam	< 0.1
Paracetamol	Not detected (<0.1)
Salicylic acid	5

Exhibit : Liver (Lab No. 85G59)

Marks : Liver 2.1.85

Result of examination :

	mg/kg
Amylobarbitone	195
Pentobarbitone	75
Carbamazepine	45
Codeine	4
Morphine	0.08
Dextropropoxyphene	0.2
Diethylpropion	0.1
Diazepam	6
Desmethyl diazepam	3
Oxazepam	< 0.1
Paracetamol	Not detected (< 0.1)
Salicylic acid	Detected

Exhibit : Stomach and contents (Lab No. 85G60)

Marks : Stomach and contents 2.1.85

Result of examination :

	mg (total)
Amylobarbitone	3100
Pentobarbitone	320
Carbamazepine	10
Codeine	< 1
Dextropropoxyphene	< 1
Diazepam	13
Diethylpropion	< 1

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MULTIPLE DRUG CASES.

The question of the extent of the investigation is not only pertinent in negative cases but also in positive cases where a drug cocktail has been used as a means of suicide. In one case last year, a pharmacist left nothing to chance and was responsible for a very complex report from the Laboratory. Overdose levels of amylobarbitone, pentobarbitone, carbamazepine and codeine were determined together with therapeutic levels of morphine, dextropropoxyphene, diethylpropion, diazepam, desmethyldiazepam, oxazepam and salicylic acid and a blood alcohol level of 0.107 % (table III).

With increasing pressure due to oppressive workloads, it is becoming more tempting to succumb to a procedure whereby, having found one drug present at fatal levels, no additional is proceeded with. Pathologists, however, are not generally happy with this approach. I would be interested to get feedback from toxicologists at this meeting on how they would approach cases such as this.

POSITIVE IDENTIFICATION.

Prior to the advent of the GC-MS, drugs were identified by a combination of TLC data on a number of systems, Kovat's Indices, UV spectra and if sufficient drug could be extracted, an IR spectrum. Although not the norm in Australia, GC-MS is used in our Laboratory routinely for toxicological work as the means of identification and is part of our screening and detection. Problems arise when the GC-MS is down because the new breed of toxicologists in our laboratory have rarely had recourse to IR as a means of identification and have hardly ever used the technique. The younger staff also would not appreciate the importance of UV in pre GC-MS days and the only time they use UV is for the determination of CO.

It is of interest to note that whereas in the past our more senior staff would have been confident in reporting the identity of a drug by the combination of the older procedures, chemists today are not confident in any identification which does not include a full EI mass spectrum, or at least SIM and regard this as the basis to a positive drug identification.

SCOPE OF THE REPORT.

Due to tradition and the lack of formal training in pharmacology of most of our toxicologists, it was the practice in the past to report only the results of analysis. Indeed, at least one forensic pathologist was very much opposed to the toxicologist expressing any comment on the drug levels on the basis that it was only the pathologist who could interpret the levels with his knowledge of all the facts, such as the histopathology and the post-mortem examination and the circumstances surrounding the death.

Our current policy is to quote published literature levels and in particular Osselton's collation in the TIAFT Bulletin and report that the levels are consistent with reported literature levels for a therapeutic, toxic or fatal dose or overdose. In addition we are now starting to report from our own experiences since the data on some drugs is now mounting to a considerable level. The local coroner has expressed enthusiasm for the increased involvement of the toxicologist in this matter over the last few years.

CONCLUSION.

In this paper I have attempted to give a brief resume of the activities relating to forensic toxicology in our Laboratory raising a few issues which I hope this meeting will take up for discussion.

APPENDIX I

Extract from a laboratory report indicating reference to local and published data

Comments :

The blood and liver dextromoramide concentrations are consistent with a fatal overdose.

Fatal blood dextromoramide concentration (1)		0.2	mg/l
Fatal blood dextromoramide	range (2)	0.1 to 6	mg/l
	mean	1.07	mg/l
Fatal liver dextromoramide	range (2)	0.1 to 14	mg/kg
	mean	2.05	mg/kg

References :

1. OSSELTON M.D. TIAFT, February 1983, 17 (1).
2. Thirteen Case Studies of Dextromoramide Deaths Associated with Drug Addicts and Palfium Overdoses. Reported : Government Chemical Laboratories, Forensic Science Laboratory, 1976-1985.

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APPENDIX II

Principal causes of death in drug / poison overdoses - 1985

	As a single drug overdose	*Cases involving multiple drug abuse*	*Total*		*Total number of detections in 1985*
Carbon monoxide . .	39	16	55		55
Morphine (from heroin) .	10	7	17	out of	41
Morphine (from morphine)	0	2	2		41
Codeine	0	8	8		37
Trichloroethanol . . .	2	5	7		8
Paracetamol . . .	0	6	6		21
Pentobarbitone . . .	3	2	5		7
Propoxyphene . . .	1	4	5		12
Dextromoramide . . .	1	3	4		4
Verapamil	2	2	4		6
Amylobarbitone . . .	0	3	3		3
Cyanide	3	0	3		3
Doxepin	1	2	3		5
Propranolol	1	2	3		3
Chlorpromazine . . .	0	2	2		4
Freon	2	0	2		2
Quinalbarbitone . . .	0	2	2		2
Salicylic acid . . .	0	2	2		6

One overdose from single drug overdose : ethylfenthion (1), metasystox (1), orphenadrine (1), pethidine (3), phenytoin (11), strychnine (1).

One overdose from multiple drug abuse : amitriptyline (10), carbamazepine (6), disipramine (2), dichlorvos (1), diflunisal (1), dihydrocodeine (1), methadone (1), oxycodone (2), paraquat (1), petrol (1), ritalinic acid (1) and trichlorphon (1).

Brackets () denote total number of detections in 1985.

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First aid treatment as an important source of drugs in serum and urine of traffic victims*

by E. HAUSMANN [1], M.R. MOELLER [2] and D. OTTE [3]

SUMMARY.

Serum and urine samples of 634 drivers injured in traffic accidents were obtained from 6 clinics located in the Saarland and the Hannover area. The samples were screened by radioimmunoassay, thin layer chromatography, gas chromatography and mass spectrometry for about 100 drugs suspected to impair driving performance. Additionally, all patients were interviewed for drug use prior to their injury. — Toxicological analysis indicated in 188 patients the presence of drugs. Benzodiazepines, barbiturates, and strong analgetics prevailed to an unexpected extent. On closer inspection, most of the barbiturates were identified as thiopental, and most of the benzodiazepines could be traced back to diazepam. As thiopental is available in Germany for intravenous injection only, the suspicion arose that thiopental, benzodiazepines, or strong analgetics were given to most of the drug-positive patients in the course of first-aid treatment. No confirmation of this suspicion could be obtained on checking with the hospitals. However, it became clear from the evaluation of the data-sheets completed in the ambulance cars or helicopters that indeed the injection of thiopental, diazepam and strong analgesics is common practice and has to be considered as the main source for the occurrence of these drugs in urine and serum samples of many traffic victims.

* This study was supported by the Bundesanstalt für Strassenwesen, Bergisch-Gladbach, FRG, and the National Institute of Drug Abuse, Rockville, Maryland, USA.

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Postmortem changes in drug levels A common phenomenon ?

by Graham R. JONES

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INTRODUCTION.

It is often assumed that drug levels in blood drawn at autopsy approximately reflect the levels present at the moment of death. While that is often true, there is growing evidence that blood levels of some drugs can increase substantially post-mortem. Several years ago, Vorpahl and Coe (1) noted that heart blood levels of digoxin tended to increase after death. Furthermore, Rouzioux and co-workers (2, 3) observed that blood levels of several drugs, and in particular imipramine, increased substantially after death, compared with levels measured in blood drawn just before death. More extensive supporting data has been presented recently (4-7) indicating that (i) blood levels of many drugs increase after death and that (ii) the post-mortem concentrations of many drugs are site dependent, with higher concentrations normally found in abdominal/thoracic blood (eg. cardiac) and lower levels found in peripheral blood (eg. femoral).

Further data in support of these findings is presented here and possible explanations for the phenomenon of post-mortem change discussed.

METHODS.

Case material.

All presented in this paper came under the jurisdiction of the Alberta Office of the Chief Medical Examiner. If a person had been hospitalized prior to death, antemortem blood or serum specimens were obtained. Post-mortem specimens were obtained

at autopsy or during external examination of the body. Femoral blood specimens were drawn from the groin area without tying off the vessel and are presumed to be venous. « Cardiac » blood samples were obtained as part of the standard autopsy procedure. The pericardial sac was opened, the heart lifted, the major vessels cut (eg. vena cava, aorta, pulmonary vein and artery) and the resulting pooled blood samples.

Drug analyses.

The antidepressants (amitriptyline, nortriptyline, desipramine, trimipramine and maprotiline), diphenhydramine and propranolol were analyzed by gas chromatography (GC) with nitrogen selective detection (NPD) using either a packed column (3 % SP-2250 DB, Supelco) or fused silica capillary column (cross-linked SE-54, Hewlett Packard). Benzodiazepines (diazepam, nordiazepam, desalkylflurazepam, triazolam) were quantitated by GC with electron capture detection (EC) using a 3 % SP-2250 DB column. Pentobarbital and primidone were analyzed by reverse phase (C_{18}) high performance liquid chromatography (HPLC) with UV detection. Ethanol was measured by headspace GC with flame ionization detection (FID) using a 5 % Carbowax 20M on Carbopack B column (Supelco).

For the sake of brevity, precise analytical details are not given here. However, all drugs were extracted with an appropriate solvent, after the addition of structurally related drugs as internal standards and adjustment of the pH. Acidic back-extractions were employed where possible, to separate basic and neutral substances. The secondary amine antidepressants and propranolol were chromatographed as trifluoroacetyl (TFA) derivatives.

All calibrations were performed with a blank and a minimum of five standards. Where available, a commercial control was run. If this was not possible, an in-house control was independently prepared in out-dated Red Cross blood using a different stock standard. All specimens were run in duplicate.

RESULTS AND DISCUSSION.

Two groups of cases are presented. Five cases involve drug overdoses (table I) and in four other cases death was attributed to non-drug causes but drugs were detected at therapeutic levels

TABLE I

Drug and metabolite concentrations in antemortem and postmortem specimens from five drug overdose cases

	Drug/ Metabolite	Ante-mortem (1) Se or Bl	Postmortem blood		Liver	Post-mortem (2) Interval
			Femoral	« Cardiac »		
Case 1 :	Pentobarbital	—	7.5	20.5	112	+ 26 h
	Triazolam (3)	—	29	82	1220	
	Ethanol (3)	—	2.6	2.7	—	
Case 2 :	Trimipramine	—	3.6	16.5	—	> 48 h
	Nortrimipramine	—	1.1	4.1	—	
	Nortriptyline	—	1.0	2.9	—	
	Nordiazepam	—	2.3	3.5	—	
	Desalkylflurazepam	—	0.28	0.42	—	
Case 3 :	Desipramine	0.47 (Se)	—	10.9	370	+ 18 h
	Primidone	31 (Se)	—	30	32	
Case 4 :	Amitriptyline	2.8 (Bl)	—	10.0, 7.5 (4)	200	+ 3.5 h
	Nortriptyline	0.65 (Bl)	—	3.1, 2.8	83	
Case 5 :	Propranolol	5.7 (Bl)	8.2	19.8	93	+ 6 h

(1) All antemortem specimens were drawn within one hour prior to death. Se = serum, Bl = blood, « — » = not available or not analyzed.

(2) The interval between the estimated time of death and autopsy.

(3) Triazolam expressed as µg/l or µg/kg and ethanol as g/l. All other concentrations are in mg/l or mg/kg.

(4) Determinations of two separate tubes of « cardiac » blood.

(table II). Death in the 10th case was attributed to morphine intoxication, but the other drugs were present at therapeutic levels (table II).

Case 1 is typical of our findings in overdose cases where « peripheral » and cardiac blood is available. Cardiac blood levels of pentobarbital and triazolam are nearly three times the femoral blood levels, with extremely high levels of each drug in the liver. The body was not decomposed. The similar levels of ethanol in both blood samples indicates that the femoral blood has not been contaminated or diluted with another fluid.

Case 2 involved a multiple drug overdose where the body was not discovered for 1-2 days and decomposition was moderately advanced. Trimipramine cardiac blood levels were 4-5 times the femoral blood levels whereas the benzodiazepine levels differed by less than 2-fold. Subclavian blood was also drawn and had intermediate drug levels (trimipramine 7.7 mg/l, nortrimipramine 1.4 mg/l, nortriptyline 1.2 mg/l, nordiazepam 2.3 mg/l and desalkylflurazepam 0.28 mg/l). The nordiazepam resulted from

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TABLE II

Drug and metabolite concentrations in antemortem and postmortem specimens from cases involving therapeutic dosage

	Drug/ Metabolite	Ante-mortem (1) Serum	Postmortem blood		Liver	Post-mortem (2) Interval
			Femoral	« Cardiac »		
Case 6 :	Amitriptyline	0.23	—	1.1	9.3	+ 31 h
	Nortriptyline	0.17	—	0.70	7.6	
	History : Hospitalized for 3 days with anorexia. Developed a severe electrolyte imbalance and died. She was originally prescribed amitriptyline but had not received any for at least 24 hours previously.					
Case 7 :	Diphenhydramine	0.17	0.85	—	—	+ 18h
	Diazepam	0.11	0.11	—	—	
	Nordiazepam	0.60	0.68	—	—	
	Ethanol (3)	2.7	2.7	—	—	
	History : Suicidal gunshot wound. No evidence of drug overdose.					
Case 8 :	Maprotiline	—	0.08	0.31	3.0	+ 12 h
	Nortriptyline	—	0.37	1.2	5.6	
	History : Sudden collapse at work, of a 49 year old man with severe coronary heart disease.					
Case 9 :	Amitriptyline	—	1.1	1.2	22	+ 30 h
	Nortriptyline	—	0.43	0.43	11	
	History : 55 year old man found dead at home. No evidence of a drug overdose. There was a history of chronic alcoholism and diabetes.					
Case 10 :	Amitriptyline	—	0.96	0.42	6.5	> 24 h
	Nortriptyline	—	0.53	0.23	4.8	
	Morphine (unconj.)	—	0.30	0.41	1.2	
	Nordiazepam	—	1.8	1.5	5.5	
	History : 43 year old man found dead at home. Suffered chronic back pain and depression following a serious motor vehicle accident. He was prescribed quite large doses of morphine. Death was presumed to be due to morphine intoxication. Amitriptyline levels are consistent with therapeutic doses.					

(1) All antemortem specimens were drawn within one hour prior to death, except for case 6 where it was drawn three hours prior to death.

(2) The interval between the estimated time of death and autopsy.

(3) Ethanol concentrations are expressed as g/l ; all other levels are in mg/l or mg/kg.

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clorazepate (« Tranxene ») ingestion and the desalkylflurazepam from flurazepam (« Dalmane »).

Case 3 is particularly interesting because it involves an overdose of two drugs — one of which is markedly subject to postmortem redistribution (desipramine, with a 20-fold increase in this case) and one which has not been subject to redistribution (primidone). There were negligible levels of phenobarbital (primidone metabolite). Serum separated from the post-mortem cardiac blood had a desipramine level of 7.4 mg/l. This indicates that desipramine concentrates in red cells to a limited extent.

However, this effect is not important enough to explain the massive post-mortem increase shown in table I.

Post-mortem increases of 3-4 fold are also evident in cases 4 and 5 (amitriptyline, nortriptyline and propranolol). The results on case 4 demonstrate one of the problems encountered in post-mortem toxicology. That is, post-mortem blood is not homogeneous. Two apparently identical tubes of blood were received, taken from a « cardiac » site, but each had different levels of amitriptyline and nortriptyline.

It might be argued that some of the increase evident from the post-mortem values might be due to continued absorption before death, even though most of the antemortem specimens were drawn from the overdose patients no longer than 1 hour before death. However, the difference in ante-mortem and post-mortem blood levels in cases 3 and 4 is so large that true post-mortem redistribution is probably a major factor.

This hypothesis is supported by data from cases where drugs have been taken at therapeutic doses and death was attributed to other causes (table II). For example in case 6, involving a young woman with anorexia, amitriptyline had been prescribed for depression, but was not given for at least 24 hours prior to death. However, cardiac blood levels of amitriptyline and nortriptyline were 4-5 times greater than the corresponding levels in serum drawn 3 hours before death. Amitriptyline was in the elimination phase and therefore the antemortem-postmortem difference in blood levels cannot be attributed to continued drug absorption.

In case 7, the person died from a suicidal gunshot wound. The levels of ethanol are obviously toxic (especially in combination with the other drugs) but there was no evidence of a drug overdose. Yet the diphenhydramine level in the postmortem blood was 5 times the antemortem serum level. This is in contrast to the nearly equal antemortem and postmortem levels of diazepam, nordiazepam and ethanol.

It is our experience that where site dependent differences exist, cardiac blood levels of drugs are invariably higher than femoral blood level ; subclavian blood levels are generally intermediate in this respect. An example is given in case 8 where cardiac blood levels of maprotiline and nortriptyline are 4 times greater than femoral blood levels. However, such differences are not

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always seen (eg. case 9). Indeed post-mortem femoral blood levels of tricyclic antidepressants may sometimes be greater than the cardiac blood level (eg. case 10), although this is unusual. In case 10 the post-mortem amitriptyline levels in the blood and liver are consistent with therapeutic doses, even though the unconjugated and total morphine levels are high (total morphine cardiac blood level was 1.7 mg/l).

Our laboratory has now collected data on over 50 cases where antemortem blood and/or blood from two or more sites was collected. Even so, it is difficult to make firm rules regarding the predictability of post-mortem changes in drug levels. One major problem is the nature of post-mortem blood itself — at the same time a « continuous » but a non-homogeneous fluid which cannot be precisely sampled. For example, unless very careful dissections are made and appropriate blood vessels isolated by tying-off, syringing blood from one of the femoral veins is likely to draw blood down from the iliac vein and inferior vena cava (IVC), ultimately obtaining « liver blood » with falsely elevated drug levels. The same caution applies to sampling blood from the subclavian veins. If too much blood is taken, it will be drawn up from the superior vena cava and eventually from the IVC and the liver. It is also probable that some « mixing » of blood occurs prior to autopsy due to the process of rigor mortis and perhaps gas production. It will certainly occur as the body is moved during transportation to the morgue, during placement on the autopsy table and during the external examination.

The phenomenon of post-mortem change or redistribution seems to apply mostly to drugs which are highly protein bound, have a large volume of distribution and which tend to concentrate in the liver and lungs (eg. tricyclic antidepressants). Consequently, one of the major mechanisms which might account for post-mortem increases in blood levels of drugs is their release from major organs such as the liver and lungs, due to tissue autolysis. Site dependent differences in drug concentrations in blood have been noted for other drugs such as benzodiazepines and barbiturates in overdose situations. However here, incomplete distribution prior to death is probably a significant factor. In these circumstances, lower peripheral venous blood levels of drugs, than cardiac or IVC levels, would be expected because of drug deposition in peripheral tissues (eg. muscle, fat) as the blood circulates.

CONCLUSIONS.

Blood levels of many drugs will increase after death and may be site-dependent. Furthermore, the magnitude of post-mortem change is likely to increase with lengthening post-mortem interval, be greater when blood is drawn from cardiac, thoracic or abdominal vessels than from a « peripheral » site (eg. femoral vein) and is likely to be greater in overdose situations than after therapeutic doses. However, post-mortem change is probably more important in « therapeutic » or « prescription misuse » situations than in suicidal overdose cases (where the levels are usually very high, wherever they are sampled from). Postmortem femoral blood levels of drugs do not necessarily reflect the levels that were present in blood at the moment of death.

It is suggested that small (2-10 ml) volumes of femoral vein blood are routinely obtained and saved for quantitative work only. Larger volumes of « cardiac » blood may then be used for routine screening and preliminary drug estimation. One or more tissues (eg. liver, brain) should also be collected for quantitative drug analysis.

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Why should human toxicologists perform veterinary toxicological analysis?

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SUMMARY.

During the last few years, besides our normal case work in clinical and forensic toxicology our laboratory has been requested to investigate some 209 cases of suspected poisoned animals (mostly dogs, cats, but also cattle, birds, ducks, etc., as well as poisoned food and baits). In 27.3 % of these cases we were able to detect poisoning by our analytical screening methods used. A summary of the analytical methodology used is outlined, and the results of some 670 analyzed samples are discussed. It may seem at a first sight, that this may be a waste of our precious lab-time, but at a closer look, it must be said that these investigations are very useful for our other work. We feel that these exercises constitute an excellent training for intoxications rarely seen (or discovered) in man. On the other hand, the fact that there are quite a lot of criminal poisoning in animals, must give us some concern about the criminal poisoning in man.

INTRODUCTION.

It is well known that in human toxicology most acute poisoning cases are of suicidal (adults) or accidental (children) origin (1). Chronic intoxications from workplace or environmental exposure are also observed. But criminal poisoning of humans by humans are nowadays rarely seen in toxicology laboratories. This is not the case in veterinary toxicology, where we do still have quite a lot of criminal poisoning of animals by man. Cases of accidental poisoning may also be encountered, but not quite so fre-

quent as intentional poisoning. An other difference with human toxicology is that no or only little reliable background information on the circumstances of poisoning is available to the toxicologist. Some good books and review articles, but without great emphasis on the analytical methodology used have been published in the past (2, 3, 4, 5, 6).

MATERIAL AND METHODS.

The veterinary analytical toxicology covers specimens coming from different categories of animals such as domestic animals (dogs, cats), animals from the farmstock (cattle, sheep, ducks, pigeons, horses, etc.) animals from wildlife (birds, wild boars) and animals from the zoo (lions, llamas, dolphins, etc.). The autopsies of these animals are generally performed by the veterinarians of the Government Veterinary Laboratory in Luxembourg. After exclusion of common diseases, the organs or the other items are sent to our institution for toxicological investigation.

In most cases we screen for poisons in the gastric content. But other samples like liver, blood, urine, baits, etc., may also be examined. Liver usely serves for quantitative analysis, as blood is not always available. Routine screening in most circumstances includes : chlorates, cyanides, paraquat, phosphides, heavy metals, chlorinated solvents, aldehydes, anticoagulants, normal drugs encountered in human toxicology, alcaloids and organophosphorus pesticides. On request screening tests for other substances like ammonia, terpenes, chlorinated pesticides, different herbicides, etc., can also be performed.

The analytical methods are roughly the same, than those used in human toxicology (7, 8, 9, 10). The analytical methods used in this particular study can be summarized as follows :

The volatile poisons (solvents, aldehydes) are isolated by steam distillation using a Clevenger device (continuous solvent extraction during steam distillation) and preliminarily identified by Fujiwara or/and Schiff reaction. Confirmation is made by gas chromatography using the head-space technique.

Non volatile drugs are isolated by solid-liquid extraction using commercially prepared diatomaceous earth columns for clean up (11). Classical spot tests are performed either on filtrated aqueous extracts (diphenylamine, dithionite, Schiff reagents for chlorates, paraquat and aldehydes respectively) or by blowing

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nitrogen through the acidified sample and a Dräger test tube (phosphides, cyanides).

Heavy metals (in acute poisoning) are detected by X-ray fluorescence spectroscopy and quantified by atomic absorption spectroscopy*.

The drugs and basic compounds are screened by thin layer chromatography (THC) using the classical developing systems. Detection is made either by different colour reactions or by UV-spectrum registration of the spots on the TLC plates with a densitometer with data processing for the anticoagulant drugs for instance.

The organophosphorus pesticides are examined by TLC and visualized by the 4-(4-nitrobenzyl) pyridine reagent. Quantitative work or confirmation is done by gas chromatography with NPD detector or by gas chromatography coupled to mass spectrometry (12).

In some cases other methods like high performance liquid chromatography, flame photometry, etc. may be necessary.

RESULTS.

During the last 8 years (1978-1985) we were asked to investigate 209 cases of suspected poisoning (this represents some 607 samples) in animals. In 57 cases (27.3 %) we did find a poison. The most commonly used poison was strychnine (11×) clearly only in criminal poisoning of domestic animals.

Parathion (10×) and other organophosphorus pesticides (5×) were found as well in criminal as in accidental poisoning. Sodium chlorate (4×), thallium salts (3×) and zinc phosphide (3×) were also used only in intentional intoxications.

An acute lead poisoning was observed in 4 calves which licked the doors of the cattle-shed painted with 2.2 % lead containing paint.

The Pb-content of the liver was :	75 mg Pb/kg (wet weight)
	34 mg Pb/kg
	13 mg Pb/kg
	38 mg Pb/kg
in the controls :	< 0,1 mg Pb/kg

* The author likes to thank Dr. N. Kirsch and his team from the Government Environmental Laboratory for performing the different a.a.s.-analysis.

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A study on cattle killed by environmental molybdenum pollution was published elsewhere (13). Many other elements of the periodic chart of elements were also found in these cases.

DISCUSSION AND CONCLUSION.

From this study it is clearly seen that with only slightly modified analytical methods, it is possible to perform veterinary toxicology. The single items from an exhibit can be stored in the deep-freezer until enough samples are available to run a series, besides the normal forensic and clinical case work. The toxic compounds seen in veterinary toxicology are more rarely seen in man nowadays and thus this constitutes a good training possibility for human cases.

It constitutes also a warning, that criminal poisoning is easily used to kill animals, and it is likely that those persons do probably not hesitate to kill also human beings in the same way.

A question mark remains : are all fatal human intoxications discovered by the physicians or the police ?

It is quite astonishing that today with all the available poisons, we only rarely see homicides by intoxication in our case work.

I am convinced, that from this point of view, this investigation in veterinary toxicology is by no mean a waste of time and money.

***

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Piracetam in drug abuse

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SUMMARY.

Over a year ago, a substance which turned out to be piracetam was detected in samples of street drug heroin. The identification of this substance was carried out with application of techniques of Gas Chromatography, Mass Spectrometry, I.R. and U.V. Spectrophotometry, and Nuclear Magnetic Resonance.

Since then, piracetam has been found in a high percentage of samples of street drugs.

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In a consignment of street drugs, a substance which was not identifiable as an active product or additive usually found in this type of sample, was detected by gas chromatography. A thorough study by means of U.V. spectrophotometry, chromatography and mass spectrometry and afterwards by spectrophotometry I.R. and nuclear magnetic resonance, permitted the identification of the product as piracetam.

Piracetam (2-oxo-1-pyrrolidine-acetamide), synthesized by Morren in 1966, is a stimulant of the central nervous system. It is affirmed that it acts as a specific activator of the cellular metabolism of the neurones of the cerebral cortex, for which it is employed in the treatment of acute and severe syndromes due to dismetabolism or suffering of the corticoidal neurones of different aetiologies, whether from toxic causes (carbon monoxide, drug addiction, alcoholism, etc.), hypoxia (a consequence of vascular accidents) traumatic (concussion) or metabolic (involute syndromes).

It is accepted that its acute toxicity is low, as the i.v. LD_{50} in the mouse is over 10,000 mg/kg.

In the first half of 1986, we identified piracetam in 38 (**27.14 %**) of 140 samples of street drugs (table I), and although in some cases (1.43 %) piracetam was the only component, in general the proportion of this product in the sample ranged between 20 and 30 %. We have observed that piracetam is added both to heroin and caffeine and to cocaine and local anaesthetics ; the latter are frequently added to cocaine as a fraudulent additive, which produces anaesthesia in the taste buds in tests on the quality of the drug.

TABLE I

Piracetam in street drugs

1986	N = 140
Pireacetam present in	27.14 %

	Samples %
Pure piracetam	1.43
Piracetam + caffeine	21.42
Piracetam + heroin	20.71
Piracetam + mam	20.71
Piracetam + cocaine	7.85
Piracetam + procaine	7.14

TABLE II

Pirecetam in blood samples

1986	N = 184
Piracetam	N = 1
Piracetam + cocaine	N = 1
Piracetam + procaine	N = 1

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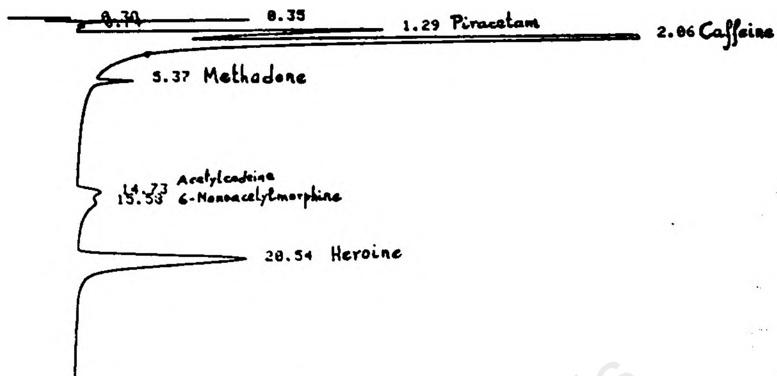
As for the biological samples analysed (n = 184), in only three blood samples (table II) was piracetam found, in one of them as the only product, in another together with cocaine (as benzoilecgonine) and in the one remaining with procaine.

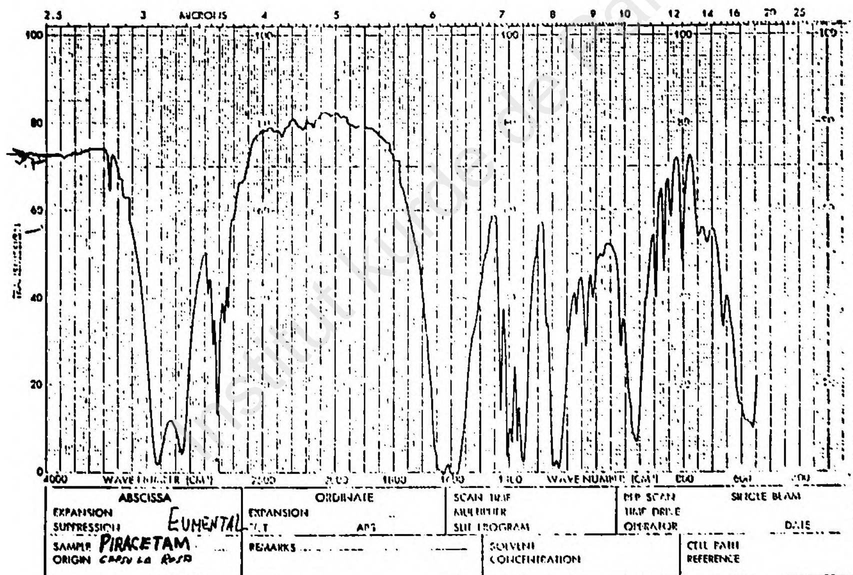
The analytical method used consists in dissolving the solid sample in methanol, or extraction from blood serum, adjusted to pH 9.2 with phosphate buffer, by means of methylene chloride.

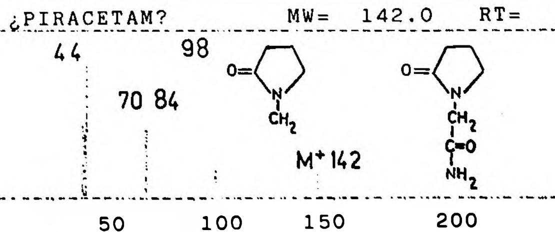
Two Hewlett-Packard chromatographs with the following characteristics and operating conditions, were used :

A) H-P Instrument, 5710 A.

— Column OV-1 2 % over Chromosorb G HP 100/120 : length 2 m, I.D. ¼".
— Column Temperature : 220°C.
— Temperature, Injector and Detector Blocks : 250°C.
— Carrier Gas : He ; 45 ml/m.
— Detector : N-P.
— Retention Time : 1.07 m.







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B) H-P Instrument, 5890.

- Column HP-1 Methyl-silicone 530 u, length 10 m.
  Programmed Temperature.
- Initial Temp.: 150°C — Initial Time: 2 min.
- Ramp: 10°C/min.
- Final Temp.: 250°C.
- Final Time: 5 min.
- Detector: N-P — Temp. Det.: 250°C.
- Injector Temp.: 250°C.
- Carrier Gas: He; 15 ml/m.
- Retention Time: 1.70 m.

The I.R. and mass spectra were carried out with samples of the greatest purity. In the first case a Perkin-Elmer instrument model 1420 was used, and in the second a Hewlett-Packard GC-MS system, model 5995 B.

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N-Acetylation phenotype of patients with bladder cancer

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INTRODUCTION.

Most cancers are associated to some degree with environmental factors. It is clear, perhaps to a greater extent than with other epithelial cancers, that the occurrence of urinary bladder cancer is the result of chemical carcinogenic hazard (1). Since the initial clinical observations of bladder cancer cases in the German chemical-dye industry in 1895 (2), Huper and associates found that 2-aminonaphthalane could cause bladder cancer in dogs reproducing the lesions seen in man (3). So far many epidemiological investigations show that a significant portion of the incidence of human bladder cancer can be associated with exposure to various arylamines, many of which represent established human arylamines, many of which represent established human carcinogenes (4, 5, 6, 7).

Some arylamines are acetylated by N-Acetyltransferase in both man and animals (8, 9). N-Acetylation has come to be regarded as a detoxification of N-substituted aryl compounds in human bladder cancer (10, 11, 12). In man N-acetylation is under polymorphic genetic control with the rapid acetylation phenotype dominant (13, 14). These considerations have led to the suggestion that two human acetylator phenotypes may differ in their predisposition to develop neoplastic disorders which are believed to be caused by arylamines. Many studies support this hypothesis (11, 15, 16).

The present study was undertaken to investigate the relation between acetylator phenotype and bladder cancer in Turkey.

PATIENTS AND METHODS.

Twenty-three patients with biopsy-proved traditional cell bladder cancer who were not undergoing chemotherapy were studied after physician consent in the Department of Urology. Faculty of Medicine, Ankara University. Their ages ranged from 45 to 70 years and there were six females and 17 males. A total of 109 healthy adult volunteer subjects. Fifty-seven men and 52 women, between the ages of 18 and 52 years, were our control group. The control group was held large to determine the acetylation profile in the Turkish population. All our subjects were Turkish by parentage and birth.

Samples of plasma were obtained from each individual before ingestion of the drug and 6 h after ingestion by venepuncture. Dosage was 40 mg of sulphamethazine kg (17). Samples were put in to Vacutainer tubes containing ethylenediaminetetra-acetic acid (EDTA). Samples were stored frozen at —20°C until assays were carried out. Analysis of unacetylated and total (unacetylated plus acetylated) sulphamethazine were measured by the modified Bratton Marshall procedure (18) as follows ; 0.5 ml of plasma was added to a mixture of 5 ml of distilled water and 1.66 ml of 20 % (w/v) trichloracetic acid, mixed and allowed to stand for 5 min. at room temperature. This was followed by centrifugation at 1500 g for 5 min. Two 1.5 ml portions were taken from the supernatant. One portion was used for determination of sulphamethazine, the other was used for determination of total sulphamethazine by adding 0.2 ml 4N-HCl to each tube and heating in boiling water for 1 h. After this step the procedure was identical for both determinations.

Freshly prepared 0.1 % sodium nitrite (0.2 ml) was added to each tube and mixed and allowed to stand for 3 min. and 0.2 ml of 0.5 % ammonium sulphamate was added, mixed and allowed to stand for 3 min. One millilitre of 0.05 % N-ethylenediamine dihydrochloride was then added and allowed to stand for 10 min. for colour development. Absorbance readings were determined at 540 nm in a Shimadzu Spectrophotometer. If blank blood samples showed any interference from endogenous substances, they were disregarded. The percentage of sulphamethazine (SMZ) was calculated as :

$$\% \text{ SMZ} = \frac{\text{total SMZ-free SMZ}}{\text{total SMZ}} \times 100$$

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Most efficient percentages for distinguishing between rapid and slow acetylators of sulphamethazine were taken as 40 % (17, 19). A value of 40 % and higher acetylated in the plasma denoted that the patient was a rapid acetylator and those lower denoted slow inactivators.

Results.

N-Acetyltransferase phenotype of the patients with bladder cancer and of control subjects are shown in table I.

TABLE I

Acetylation phenotype distribution between patients with bladder cancer and control subjects. (Means ± SD)

Group	*n*	*% acetylsulphamethazine in blood*	*% acetylator phenotypes*
Patients :			
— Slow	9	31.8 ± 1.8	39.1
— Rapid	14	65.5 ± 3.4	60.9
Control subjects :			
— Slow	67	18.9 ± 0.8	61.5
— Rapid	42	70.4 ± 1.7	38.5

NS = not significant.

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Among 23 patients with bladder cancer 39 % were slow acetylators and 61 % were rapid acetylators. The distribution in 109 control subjects was 62 % slow and 39 % rapid. The difference between patients and control subjects with regard to acetylator phenotype was not statistically significant ($P > 0.05$, $X^2 = 3.743$). The former group tended to have more fast acetylators than slow phenotypes.

DISCUSSION.

The results in the present study differ from those in studies published previously (15, 16, 20) which found a larger percentage of slow acetylator phenotypes among patients with bladder cancer than among control subjects. In our study, there, actually, was a greater percentage of rapid acetylator phenotypes in the patients with bladder cancer than in the control subjects, al-

though the number in the patient group is small. An association is remarked in studies involving chemical workers in the dye-manufacturing industry and slow acetylator phenotype. In this study, we could not examine this association, since none of the patients had occupations which exposed them to carcinogenic chemicals.

However, our results are in accordance with those of Miller and associates, who studied the acetylator phenotype in 26 cases of bladder cancer from New York using sulphamethazine. They found that 12 of the patients with bladder cancer had the slow acetylator phenotype compared with 18 of the 26 control subjects (21). Furthermore, isoniazid was used instead of sulphamethazine to study the acetylator phenotype in 30 patients with bladder cancer and in 27 control subjects ; 59 % of the control subjects and 70 % of patients with bladder cancer were phenotypically slow acetylators, the difference of which was not significant (22).

We therefore conclude that in our population N-acetylation is not a major determinant of bladder cancer. Further investigations are warranted with particular emphasis on occupational exposure.

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Drugs of Abuse and Doping Analysis

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Gas chromatographic determination of codeine in whole blood

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INTRODUCTION.

In forensic toxicology a sensitive assay for codeine in whole blood is often needed.

This work describes a gas chromatographic method for codeine quantitation and verification using derivatization, capillary column and a nitrogen selective detector.

EXPERIMENTAL.

Instrumentation.

Apparatus : Hewlett Packard 5890 gas chromatograph equipped with a nitrogen phosphorus sensitive detector.

Column : 12.5 m crosslinked methyl silicone fused silica capillary column, i.d. 0.22 mm, film thickness 0.33 μm (Hewlett Packard).

Gas : Carrier gas : helium 1.4 ml/min. ; makeup gas : helium 28.5 ml/min.

Temperatures : Injector 250°C, detector 300°C, oven 120°C for 1.5 min., 120-240°C at 40°C/min., 240-260°C at 5°C/min., 260-300°C at 30°C/min.

Injection : 1 μl injected. Splitless injection, 1 min. delay before split opening.

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Reagents.

Codeine phosphate was obtained from Norwegian Medical Depot, Oslo. O-methyl nalorphine was a generous gift from Weider Pharmaceuticals, Oslo. The derivatization reagents used were pentafluoropropionic anhydride (PFPA) and bis(trimethylsilyl)-trifluoroacetamide (BSTFA) in 1 ml ampoules from Supelco, Bellefonte, USA. All other chemicals were of analytical grade. The glass tubes used for extraction and derivatization had been silanized with dimethylchlorosilane (Pierce, Rockford, USA).

Blood.

Blood was drawn form healthy, drug-free volunteers into glass flasks containing sodium fluoride.

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Standards.

Stock solutions of codeine and methyl nalorphine were prepared in methanol ; working solutions were prepared by diluting stock solutions with water. Blood standards were prepared from the working solutions.

Extraction.

To 2.0 ml of blood were added 1.0 ml internal standard solution (3-O-methyl nalorphine in water) and 1 ml 1M NaOH in a stoppered centrifuge tube. The mixture was extracted with 8 ml ethyl acetate with 10 % butanol and the organic layer transferred to another glass tube containing 1 ml 0.05M H_2SO_4. After shaking and equilibration, the aqueous phase was transferred to a third tube containing 400 μl 1M NaOH and extracted with 3 ml chloroform with 10 % butanol. The organic phase was transferred to a small, tapered glass tube and evaporated to dryness under a stream of nitrogen at 40°C.

Derivatization.

PFPA (50 μl) (for quantitation) or 50 μl of a mixture of BSTFA in acetonitrile (1+2) (for verification) was added to the dry residue. The tube was stoppered and heated at 60°C for 20 min. The

solution was then evaporated to dryness at 40°C under a stream of nitrogen, and the derivatized extract was dissolved in 50 μl butyl acetate for the chromatographic analysis.

Standard curves.

Calibration curves were determined from plots of peak height ratio of the PFPA-derivatives of codeine and internal standard against known concentrations of codeine.

Range 0.05 to 10 nanomol/ml (0.015 to 3 micrograms/ml).

RESULTS AND DISCUSSION.

TABLE I

Recovery from spiked blood samples (derivatized with PFPA) at two different concentrations.

	0.1 nanomol/ml	1 nanomol/ml
Codeine	94 ± 2.8 % (n=10)	84 ± 6.2 % (n=10)
Methyl nalorphine (I.S.)	68 ± 5.9 % (n=10)	75 ± 5.5 % (n=10)

Sensitivity : Better than 0.05 nanomol (15 nanograms)/ml (both derivatives).

Precision (PFPA-derivatives) : Within-run coefficient of variation was 6.4 % (n=10, concentration 0.5 nanomol/ml blood).

Linearity : The standard curve was linear between 0.05 and 10 nanomol/ml. Coefficient of correlation was 0.998.

Underivatized codeine and methyl nalorphine gave tailing peaks and low sensitivity.

The PFPA- and BSTFA-derivatives, on the other hand, showed good peak symmetry and better sensitivity.

Nitrogen sensitive detector gave fewer interfering peaks than electron capture detector and was best suited for codeine analysis.

From these chromatograms it is clear that no peaks in the blank sample interfered with codeine or with internal standard.

The main metabolites of codeine in whole blood : morphine and norcodeine, were separated from codeine under these conditions as BFPA-derivatives.

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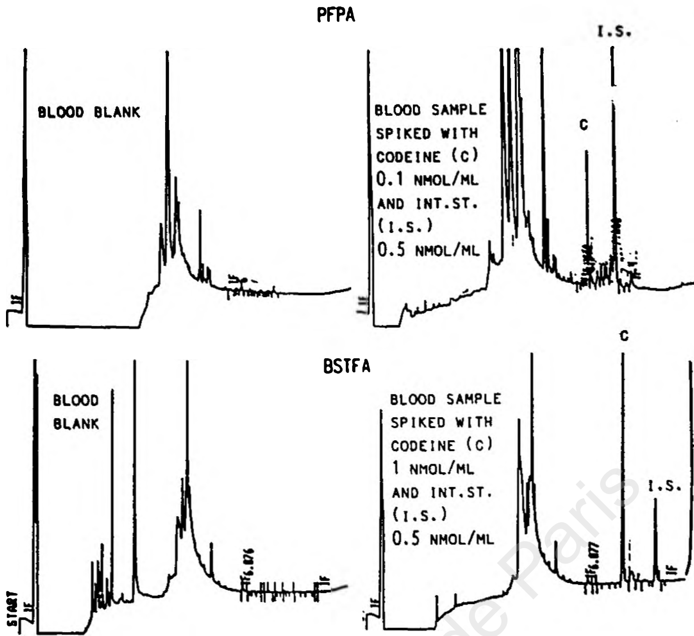

FIG. 1. — Chromatograms from an unspiked blood sample and a sample spiked with codeine and derivatized with PFPA and BSTFA.

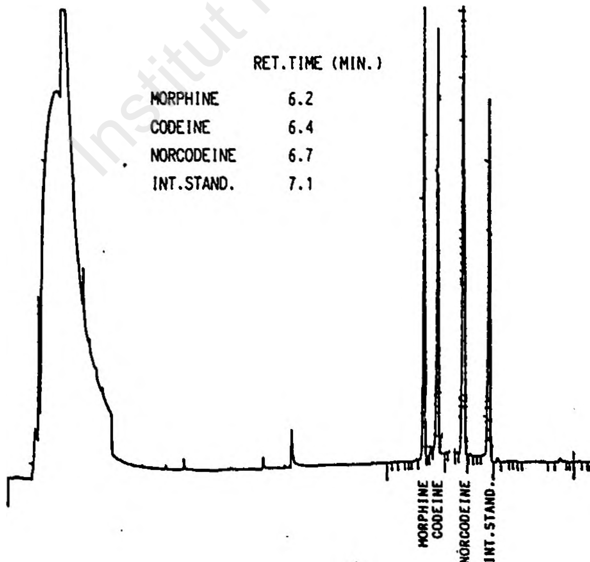

FIG. 2. — Chromatogram of codeine, morphine, norcodeine and int. standard derivatized with PFPA.

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The BSTFA-derivatives of codeine and norcodeine were not separated. For this reason and the low stability of the BSTFA-derivatives, the PFPA-derivatives were not useful in the quantitative analysis.

In order to obtain a reasonable yield of codeine, a more polar extraction solvent than diethyl ether or chloroform was necessary. Diethyl ether and chloroform were used previously in this laboratory.

According to Renzi (1), an addition of 10 % butanol to chloroform gave recovery of more than 80 % of codeine from plasma. This extraction solvent is unsuitable for the extraction of codeine from whole blood because of poor separation of the organic phase from the aqueous phase.

In the present work ethyl acetate with 10 % butanol was used for extraction of codeine from blood. Chloroform with 10 % butanol was used in the second stage. These solvents gave clear extracts and good separation of the phases.

Extraction was performed from pH 11 to obtain a reasonably high recovery of codeine (1). This gave poor recovery of morphine, indicating that it is difficult to obtain a satisfactory quantitation of both codeine and morphine by the same procedure.

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Incidence of drugs of abuse in newborns in Los Angeles

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INTRODUCTION.

The Child Abuse Prevention Program of the County of Los Angeles has been in effect only since 1981. This program has many functions one of which is to monitor infants born to mothers who are drug abusers. There is by law a requirement for all hospitals and clinics to report cases of infants suspected of being under the influence of drugs of abuse at birth. A Child Protective Services worker then investigates and a decision is made on the disposition of each individual case.

Los Angeles County has a population of over 8 million. There were 144,000 infants born in L.A. County in 1984. Statistics compiled by the Drug Abuse Program office for 1984 gives the number of female drug abusers in the County of Los Angeles of child bearing age as 10,990. Data for the number of these who had children are not available but the number of reported cases of neonatal drug withdrawal for this period was 240. Data for the first 10 months of 1985 show over 400 reported cases of neonatal withdrawal for the entire County. In the County of Los Angeles reports are received from 39 hospitals 5 comprehensive health centers and 41 clinics. Some of these institutions do not have laboratory facilities for analysis of samples so these reports are by history only. The Los Angeles County - University of Southern California (LAC-USC) Medical Center is the largest hospital in the County system. An average of 40 infants are born each day in the maternity wards of our hospital which is over 10 % of the total births in the County. The distribution of obstetrical cases by race in our hospital population and the percent of drug cases for each of these races is given in table I.

TABLE I

Distribution of obstetrical cases by race

Race	Percent of total OB patients	Percent of total drug cases
Hispanic	85	40
Black	10	40
Caucasian	5	20

The distribution shows the majority of maternity patients in our hospital are of hispanic origin but the percent of drug cases in this population is proportionally much less than in the other races.

A urine sample is obtained from the infant when it shows signs of being hyperactive or « jittery » shortly after birth. Samples are also obtained from infants when the case history of the mother shows prior use of drugs during registration in County programs such as the prenatal care program, the methadone maintenance program, etc. In other cases the drug abuse by the mother may not be known but during delivery she may be combative, she may demand to leave with the baby immediately after delivery, or she leaves AMA, etc. A medical judgment is made shortly after birth whether a sample should be submitted for drug analysis. The pediatric staff requires a short turn-around time for the laboratory to report results on these infants in order to process discharges or refer the infants to regulatory agencies.

The efficiency of the program directly involves the nursing staff in the newborn service. There are two important factors that must be considered. One is early recognition of the need for an analysis and the other is collection of sufficient sample. In some infants the symptoms of addiction appear very soon during the first day. In others it is only when withdrawal symptoms become recognizable after 3 or 4 days and by this time it may be too late for the analysis to be of value since they will usually be negative. Thus, every effort is made to obtain a specimen during the first 24 hours if drugs are suspected. Collection of the sample requires a dedicated effort by the personnel who perform this service. In most cases a sufficient amount of urine, approximately 5 ml, can be obtained in the first 24 hours after birth without difficulty. This amount may be difficult to obtain on those infants who were immediately started on breast feeding, if there was strangulation during delivery or if improper collection technique is used, particularly on female infants.

METHODS.

Collection of Urine Sample.

The urine specimens in our hospital are collected by personnel who have been given specific instructions in following a protocol designed to ensure a sufficient volume. The urine is collected in a specially designed bag, a U-Bag, newborn size, and after collection is removed by a syringe to prevent loss. The equipment required includes : Betadine swabs, cotton balls, a U-Bag newborn size (Hollister) and a 5 ml syringe with 21 gauge 1 ½ inch needle. In collecting the specimen the perineal area is washed with betadine and tap water using cotton balls. The area is patted dry from top to bottom. A U-Bag is applied according to instructions on the package. The bag contains an adhesive which binds its opening to the area around the vulva and through which the penis is inserted. The U-Bag contains a reservoir into which the urine flows and is collected. Care must be taken in positioning the bag to ensure gravity flow into the reservoir. The urine is removed from the reservoir by inserting the needle of the syringe. If insufficient urine is obtained on the first attempt the infant is rebagged and collection continued.

Analytical Procedures.

The analytic methods selected are those which the laboratory can offer to meet the short turn-around requirements of the program. Enzyme immunoassays (Syva EMIT) are used for the initial

TABLE II

Methodology for drugs of interest

Drug	Method
Cocaine	Immunoassay
Phencyclidine	Immunoassay
Morphine	Immunoassay and TLC
Codeine	Immunoassay and TLC
Methadone	Immunoassay and GLC
Amphetamines	Immunoassay and GLC
Alcohol	Dichromate and GLC

screening for all except alcohol which is assayed by Widmark dichromate oxidation. The drugs of interest in the program, the screening and confirmatory methods are listed in table II.

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Cocaine, PCP and the opiates (morphine and codeine) are routinely assayed on all urines. Alcohol, amphetamine and methadone are done only when specifically requested. All positive opiates are hydrolyzed and confirmed by TLC to distinguish between morphine and codeine. Confirmation of the immunoassay is not done for benzoylecgonine and phencyclidine.

RESULTS.

The total number of positive samples for each drug for both the baby and mother are given in table III. The table also lists the number of times in which a positive and negative were obtained in paired samples.

TABLE III

Drugs found in positive urine samples

Drug	*Babies*	*Mothers*
Cocaine	119	55
Morphine	62	25
PCP	40	8
Methadone	16	4
Codeine	9	3
Amphetamine	3	1
Alcohol	2	1

Baby + Mother — 2
Baby — Mother + 8

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The number of positive samples found by year are given in table IV.

TABLE IV

Number of positive samples by year

Year	*Babies*	*Mothers*
1983	29	1
1984	45	7
1985	118	54
1986 (4 months)	63	35

The distribution of the individual drugs by year found in newborns are listed in table V. The 1986 data are for the first 4 months.

Multiple drugs are found in some newborns. The combinations found in these cases are given in table VI.

TABLE V

Drugs found in newborns by year

Drug	*1983*	*1984*	*1985*	*1986*
Cocaine	0	15	60	44
Morphine	9	11	40	13
PCP	14	8	15	3
Methadone	3	7	4	0
Codeine	3	2	4	1
Amphetamine	0	0	1	2
Alcohol	0	0	1	1

TABLE VI

Multiple drug combinations in newborns

Cocaine and morphine	11
Cocaine and PCP	4
Morphine and methadone	2
Morphine and PCP	1
Codeine and PCP	1
Cocaine and alcohol	1
Cocaine, morphine and methadone	3

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TABLE VII

Distribution of drugs in newborns by race

Race	*Cocaine*	*Morphine*	*PCP*
Black	63	9	13
Hispanic	25	33	16
Caucasian	31	20	11

The popularity of the drug varies with race. The distribution of the three most popular drugs in newborns by race are given in table VII.

DISCUSSION.

Although the Child Abuse Program was instituted by the County of Los Angeles in 1981, the division responsible for the collection of data on drugs of abuse in newborns was not active until 1983 as seen in table IV in which the number of positive cases are listed by year. Prior to 1983 very few analyses for drugs were requested on newborns in our hospital. The increase in the positive findings is not thought to be an increase in drug use by mothers. It may be considered to partially reflect the increase

in efficiency and educational efforts of the Child **Abuse** Program and also advances in the collection of urine samples from the infants. In the initial phase of the program an observation was made by the laboratory that negative reports were being challenged since the babies were having withdrawal symptoms and the mothers had been addicts. Most of these samples were not being obtained until after the onset of the withdrawal symptoms 3 or 4 days after birth when the drug concentration would be too low to detect. In other cases insufficient sample was obtained when urine was being extracted from the diaper. A change in collection procedure in which the samples were obtained within the first 24 hours resulted in the increase in positive findings.

Table III presents some interesting observations. The low number of alcohol cases is surprising in view of the fact that Fetal Alcohol Syndrome is considered to be one of the leading causes of birth defects in children. The almost absence of amphetamines reflects the disappearance of these drugs from the general drug scene. The dramatic increase in use of cocaine reflects its popularity as a recreational drug. There were a small number of cases in which the drug is found in the mother and absent in the baby or vice versa. These findings may be partially explained by the differences in the pharmacokinetics of drug clearance by the mother and infant. Another explanation may be that a drug may be reported negative because the concentration is below the « cut-off » calibrator in the immunoassays and thus reported as absent. In table VII the observation is made that morphine (heroin) use is more popular with the hispanics and cocaine is the more popular drug with blacks. Multiple drugs were found in some infants. The combination found most often was cocaine and morphine.

The yearly increase in reportings can be attributed to either, more drug abuse by mothers, better urine sampling by personnel, more efficient case reporting procedures or any combination of these. The long term effect of neonatal drug abuse is not known at this time and can only be assessed by following the progress of the infant in future studies. The data collected in our hospital program is fully documented and will be very valuable for future studies. Copies of all original request forms containing pertinent information suitable for these follow up studies on infants born in our hospital have been saved for such a purpose.

Analysis of data from a drug abuse screening service

by B. WIDDOP and R. CALDWELL

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INTRODUCTION.

The Poisons Unit laboratory has provided a service for the detection of abused drugs in urine specimens since its inception in 1967. Treatment centres make use of this service in the following context :

a) To verify or disclaim the drug-taking history of newly presenting patients. This guards against the issuing of prescriptions of controlled drugs to patients who are not in fact dependent on these, but intend to dispose of them subsequently for financial gain.

b) To check that established patients are taking the drug(s) prescribed and to detect any other drug misuse which he or she has not reported to the psychiatrist.

In addition, psychiatric clinics refer samples from patients whose disturbed behaviour may be attributed wholly or in part to recreational drug misuse. Symptoms range from the drowsiness associated with barbiturate and cannabis usage, the restlessness and agitation induced by stimulant drugs such as amphetamines and cocaine through to overt psychosis as a result of heavy and prolonged misuse of amphetamine and some of its derivatives and possibly of cannabis. LSD abuse is also indicated as a cause of psychotic behaviour, although no reliable test for this compound is available at the present time.

Previously it has not been possible to analyse the large volume of data generated by this service and to assess its value as an aid to the diagnosis and treatment of drug misuse.

From April 1984 these data have been entered and stored on a micro-computer and this paper presents findings over the period April to December 1984 and January to December 1985.

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Analytical methods.

All samples were tested for the presence of opioids and barbiturate drugs by thin-layer chromatography. Where appropriate positive results were checked by gas-liquid chromatography. Throughout 1984 amphetamines and related stimulants were detected using the EMIT Autolab system and positive samples reanalysed by gas-liquid chromatography. During 1985 these compounds were analysed solely by gas-liquid chromatography. Those samples referred for a « drug-induced psychosis screen » were also tested for cocaine by the EMIT system and cannabis by EMIT or radioimmunoassay. Details of the methods applied have been reported elsewhere (1).

Data Storage.

Data was stored on an IBM PC XT micro-computer fitted with a 10 MB hard disk. A commercial database (R BASE 4,000) was

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Name : Williams John — Hospital : St Giles

Sample date : 14-04-1986 — Lab number : 10268

New patient : Y — Screen type : A

Prescribed drugs : Methadone... Heroin... Others...

+ Morphine	+ Amphetamine
— Monoacetylmorphine	— Methylamphetamine
— Codeine	+ Ephedrine
— Methadone	+ Mephentermine
+ Dihydrocodeine	— Diethylpropion
— Diconal	— Barbiturates
— Dextropropoxyphene	— Phenothiazines
— Cocaine	— Phentermine

Other drugs detected : ....

Results from other tests performed

Cannabis + ... Benzodiazepines + ... Haemagglutination test — ......

FIG. 1. — Example of a patient record.

used and storage space was available for up to 10,000 records. Each record carried details of the patient's name, age, sex, the referring hospital or clinic, sample date, drug(s) prescribed and all analytical results. An example is shown in figure 1.

Sources of samples.

During the nine-month period, April to December 1984, 4,463 urine samples were analysed, 246 of these being from patients with suspected drug-induced behavioural disturbance. In 1985 a total of 7,581 samples were processed with 388 of these coming from patients with behaviour problems. Although samples were referred from over 100 hospitals and clinics, the bulk originated from two of the major London Drug Dependence Treatment Centres.

RESULTS.

Overall incidence of drug detection.

Table I lists the incidence of detection of opioid drugs in terms of the percentage of samples out of the totals which were positive. Diamorphine (heroin) is rapidly degraded to morphine and

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TABLE I

Incidence of opioid drug detection (%)

Drug	*1984 (n = 3833)*	*1985 (n = 7193)*
Total morphine	33.6	32.7
« Street » heroin	14.9	14.9
Pharmaceutical heroin	8.0	7.9
Morphine (origin unknown)	11.5	8.7
Methadone	48.0	50.1
Codeine	3.7	3.9
Dihydrocodeine	4.9	5.0
Dextropropoxyphene	2.9	1.9
« Diconal »	0.8	0.7
Pethidine	< 0.1	< 0.1
Pentazocine	< 0.1	< 0.1
Dextromoramide	< 0.1	< 0.1

monoacetylmorphine, both of which are detectable in urine by TLC. Impure or « street » heroin often contains acetylcodeine and this preparation gives rise to morphine, monoacetylmorphine and a small amount of codeine in the urine. This was used as a means of differentiating between the use of pharmaceutical heroin and « street » heroin. Total morphine encompasses samples positive for either type of heroin or for morphine alone.

As anticipated methadone was by far the most commonly encountered drug due to its widespread prescription as a heroin substitute. The table also illustrates the predominance of heroin over all other opioids as a drug of abuse. Virtually no change in the overall pattern of opioid drug abuse was discernable between the two study periods.

Table II gives a breakdown of the incidence of detection of amphetamines and related sympathomimetic amines. All these compounds are known to be sold illicitly as amphetamine due to their essentially similar pharmacological effects.

TABLE II

Incidence of detection of amphetamine and related stimulants (%)

Drug	*1984 (n = 4463)*	*1985 (n = 7193)*
Amphetamine	9.9	10.8
Methylamphetamine	1.1	2.0
Mephentermine	0.9	0.3
Phentermine	0.2	0.7
Diethylpropion	0.2	0
Ephedrine	1.4	1.1

Here again, little change in the pattern of sympathomimetic amine abuse was found with amphetamine being by far the most important drug in this group.

It is not the policy of the laboratory to screen all samples routinely for the presence of cocaine, cannabis and benzodiazepines due to the prohibitive costs of immunoassays. Instead, these drugs are sought only when the psychiatrist specifically requests the test. Cocaine is very rarely detected by TLC as the parent compound, although the degradation product, benzoyl ecgonine is sometimes discernable and it's presence is confirmed by an EMIT assay. Table III shows the incidence of cocaine detection by TLC as being 0.5 and 1.0 % for 1984 and 1985 respectively. It is most likely that this is an underestimate since in a more recent survey on samples from 500 patients, 21 (4.2 %) were positive by EMIT, but only 9 (1.8 %) were positive by TLC.

Cocaine was detected at a much higher rate by EMIT in those patients suspected to be abusing the drug by the psychiatrist (table III).

No significant change in the overall incidence of detection of cannabinoids, benzodiazepines or barbiturates was discernable from these data.

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TABLE III

Incidence of miscellaneous drugs (%)

Drug	*1984*	*1985*
Cocaine	0.5 (n = 3833) TLC 8.1 (n = 210)* EMIT	1.0 (n = 7193) TLC 15.6 (n = 250)* EMIT
Cannabis	33.8 (n = 400)*	31.6 (n = 1057)*
Barbiturates	3.2 (n = 3833)	3.7 (n = 7193)
Benzodiazepines	54.8 (n = 254)*	41.4 (n = 358)*

* Analysis carried out by specific request.

Temporal variations in the incidence of drug detection.

The computer system enables a scrutiny of the monthly detection rate of each drug to be made with ease (fig. 2).

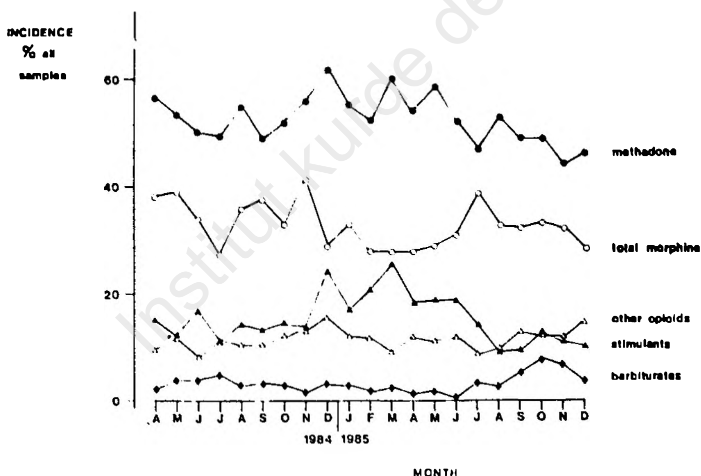

FIG. 2. — Monthly detection rate of drugs.

The data indicate a significant increase in stimulant detection from November 1984 declining to previous levels from June 1985 onwards. Closer analysis showed that this was attributable almost entirely to the seasonal use of ephedrine and pseudoephedrine in proprietory cold cures and anti-allergic preparations. The rise in barbiturate detection proved to be more sinister and continued dramatically into 1986 (fig. 3).

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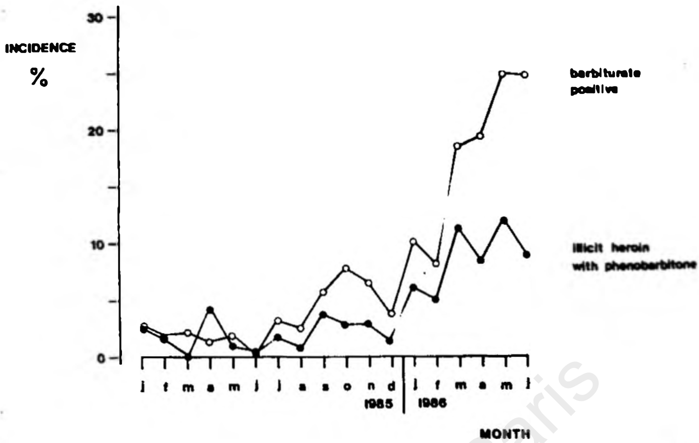

FIG. 3. — Monthly detection rate of all barbiturates and illicit heroin in combination with phenobarbitone.

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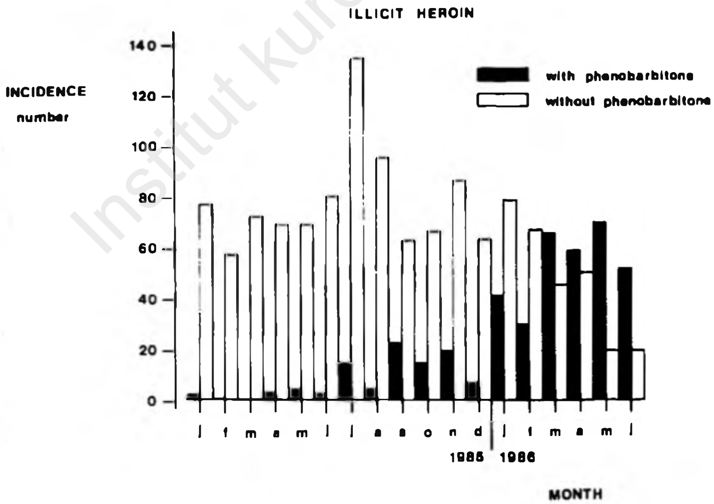

FIG. 4. — Increase in detection rate of illicit heroin in combination with phenobarbitone.

Over this period large quantities of illicit heroin adulterated with phenobarbitone began to appear on the streets such that by mid-1986 this was the predominant form of illicit heroin detected (fig. 4). A sample of illicit heroin powder sent for analysis was found to contain 40 % heroin and 10 % phenobarbitone.

Drug detected in acute psychosis patients.

The incidence of drugs found in this group of patients was remarkably constant (table IV).

TABLE IV

Drugs detected in acutely psychotic patients (%)

Drugs	*1984 (n = 247)*	*1985 (n = 388)*
Phenothiazines	32.4	36.1
Cannabinoids	22.3	25.5
Amphetamine	2.0	2.1
Dextropropoxyphene	2.0	1.0
Ephedrine	1.2	0.5
Dihydrocodeine	0.8	—
Morphine	—	0.3
Codeine	—	0.3
Methadone	0.4	0.8
Pethidine	0.4	0.5
Cocaine	0.4	—
Phenylpropanolamine	—	—

The predominance of phenothiazines was anticipated since these agents are often administered to patients presenting with an acute psychotic reaction. Approximately one in five patients gave positive reactions in urine cannabinoid immunoassays. Excessive use of cannabis has been implicated as a possible cause of acute psychotic reactions (2) and there is some corroborative evidence from this survey, although it should be emphasized that the urine assays were not quantitative and no detailed analysis of the clinical features of these patients compared to a matched cannabis-free control group has been possible. Samples from these patients have not been analysed for other psychotomimetic drugs such as phencyclidine and LSD. The former has never been found in seizures by U.K. government agencies or in biological samples screened by the forensic laboratories and appears to remain a problem peculiar to the USA. No routine assay for LSD was available over the period of this survey, although a test for this drug will be incorporated into future investigations.

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DISCUSSION.

Urine analysis is the only objective means of assessing a patient's drug-taking status at the time of clinical examination and its value is well-established. This paper illustrates that computerized analysis of the accumulated data can also yield useful information. The finding that heroin was far and above the most commonly abused opioid came as no surprise, but the extent of use of other opioid drugs has not heretofore been quantified. Government Departments concerned with the legislative control of drugs require firm evidence of this kind to reach rational decisions on the degree of the restriction which should be imposed, for example, on the newer opioid analgesics. Thus in a recent survey of buprenorphine incidence in 1,000 samples referred to the screening service we were able to demonstrate an insignificant detection rate of less than 1 %.

Changing trends in the pattern of drug abuse can now be monitored as illustrated here by the detection of an influx of heroin diluted with phenobarbitone. Although amphetamine is still the most common stimulant drug encountered, it is anticipated that cocaine abuse will gradually increase and we now have a means of invigilating this.

Finally, the routine service to the treatment centres has been much improved. Records for individual patients are readily retrieved and cumulative reports can be generated which enable the physician to judge the success or otherwise of his treatment.

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Gas chromatographic determination of underivatized amphetamine in whole blood

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SUMMARY.

Extraction : to 1 ml whole blood was added : 1 ml distilled water, 200 µl quinoline solution 0.01 µmol/l (internal standard), 300 ul 5M NaOH and 6 ml diethyl ether. After mixing for 10 min. and centrifugation, the organic phase was transferred to a new glass tube, washed with 2 ml distilled water and transferred to a tapered glass tube. The organic extract was acidified with 30 ul 6M HCl and evaporated (35°C) under N_2-gas. Fifty ul diisopropyl ether, 20-25 mg solid NaCl and 50 ul 5M NaOH were added. After brief vortexing, 1 ul organic phase was injected into the gas chromatograph.

Chromatographic conditions : Packed column : GP 10 % Apiezon/2 % KOH on 80/100 Chromosorb WAW (210 cm × 2 mm) was used for most routine samples. *Capillary column :* Fused silica methyl silicone (12 m × 0.2 mm, 0.32 um film thickness). Nitrogen sensitive detector was used for both systems. *Results :* Calibration graph from 0.5-8 µmol/ml (regression coefficient : 0.998). Detection limit : 0.2 µmol/l (packed column) and 0.1 µmol/l (capillary column).

Precision : Packed column : 5.8 % (2 µmol/l ; n=10), 5.6 % (5 µmol/l ; n=10).

Capillary column : 7.5 % (0.5 µmol/l ; n=10), 6.6 % (3 µmol/l ; n=10). Inter-assay variation : 10 % (3 µmol/l ; n=11).

Recovery (human drug-free whole blood with NaF and Naoxalate collected and stored on glass bottles) : Amphetamine : 91 % (2 µmol/l) and 93 % (5 µmol/l). Quinoline : 85 % (2 µmol/l).

Blood bank blood (collected in plastic bags) occasionally gave low amphetamine recovery (55 %).

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A capillary gas-chromatographic method for the identification of drugs of abuse in urine samples

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SUMMARY.

This paper describes the application of capillary gas liquid chromatography (GLC) to the analysis of urine samples from Drug Dependence Treatment Centres. A simple micro extraction into butylacetate is followed by isothermal analysis at 150°C for amphetamine and related stimulants or temperature programmed analysis from 90°C to 310°C to provide a comprehensive screen for basic drugs. Retention data are presented for 27 compounds using the isothermal analysis and approximately 250 compounds using the temperature programme.

The use of capillary GLC is compared with packed column methods used previously in this laboratory. Data on the reproducibility of retention data and sensitivity of these analyses for several commonly encountered drugs are presented.

INTRODUCTION.

The combination of splitless injection with capillary GLC has been shown to provide an excellent method for rapid drug screening (1). GLC is often used as a method of screening for amphetamine type drugs (2, 3), and also as a confirmatory method following antibody mediated techniques of detecting amphetamines. In this laboratory, large numbers of urine samples are screened for drugs of abuse and we have found that by using a capillary column isothermally we are able to exploit the re-

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solution achieved with capillary columns while keeping the analytical time for each sample to a minimum. The splitless injection technique is ideally suited to the automatic processing of large numbers of samples. This technique allows the use of normal syringes and steel needles and by injecting onto a quartz glass injector liner non-volatile contaminants accumulate there rather than on the analytical column.

The same column and machine are used to provide a back-up procedure for the thin layer chromatographic (TLC) method used routinely to identify opioids and other basic drugs.

MATERIALS AND METHODS.

Instrumentation.

All analyses were performed on a model 5890 gas chromatograph (Hewlett-Packard) equipped with a model 7673A automatic liquid sampler, a Nitrogen-Phosphorus detector and a capillary column split-splitless inlet system. The column was a wide bore (0.32 mm i.d.), thick film (0.52 µm) cross-linked fused silica SE-54 capillary column (Hewlett-Packard). A model 3392A integrator (Hewlett-Packard) was used to monitor the GC output and store work-files containing GLC parameters and autoinjector functions.

GC operating parameters.

Helium was used as the carrier gas. The column head pressure was 19 psi, which gave a flow rate of approximately 3.5 ml/min. Other flow rates were : nitrogen (make-up gas) 26 ml/min. ; air 100 ml/min. ; hydrogen 20 ml/min. ; purge flow 45 ml/min. ; and septum purge 1 ml/min.

The injector was heated to 250°C and the detector to 300°C. The bead current was adjusted to give a signal of 25 at rest. The range was set at 3, attenuation at 2^5, chart-speed at 1 cm/min., peak width at 0.04, and threshold to 6. The column temperature was set to 150°C isothermal for the analysis of amphetamine-type drugs or programmed from 90°C to 310°C to provide a more comprehensive screen for basic drugs (table I). All other GC conditions remained the same for both analyses.

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TABLE I

GLC conditions for basic drug screening

Resting oven temperature .	90° C
Equilibrium time	0.5 min
Temperature programme	
Initial value	90° C
Initial time	0.5 min
1st ramp	
Ramp rate	40° C/min
Final value	250° C
Final time	0.1 min
2nd ramp	
Ramp rate	5° C/min
Final value	310° C
Final time	0
Total run time	16.60 min

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Chemicals and reagents.

HPLC grade butylacetate was used for all extractions (Rathburn Chemicals Ltd, Walkerburn, Peebleshire, Scotland). Analar grade methanol (BDH Chemicals Ltd, Poole, England) was used to prepare 1 g/l stock solutions of all standards and internal standards. These methanolic solutions were diluted subsequently with butylacetate to provide working solutions. Urine samples were buffered using 1 M sodium hydroxide.

Internal standards.

Nicotine (5 mg/l) was prepared in butylacetate for amphetamine analyses and prazepam (5 mg/l) in butylacetate was used as the internal standard for the temperature programmed analysis.

Standards.

10 mg/l solutions of drugs and metabolites were prepared in butylacetate and chromatographed to produce a library of retention data.

Extraction procedures.

For amphetamine-type drugs 400 µl urine, 100 µl 1 M sodium hydroxide and 200 µl of nicotine internal standard solutions were

added to a clean Dreyer tube, vortex mixed for 30 sec. and centrifuged for 2 min. 100 µl of the butylacetate layer were transferred to tapered autosampler vials (Chromacol Limited, Glen Ross House, Summers Row, London N12 OLD) and 2 µl were injected onto the GC. The same extraction procedure was used for the basic drug screen, but prazepam was used as the internal standard instead of nicotine.

RESULTS AND DISCUSSION.

Initially a short temperature programme was used to analyse samples for amphetamine and related stimulants using capillary GLC. However, the « cool down » time required between injections resulted in a relatively long analysis time of greater than 10 min. per sample. By using the equipment isothermally at 150°C this time could be reduced to 5 min. per sample, enabling large batches of samples ($>$ 100) to be analysed overnight. It was anticipated that the less volatile drugs present in these samples (methadone, codeine, etc.) would elute during subsequent injections, but this has not proved to be a problem. Occasionally extracts containing large amounts of lignocaine or pethidine give rise to late eluting peaks, but these are easily recognized due to their wide peak shape.

After isothermal use, butylacetate is injected and the GC programmed to 310°C. This elutes any compounds retained on the column. The present column has been used for at least 5,000 injections and the only maintenance required has been periodic cleaning of the injection liner and occasional removal of the front 15 cm of the analytical column. This system requires far less attention than the packed column method used for detecting amphetamine previously in this laboratory. The columns used (Apiezon L KOH and Carbowax 20M KOH) needed frequent re-packing due to bleeding of the stationary phase.

Table II presents retention data obtained isothermally at 150°C. Most of the compounds on this list are adequately resolved ; those which are not can often be identified by the presence of metabolites. For example, althoug methoxyphenamine and pseudoephedrine give the same relative retention time (RRT), pseudoephedrine will normally be accompanied by its major metabolite norpseudoephedrine. Chlormethiazole is characterized by an abundance of urinary metabolites, and different patterns of these metabolites are often present in samples from individual

TABLE II

Retention data for drugs and metabolites eluted isothermally at 150° C

Compound	*Retention time*	*Retention time relative to nicotine*
β-phenylethylamine	1.51	0.467
Amphetamine	1.67	0.518
Norfenfluramine	1.69	0.523
Phentermine	1.83	0.567
Chlormethiazole metabolite I	1.84	0.570
Propylhexedrine	1.84	0.570
Chlormethiazole metabolite II	1.91	0.592
Methylamphetamine	1.92	0.594
Chlormethiazole metabolite III	1.98	0.614
Fenfluramine	2.16	0.699
Pargyline	2.20	0.681
Chlormethiazole metabolite IV	2.29	0.708
Chlormethiazole	2.34	0.724
Mephentermine	2.38	0.737
Indole	2.66	0.824
Norpseudoephedrine	2.96	0.916
Phenylpropanolamine	2.99	0.926
Nicotine	3.23	1.000
Chlorphentermine	3.36	1.040
Ephedrine	3.51	1.087
Paramethoxyamphetamine	3.56	1.102
Methoxyphenamine	3.59	1.111
Pseudoephedrine	3.59	1.111
Methylephedrine	4.09	1.266
Phenmetrazine	4.65	1.440
Phendimetrazine	5.02	1.554
Diethylpropion	5.70	1.760

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patients. Chlormethiazole is confirmed in this laboratory by reference to the routine TLC method. Figure 1 shows example chromatograms from patients using methylamphetamine (metabolised to amphetamine), pseudoephedrine and chlormethiazole.

Although TLC provides a cheap and quick method for identifying some of the commonly encountered opioids and other abused drugs, the lack of resolution limits its potential to identify the more unusual drugs encountered. TLC is also affected adversely by « oily » samples and overloading caused by the high concentrations of drugs found in drug abusers (particularly codeine and dihydrocodeine). Samples which present these problems are analysed by capillary GLC with temperature programming.

Butylacetate has proved a suitable solvent with high extraction efficiencies for methadone, amphetamine and codeine. The temperature programme starts at 90°C (below the boiling point of butylacetate which is 109°C). This allows vaporisation of the

extract in the injector and subsequent recondensation on the analytical column. A fast 40°C/min. ramp to 250°C is then followed by a slower 5°C/min. ramp to 310°C during which the majority of compounds elute. The run time is 16.60 min. with approximately 4 min. required for the machine to cool down before the next injection.

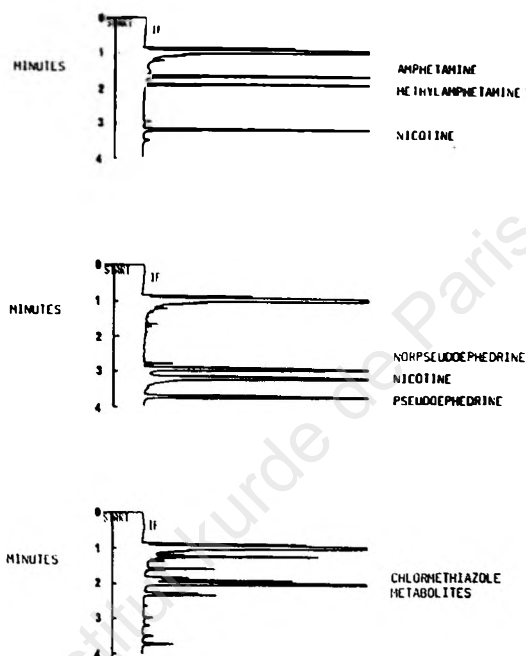

FIG. 1. — Chromatogram of sample extracts from patients abusing *a)* methylamphetamine ; *b)* pseudoephedrine ; *c)* chlormethiazole.

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Table III presents retention data resulting from injection of standards and sample extracts using this programmed analysis. The resolution and the reproducibility of RRT's allows many compounds to be identified immediately. However, some compounds elute coincidentally or very close together. Referral back to the TLC plates can sometimes rule out one or more possibilities, but more often the detection of metabolites on the GLC system enables the correct identification to be made. Compounds which appear as one peak on packed column GCs often resolve into parent and metabolite peaks on capillary GLC, for example chlorpheniramine and brompheniramine. This emphasises the need to compile data bases with RRT's not only for the parent drugs,

TABLE III

Retention data for drugs and metabolites éluted by temperature programming between 90° C - 310° C

Compound	Retention time	Retention time relative to prazepam
Mexilitine	ND	—
Lorazepam	ND	—
Erythromycin	ND	—
Chlordiazepoxide	ND	—
Morphine-N-oxide	ND	—
Trazadone	ND	—
Desmethylastemizole	ND	—
Ranitidine	ND	—
Benserazide	ND	—
Tranylcypromine	ND	—
Benzoylecgonine	ND	—
Cimetidine	ND	—
Pemoline	ND	—
Astemizole	ND	—
Buprenorphine	ND	—
Ipecacuanha	ND	—
Oxymorphone	ND	—
Meprobamate	ND	—
Hydromorphone	ND	—
Penicillamine	ND	—
Phenelzine	ND	—
Perphenazine	ND	—
Tocainide	ND	—
Labetolol	ND	—
Buclizine	ND	—
Naloxone	ND	—
Theophylline	ND	—
Cyproheptadine	ND	—
1-phenylethylamine	2.241	0.206
2-phenylethylamine	2.448	0.255
Amphetamine	2.579	0.237
Norfenfluramine	2.589	0.238
Propylhexedrine	2.731	0.251
Chlormethiazole metabolite 1	2.731	0.251
Phentermine	2.753	0.253
Methylamphetamine	2.807	0.258
Chlormethiazole metabolite 2	2.840	0.261
Chlormethiazole metabolite 3	2.872	0.264
Fenfluramine	2.970	0.273
Pargyline	2.981	0.274
Chlormethiazole metabolite 4	3.036	0.279
Chlormethiazole	3.079	0.283
Mephentermine	3.123	0.287
Indole	3.286	0.302
Phenylpropanolamine	3.362	0.309
Norpseudoephedrine	3.395	0.312
Diethylpropion metabolite 1	3.405	0.313
Nicotine	3.492	0.321
Chlorphentermine	3.503	0.322
Acetanilide	3.536	0.325
Ephedrine	3.569	0.328
Paramethoxyamphetamine	3.569	0.328
Pseudoephedrine	3.580	0.329
Methoxyphenamine	3.634	0.334

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TABLE II *(continued)*

Compound	Retention time	Retention time relative to prazepam
Methylephedrine	3.775	0.347
Diethylpropion metabolite 2	3.819	0.351
Phenmetrazine	3.852	0.354
Phendimetrazine	3.928	0.361
Diethylpropion metabolite 3	3.993	0.367
Cocaine impurity (? methyl-ester)	4.036	0.371
Diethylpropion	4.091	0.376
Methyprylone	4.178	0.384
Diethylpropion metabolite 4	4.319	0.397
Phenylephrine	4.472	0.411
Paracetamol	4.613	0.424
Fencamfamin	4.678	0.430
Cotinine	4.755	0.437
Methylphenidate	4.874	0.448
Tryptamine	4.874	0.448
Pethidine	4.972	0.457
Diphenoxylate	5.027	0.462
Norpethidine	5.103	0.469
MEGX (lignocaine metabolite)	5.212	0.479
Pheniramine	5.222	0.480
Prilocaine	5.266	0.484
Ethotoin	5.277	0.485
Benzphetamine	5.288	0.486
Caffeine	5.288	0.486
Pheniramine metabolite	5.331	0.490
Pethidine metabolite	5.407	0.497
Viloxamine	5.418	0.498
Diphenhydramine	5.429	0.499
Ethoheptazine	5.440	0.500
Glutethimide	5.440	0.500
Lignocaine	5.473	0.503
Phenazone	5.484	0.504
Oxprenolol	5.560	0.511
Meptazinol	5.614	0.516
Doxylamine	5.625	0.517
Orphenadrine	5.701	0.524
Desmethylorphenadrine	5.701	0.524
Tofenacin	5.723	0.526
Methadone metabolite 2	5.745	0.528
Phenyltoxalamine	5.788	0.532
Chlorpheniramine	6.006	0.552
Procaine	6.104	0.561
Chlorpheniramine metabolite 1	6.125	0.563
Hydroxypethidine	6.136	0.564
Cyclizine	6.169	0.567
Chlorpheniramine metabolite 2	6.202	0.570
Nefopam	6.223	0.572
Methadone metabolite 1	6.223	0.572
Norcyclizine	6.321	0.581
Carbamazepine 10,11 epoxide	6.397	0.588
Terfenadine metabolite	6.397	0.588
Carbinoxamine	6.419	0.590
Dicyclomine	6.441	0.592
Clonidine	6.441	0.592
Benzoctamine	6.484	0.596
Brompheniramine	6.561	0.603
Diphenylpyraline	6.604	0.607

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TABLE II *(continued)*

Compound	Retention time	Retention time relative to prazepam
Brompheniramine metabolite 1	6.659	0.612
Brompheniramine metabolite 2	6.735	0.619
Nifedipine	6.756	0.621
Methadone	6.767	0.622
Dextromethorphan	6.767	0.622
Levomethorphan	6.789	0.624
Propranolol	6.811	0.626
Orphenadrine metabolite	6.844	0.629
Phenindamine	6.854	0.630
Nomifensin	6.854	0.630
Methaqualone	6.898	0.634
Orphenadrine metabolite	6.909	0.635
Monodealkylated disopyramide	6.963	0.640
Procyclidine	6.985	0.642
Dextropropoxyphene	6.996	0.643
Butriptyline	7.083	0.651
Amitriptyline	7.094	0.652
Cocaine	7.137	0.656
Atropine	7.148	0.657
Timipramine	7.213	0.663
Nortriptyline	7.213	0.663
Imipramine	7.246	0.666
Mianserin	7.246	0.666
Doxepin	7.279	0.669
Levorphanol	7.290	0.670
Mepyramine	7.290	0.670
Dextrorphan	7.311	0.672
Procainamide	7.333	0.674
Phenoxybenzamine	7.344	0.675
Chlorcyclizine	7.377	0.678
Desipramine	7.409	0.681
Protriptyline	7.431	0.683
Medazepam	7.442	0.684
Triprolidine	7.442	0.684
Benzhexol	7.475	0.687
Chlormezanone	7.496	0.689
Desmethylmianserin	7.572	0.696
Pentazocine	7.583	0.697
Terfenadine metabolite	7.627	0.701
Promethazine	7.681	0.706
Bupivicaine	7.714	0.709
Trimeprazine	7.844	0.721
Benztropine	7.942	0.730
Carbamazepine	7.986	0.734
Hyoscine	8.029	0.738
Diprophylline	8.051	0.740
Promazine	8.051	0.740
Maprotyline	8.084	0.743
Chlormethiazole metabolite 5	8.106	0.745
Iprindole	8.127	0.747
Amitriptyline metabolite 1	8.236	0.757
Phenylbutazone	8.312	0.764
Amitriptyline metabolite 2	8.378	0.770
Dothiepin	8.443	0.776
Amitriptyline metabolite 3	8.486	0.780
Codeine	8.563	0.787
Dihydrocodeine	8.584	0.789

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TABLE II *(continued)*

Compound	Retention time	Retention time relative to prazepam
Clomipramine	8.617	0.792
Norcodeine	8.639	0.794
Clemastine	8.660	0.796
Procyclidine metabolite 1	8.682	0.798
Orphenadrine metabolite	8.737	0.803
Procyclidine metabolite 2	8.748	0.804
Ethylmorphine	8.814	0.810
Dothiepin metabolite	8.845	0.813
Diazepam	8.932	0.821
Morphine	8.954	0.823
Procyclidine metabolite 3	9.009	0.828
Dibenzepine (major peak)	9.041	0.831
Normorphine	9.074	0.834
Hydrocodone	9.107	0.837
Dipipanone	9.194	0.845
Possible urine artifact	9.226	0.848
Methizene	9.313	0.856
Chlorprothixene	9.368	0.861
Disopyramide	9.400	0.864
Chlorpromazine	9.422	0.866
3-monoacetylmorphine	9.585	0.881
Methotripmeprazine	9.640	0.886
6-monoacetylmorphine	9.694	0.891
Thebacon	9.738	0.895
Didesmethylchlorpromazine	9.759	0.897
Desmethylchlorpromazine	9.759	0.897
Nordextropropoxyphene	9.901	0.910
Trimethroprim	10.06	0.825
Midazolam	10.33	0.949
Chloroquine	10.34	0.950
Temazepam	10.35	0.951
Promethazine metabolite 1	10.37	0.953
Chlormethiazole metabolite 6	10.48	0.963
Metachlopromide	10.48	0.963
Flurazepam metabolite 1	10.55	0.970
Bromazepam	10.68	0.982
Diamorphine	10.76	0.989
Prazepam	10.88	1.000
Promethazine metabolite 2	10.99	1.010
N-1 (hydroxyethyl) flurazepam	11.02	1.013
Trifluperazine	11.02	1.013
Phenazocine	11.32	1.040
Dibenzepine (minor peak)	11.32	1.040
Acepromazine	11.48	1.055
Flurazepam metabolite 2	11.52	1.059
Flurazepam	12.10	1.112
Quinidine	12.42	1.142
Quinine	12.56	1.154
Chlorpromazine 7-OH metabolite	12.72	1.169
Chlorpromazine sulphoxide	13.05	1.199
Dextromoramide metabolite	13.32	1.224
Chlorpromazine metabolite 2	13.49	1.240
Dextromoramide	13.94	1.281
Haloperidol	14.10	1.296
Alprazolam	14.19	1.304
Prochlorperazine	14.38	1.322
Quinine metabolite	14.39	1.323

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TABLE II *(continued)*

Compound	*Retention time*	*Retention time relative to prazepam*
Fluphenazine	15.09	1.387
Etorphine	15.20	1.397
Dimethothiazine	15.55	1.429
Thioridazine	16.19	1.488
Verapamil	16.60	1.526
Pholcodine	16.76	1.540
Strychnine	16.80	1.544

but also for any metabolites which may be present in the samples.

A standard mixture of commonly encountered drugs was chromatographed prior to any samples (fig. 2). This standard contained

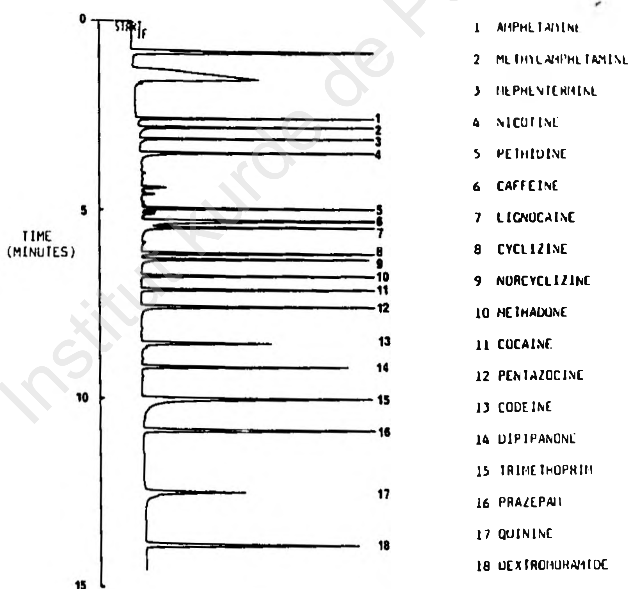

FIG. 2. — Chromatography of the standard mixture.

two polar drugs, trimethoprim and quinine. It was found that if the injection liner or column were contaminated, these peaks began to tail and even disappear completely. The chromatography was restored by immersing the injection liner in chromic acid overnight, rinsing in methanol and immersing in 10 % dichlorodimethylsilane in toluene. The liner was then heated in

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an oven at 300°C overnight before replacement. If necessary the front 15 cm of the analytical column was removed as well.

Reproducibility of retention data.

Example traces of this standard mixture were retained over a four-month period. These were then used to calculate the coefficient of variation for the relative retention times (table IV).

TABLE IV

Variability of retention times (relative to prazepam) over a 4 month period

Drug	*Mean relative retention time*	*Standard deviation*	*Coefficient of variation %*
Amphetamine	0.237	0.0009	0.37
Methylamphetamine	0.258	0.0008	0.31
Mephentermine	0.287	0.0004	0.30
Nicotine	0.321	0.0008	0.25
Pethidine	0.457	0.0006	0.13
Caffeine	0.486	0.0006	0.12
Lignocaine	0.503	0.0006	0.11
Cyclizine	0.567	0.0005	0.09
Norcyclizine	0.581	0.0005	0.08
Methadone	0.622	0.0005	0.08
Cocaine	0.656	0.0005	0.07
Pentazocine	0.697	0.0003	0.04
Codeine	0.787	0.0005	0.06
Dipipanone	0.845	0.0004	0.05
Trimethroprim	0.925	0.0027	0.29
Quinine	1.154	0.0039	0.34
Dextromoramide	1.281	0.0006	0.05

The earlier eluting drugs (amphetamine to nicotine) are more prone to variability. These drugs elute during the 40°C/min. ramp which appears to exceed the ability of the equipment to control the oven temperature precisely. Even so, there are relatively few drugs that elute during this section of the temperature programme, and the accuracy is sufficient to allow identification of these. The variation in relative retention times of other drugs is very low, the exceptions being those drugs which are relatively polar and tend to give tailing peaks. These include quinine and trimethoprim as already mentioned and, to a lesser extent, caffeine. Since compiling our library of retention data in 1984, three capillary columns have been used. We have found no need to alter the recorded retention data with each new column. Figures 3 and 4 show example chromatograms from routine cases.

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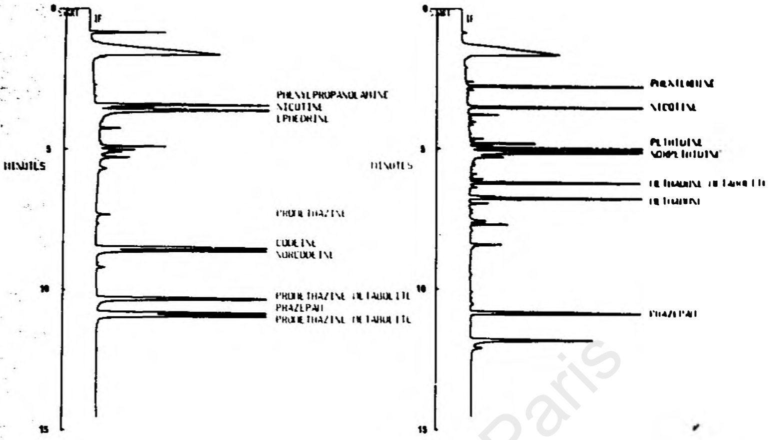

FIG. 3. — Chromatograms of sample extracts from patients taking *a)* Phensedyl (cold medicine containing ephedrine, codeine and promethazine) ; *b)* phentermine, methadone and pethidine.

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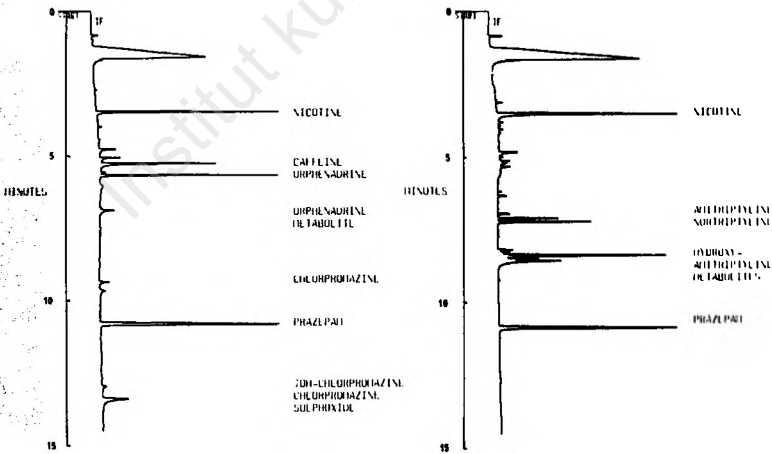

FIG. 4. — Chromatograms of sample extracts from patients with psychotic disorders and receiving treatment with *a)* chlorpromazine and orphenadrine ; *b)* amitriptyline.

Sensitivity of detection.

The quantitative limits of detection for the drugs listed in table V were determined by extracting and chromatographing blank urine samples spiked at concentrations of 0.1, 0.25, 0.5, 1.0 and 2.0 mg/l. Most drugs were detectable at the 0.25 mg/l level. Dextropropoxyphene is extensively metabolised and normally only nordextropropoxyphene and other degradation products are detected in urine. These metabolites were not available for study, and therefore no limits of detection could be assigned.

Users of drug abuse services often enquire as to how long after a dose the drug remains detectable in the urine. Accordingly, drug-free volunteers were given single therapeutic doses of drugs and asked to collect urine samples at 4, 8, 12, 24, 36 and 48 hours post ingestion. All drugs were taken orally.

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TABLE V

Sensitivity data for some drugs of abuse

Drug	*Detection limit (mg/l)*	*Dose*	*Volunteer*	*Time (hours) detectable by GLC*
Amphetamine	0.25	10 mg	1	36
			2	36
			3	48
Phentermine	0.25	30 mg	1	12
			2	48
Codeine	0.25	60 mg	1	8
			2	12
			3	12
Dihydrocodeine	0.25	30 mg	1	12
			2	12
			3	24
Methadone	0.25	10 mg	1	48
Dextromoramide	0.25	10 mg	1	ND
			2	ND
			3	ND
Dextropropoxyphene	Not known	30 mg	1	48*
			2	48*
			3	48*

* As metabolites.

Methadone and dextropropoxyphene were detectable in all urine samples including the 48 hour samples. Phentermine was detectable for 48 hours in one volunteer, but was only detected in the

4 and 12 hour samples from the other subjects. Amphetamine was detectable for between 24 and 36 hours post-dose, dihydrocodeine for 12 to 24 hours and codeine for 8 to 12 hours. Dextromoramide was undetectable in any sample from the three volunteers given this drug. This supports the findings of other workers, that very little unchanged drug is excreted in the urine (A Clatworthy, personal communication).

CONCLUSION.

Wide bore capillary GLC has proved to be an effective addition to a drug abuse screening service. The equipment requires very little maintenance and the chromatography has proved far more reproducible than that previously obtainable by using packed columns.

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Morphine/morphine metabolite ratios in blood after heroin overdose

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SUMMARY.

Morphine, the active metabolite of heroin is rapidly inactivated by glucuronidation at the 3 carbon. Unconjugated morphine was measured in blood using a coated tube radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA) and GC/MS. The RIA kit shows less than 0.2 % cross-reactivity with codeine and morphine-3-glucuronide (Matejczyk and Kosinski J. For. Sci., 1985). GC/MS analysis of non-hydrolyzed blood using a cold 10 % trichloroacetic acid (TCA) precipitate method correlated well with the RIA results (r = .98, n = 100). Total morphine (morphine and conjugated metabolites) was quantitated using the GC/MS procedure after hydrolysis of the TCA precipitated blood extract at 100 C, 30 min. with conc. HCl.

Unconjugated morphine ranged from 0 % to 100 % of the total morphine. The average ratio of morphine/morphine metabolite was 0.72 (n = 190). In cases in which survival time after heroin injection was less than 3 hours (rapid death, Garriott and Sturner New Eng. J. Med, 1973), the morphine/morphine metabolite ratio averaged 2.3 (n = 32). Exceptions to this trend were always to lower ratios suggesting the accumulation of morphine-3-glucuronide in chronic heroin users as suggested by Baselt 1982 ; or more rapid metabolism in some individuals. No case was found in which the individual is known to have survived for greater than 3 hours but continued to maintain a high ratio of morphine to morphine-3-glucuronide (i.e. slow metabolizer of morphine). These findings are in agreement with the pharmacokinetics of morphine and morphine-3-glucuronide reported by Brunk and Delle Clin. Pharm. Ther. 1974, and by Berkowitw Clin. Pharm. Ther., 1975. One or two hours after an acute dose, the blood morphine glucuronide concentration exceeds the blood morphine concentration.

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Determination of morphine in exhumed corpse

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Analysis of autopsy material obtained from exhumed corpse raises numerous difficulties. Appearance of products of decay is accompanied by chemical processes which affect poisons included in the material and make it either difficult or even impossible to identify the original substance.

Morphine is one of many substances which undergo oxidation when kept in the air. The process may lead to formation of morphine N-oxide and oxydimorphine. These substances have been often identified in injections and morphine pharmaceuticals (1, 2, 3), in the process of morphine enzymatic oxidation (4, 5), as well as in decomposed viscera (6). It is also known that progressing decay of tissue leads to complete undetectability of morphine within about six months (7, 8).

In order to diminish the influence that tissue decay products might have on the results of chemical analysis of the exhumed corpse attempts have been made to use this kind of tissue in which the process of decay is the slowest. In such cases there is applied chemical analysis of bone marrow, lung muscle (9) and stripped muscle (9, 10, 11).

Progress in science and technology has allowed for including such analytical material in morphine detection as vitreous humour and hair, both demanding application of extremely sensitive analytical methods like radio-immunoassay or gas chromatography - mass spectrometry (12, 13, 14). Here we must remember that such sensitive methods appear useless when applied to samples in advanced stage of decomposition.

However, it happens that laboratories deprived of very sensitive and consequently, very expensive equipment suddenly face the problem of giving an answer to the question whether in delivered exhumation material there is any morphine or not. The problem

arose also in our case and we decided to cope with it using widely common laboratory equipment.

In August 1985 it was reported that J.P. aged 21, known to his family and the neighbours as an abuser of « hard » drugs including « compot », that is home-made heroine, had disappeared. In February 1986 militia received a report from a registered drug-taker, who explained circumstances of J.P.'s disappearing. The incident was said to have happened as follows. One day, about mid-August 1985, the testifier and J.P. took a « compot » injection (J.P. was said to have taken a « large » dose of the drug) and fell in narcotic sleep. When the testifier came to his senses he realized that J.P. did not show any signs of life. Afraid of criminal liability he stripped clothes off the corpse, sewet it up in two jute bags, and transported it out of the city on his bicycle. There he buried the body half a meter deep in the ground. The testifier showed the place to the militia.

The corpse was exhumed and underwent autopsy six months after death. Toxicological laboratory was delivered tissue fluid, the brain, the liver, a kidney, a lung, the stomach, hair and a rib bone. All tissues were in a relatively good state (they were compact, dark red in section, the stomach was filled with pulpy, fermented content).

METHOD.

Because we have mentioned above we carried out toxicological analysis using classical methods of extraction, thin-layer chromatography and ultra-violet spectrophotometry.

In order to obtain fluid phase of the tissues, they were freed of protein with various methods, hair and the bone underwent some additional treatment.

Hair was washed in detergent, degreased with acetone and hydrolyzed first in concentrated sodium hydroxide and then in concentrated hydrochloric acid. After alcalizing up to pH 9 with the use of carbonate buffer the sample was extracted with amyl acetate.

The bone was mechanically crumbled and next dissolved in concentrated hydrochloric acid while slight heating of the solution. After alcalization with carbonate buffer up to pH 9 the sample was extracted with the use of organic solvents.

Chromatographic analysis included the one-direction and two-dimensional techniques applied in several solvent systems.

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TABLE I

Protein separation and extraction methods

Amount of tissue		*Method*	*Solvent*
50 g	tissue fluid liver kidney brain lung stomach	Ammonium sulphate	Chloroform « rolling »
20 g	tissue fluid liver kidney brain lung stomach	Acid digest	Chloroform 3 × 20 cm^3 ——— Chloroform Isopropanol 9 + 1 3 × 20 cm^3
10 g	liver kidney	Ammonium phosphosate	Chloroform 40 + 20 cm^3 Extrelut®
2 g	hair	Hydrochloric acid concentration	Amyl acetate 3 × 20 cm^3
9.7 g	rib bone	Hydrochloric acid concentration	Chloroform 3 × 20 cm^3 ——— Amyl acetate 3 × 20 cm^3

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TABLE II

Chromatographic analysis systems

Adsorbent	*Solvent systems*
• Kieselgel 60 G • DC Plastikfolien Kieselgel 60 • DC-Alufolien Kieselgel 60 F_{254} o Pre-coated TLC Plates Silica gel 60	One direction $CHCl_3$-Iso PrOH-NH_4OH 9+11+2 $CHCl_3$-Iso-PrOH-diethylamine 18+2+1 MeOH-NH_4OH 9+1 CCl_4-n-BuOH-MeOH-NH_4OH 4+3+2+0.2 Two dimensional chromatography I $CHCl_3$-Iso-PrOH-NH_4OH 9+11+2 II CCl_4-n-BuOH-MeOH-NH_4OH 4+3+2+0.2 I $CHCl_3$-Iso-PrOH-NH_4OH 9+11+2 II MeOH-NH_4OH 9+1

In order to visualize spots on plates we used ultra-violet light 254 and 366 nm, reagents of Dragendorff, Marquis, and Mandelin, as well as acidified iodoplatinate spray.

At a later stage of our research we found it necessary to use the ultra-violet spectrophotometry method to confirm the presence of morphine in the analysed material.

RESULTS.

Chromatograms prepared of tissue extract solutions in the ultra-violet light 254 and 366 nm revealed various spots characterized by different shades of fluorescence. They represented among others products of tissue decomposition. After spraying the samples with colouring reagents the extracts of liver and kidney showed morphine spots of Rf value equal to I 0.6, II 0.4. However, in other tissue samples we did not detect any traces of morphine.

Spectrophotometric quantitative analysis proved the existence of morphine at the approximately 20.0 mg % level in liver and confirmed its presence in the kidney.

In standard morphine solution previously stored for six months in room temperature we detected a spot of Rf value I 0.2, II 0.3 which, when treated with Marquis test, coloured green. Its absorption spectrum in 0.1 N sulphuric acid showed maximum absorption at 260 nm, which is characteristic for oxydimorphine. On the contrary, the substance was not detected in the examined tissues.

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DISCUSSION.

Employing several methods of extracting morphine out of the autopsy material let us isolate it from the tissue of liver and kidney.

Among the employed methods of isolating the substance the best one turned out to be freeing samples of protein, ammonium sulphate method and chloroform « rolling » extraction.

Extracts obtained in this way contained the greatest amount of morphine. However, the technique has its disadvantages, namely, apart from the basic analysed substance, there are extracted large quantities of extraneous material which blures the chromatographic picture.

Acid digest method did not improve the effectivity of extraction.

In our case Extrelut® also turned out useless as the obtained extracts were chromatographically undistinguishable and formed streaks from start to the head of the solvent.

The fact that we applied only ultra-violet spectrophotometry in order to confirm the presence of morphine in analysed tissues and later to determine its quantity significantly affected the obtained results. Limitations springing from low sensitivity of the method, particularly in case of determining compounds with low values of absorption coefficient, did not allow for giving an explicit answer to the question concerning the presence or absence of morphine in the remaining testing material, except for liver and kidney.

Despite obtaining negative results of analysis of hair and bone, which in our case could have been caused by possibly inappropriate preparation of samples for examination or by limitations of the accessible analytical methods, this material should still remain within the interest of forensic toxicologists as it undergoes decay to the smallest extent.

Moreover, it is worth considering that in the standard morphine which had been stored in room temperature for six months we detected oxydimorphine, whereas the substance was not identified in the examined tissue samples.

CONCLUSIONS.

Summing up the obtained results we believe that even in laboratories deprived of highly specialized equipment (radioimmunoassay, mass spectrometry and so on) it is possible to identify morphine in exhumed corpse provided it is well preserved and concentration of the substance in the tissue is relatively high.

In such cases it would be useful to apply a method freeing the material of protein, the ammonium sulphate method, the chloroform « rolling » extraction and two-dimensional thin-layer chromatography.

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A review of fatal cases involving drugs of abuse

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INTRODUCTION.

In the last few years there has been a general increase in drug abuse. This is evident from the fact that between 1980 and 1984 there was a 200 % increase in the number of new addicts registering with the Home Office (1,600 new addicts registered in 1980 compared with 5,415 in 1984). Secondly, during the same period there was an 800 % increase in the amount of heroin seized by H.M. Customs. The street value of the total drugs seized in 1984 exceeded £ 100 million (1).

The serious concern there is in the increase in the number of people abusing drugs prompted us to review fatal cases involving drugs of abuse.

The department covers North and West London and carries out toxicological analysis on any fatal cases in this area reported to the Coroner where death was thought to involve drugs. During the period 1975-1985, there were a total of 98 cases involving drugs of abuse.

METHODS.

The case histories and causes of death were taken from the post-mortem reports. All the cases were subjected to a full toxicological screen. Qualitative analysis was carried out using the standard methods described in any practical toxicological text book (2).

Quantitative analyses for methadone, cyclizine, dipipanone and ethanol were carried out using gas liquid chromatography (GLC) (3). Morphine and dihydrocodeine until 1984 were also measured

using GLC, morphine being first acetylated using acetic anhydride and pyridine. Levels of these two drugs in 1985 were measured by high performance liquid chromatography using electrochemical detection.

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RESULTS.

An analysis of fatal cases involving drugs of abuse.

The total number of deaths involving addicts has steadily increased over the period 1975-1985 as shown in figure 1.

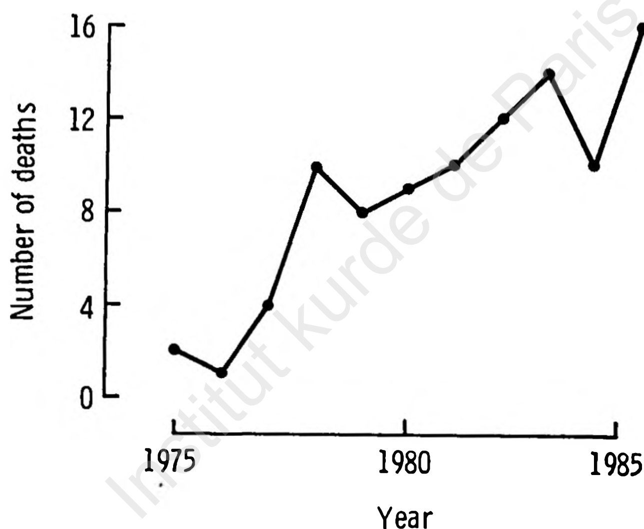

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FIG. 1. — Total number of deaths amongst addicts/year.

The number of females involved was 13 %. Figure 2 shows that with the exception of one case, the age range was 18-41 with the peak in the 25-29 age group. The exception was a 62 year old who had been an opium addict for many years. The causes of death showed that 56 of the addicts died directly from drug overdose, 39 from complications of the drug overdose, 1 was a natural death and 2 were unascertained due to decomposition. Those dying from complications of the drug overdose could be divided into those dying due to associated findings in drug overdose and those due to indirect complications following overdose. These results are summarized in table I.

An analysis of the case histories showed that 35 out of the 98 cases involved people who were registered addicts receiving treatment. A further 54 cases involved people who had a history of being drug users. In a further 6 of the cases there was no history of drug abuse but the scene of death revealed syringes, needles, etc. In only 3 cases was there nothing in the histories or at the scene to suggest that drugs of abuse were involved.

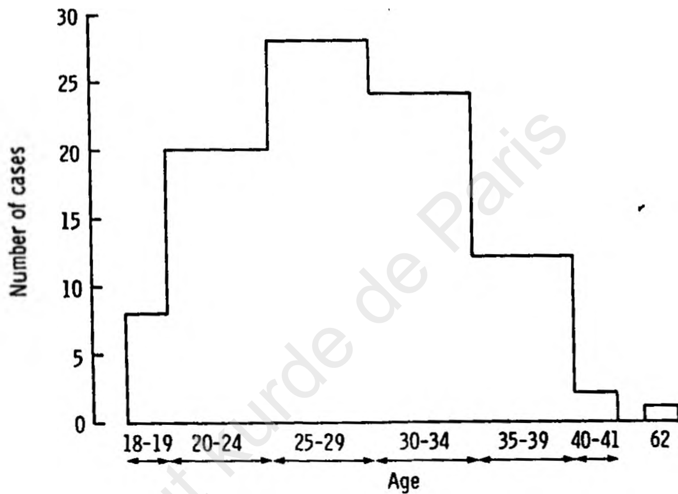

FIG. 2. — Age distribution.

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TABLE I

Death due to complications of drug overdose

a) Associated findings in drug overdose.

	No. of cases
Inhalation of food/vomit	11
Asphyxia	4
Haemorrhage	4
Drug addiction	3
Multiple injuries	2

b) Indirect complication following overdose.

	No. of cases
Bronchopneumonia	8
Myocarditis/endocarditis	3
Acute Pulmonary Oedema	2
Acute liver failure	1
Pyaemia	1

An analysis of the drugs detected in fatal cases involving drugs of abuse.

These findings are summarized in table II. The main drugs we encountered were morphine, methadone and diconal. A breakdown of the number of cases per year of each of these drugs is shown

TABLE II

Summary of drugs detected

Drug	*Total cases*	*Causing death alone*
Morphine	38	9
Diconal*	24	11
Methadone	24	4
Barbiturate	7	0
Dihydrocodeine	7	5
Amphetamine	5	0
Dextromoramide	3	1
Cocaine	2	1

* Diconal = 10 mg dipipanone HCl + 30 mg cyclizine HCl.

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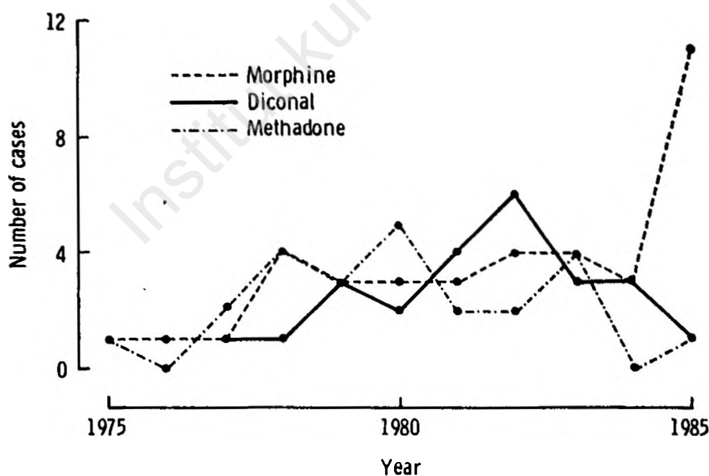

FIG. 3. — Number of cases per year.

in figure 3. In un-registered addicts, 49 % of cases involved morphine, 25 % methadone and 24 % diconal compared with registered addicts where 31 % of cases involved morphine, 29 % methadone and 29 % diconal.

Drug levels found in fatal cases involving drugs of abuse.

These are summarized in table III.

TABLE III

Drug levels in fatal cases

Drug	Fatal levels (μg/ml)			Non-fatal levels (μg/ml)		
	Number	Mean	Range	Number	Mean	Range
Methadone	4	2.7	1.6 - 3.6	7	1.76	0.1 - 6.2
Dihydrocodeine	5	4.39	2.35 - 7.20	1	1.2	
Dipipanone*	11	1.28	0.1 - 6.2	2	0.55	0.4, 0.69
Cyclizine*	11	2.91	0.5 - 14.5	2	1.88	0.8, 2.95
Morphine	9	0.83	0.16 - 21	4	0.29	0.04, 0.82
Morphine**	7	0.56	0.13 - 11	2	0.15	0.26, 0.04
Ethanol (mg %)	7	116	52 - 151	2	83	126, 40

* Taken together in the preparation Diconal.

** Morphine and ethanol taken together.

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DISCUSSION.

The age range and sex ratio found in this study can be compared with the results from a similar study carried out in Denmark in 1982 (4). This Danish study found 20 % of the deaths amongst addicts involved females which compares with our figure of 13 %. The age range in the Danish study was found to be 19-35 with a peak at 28, which can be compared with the range we found of 18-41 with a maximum in the late 20's. The sex ratio amongst dead addicts is not reflected in the sex ratio seen in the addicts attending the Charing Cross drug dependency unit where since 1980, out of an average of 194 new patients registering each year 38 $\pm$ 4 % were female. The age ranges are similar, the majority of addicts attending the unit are also in their late 20's.

Over the period 1975-1985 we have seen a steady increase in the number of deaths involving drugs of abuse. The main drugs encountered have been morphine, methadone and diconal. Cases involving diconal steadily increased from the time when the first case was encountered in 1977 until 1982. Since then this trend has been reversed, probably due to closer supervision of general practitioners prescribing this drug. Only one case involved diconal in 1985. This trend has also been reflected in a low incidence of cyclizine and dipipanone detected in urine

samples received from the drug dependency unit. There was evidence to show that in 9 out of 11 fatal cases of diconal the addicts had crushed up the tablets and injected the residue.

CONCLUSION.

Our survey has shown that in North and West London the number of deaths involving drugs of abuse are steadily increasing. The majority of these deaths involve men in their late 20's. The major drugs involved were morphine, methadone and diconal. The ratio of un-registered to registered addicts was 2:1.

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Some problems concerning narcotic drugs used in Poland

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Different pieces of evidence connected with drug addiction have been used as materials for studies carried out in the Institute of Forensic Research since the end of the sixties, the number of expertises concerning these questions being constantly on the increase. The activity of the Institute in this field permits the listing of the stupefacient drugs used by addicts (1), the following of the methods of their illicit production (2) and the ways and usable forms in which they are introduced into the system. The objective of such studies is to identify the substances received (3) and to analyze the biological material obtained from living people and, in cases of fatal poisonings, from corpses. The results of these examinations constitute the basis for the opinions pronounced in connection with the cases for drug addiction.

While surveying the expertises made in the Institute, we can find that in Poland drug addiction consists in applying various substances as substitutes for genuine narcotic drugs the use of which is characteristic of the wertern countries. Since the smuggling of and illicit traffic in genuine narcotics occur only sporadically, a practice was made of using substitutes. A detailed discussion of the substitutes used by addicts in this country till 1978, their classification and the legal problems connected with their use have been presented in an earlier paper (4). Since then the use of some substitutes has been given up, mainly owing to effective preventive actions (e.g. the withdrawal of some substances and preparations from sale and the dispensation of some medicines exclusively on the basis of physicians' prescriptions).

As regards the use of various substances in order to evoke the symptoms of stupor, the ingenuity of drug-addicts is practically without limit.

Some of the products widely used in everyday life, housekeeping and industry are adopted as substitutes for stupefacient drugs. They are generally characterized by low toxicity and their inclusion among such substitutes is caused by the unusual method of their preparation and the way of their introduction into the system, inappropriate for such preparations. As examples of the use of these substitutes we may mention, among others, the practice of drinking a concentrated infusion of tea (a 50-gramme package of tea in less than a glassful of water) brewed with an addition of tobacco and, earlier, also with the herb mixture Astmosan (consisting of Folium Stramonii, Folium Salviae, Folium Belladonnae and Herba Lobeliae). The sniffing of vapours of roasted detergent powder and the use of roasted banana peels are being slowly abandoned.

A significant group of substitutes comprises medicines, which taken incompatibly with medical recommendations bring about symptoms identified with intoxication. It includes Trihexyphenidyl and benzodiazepines taken together with alcohol and also Dimenhydrinate.

Another group of substitutes contains substances characterized by high toxicity. These are different sorts of organic solvents (e.g. trichloroethylene, chloroform, toluene, etc.). Some of them have narcotic properties and it is just for these properties that the addicts use them to drug by breathing in their vapours. Not only pure substances but also ready preparations, e.g. various glues made on the basis of solvents, are used for inhaling. Drugging is started immediately after the opening of a package containing such a preparation or by placing it in a plastic bag and pulling the bag over the head. This procedure often leads to death by suffocation.

The use of substitutes prepared from various vegetable raw materials has been increasingly often recorded recently.

Decoctions of the fungi Amanita muscaria and Amanita pantherina, very common in our forests, are used as substitutes for fungi of the genus Psylocybe, broadly described in literature and unavailable in Poland. Both these species contain two groups of antagonistic active substances : muscarine of parasympathomimetic action in one group and psychotropic derivatives of

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3-hydroxyisoxazol (ibotene acid, muscymol and muscazon), the effect of which resembles that of atropine, in the other.

A search for substances producing states of excitation, including coloured visual hallucinations (like Lophophora williamsi), led to the administration by direct intravenous injections of the sap of various cacti grown in Poland and void of mescaline content.

Parallel to all those substances or products mentioned above, we have been observing the use of genuine narcotics, such as derivatives of morphine and products of Cannabis sativa, obtained by unconventional methods of home manufacture. Cannabis sativa plants are most frequently provided for examination in the form of fragmented and classified dried plants, inflorescences, small leaves, seeds and stalks. These plants are grown in kitchen gardens, flowerpots and greenhouses. Drug-addicts know which parts of plants contain the largest amounts of biologically active substances and that their content depends also on the methods of growing. Even to 0.4 % Δ 9THC was found for marihuana derived from the thus grown hemp. A form called Polish hashish has also been met with. It is the product of the soaking of dried material obtained from whole plants in organic solvents and, after the evaporation of the solvent, suitably shaped, e.g. into globules. In this form it is used for drugging by smoking.

The ever increasing use of the products of Papaver somniferum, notably of poppy straw or sap of unripe poppy heads, has been noted for six years. This very practice constitutes the greatest danger to health and even life as regards the youth of our country. The brown clouded and non-sterile products of such primitive home processing of Papaver somniferum usually contain variable amounts of morphine, codeine and other alkaloids, like narcotine, narceine, papaverine and thebaine. The most important of them, morphine, occurs in amounts from 2.5 to 20 mg per 1 ml of a solution of this product. The addicts subject these products to acetylation with acetic anhydride, and after this process the presence of heroin and monoacetylmorphine is found in them. They also add diazepam to some of these products.

Up to 1983, in the evidential materials provided to the Institute, i.e. final products of the primitive home manufacture of stupefacient drugs from poppy straw and poppy milk, the occurrence of heroin or monoacetylmorphine had been detected only in single cases. In 1983 heroin or monoacetylmorphine or both these substances together were found in 11 % of the cases of this type, especially in slightly acid products or in dry residues.

However, the heroin content was more than ten times lower than the morphine content. As a result, the necessity arose to examine heroin for its stability in such products.

Final products of the treatment of poppy straw by the production techniques for stupefacient drugs, recurring most frequently in the descriptions given by addicts, were used in the studies of heroin stability carried out in the Institute (5). Poppy straw was macerated in hot water. The solution obtained was decanted and poured out on to ion-exchange resin. After the adsorption of organic substances from the solution on the resin, their desorption into an ammonia solution was made. The ammonia solution was evaporated next and the dry residue obtained was purified by flooding with an organic solvent and stirring with a cotton-wool twist.

After the evaporation of the solvent, acetic anhydride was added to the residue and heated in order to acetylate the morphine. The productivity of the process of acetylation reached as high as 90%.

The final products of the process were aqueous solutions of the dry residue obtained after acetylation. They were yellow and brownyellow in colour and showed an acid reaction (pH 4-6) and so such as was observed in some solutions produced in evidence in law-suits for drug dependence. The solutions were divided into halves, of which one was put to tests directly and the other, prior to its analysis, was alkalized with acid sodium carbonate to pH 7-8, or the value found in the solutions impounded from drug-addicts.

The separation and identification of alkaloids contained in the products obtained were performed by the method of thinlayer chromatography. After its separation from other alkaloids by thin-layer chromatography heroin was identified by the infrared spectrophotometric method using a FTS-15B/D spectrophotometer of Digilab production.

The results of these studies indicate that heroin contained in the products of the home processing of poppy straw decomposes while being stored and it may undergo a complete decay if its storage is unduly prolonged. Its stability depends also upon storage temperature. The storage of heroin-containing products in a refrigerator delays its decomposition, which also proceeds more slowly in slightly acid and pure solutions. However, it is most stable in a non-aqueous environment.

In this connection a failure in the demonstration of the presence of heroin in law-court exhibits which are final products of the

processing of poppy straw does not exclude its previous occurrence there, for it may have undergone a decomposition in the period preceding the delivery of these materials for analysis.

The products of the primitive processes described are most often introduced into the system by means of intravenous injections. The raw material used for production is of unknown origin and of unknown active-substance content. The product obtained by such a primitive technique contains not only alkaloids but also other constituents of poppy straw or milk. It may also be infected with bacterial and fungal floras. Its unknown qualitative and quantitative composition causes that doses taken differ from each other. It is therefore hard to estimate the action of the dose administered upon the human organism in respect of both its stupefying power and toxicity. Drugs are often injected using a common needle, which results in the transmission of diseases. A high morbidity rate is observed among drug-addicts as regards infectious jaundice and implant syphilis.

The above-mentioned factors constitute one of the main causes of high mortality among the drug-addicts in Poland.

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Fatal cases of poisoning in Denmark following ingestion of morphine from opium poppies

by A. STEENTOFT*, K. WORM* and E. KAA**

SUMMARY.

In Denmark it is legal to grow opium poppies and to sell dried Danish poppy capsules.

This has resulted in a misuse of morphine from both fresh and dried poppy capsules, and several deaths occurred among narcotic addicts solely or partly caused by these opium poppies.

In this paper the deaths caused by morphine from opium poppies in Denmark in the period 1982-1985 will be presented.

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The cross reactivity of some opiate immunoassays to non-prescribed narcotics

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INTRODUCTION.

Immunoassays for the detection of drugs of abuse have become a widely used technique. They have proved particularly advantageous in drug screening programmes run outside the laboratory (1). The increasing demand for drug abuse screening programmes within general occupational health schemes is likely to lead to their increasing use. The opiate assays, in particular, are often only group specific and will react with a wide range of structurally similar compounds. Some of these compounds are found in proprietary non-prescribed analgesics and cough medicines available in the United Kingdom. Previous studies have shown considerable reactivity toward some opiate immunoassay by some of these compounds (2, 3). This lack of specificity will therefore cause considerable problems in interpreting a positive result. This study looks at the reactivity of four preparations given either in single doses or in doses representative of a whole days dosage. Samples were taken and analysed by various commercially available immunoassay techniques and thin layer chromatography.

MATERIALS AND METHODS.

One proprietary analgesic and three proprietary cough medicines were studied. The analgesic was Paracodol [8 mg Codeine Phosphate, 500 mg Paracetamol (Fisons)]. The cough mixtures were Venos Cough Medicine [1.5 mg/ml Noscapine (Beecham)], Phol-

codine Linctus [1 mg/ml (Boots)] and Actifed Compound Linctus (2 mg/ml Dextromethorphan, 6 mg/ml Pseudoephedrine).

The total dose, dosage, number of subjects in each trial and the time of collection of urine samples is given in tabel I. All subjects were healthy volunteers, chosen from four males and three females aged 24-34 years. No dietary restrictions were imposed while on the trial. All urine samples were stored at 4°C prior to analysis.

TABLE I

Trial protocols

Drug	*Total dose (mg)*	*Dosage*	*Number patients in trial*	*Time of urine samples*
Pholcodine	5	Single	5	0, 1, 2, 4, 6, 8, 12 then every 12 hours
	20	10 mg every 2 hours	3	0, 2, 24 then every 12 hours
Codeine	8	Single	5	0, 1, 2, 20, 24 hours
Noscapine	60	15 mg every 4 hours	4	0, 8, 24 hours
Dextromethorphan/ Pseudophedrine	60/180	20/60 mg every 8 hours	4	0, 2, 8, 24, 48, 56, 72, 96, 108 hours

TAKE
10 mls Urine
2 mls 1M Acetate Buffer (pH 4.5)
0.05 mls β-Glucuronidase/Aryl Sulphatase

↓ 37° C Overnight

ADD
2 ml pH 9.0 Buffer (Saturated NH_4Cl/NH_3)
NaCl (to saturate) and 200 μl NH_3 (0.88 SG)
7 ml Chloroform/Propan-2-ol (9 : 1)

DISCARD AQUEOUS
↓ MIX, CENTRIFUGE

Evaporate Chloroform/Propan 2-ol to dryness
Reconstitute in 0.1 ml Methanol for TLC

FIG. 1. — *Sample preparations for TLC.*

All urine samples were analysed by Thin Layer Chromatography (TLC) and the extraction procedure is shown in figure 1. The extract was divided between two TLC systems : *a)* Ethyl Acetate/Methanol/Ammonia (0.88SG) (85/10/5) on Silica plates and *b)* Chloroform/Methanol (4/1) on Silica plates washed with 0.1M methanolic sodium hydroxide. Samples were also analysed

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by EMIT-dau and EMIT-st (Syva) until consistently negative. Based on this information, selected analyses were made using Agglutex (Roche) and two Haemaglutination Inhibition (HI) assays - Drug Test Opiates (Boehringer) and the HI-M test (American Drug Research Institute).

All immunoassays were run according to the manufacturers instruction. Detection limits of the various assays were determined on drug free urine which had been spiked with the test drug.

RESULTS.

These are given in tables II and III. The detection limits of Morphine, Codeine, Pholcodine, Dextromethorphan and Noscapine by TLC, EMIT, Agglutex and Drug Test Opiates are given in table IV.

TABLE II

Drug	*Number of subjects*	*Assay*	*Time (hours)*		
			Initially —ve	*+ve*	*—ve*
Codeine	5	T, Ed Es	0	1 20	24
Noscapine	4	T, Ed, Es HI, Ag	0-24		
Dextromethorphan . .	4	T, Ed Es, Ag	0-56		
Pseudophedrine . .	4	Ed	0	2-72 (n = 2) 2-96 (n = 2)	96 (n = 2) 108 (n = 2)

TABLE III

Drug and assays	*Subject*	*Time (hours)*													
		0	*1*	*2*	*4-12*	*24*	*36*	*48*	*60*	*72*	*96*	*108*	*120*	*136*	*144*
Pholcodine	a	—	+	+	+	+	+	+	+	+	—				
(5 mg)	b	—	—	—	+	—	—								
T, Ed, Es	c	—	+	+	+	+	—	+	—						
HI, Ag	d	—	+	+	+	+	+	—	—						
	e	—	+	+	+	+	—	—	—						
Pholcodine	a	—	NT	+	+	+	+	+	+	+	—	+	—	+	—
(20 mg)	b	—	NT	+	+	+	+	+	—	+	+	—	—		
T, Ed	c	—	NT	+	+	+	+	+	+	+	+	—	—		
Es, HI, Ag															

Key to tables II and III. — T = TLC, Ed = EMIT-dau, Es = EMIT-ST, HI = Haemaglutination Inhibition (Drug Test Opiate), Ag = Agglutex, NT = Not Tested.

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TABLE IV

Detection limits (mg/l)

	Morphine	*Codeine*	*Pholcodine*	*Dextro-methorphan*	*Noscapine*
TLC	1	1	0.5	1	4
EMIT	0.3	0.3	0.5	> 5	> 5
Agglutex	0.3	0.2	0.5	> 5	> 5
HI (ADRI)	0.2	ND	ND	ND	ND
HI (DTO)	0.2	0.2	0.5	> 5	> 5

Key. — HI (ADRI) = Haemaglutination (ADRI), HI (DTO) = Haemaglutination (Drug Test Opiate), ND = Not determined.

DISCUSSION.

It is important to know the extent that proprietary preparations containing narcotics and stimulants interfere with commercial immunoassays. This enables better interpretation of results. It reduces the chance of false positives whilst screening for drugs of abuse either in the hospital or general population.

It has previously been shown by Posey *et al.* (4) that a total dose of 150 mg Codeine, taken in 5 doses of 30 mg every 4 hours could be detected for up to 58 hours after the last dose. This was by RIA (Roche Abuscreen, cutoff 0.3 mg/l Morphine equivalents). This represents a large analgesic dose as the British National Formulary (BNF) suggests 200 mg as the maximum daily dose. This study shows that even the smallest dose normally given (i.e. 8 mg) would result in positive opiate assays for 24 hours, commencing from one hour after dosing. The dosage of two proprietary cough medicines was chosen to represent a maximal daily dose. Under these conditions, neither Noscapine or Dextromethorphan interferred in any of the opiate assays. However, Pholcodine was detectable in the EMIT Amphetamine assay for 2 hours up to 72 hours, extending to 96 hours in one subject.

Pholcodine excretion has been studied by Svenneby *et al.* (1983), who could detect an oral dose of 70 mg for 9-10 days by EMIT (cut-off 0.3 mg/l) but in one individual for up to 40 days by RIA (Roche Abuscreen, cut-off 0.04 mg/l). This however, is in excess of the maximal daily dose of 60 mg recommended in the BNF. Cordonnier *et al.* (1984) gave an oral dose of 20 mg and detected Pholcodine for over two weeks by RIA (Roche Abuscreen,

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cut-off 0.3 mg/l). This compares to 96-136 hours over which it was detected in this study when given at the same dose, but using EMIT (cut-off 0.3 mg/l). This may reflect different cross reactivities of the antibodies. It was hence significant that even a small dose of 5 mg was positive within the first 1-4 hours and was detectable between 12 and 72 hours. Care is needed in distinguishing between Codeine, Dihydrocodeine and Pholcodine on the TLC system used, as the Retention Factors are similar.

Fluctuation in the ability to detect Pholcodine are seen. In one subject [subject (b), table III] after 20 mg, the urine went negative at 60 hours, but was subsequently positive up to 96 hours, while in a second subject [subject (a), table III] after 12 hours the urine was alternatively positive then negative three times between 72-144 hours. This may represent a fairly constant excretion affected by a variable urinary volume.

The immunoassays used all gave very similar results. There was one discrepancy between EMIT-st and EMIT-dau on a 24 hour sample on the Codeine trial, due to the readings being closely distributed on either side of the calibrator. The Agglutex gave a false positive reaction on one blank urine, and the cause of this is being investigated.

This study shows that there can be significant interference in immunoassays from proprietary products. This has two implications :

1. *Clinical.*

The confidential nature of a patient undergoing treatment for drug addiction where the emphasis is on helping and caring for the patient, has meant that it is often clinically more useful to have a rapid, non-specific result, than a delayed result. Consequently, clinicians using rapid drug assay systems in this way must be aware of these likely interferences and have the facility to subsequently confirm positives.

2. *Medico-legal.*

The use of drug testing in occupational medicine and sports medicine necessitates that result must be confirmed by a method of sufficient sensitivity. TLC, with a typical detection limit of 1-2 mg/l may not be sensitive enough, and further analysis by Gas or Liquid Chromatography will be required.

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Pharmacokinetic studies of non-steroidal anti-inflammatory drugs in the racing greyhound

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SUMMARY.

Non-steroidal anti-inflammatory drugs are prohibited by the rules of the U.K. National Greyhound Racing Club during the racing of greyhounds and it is therefore essential to be able to detect and quantify these drugs in the urine of the dogs. In addition, it is important to know the metabolic fate of the drugs in the greyhound as both the rate of excretion and the metabolite composition are likely to be different from those established in man or in other species of dog.

In the present study, the metabolism and excretion of mefenamic acid, ibuprofen and phenylbutazone were studied in the greyhound following oral administration of the drugs at therapeutic levels. Four dogs were housed in a metabolic unit and serial samples of urine and blood were collected and analyzed by GC-MS and HPLC. Pharmacokinetic profiles were obtained for the drugs and compared with those for other species.

Using this data the time of dosing and the quantity of these drugs administered can be estimated. The value of using the diurnal creatinine excretion to correct for variation in urine volume was also assessed. This is likely to be of importance in the interpretation of concentrations of drugs and their metabolites in urine, given that it is impossible in practice to collect 24 hour urine specimens in racing dogs.

Systematic Toxicology, Standardization and Criteria for Data

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Screening by gas chromatography using wide-bore fused silica capillary columns

Temperature dependent behaviour of retention indices

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INTRODUCTION.

For systematic toxicological analysis, gas chromatography (GC) on packed columns with SE-30 or OV-1 as liquid phase and using Kovats retention indices (RI) is a recommended method for which data are available for over 1,700 substances (1). As retention indices are temperature-dependent, their documentation would require information about the temperature at which they were measured (2). This temperature dependent behaviour can be ignored, however, by using a sufficiently large search window of ± 50-60 units. Under these circumstance RI-values can also be determined under temperature programmed conditions (1).

Wide-bore fused silica (WFS) capillary columns, with an internal diameter of 0.53 mm and with a methylsilicone phase offer some important advantages over packed columns, such as higher separation power and inertness at a load capacity that is adequate for toxicological purposes.

Fortunately, RI's determined on these capillary columns show reasonably good agreement with those determined on packed columns and vice versa. Also under temperature programmed conditions retention indices on capillary columns show an inter-laboratory variability comparable with packed columns which is relatively large (3). Thus, in this way the advantage of capillary columns, the high separation power, is not utilized to its full extent.

The purpose of this study was to investigate the effects of some variables on the reproducibility of RI's using WFS columns.

EXPERIMENTAL.

Instrumentation.

A HP 5880 gas chromatograph (Hewlett-Packard) with a splitless capillary injection system was used in conjunction with a HP 7671 A automatic injector.

Three different fused silica capillary columns with an inner-diameter of 0.53 mm and with a chemically bonded methylsilicone phase were used :

A. Hewlett-Packard (HP-1) : 10 m in length, df = 2.65 μm.
B. Chrompack (CP-sil-5) : 10 m in length, df = 5.4 μm.
C. SGE (BP-1) : 25 m in length, df = 3.0 μm.

Helium was used as the carrier gas.

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Procedure.

Aliquots of 3 μl of solutions of individual substances or of a mixture of straight chain alkanes were injected. The substances used were obtained from commercial suppliers and dissolved in hexane to a final concentration of 0.5 g/l.

Dependent on carrier gas flow rate and column a temperature program was chosen so that a nearly straight line was obtained when the carbon numbers of the reference alkanes were plotted versus their times, and so that the C32 alkane eluted in about 25 min.

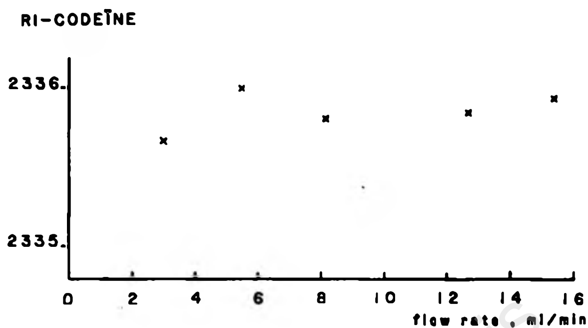
RESULTS AND DISCUSSION.

In order to investigate the variability of retention indices the following parameters were investigated : the carrier gas flow rate, elution temperature, and column characteristics such as length, liquid phase and its film thickness.

Carrier gas flow rate.

At an oven temperature of 200°C the retention index of codeine was measured at different flow rates. The substance was co-injected with the C_{23} and C_{24} straight chain alkanes. The retention

FIG. 1.



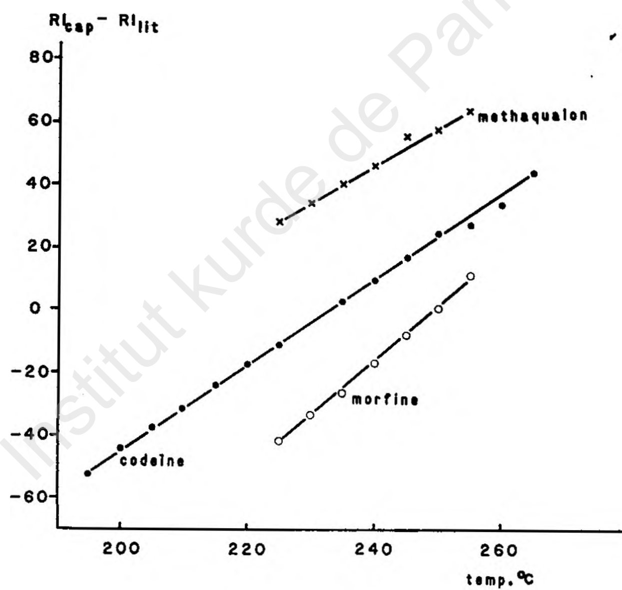


FIG. 2.

FIG. 1. — Retention index of codeine versus carrier gas flow rate.

FIG. 2. — Relationship between retention index found relative to literature values and column temperature in isothermal runs.

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indices found were very reproducible with a range of 0.33 retention index units (fig. 1), so that it can be stated the retention indices are virtually independent on the flow rate of the carrier gas.

Temperature.

In isothermal runs, but at different oven temperatures, retention indices were measured for three drugs : codeine, methaqualon and morphine. Figure 2 shows that an almost linear relationship is found between RI and oven temperature over a wide temperature range. With methaqualon and codeine some small deviations from the straight line were found, due to overlapping of the drug peak with one of the straight chain alkanes, which were injected together. The overlapping of the peaks prevented an accurate measurement of retention times.

It can also be seen that the slope of the lines are substance-dependent and that they are relatively steep. For codeine, for example, an increase in retention index of about 100 retention index units was observed, which makes it clear that temperature is a main cause of variability of retention indices.

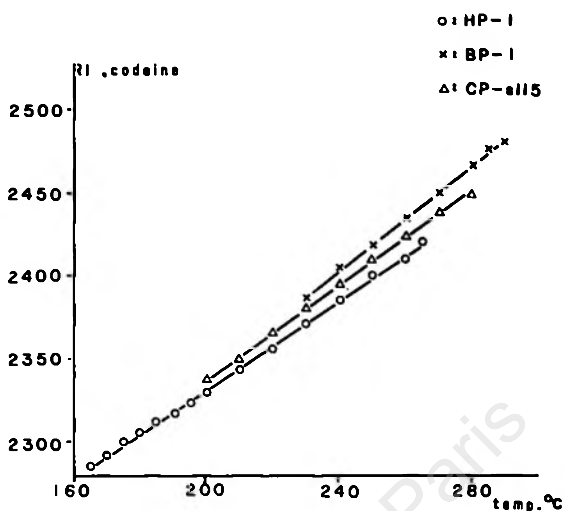
Column characteristics.

In order to investigate the potential variability of retention indices caused by differences in column, 3 columns were tested from 3 different manufactures : 2 with a length of 10 meters and 1 of 25 meters ; 2 columns had a film thickness of about 3 μm and 1 column a film thickness of 5 μm.

Retention indices for codein were measured on all three columns using isothermal runs, but at different temperatures. The results are depicted in figure. 3. It can be concluded that for the 3 columns a linear behaviour of retention index with temperature is found. Also, at a given temperature, the three columns yield slightly different retention indices. From these limited data, it can *not* be concluded whether these differences are caused by the column length, inertness, film thickness or the chemical composition of the liquid phase. Fortunately, at a given temperature the differences are so small in relation to the temperature effect that they can be neglected in practice.

From figure 2, it appeared that the slope of the line which relates RI and temperature, is substance-dependent. Figure 4 shows

FIG. 3.



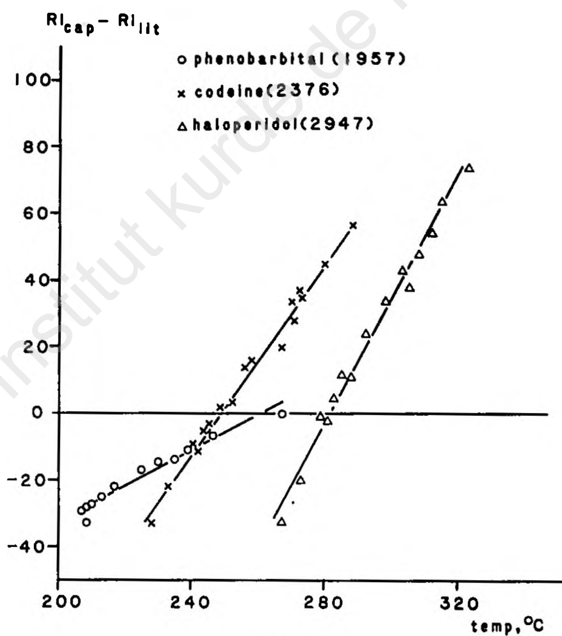


FIG. 4.

FIG. 3. — Relationship between retention index and column temperature of codeine for three different columns.

FIG. 4. — Influence of retention index on elution temperature during temperature programmed runs.

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that this effect is stronger visible for other drugs. It must be noted that in figure 4 the elution temperature was derived from temperature programmed runs and that data for the 3 columns were combined, so that the spread around the lines is slightly enhanced. It can also be seen that the slope (and thus the variability) is larger with increasing retention index.

FIG. 5. — Relationship between retention time and retention index of the 14 drugs of table I.

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Standardisation.

Due to the temperature dependent behaviour of retention indices a decrease in variability can be achieved by applying a series of isothermal runs at some fixed oven temperatures. However, in practice, temperature programmed runs are much more convenient. Therefore, we investigated another way to diminish interlaboratory variability, namely by using a set of drugs with RI values determined under standardized conditions and that cover a wide RI range. Figure 5 shows a plot of retention times of a mixture of 14 drugs versus their retention index, obtained by a temperature programmed run. The difference in retention index between each drug is about 150 units (table I).

TABLE I

Mixture of reference substances for the determination of retention indices on wide-bore capillary columns

	RI		*RI*
Amphétamine	1108	Methaqualon	2130
Ethinamate	1350	Trimipramine	2220
Barbital	1463	Codeine	2363
Allobarbital	1573	Nalorphine	2552
Pentobarbital	1715	Quinine	2779
Diphenhydramine	1856	Haloperidol	2929
Tripelennamine	1971	Strychnine	3114

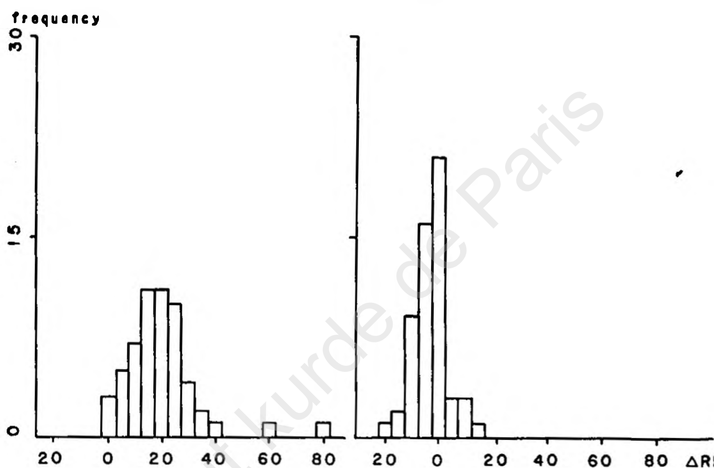

FIG. 6. — Frequency distribution of difference in retention index obtained for 57 drugs using 2 temperature programs.
Left : Retention indices calculated using straight chain alkanes.
Right : Retention indices calculated using figure 5.

As the curve is nearly a straight line, the retention index of an unknown peak can be determined accurately by interpolation between 2 bracketing drugs.

In this way, variability can be diminished as shown in figure 6 in which the frequency distribution of retention indices for 57 drugs are depicted, using straight chain alkanes (a) and using the mixture of drugs (b).

On the X-axis the difference in retention index between 2 different temperature programs on 1 column (HP-1) are depicted. When the drug mixture is used for measuring RI's, a smaller distribution is obtained then when using alkanes. Moreover, the mean value using the drug mixture is close to zero.

In order to simulate interlaboratory conditions the 3 columns were each used with 3 different temperature programs and reten-

tion indices were determined for 103 drugs based on the drug mixture. Figure 7 shows the frequency distribution of the standard deviations of the individual drugs (n = 9 ; 3 temp. programs × 3 columns). From this figure and from the ranges of retention index data per drug it can be concluded that with a search window of ± 15 RI units about 90 % can be found. However, when substances with RI greater than 3000 are excluded, about 95 % of the test population can be retrieved. The sub-

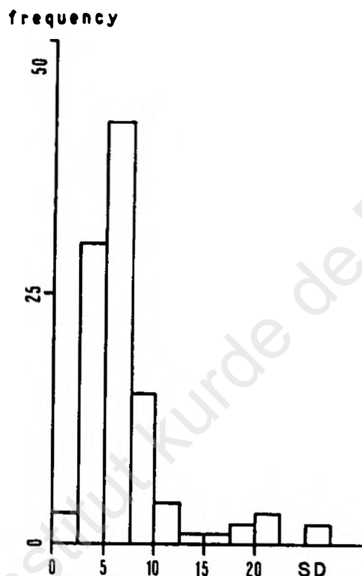

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FIG. 7. — Frequency distribution of standard deviation of retention index values for 103 drugs obtained under 9 different conditions.

stances with greater standard deviations are in general substances with bad chromatographic characteristics like underivatized benzoic acid and paracetamol.

CONCLUSION.

In capillary GC for screening temperature programmed runs are normally hard. Under these conditions, the use of a drug mixture, with known RI values for each drug, to determine retention indices of other drugs can produce data which are transferable from laboratory to laboratory with a variability much lower than

retention index data for drugs on packed columns measured with straight chain alkanes.

*
* *

Acknowledgment. — The authors wish to thank Techmation (The Netherlands) for their support by putting the SGE fused silica capillary column to their disposal.

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Study on the factors influencing the variability of retention index values in high pressure liquid chromatography

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SUMMARY.

Two HPLC columns were filled with different batches of the same 5 um ODS Hypersil stationary phase. The columns were used for analysis of selected acidic and basic drugs in one laboratory (Groningen) and then installed in the second laboratory (Heidelberg), where the same drugs were analyzed under the same conditions. As acidic drugs the barbiturates were analyzed (amobarbital, aprobarbital, barbital, butobarbital, methohexital, pentobarbital, secobarbital and talbutal) using the mobile phase methanol-phosphate buffer (40:60, pH 8.5). The basic drugs caffeine, chlordiazepoxide, codeine, methaqualone and strychnine were separated in the mobile phase methanol-water (75:25) containing 0.05 % ammonia (min. 25 %). The retention index (RI) values were calculated against a series of alkyl-aryl-ketones. Sodium nitrate was used as the dead time marker. The study showed that when the same column was used, instrumental conditions affected the value of the RI as well as the day-to-day variability. Batch-to-batch differences also occurred, particularly with the basic drugs. The results indicate that the combined effect of instrumentation differences and batch-to-batch differences can result a large overall variability of RI values in HPLC, thus necessitating large search windows in systematic toxicological analysis.

Recommended thin layer chromatographyc systems for systematic toxicological analysis with a data bank of R_F values on some 1100 toxicologically relevant substances

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SUMMARY.

Thin layer chromatography (TLC) is a very frequently applied technique to detect and to identify potentially harmful substances. In order to utilize the technique to the best advantage, optimum systems should be selected on the basis of their discriminatory power and reproducibility. Furthermore, to obtain reliable identification and to allow comparisons between data measured in different laboratories, it is of paramount importance that a collection of reference Rf-values be available, measured under well-defined conditions.

The STA-Committee has assessed the potential value of various TLC-systems with regard to their usefulness for screening purposes, based on their discriminatory power when used alone as well as when used in combination, and on their interlaboratory reproducibility. The following systems were finally selected as recommended systems : For acidic and neutral drugs : Chloroform-Acetone 80+20 ; Ethyl Acetate ; Chloroform-Methanol 90+10 ; Ethyl Acetate-Methanol-25 % Ammonia 85+10+5, all on silica gel plates. For basic drugs : Methanol ; Methanol-Butanol 60+40, 0.1 M/L sodium bromide ; Ethyl Acetate-Methanol-25 % Ammonia 85+10+5 ; Methanol-25 % Ammonia 100+1.5 ; Cyclohexane-

Toluene-Diethylamine 75+15+10 ; Chloroform-Methanol 90+10 ; Acetone, all on silica gel plates. The latter 4 systems use plates impregnated with 0.1 M KOH.

For these systems Rf-values were determined for a large variety of toxicologically relevant compounds, the values found were corrected by means of reference substances run at the same plate and the mean values were calculated on an inter- or intralaboratory basis. The thus obtained data base now contains mean corrected Rf-values for more than 1100 substances.

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A computerized colour-coded TLC system for the identification of unknown compounds in biological fluids

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SUMMARY.

The possibility of increasing the discrimination of a dual solvent TLC system by consideration of colour responses is examined. Methods of encoding and processing the data and problems encountered are described. The modified system has improved the incidence of identification of unknowns in emergency situations, whilst maintaining the simplicity and speed of the original technique.

INTRODUCTION.

Thin layer chromatography (TLC) has wide application in the detection and identification of drugs and poisons in both clinical and forensic settings. However, the collation and documentation of TLC data can be a problem in what is otherwise a simple and inexpensive analytical technique. A number of compilations of Rf value (distance migrated by the sample relative to the solvent front) have already appeared in the literature (1, 2) but contain only limited information on colour reactions. We believe these to be sufficiently important to warrant attention in their own right. The value of colour is assessed here as an additional discriminatory factor to Rf in a dual solvent system on the premise that, if there is a negligible correlation between colour and Rf value, further discriminatory power should be achieved.

The initial problem was in transforming highly subjective colour observations into a format which would provide reproducible and unambiguous data. Photographic recording (3, 4, 5) and preservative fixing (3) of TLC plates have been used previously as means of recording colour response. However, the need for carefully controlled lighting conditions and additional capital outlay for photographic equipment, or the difficulty in storing and indexing a large number of preserved plates make these approaches less practicable. An alternative strategem would be to match colour reactions to a pre-coded range of options, which could be easily computerized to facilitate processing and searching for unknowns (a particularly important consideration in an acute screening service such as that offered by this laboratory). In terms of speed and simplicity this appeared to be the most viable solution to the problem.

MATERIALS.

Equipment.

Schleicher and Schuell 20 cm × 20 cm polyacetate-backed silica gel TLC plates (Anderman and Co., Kingston-upon-Thames, Surrey, U.K.).

pH 9.0 buffered ToxElut extraction columns (Jones Chromatography Limited, Llanbradach, Mid. Glamorgan, U.K.).

Shandon S/P Chromotank 20 cm glass TLC tanks (Scientific Supplies, Vine Hill, London, U.K.).

One hundred μl spotting syringe [S.G.E. (U.K.) Limited, Milton Keynes, U.K.]. Quickfit spraying bottles (A.W. Dixon Limited, Beckenham, Kent, U.K.).

Ultra-violet viewing box (Ultra-violet Products Limited, Cambridge, U.K.).

IBM-XT personal computer with 10 Megabyte hard disk and single floppy disk drive linked to an IBM Proprinter (International Business Machines Corporation, Florida, U.S.A.).

« pfs : file » and « pfs : report » database components (IBM as above).

Reagents.

Chemicals, unless otherwise stated, were Analar grade supplied by BDH Chemicals Limited, Poole, U.K.

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Acetic acid, 35 % (880) ammonia solution (Aristar), ammonium metavanadate, bismuth subnitrate, chloroform (May and Baker Limited, Dagenham, U.K.), chloroplatinic acid (GPR), concentrated sulphuric acid, ethyl acetate, 37 % formaldehyde solution, isopropanol (propan-2-ol), mercurous nitrate, methanol (Rathburn Chemicals Limited, Walkerburn, U.K.), potassium iodide and potassium permanganate.

Drugs and metabolites were generously provided by their respective manufacturers.

The following spraying reagents were prepared and stored according to established methods (8) :

— Iodoplatinate.
— Mandelin's reagent.
— Mercurous nitrate.
— 50 % sulphuric acid.
— Concentrated sulphuric acid.
  37 % Formaldehyde solution.
— Dragendorff's reagent.
— 10 % Potassium permanganate.

METHOD.

Approximately 20 ml urine samples were eluted with 30 ml chloroform : isopropanol (9:1) through pH 9.0 buffered ToxElut columns into 50 ml capacity solvent-washed glass tubes. After evaporation under air at 60°C, residues were reconstituted with about 70 µl chloroform:isopropranol (9:1) and applied in six equal aliquots to one and one half scored thin layer plates according to the illustration in figure 1. For each of the six columns a solution of mixed standards was chromatographed alongside the sample extract. Each of these solutions contained four or five drugs with characteristic colour and/or shape responses which were discriminated from each other under the chromatographic conditions used.

Plates were chromatographed for 10 cm, two columns in methanol:ammonia (100:1.2) (MA) and four columns in ethyl acetate:methanol:ammonia (85:10:6) (EMA), and air dried for 30 minutes prior to spraying.

Saturation was maintained by lining the sides of the tanks with filter paper. Plates were run singly and solvents were renewed after 5 plates in MA, 3 plates in EMA or 2 days overall. For each

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drug in the standard mixtures the mean of 200 Rf values was used to calculate the expected value with a range of ± 2 standard deviations, which equated to ± 3 mm on the MA system and ± 5 mm on the EMA system in practical terms.

Each of the marked columns was sprayed according to the sequence below.

Columns 1 and 3 : Iodoplatinate reagent. This provided a colour-related cross reference between the EMA and MA systems. These were then oversprayed with Dragendorff's reagent.

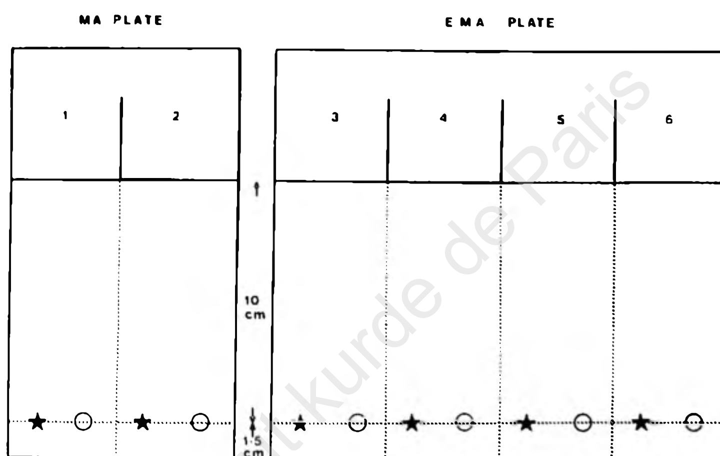

FIG. 1. — Illustration of plate scoring and sample application for TLC plates in MA and EMA chromatographic systems.
(★ = 5 μl methanolic mixture of standard compounds ; ○ = 1/6th portion of reconstituted sample extract).

Column 2 : Mandelin's reagent. The fluorescent properties of compounds were shown by subsequent examination under long wave (365 nm) UV light.

Column 4 : Mercurous nitrate. This is a good locating reagent for barbiturate-type compounds. Saturated and unsaturated ring structures could be distinguished by overspraying with potassium permanganate.

Column 5 : 50 % sulphuric acid. This is a good locator for phenothiazine-type drugs and their metabolites. The fluorescent properties of compounds could be examined under short wave (270 nm) UV light.

Column 6 : a modified Marquis reagent was used : very lightly sprayed formaldehyde solution heavily oversprayed with concen-

trated sulphuric acid. Fluorescent reactions were examined under short wave (270 nm) UV light.

Plates were read when strychnine (in the Mandelin's standard mix) changed from purple to orange. This was about 4 minutes after spraying. Rf was measured, to 1 mm precision, from the application point to the centre of any given spot using a standard ruler.

For reference data, methanolic solutions of compounds (1 g/l ; stored at —20°C, were spiked into 20 ml blank urine to concentration 5 mg/l, extracted and chromatographed as described above. If no responses were observed the compound was chromatographed again at a concentration of 25 mg/l. The methanolic solutions were also chromatographed directly to characterize those compounds which did not extract at pH 9.0. Since authentic patient urine samples reflect the metabolic profiles of drugs these were also included in the library whenever possible.

A reference chart was compiled to code colours and shapes. A range of 20 colours was chosen with regard to common responses to the spraying reagents used, each colour being coded as a two digit number — 01, 02, 03,...20. The overall spot colour was coded as a four digit number, the first two digits representing the outer colour of the spot and the second two digits the inner colour. Shapes were coded from a range of 11 initial letters e.g. round (R), streak (S), bar (B), etc. Observed data were matched to their *closest equivalent* on the chart. All responses were recorded on a data sheet as shown in figure 2. Within each spraying group Rf was recorded in mm (effectively a percentage figure) and the encoded visual data were entered under (where appropriate) primary and secondary location. Any unusual features were noted in the comment section.

These record sheets were filed alphabetically and the data were then entered onto the computer. As well as TLC data additional information was stored in the database. This included : GC data [OV1 retention time relative to tetraphenylethylene, Poly A 103 retention time relative to tetraphenylethylene and SE54 capillary retention index (6)] ; HPLC data (silica column retention time relative to imipramine and electrochemical/UV detector response ratio) (7) ; pKa and comments on metabolism and pharmaceutical usage. Figure 3 shows a database record completed for nortriptyline.

Selective lists were printed in alphabetical compound order, ascending order of MA Rf and ascending order of EMA Rf to

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REFERENCE

Acceptable Rf variation: MA ±3 EMA ±5

UNKNOWN ☑ *Example*

PATIENT SAMPLE ☑ PURE DRUG ☐

MIX	COMPOUND	Ref Rf	Act Rf	1st Location Rf	1st Location Col.	1st Location Shape	2nd Location Rf	2nd Location Col.	2nd Location Shape	COMMENTS
1	Dextromoramide	74	75	60	0216	R		0217		
	Cyclizine	61	55							
	Methadone	50	47							
	Morphine	37	35							
	Atropine	21	20							
2	Mianserin	61	60	60	1717	E				
	Imipramine	48	47							
	Nortriptyline	33	31							
	Strychnine	24	22							
3	Lignocaine	79	75	49	0216	R		0213		
	Disopyramide	69	65							
	Codeine	43	40							
	Indomethacin	10	09							
4	Glutethimide	78	75	49	1519	R		1519		
	Quinalbarbitone	60	56							
	Barbitone	43	40							
	Phenobarbitone	35	33							
	Diflunisal	19	18							
5	Trimipramine	82	50							
	Thioridazine	75	72							
	Trifluoperazine	67	62							
	Perphenazine	47	43							
6	Orphenadrine	75	71	49	1515	R				
	Pericyazine	60	56							
	Protriptyline	44	39							
	Morphine	26	23							

	MIX	FIRST LOCATION	SECOND LOCATION
MA	1	Iodoplatinate	Dragendorff
	2	Mandelin's	U.V. 365nm
EMA	3	Iodoplatinate	Dragendorff
	4	Mercurous Nitrate	Potassium Permanganate
	5	50% Sulphuric Acid	U.V. 270nm
	6	Marquis	U.V. 270nm

DATE: 13th August 19[illegible]

ANALYST: [illegible]

SAMPLE VOLUME: 20

ToxElut: ~~18~~/20

FIG. 2. — An example of a completed TLC data form (note composition of standard mixtures for each column).

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provide an accessible hard copy of the data in the laboratory. If a computer search was required to identify an unknown, the Rf values were entered as ranges dictated by the predetermined error windows (± 3 mm for MA and ± 5 mm for EMA) provided the Rf values in the standard reference mixture were also within these limits. For example, an unknown running at 40 mm in MA and at 56 mm in EMA would be entered as 37-43 and 51-61 respectively. Since the database is capable of simul-

RT/OV1: 0.50	MA/Rf: 28	EMA/Rf: 52	
RT/POLY A: 9999			
	1C1: 0302	3C1: 0302	5C1:
RI/SE 54: 2264	1Sh: A	3Sh: R	5Sh:
	1C2: 0102	3C2: 0102	5C2:
	2C1: 1919	4C1: 1919	6C1: 1717
	2Sh: A	4Sh: R	6Sh: R
	2C2: 1619	4C2: 1919	6C2: 1818
RTSil: 0.58	EC/UV: 18		

COMMENT: AMITRIPTYLINE METABOLITE; DRUG IN OWN RIGHT; ANTIDEPRESSANT

NAME: NORTRIPTYLINE pKa: 9.7 CODE: N12

FIG. 3. — A data record for nortriptyline, illustrating the data field format. (RT = Retention time relative to an internal standard [tetraphenylethylene on OV1 and Poly A 103 and Imipramine on Silica] ; RI = Retention Index ; 1C1 = 1st location colour, 1Sh = Shape and 1C2 = 2nd location colour on column 1 [similarly for columns 2 - 6]).

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taneous multi-field searching, any number of colour responses could also be entered and the programme would produce a list of matches to the overall data.

RESULTS AND DISCUSSION.

The reference library currently contains data from nearly 600 acidic, basic and neutral drugs, metabolites and associated compounds from which an extract is shown in figure 4.

Stead *et al.* (1) demonstrated that any two chromatographic systems with low Rf correlation could show greater discriminatory power together than individually, but that little subsequent improvement could be obtained by using further additional systems. Of the 586 compounds chromatographed in the present work,

100 were not located at all. The majority of the 62 compounds which responded only on EMA were located by just one of the spraying reagents used on that system, for example barbiturate-type compounds were located only with mercurous nitrate. Similarly, 11 compounds were located just on the MA system. Overall, the difference in selectivity between the spraying reagents may be regarded as the main reason for such single system observations. The linear least squares regression correlation coefficient, r, for MA Rf on EMA Rf for the 411 compounds which

Extract from TLC Reference Library. 30th Sept 1986

COMPOUND	MA	1C1	1C2	2C1	2C2	EMA	3C1	3C2	4C1	4C2	5C1	5C2	6C1	6C2
ACEBUTOLOL	45		1414	2020		34	1010	1414	2020	1313			1616	
ACEPROMAZINE	44	[illegible]	1818	1014		67	0304	1813	[illegible]	1313	0208		0808	1515
ACETAZOLAMIDE														
ACETOHEXAMIDE														
ACETOPHENAZINE	55	[illegible]	0404	[illegible]		43	[illegible]	0304	1313	[illegible]	[illegible]		[illegible]	1616
ACROSOXACIN	[illegible]	[illegible]	0202			[illegible]	0202	0202	2016	1313		0606		0505
ALCLOFENAC														
ALETHOL						00								
ALFENTANIL	76	[illegible]	1010			70		1010						
ALLOPURINOL						71		1010	2020	2020			1313	1212
ALOXIPRIN														
ALPHADOLONE	78	[illegible]		2020		[illegible]	1010	1010	2020	2020			1313	1212
ALPHAXALONE														
ALPHAPRODINE	57	0202	0201	1919	[illegible]	65	[illegible]	0202	1919	1319				
ALPRAZOLAM	67	1616	[illegible]			38	1616	[illegible]				[illegible]		1212
ALVERINE	57	0303	0303	[illegible]		79	0303	0301	1919				0808	
AMANTADINE	28	[illegible]	1019			44	1919	1919	1818					
AMETHOCAINE	61	0303	0302			69	[illegible]	0201	1818	1318				
AMIKACIN														
AMILORIDE														
AMIODARONE	72	1919	1919	1919		[illegible]	[illegible]	[illegible]					1313	
AMITRIPTYLINE	52	0303	0302	1919	1619	71	0104	0101	1919	1919			[illegible]	0606
AMODIAQUINE	64	0101	0101	1515		73	0302	0101	1616	1313	1616			
AMOXYCILLIN														
AMPHETAMINE						46							1515	0909
	37	1919	0303			48	2019	1919					1414	1502

FIG. 4. — An extract from the database file.
1C1 = 1st location colour on column 1.
1C2 = 2nd location colour on column 1.
2C1 = 1st location colour on column 2, etc.

located on *both* systems was +0.53. This is higher than suggested by Stead and his group, but in their work only neutral drugs were chromatographed on both EMA and MA (acidic compounds on EMA only, and basic compounds on MA only). In this paper the chromatography of all the compounds were examined on both solvent systems.

The MA solvent mixture was chosen since its use was already well documented in a number of sources. The EMA solvent mixture was then chosen to complement this as it discriminated between the barbiturates particularly well.

Rf reproducibility is highly influenced by chromatographic conditions so it is important that they be carefully controlled. This was mostly achieved by fully saturating the tanks, and by re-

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placing the running solvent at regular intervals. The inter-run Rf coefficient of variation (cv) in MA ranged from 3 % to 5 % (inversely with Rf) and the corresponding range in EMA was 5 % to 9 % (mean of 200 runs with 9 compounds on MA and 17 compounds on EMA). Table I outlines the Rf distribution of compounds in the two chromatographic systems, and shows that the lowest and highest sectors of the plate contain a much lower percentage of the observations. Thus, the cv of each system overall will approximate to the middle of each range (i.e. 4 % for MA and 7 % for EMA) which is acceptable for this technique.

TABLE I

Percentage distribution of Rf on EMA and MA chromatographic systems

Rf (mm)	*MA Percentage Distribution*	*EMA Percentage Distribution*
0 - 10	2.6	4.2
11 - 20	5.0	5.5
21 - 30	10.2	6.1
31 - 40	9.2	9.7
41 - 50	16.4	17.1
51 - 60	16.6	15.0
61 - 70	17.8	21.1
71 - 80	20.1	19.2
81 - 90	2.1	1.9
91 - 100	—	—

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There is an inherently poor discrimination of Rf value in TLC because most spots are at least 3 mm in diameter and ruler measurements to 1 mm precision over a 10 cm band give only 100 discrete divisions. A number of compounds would therefore remain undiscriminated even using a dual system such as described here. This paper suggests that the use of an unrelated parameter such as colour should confer further discriminatory power since undiscriminated compounds might well produce distinctly different reactions with at least one of the locating reagents used.

Selecting the method for colour coding was not a straightforward task. The system was intended to be reproducible and easily computerized and therefore demanded unambiguous colour options. Continuous colour spectra were considered as a possible solution but reproducible matching was difficult. Moreover, since concentration-dependent shifts in colour do not follow spectral sequences (eg lignocaine when sprayed with iodoplatinate

changes from grey/blue to purple to yellow as concentration increases), this method was abandoned. By presenting a range of only 20 discrete colours and matching observed colours to the closest equivalent on the chart, reproducibility was improved and the computer search parameters were much easier to define. Although similar colours were grouped for clarity, overall they were independent of the numercial sequence on the chart. Reproducibility was investigated by a short experiment in which 30 analysts with normal colour vision were asked to match a random selection of 25 independently produced colours to those on the chart. More than 70 % of the participants agreed on *one* particular match for 21 of the 25 colours (> 90 % agreement for 15 of the colours and 100 % agreement for 8 of the colours) and for the remaining 4 colours the choice was clearly divided between *two* alternatives, although there were instances of solitary disagreement in addition to these, which may be regarded as statistically unimportant.

Colour responses are subject to a number of obvious but nevertheless important influences. Firstly, if plates are not documented at a standard time after spraying reproducibility will be affected by any chronological colour changes. For example, trazodone does not appear as a red spot until 4 minutes after spraying with Mandelin's reagent and thereafter gradually bleaches from the centre. If the initial response is recorded at the set reading time (when strychnine has changed to orange) and the latter part of the reaction is recorded as a comment, reproducibility will be improved. Secondly, located spots may be expected to vary radially in colour intensity, but a number of compounds were found to produce spots composed of two distinct colours (an outer colour and an inner colour), particularly at higher concentrations, and dichromatic coding was introduced to account for this. The comment section was used to record instances of more unusual colour responses, such as that of meprobamate, which with Marquis reagent produces a white speckled spot only after 20 mins. Thirdly, experience has shown that colour response varies with concentration and that the range of drug concentrations encountered in patient urine samples can vary by several hundredfold. Consequently, different concentration entries (when appropriate) are included in the database to facilitate reliable matching over a wider concentration range. Fourthly, coincidence of spots may be another source of confusion, especially with regard to the aggregate colour. For example, codeine and 10-hydroxynortrip-

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tyline, located with Marquis reagent, produced a colour reaction unrelated to the original component colours (codeine purple, 10-hydroxynortriptyline green, aggregate colour turquoise). Alternatively, one of the components could mask the presence of the second if the aggregate colour resembled that of the first component (e.g. clomipramine and diphenhydramine sprayed with Mandelin's reagent give an aggregate colour which is effectively the same as that for clomipramine). In the majority of cases, however, the presence of metabolites or reactions with the other locating sprays clarified the situation.

Located shape was found to be of little use as a discriminatory parameter. Some drugs (for example nordextropropoxyphene) did produce distinct shapes which were usefully recorded, but spot concentration and the presence of other adjacent spots had a noticeable and unpredictable effect on responses in general.

The variability in Rf values between urine-extracted and spotted compounds increased with Rf. This « lagging » phenomenon was attributed to the fact that a number of the methanolic standard solutions contained a drug salt rather than a free base. However, it was subsequently shown that the chromatography of free base and salt were identical (lignocaine hydrochloride, atropine sulphate, chlorpheniramine maleate, codeine phosphate and prochlorperazine mesylate showed no measurable difference from their free base on either solvent system). It could therefore be assumed that endogenous components of the urine retarded the chromatography. All Rf data recorded are consequently relating to compounds extracted from urine rather than those directly spotted onto the plate. Another factor affecting Rf is the applied concentration. For example, in MA the Rf of the methadone metabolite EDDP changes from 13 mm to 19 mm as the concentration increases from 5 μg to 40 μg on the plate. This problem was overcome by making separate entries in the database at 13 mm, 16 mm and 19 mm as these effectively covered the observed span when the search errors of $\pm$ 3 mm were entered and ensured correct matching.

The database could be used in several ways. Printed lists in alphabetical compound order, ascending MA Rf and ascending EMA Rf were used for quick reference in the laboratory. Additionally, given an unknown spot, the computer could be used to generate a short list, based on Rf alone, of the most likely matches, which could then be validated by reference to other parameters (TLC colour, GC characteristics, etc.). However,

if a distinct colour reaction was observed a search could be based on this alone, and the Rf data associated with this colour examined subsequently. Unmatched spots were entered into a file of « unknowns » together with a cross reference to files holding the pertinent clinical details on the sample for further investigation.

The judgement of the analyst is obviously influenced by clinical or analytical expectations. Knowledge of the most commonly prescribed drugs and their likely concentration in urine, for example, will be helpful in the interpretation of an unknown. This system attempts to consolidate extensive TLC data, which could only otherwise have been accumulated by many years experience, into a form which is easily and rapidly referenced by analysts of all abilities. The colour reference chart has proven easy to use in the laboratory and takes very little time to apply, in contrast to other methods of recording TLC data. The cost involved in setting up and using the system is comparatively very small, especially taking into account that the computer may also be used for a number of other functions. The efficiency of the TLC system has thus been greatly improved, although it should still be regarded as only one of a number of possible analytical techniques to be applied to the identification of an unknown compound.

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Correction of HPLC-RI values enables interlaboratory comparison of data obtained different ODS phases

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SUMMARY.

The series of barbiturates was analyzed by means of HPLC using the columns filled with different ODS phases (Hypersil 5, Partisil 10, Spherisorb 5, Serva Polyol 5) and the mobile phase methanol-buffer (40:60) acc.to (1). The retention index (RI) values were calculated against the series of alkyl-aryl-ketones acc.to (1). Marked differences between the RI values obtained for the same substance on different phases were noted. Then the correction of the apparent RI values was made, basing on the same principle as the correction of Rf values in TLC (2). The phase Hypersil ODS-5 was used as a reference phase, and phenobarbital, talbutal and methohexital as correction standards. A good agreement of RI data was observed after correction. Also, the discrepant data observed for various columns by Smith *et al.* (1) became very similar after correction.

The method enables the comparison of results obtained with different ODS phases for barbiturates and probably may find more universal application in HPLC screening procedures.

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Systematic analysis of volatile substances by gas chromatography

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INTRODUCTION.

Ramsey and Flanagan (1) developed a gas chromatographic method for the detection and preliminary identification of volatile compounds in a biological matrix which uses 0.3 % Carbowax 20M on Carbopack C, 80-100 mesh. For this column they gave retention times for about 185 solvents and other volatile substances under temperature programmed conditions.

The Committee on Systematic Toxicological Analysis of TIAFT decided that this data collection can be used as a basis for the systematic analysis of volatiles. However, the transferability of these data to other laboratories needed to be investigated.

As unambiguous identification of volatile substances cannot be performed satisfactory by using one type of column, a second type of column with a different separation ability needed to be chosen as well. Packing materials with a 5 % loading of Carbowax 20M were investigated. This selection was based on the fact that the latter type of columns has been in use for blood alcohol analysis for many years and represent another separation mechanism.

EXPERIMENTAL.

Instrumentation.

In Groningen a HP 5880 gas chromatograph (Hewlett Packard) was used with a FID and with a HP 7671A automatic injector. The injection temperature was 225°C, the detector temperature was 250°C.

Four different columns were used :

1. Glass column, i.d. 4 mm, length 130 cm, packed with 0.3 % Carbowax 20M on Carbopack C (80/100). The following temperature program was used : 2 min. at 50°C, 8°C/min. to 194°C and 5 min. at the final temperature. The helium gas flow was 15 ml/min.

2. Glass column, i.d. 2 mm, length 150 cm, packed with 50 % Carbowax 20M on Carbopack B, GP 60/80 and used with the following temperature program : 3 min. at 65°C, 5°C/min. to 165°C. The carrier gas flow rate was 10 ml/min.

3. Glass column, i.d. 3 mm, length 150 cm. Other conditions as with column 2.

4. Glass column, i.d. 2 mm, packed with 5 % Carbowax 20M on Carbopack B AW(80/120) and used with the following temperature program : 3 min. at 65°C, 5°C/min. to 200°C and 12 min. at the final temperature.

Columns 1 and 2 were used in Groningen, while columns 3 and 4 were used in Homburg.

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Procedure.

The substances were dissolved in water in a concentration of about 1 g/l. When the substances did not dissolve in the water phase, small amounts of methanol were added. The samples were directly injected into the gas chromatograph (1 µl) or determined by head space analysis.

RESULTS AND DISCUSSION.

Ramsey and Flanagan (1) used a temperature program which starts at 35°C. As special instrumentation is needed to obtain such a low temperature, it was decided to change the temperature program in that the initial temperature was set at 50°C. However, by changing the temperature program the retention data of Ramsey and Flanagan could not be compared anymore with our results. Moreover, it is known for non-volatile substances that retention behaviour can be transferred better from laboratory to laboratory in the form of retention indices based on straight chain alkanes (2). The same way of standardization was also used by Rübel (3) for solvents and volatile substances.

However, for volatiles the use of straight chain alkanes gave some problems in that the alkanes are not soluble in water and that the lower homologues such as propane and butane are not easily available in a pure form. Therefore, it was decided to standardize the retention behaviour of the substances by using 1-hydroxy-n-alkanes. These homologous alcohols are readily available in a pure form and easy to handle.

Data on Carbopack C ; 0.3 % Carbowax 20 M.

In order to be able to compare the data of Ramsey and Flanagan with our data set, their data collection was transformed into re-

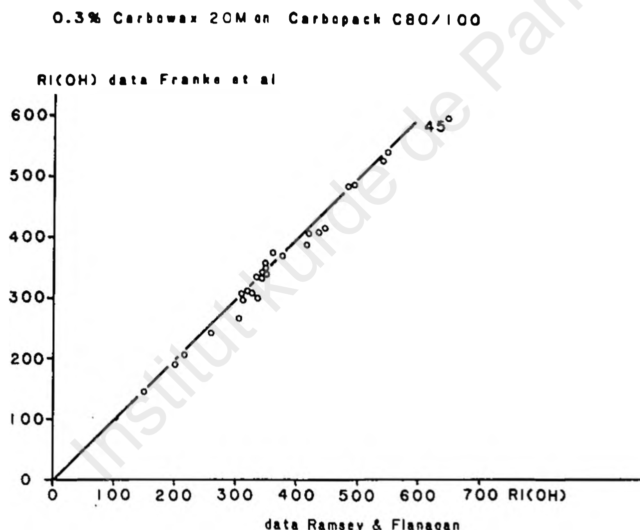

FIG. 1. — Correlation between data obtained on two 0.3 % Carbowax 20 M on Carbopack C columns.

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tention indices based on 1-hydroxy-n-alkanes. Figure 1 shows a plot in which the correlation between the two data sets are depicted. It can be seen, that there is a good correlation ($r = 0.990$) for the test set of 25 volatile substances. From these observations it can be derived that for data retrieval a standard deviation of 10 retention index units or a window of $\pm$ 30 units is adequate.

It must be noted that, although the same packing materials was used in our study, the conditions were different, with regard to

temperature programs (retention indices are temperature dependent), injection techniques (liquid injection versus headspace), the column dimensions and carrier gas flow.

Data on Carbopack B ; 5 % Carbowax 20 M.

In an interlaboratory situation for a test set of substances, retention index data were collected using a column packing material consisting of 5 % Carbowax 20M on Carbopack B. Figure 2 shows an overview of the results, which indicates that despite the

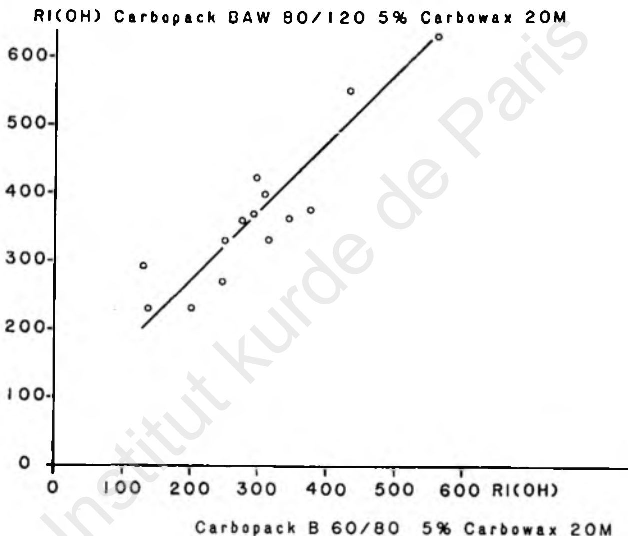

FIG. 2. — Correlation between data obtained on two 5 % Carbowax 20 M columns with slightly different support material.

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reasonable correlation the spread was much too large to be of any use. It appeared that the support material used in both laboratories was different resulting in a different retention behaviour.

We assumed that this could be due to the differences in the support materials (Carbopack B.3 60/80 mesh versus Carbopack B AW 80/120 mesh). When we repeated the experiments with the same packing material, results were found as depicted in figure 3. The interlaboratory reproducibility of these data is much better now and of the same magnitude as with the 0.3 % Carbowax 20M on Carbopack C column, so that also with a high loading of Carbowax a search window of ± 30 units can be used.

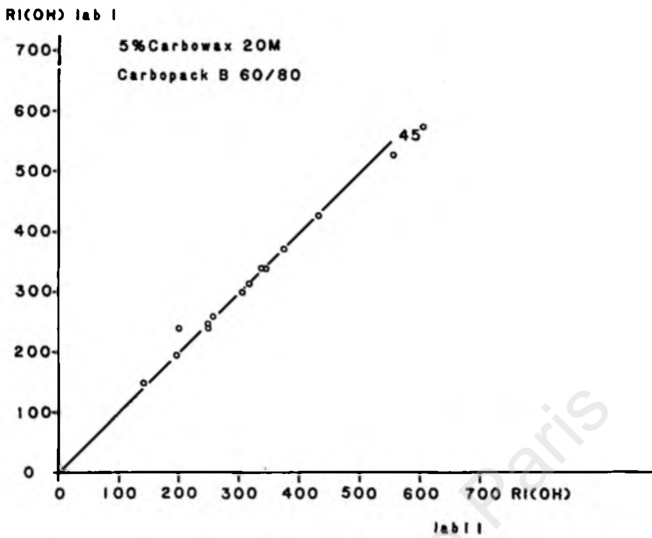

FIG 3. — Correlation between two laboratories.
Data on 5 % Carbowax 20 M on Carbopack B 60/80.

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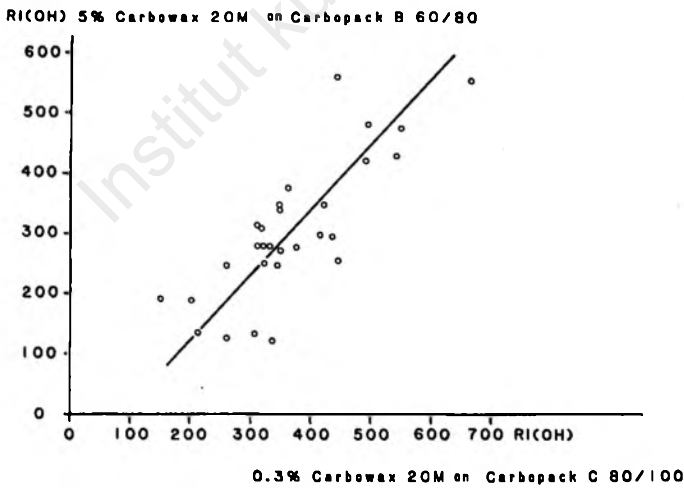

FIG. 4. — Correlation between data obtained on the two recommended column materials.

Combination of the two types of columns.

For the combination of the 2 columns the results are shown in figure 4. The correlation between the systems was found to be 0.80 which is relatively low for gas chromatography. Table I gives some data about the calculations of the Mean List Length and the Discriminating Power. From these calculations it can be

TABLE I

Identification Power calculations

	MLL*	DP**
0.3 % Carbowax 20M — Carbopack C 80/100	4.2	0.84
5 % Carbowax 20M — Carbowax B 60/ 80	4.0	0.85

* Mean list length.
** Discriminating power.

seen that the identification power for each system is comparable. For the combination of the two systems a Mean List Length value of 1.6 was calculated, so that the use of two systems results in a considerable enhancement in identification power. However, it should be noted that even with 2 columns an unambiguous identification of substances, based on interlaboratory data, can not be performed.

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CONCLUSION.

The use of the proposed columns which represent distinctly different separation mechanisms, namely adsorption vs partition chromatography, in combination with the use of retention indices calculated by means of n-alcohols as reference substances, provides a rational approach to the systematic analysis of volatile substances.

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A statistical study on the results of toxicological analyses carried out at the Department of Pharmacology and Toxicology from 1976 to 1983

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SUMMARY.

For many years, the Department of Pharmacology and Toxicology has been examining animal viscera, feeds, foods and miscellaneous materials suspected of containing poison.

The exact incidence of poisoning in livestocks in Turkey is not known.

The purpose of this statistical investigation is to determine the main causes of poisoning in domestic animals in Turkey.

This statistical investigation was carried out on 594 samples received by the Department of Pharmacology and Toxicology from 1976 to 1983.

According to our laboratory records, 318 of them were found positive and 276 were negative. The main causes of the intoxication were found to be chlorinated organic insecticides (118 cases), organic phosphate insecticides (108 cases), arsenic (23 cases), mercury (18 cases), strychnine (17 cases), aflatoxin (15 cases) and miscellaneous (26 cases). With this investigation was pointed out that 69.19 % of poisonous substances found in the suspected materials were pesticidal compounds.

INTRODUCTION.

Toxicology is the study of poisons and their action in the human and animal body. Since most drugs are also poisons if they are

used in excess. Therefore, toxicology is closely allied to pharmacology (3, 5).

Veterinary Toxicology deals with the study of poisons responsible for intoxication of animal body. This involves the investigation of, both the properties of poisons and their action on different species of animals, and the causes of poisoning and the conditions under which animals are poisoned. Detailed knowledge of the complex causes and conditions of poisoning is essential for effective prevention (2).

It is evident that a study of origin, properties and effects of poisons, their detection, and treatment of their effects demands a knowledge of botany, biochemistry, physiology and pathology, as well as animal husbandry and veterinary medicine (3).

POISONING OF ANIMALS AND ITS AETHIOLOGY.

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Poisoning (Toxicosis) is the action of an absorbed poison and the organism's response to its effect.

In countries with the advanced industry and agriculture, farm animals are exposed to the danger of poisoning by a number of different chemicals used in agriculture production, in industry and in households. Thus poisoning results mainly from industrial activities, from the use of chemicals for higher yields and higher performance of farm animal diseases.

Many new toxic substances have become available to farmers. Their inappropriate use, inexpert handling and the failure to respect the instructions for safe use may lead to the poisoning of farm animals.

The risk of poisoning may also arise some substances used as replacers of natural sources of nutrient for example urea.

Another important range of problems arises from the use of commercial fertilizers, particularly nitrates, superphosphates.

Fish may be poisoned by toxic effluents released to rivers.

Possible contamination of feed or of the animals with the industrial smoke and fumes must also be considered.

Each season has its specific toxicological problems.

The majority of substances used for plant protection are poisonous to bees. Bees are mainly poisoned when insecticides are applied at the flowering time (2).

For many years, the Department of Pharmacology and Toxicology has been examining animal viscera, feeds, foods and miscellaneous materials suspected of containing poison.

The Department of Pharmacology and Toxicology got into the work because the farmers and people requested it and there was no other agency to do it.

The exact incidence of poisoning among in domestic animals in Turkey is not known. There is only one publication on this matter. According to Ceylan and Sener (1) during the period of 1966-1975 a total of 921 biological materials obtained animals and various feed and food samples were subjected to toxicological examinations. The results of their study was shown below. Two hundred seventy-one samples were found positive and 618 were negative. Thirty-two specimens were not analysed for various reasons. Main causes of intoxications are shown in table I.

TABLE I

The compound detected from suspected samples

Compounds	*Number of cases*
1. Chlorinated organic insecticides	100
2. Organic phosphate insecticides	92
3. Strychnine	35
4. Arsenicals	26
5. Others	18
Ceylan and Sener (1)	271

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They pointed out that 93.3 % of the poisonous substances founded in the suspected materials were pesticide compounds.

MATERIALS AND METHODS.

The purpose of this study is to determine the causes of poisoning in domestic animals in Turkey.

This statistical investigation was carried out on 594 samples received by the Department of Pharmacology and Toxicology.

RESULTS.

During the period of 1976 to 1983 a total of 594 biological materials obtained from poisoning animals and various feed and food and also other samples were subjected to toxicological examinations. Depending on our laboratory records, 318 of them were found positive and 276 were negative.

I have tabulated the results of examinations (the toxicological analyses) from 1976 to 1983 in table II, table III and in table IV.

Table II shows the compounds founded in the suspected samples. The most common poisons, in their order of predominance were as follows (see table II).

TABLE II

The compounds founded in the suspected samples

Compound	Number	Total
1. *Chlorinated organic insecticides :*		
1. Aldrin	35	
2. DDT	29	
3. Endrin	19	
4. Lindane	12	111 cases (34.90 %)
5. Heptachlor	8	
6. Dieldrin	6	
7. Metasystox	2	
2. *Organic phosphate insecticides :*		
1. Trithion	34	
2. Parathion	23	
3. Malathion	21	
4. Lebaycid (fenthion)	13	108 cases (34.29 %)
5. Dursban	10	
6. Gusathion	4	
7. Ethyl. Methyl. Parathion	3	
3. Arsenic	23	23 cases (7.23 %)
4. Mercury	18	18 cases (5.97 %)
5. Strychnine	17	17 cases (5.30 %)
6. Aflatoxin	15	15 cases (4.70 %)
7. Trichlorethylene	9	
8. Sodium hydroxide	4	
9. Ethylene glycol	4	
10. Nitrite	3	26 cases (7.63 %)
11. Tannin	2	
12. Urea	2	
13. Ammonium hydroxide	1	
14. Copper sulfate	1	
Total	318	100 %

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These figures are of cases submitted for toxicological analysis and do not necessarily give a correct impression of frequency of the various types of poisoning encountered in general practice. It is very likely, for instance, that plant poisoning occurs much more often than is suggested by these statistic, which also give no indication of the incidence of toxic effects arising from industrial contamination of water and herbage.

The modern toxicology laboratory can play an important role in the evaluation of poisoning and in the treatment of acute poisoning (7).

TABLE III

The origin of the samples were analysed in different years

	1976	*1977*	*1978*	*1979*	*1980*	*1981*	*1982*	*1983*	*Total*
Feed	9	10	6	21	12	22	18	—	98
Sheep	11	15	9	15	16	15	11	1	93
Hen	12	6	15	12	11	15	11	5	87
Cat - dog	11	5	8	8	5	13	13	2	65
Cow	7	6	10	3	12	10	8	2	58
Bull - cattle - calf	9	5	4	4	8	14	9	—	53
Goat	4	7	10	8	5	10	5	1	50
Fish	—	—	2	2	3	4	6	—	17
Turkey - goose	—	1	2	—	5	6	2	1	17
Water	1	—	3	3	3	2	2	1	15
Plant	1	—	—	2	3	3	3	1	13
Horse	—	1	1	3	3	—	—	1	9
Bee	2	1	—	2	—	2	—	—	7
Fox - rabbit	1	—	—	—	1	—	—	5	7
Bird	—	—	—	—	—	2	3	—	5
Positive	39	22	43	34	52	69	45	14	318
Negative	29	35	27	49	35	49	46	6	276
Total	68	57	70	83	87	118	91	20	594

TABLE IV

The number of poisons detected from samples in different years

	1976	*1977*	*1978*	*1979*	*1980*	*1981*	*1982*	*1983*	*Total*
Aldrin	8	7	6	2	9	3	—	—	35
Trithion	4	1	1	—	8	13	5	2	34
DDT	5	—	9	4	3	2	5	1	29
Arsenic	—	—	1	4	6	7	3	2	23
Parathion	7	2	3	—	4	5	—	2	23
Malathion	5	1	—	2	2	9	2	—	21
Endrin	4	3	4	3	2	2	1	—	19
Mercury	—	—	1	—	3	4	9	1	18
Strychnine	2	2	6	2	—	2	2	1	17
Aflatoxin	—	—	—	1	4	6	3	1	15
Lebaycid (Fenthion)	—	—	—	—	2	9	2	—	13
Lindane	1	1	6	2	—	1	—	1	12
Dursban	—	—	—	—	—	1	7	2	10
Trichloroethylene	—	—	—	9	—	—	—	—	9
Heptachlor	1	2	4	—	—	—	—	1	8
Dieldrin	—	1	1	—	1	2	1	—	6
Gusathion	—	—	—	1	1	1	1	—	4
Sodium hydroxide	—	—	1	2	1	—	—	—	4
Ethylene glycol	—	—	—	—	—	1	3	—	4
Ethyl - methyl - parathion	—	1	—	1	—	—	1	—	3
Nitrite	1	1	—	—	1	—	—	—	3
Tannin	1	—	—	—	1	—	—	—	2
Meta - systox	—	—	—	—	2	—	—	—	2
Urea	—	—	—	—	1	1	—	—	2
Ammonium hydroxide	—	—	—	1	—	—	—	—	1
Copper sulphate	—	—	—	—	1	—	—	—	1
Total	39	22	43	34	52	69	45	14	318

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The work of our laboratory in testing samples for poisons was facilitated in 1970 by our acquisition of new equipments.

The use of other new physical instruments offers possibilities of identifying organic drugs, alkaloids, other poisoning substances with a great rapidity than is possible by traditional chemical methods, and often with greater certainty.

DISCUSSION.

There are animal poisoning in many parts of the world, but there are a few literature about on animal poisoning incidence. Few reports of poisoning have been published in the literature. Ceylan and Sener's study is the only one study found in the literature on this subject in Turkey (1). Our statistical investigation is the second one. We studied on 8 years specimens received by the Department of Pharmacology and Toxicology, but Ceylan and Sener (1) studied on 10 years samples. Their results was found close to our results.

During the period of 1976 to 1983 a total of 594 biological materials obtained from the poisoning animals and various feed and food, and also other samples were subjected to toxicological analyses. Percentage of all positive specimens positive for chlorinated organic insecticides, organic phosphate insecticides and others are shown in table II. Garner (5) has been given some data on poisoning incidence in England. Fisher (4) mentioned some data for Connecticut.

CONCLUSION.

Everything is poisonous, if dosage and route of entry into living organism are not restricted, no substance is completely free of toxic effects. One of the problems is to determine where the pharmacology leaves off and toxicology begins (6).

Finally, if we know the main causes of poisoning we can be able to find very easily the measurements that we protect animals from poisining. This way we never loose our food originated from animals. Animals have a high economic value for every country in the world.

GENERAL PRINCIPLES OF PREVENTION OF ANIMAL POISONING.

Many deaths occur annually due to accidental, suicidal, homocidal or industrial poisonings. Some of these deaths could have been avoided with an accurate diagnosis and adequate treatment. Early recognition of characteristic signs and symptoms of poisons is therefore a very important factor in the prevention of death due to poisoning (8).

Strict observance of the rules concerning poisons and other harmful substances is an important principle in the prevention of poisoning of farm animals. These rules include :

1. The obligatory recording of poisons, their correct storage and handling.

2. The extensive use of poisonous substances in agricultural production is particularly important ; some of these preparations are highly toxic. If there are a variety of preparations from which to choose, those of lower toxicity and without undesirable properties such as accumulation in the organism, are preferable since they are generally less dangerous.

3. Due attendance to animal husbandry and observance of technological processes of feed preparation and Zoohygienic rules are also important factors of prevention of animal poisoning.

4. Adherence to rules governing the release of industrial airborne pollutants and effluents is also a preventive measure in controlling the occurrence of toxicosis and in the protection of the environment.

5. Further preventive measures include the observance of all hygienic standards by regular disinfection, disinsection, and deratization of stables, or in mass treatment campaigns.

6. Natural conditions should also be mentioned as important factors underlying the occurrence of animal poisoning, for instance the excessive occurrence of poisonous plants in the fields or a high content of nitrates in some crops caused by unfavourable climatic conditions during their growth.

7. The prevention of toxicosis in farm animals require close cooperation of veterinarians and farmers in joint efforts towards averting losses from poisoning of farm animals and keeping the

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animals at a high level performance in the production of wholsome and biological valuable foods (2).

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Analytical Methods in Toxicology

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Solid-phase extraction using C-18 columns in the HPLC determination of drugs in biological fluids

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SUMMARY.

The sensitivity and precision of methods for the determination of drugs in plasma and serum is mainly limited by the extraction procedure applied. In particular in HPLC analysis, a substantial improvement of the specifications of variable-wavelength, fluorescence and electrochemical detectors has been achieved during the last decade. Concomitantly, the isolation of the drugs of interest from their biological matrix has become the factor which determines the final result.

The introduction of solid-phase extraction using bonded-silica sorbents has opened new possibilities for the development of selective extraction procedures. Several analytical procedures were developed in our laboratory based on solid-phase extraction using Baker-10 SPE C-18 columns. In addition, by application of this technique the detection limits of existing analytical procedures were improved. In some cases, considerably cleaner extracts were obtained by a selective dissolution of the residue after solid-phase extraction, in an inmiscible solvent mixture. In this way, an improvement in selectivity and sensitivity was achieved, resulting in lower detection limits.

At present, analytical methods based on solid-phase extraction have been developed in our laboratory for the determination in plasma, serum or blood of drugs such as benzodiazepines, phenothiazines, anticonvulsive and beta-blocking drugs, indapamide and doxorubicin. During application of these procedures it has been shown that solid-phase extraction procedures are time effective and are suited for the application to large series of samples.

In this presentation, some examples of analytical methods based on solid-phase extraction procedures will be presented. The consecutive steps that are part of the extraction procedures will be discussed.

Maprotiline intoxications

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SUMMARY.

A method for the determination of maprotiline in autopsy material is described.

Fatal, toxic and therapeutic maprotiline concentrations in whole blood and tissue from autopsy cases are presented, and the whole blood levels compared to whole blood levels from persons alive taking maprotiline.

INTRODUCTION.

Maprotiline is a tetracyclic antidepressant, belonging to the dibenzo-octadienes (fig. 1). It differs chemically from the tricyclic antidepressants by an ethylene bridge across the central ring.

Maprotiline has been marketed in Denmark since 1975 as Ludiomil from Ciba-Geigy, and during the last decade a number of cases, where maprotiline was involved, have been seen in our laboratory, The Institute of Forensic Chemistry in Copenhagen. As only few fatal cases with maprotiline have been described in the literature, we found our findings might be of interest, and autopsy cases with fatal, toxic or therapeutic concentrations of maprotiline shall be presented and compared with therapeutic blood concentrations of maprotiline from persons alive, and also with the findings of other authors.

MATERIALS AND METHOD.

Materials.

The cases come from the eastern part of Denmark with Copenhagen included, and represent a population of about 2.4 million.

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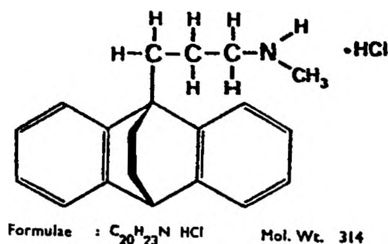

MAPROTILINE

FIG. 1.

SCHEME FOR EXTRACTION

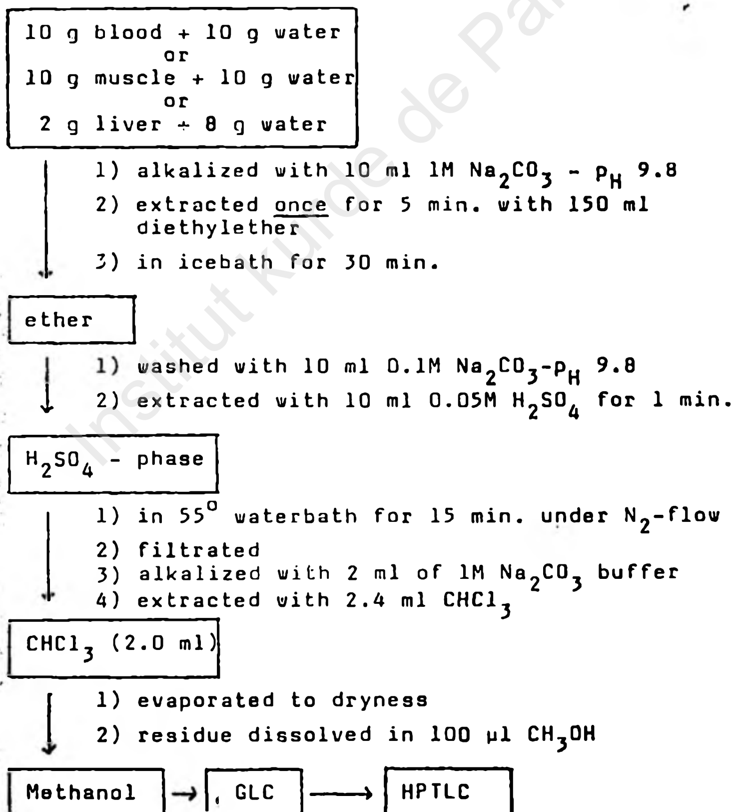

FIG. 2.

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When available, liver and whole blood were analysed. In cases, where no blood was available, extremity muscle was used instead, it being our experience, that drug concentrations in whole blood and extremity muscle are of the same level (1).

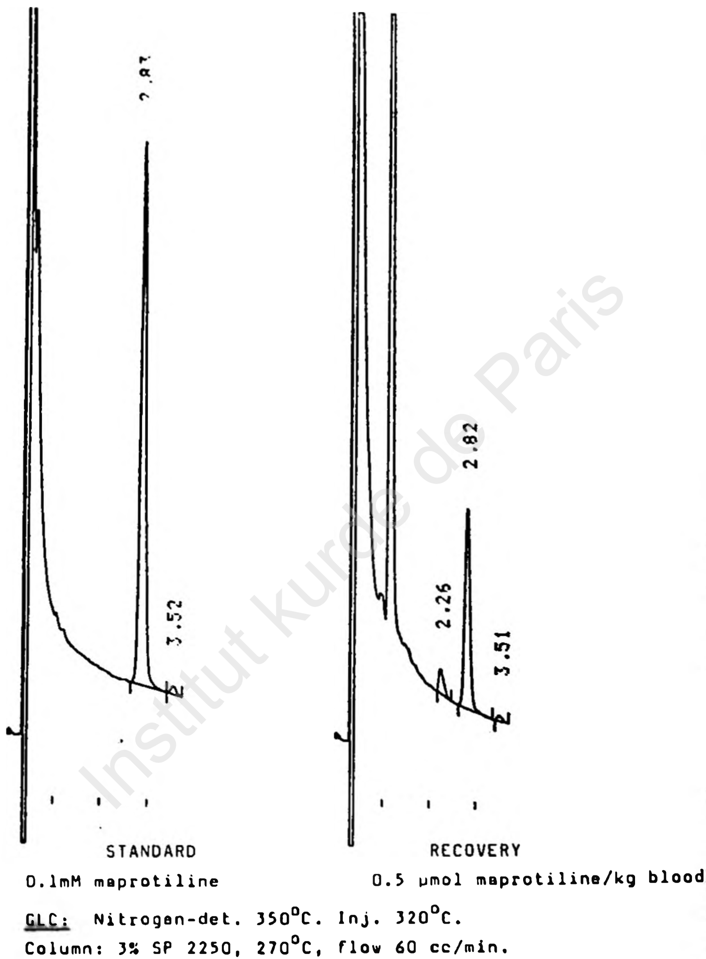

FIG. 3.

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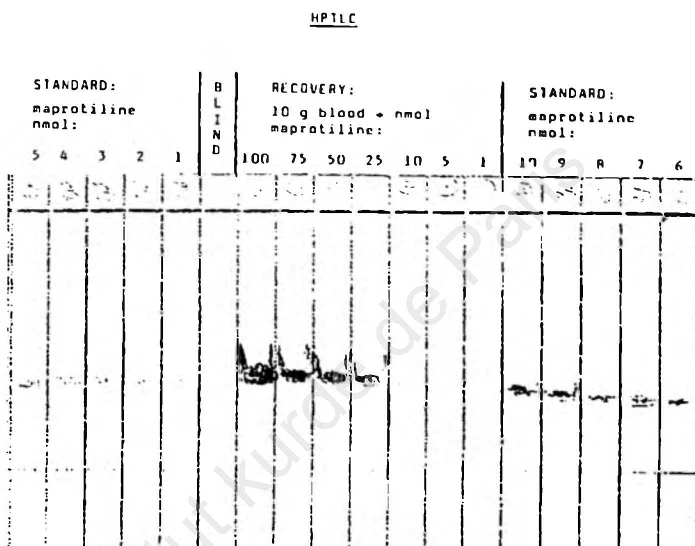
Method.

The method is outlined in figure 2. The material was diluted or homogenized with water and a single extraction af p_H9.8 performed with a large volume of ether, which has proven to give both higher yields and cleaner residues than repeated extraction. After separation in ice bath for 30 min., the ether phase was cleaned

by washing with 10 ml 0.1M Na_2CO_3 buffer solution, and then extracted with 10 ml 0.05M H_2SO_4. In order to remove residual ether, the acid phase was placed in a 55° waterbath for 15 min. under a flow of nitrogen. It was then filtrated, alkalized with 2 ml of 1M Na_2CO_3 buffer and finally extracted with 2.4 ml of chloroform, after which 2.0 ml of the chloroform was evaporated gently to dryness under a flow of nitrogen, and the residue dissolved in 100 µl of methanol.



Whatman, LKP-KDF, cat.no. 4806-711
20 x 10 cm, 200 µ.
Impregnation: Sprayed with 0.1M NaOH in methanol and dried 15 min. by 80°C.
Solvent: Methanol.
Spray-R.: Marquis-R.

FIG. 4.

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The methanol extract was used, first for GLC and finally for HPTLC.

No correction for losses in extraction was performed.

GLC. A Perkin-Elmer 3920 B with a nitrogen-phosphorous detector was used. The detector temp. was 350°C and the injector temp. 320°C. A glass column 2 m long with an inner diameter of 2 mm and filled with 3 % SP2250 was used, temp. 270°C.

In figure 3 is shown a gaschromatogram of an 0.1 mM maprotiline standard and of a recovery experiment with 0.5 µmol/kg blood.

HPTLC. Whatman plates, LKD-KDF, cat. no. 4806-711, 20 × 10 cm, 200 μ, were used for the TLC. They were impregnated with 0.1M NaOH by spraying, and subsequently dried for 15 min. at 80°C. 5 nmol of maprotiline standard and the methanol solutions from each sample were applied, and the plate was run in methanol. After drying, the maprotiline spots appeared as wine-red spots when using Marquis-R.

In figure 4 is shown the HPTLC of maprotiline standards from 1-10 nmol, together with methanol extracts from the recovery experiments, which shall be described below.

RECOVERY EXPERIMENTS.

To 10 g of whole blood was added from 100 down to 1 nmol of maprotiline, and the blood samples were analysed as described.

The results are shown in table I and give a mean of 89 ± 2.7 % (SD). The from day to day recovery, adding maprotiline to 10 g of whole blood to give 5 μmol/kg yielded a mean of 90 ± 7.5 % (SD).

TABLE I

Recovery experiments

Maprotiline added to 10 g of whole blood to give μmol/kg	*Recovery in %*
10	91
7.5	90
5	90
2.5	88
1	87
0.5	86
0.1	94

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Results

In 12 cases the maprotiline concentrations were considered fatal. The distribution of sex and age are shown in figure 5. There were 8 women and 4 men. The mean age of the women was 44 years and the mean age of the men was 33 years.

The maprotiline concentrations in blood and tissue of the 12 cases are shown in table II. In the first 9 cases there were no other findings or only therapeutic concentrations of other drugs or a BAC < 1 ‰. In the last 3 cases there were competing causes

FIG. 5.

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TABLE II

Fatal maprotiline concentrations in post mortem specimens

Case number	Hours since last seen alive	Maprotiline µmol/kg			BAC in ‰	Other findings (in blood, µmol/kg)
		Blood	Muscle	Liver		
1	21	51	38	330	0	None, or only therapeutic concentrations of other drugs
2	8	44	13	1000	0.86	
3	72	21	n.a.	530	0	
4	?	18	n.a.	980	0	
5	48	n.a.	16	740	n.a.	
6	?	14	n.a.	n.a.	n.a.	
7	25	7.3	16	640	0	
8	?	6.9	n.a.	n.a.	n.a.	
9	8	3.0	4.2	110	0.08	
10	3	6.8	2.4	22	3.66	Dead by gunshot
11	?	26	n.a.	800	0.04	Amitriptyline 21
12	?	53	n.a.	n.a.	n.a.	Nortriptyline 14

n.a. = not analysed.

of death, but still the maprotiline concentrations were so high, that they alone might have been the cause of death. Where informations were available, there were clear indications of suicide, except in case 9, which more seemed to be accidental. The range of the blood or muscle concentrations considered to be fatal were from 3.0-53 μmol/kg with a mean of 22 μmol/kg.

Three cases, where the maprotiline concentrations were considered toxic, are shown in table III. In case no. 13 it seemed as

TABLE III

Toxic maprotiline concentrations in post mortem specimens

Case number	Hours since last seen alive	Maprotiline μmol/kg			BAC in ‰	Other findings in μmol/kg blood (B), muscle (M)
		Blood	Muscle	Liver		
13	60	n.a.	1.7	83	n.a.	
14	4	1.4	2.6	260	0	Diazepam 5.4 (B) (drowned)
15	?	1.0	n.a.	34	n.a.	Desmethylclomipramine 0.8 (M) Salicylic acid 220 (M)

n.a. = not analysed

if suicide was planned, as all appointments etc. were cancelled. The deceased was found 60 hours after last seen alive, and the findings are most probably not peak levels but residual concentrations. Case no. 14 was a suicide with farewell letter and the person drowned herself. Case no. 15 seemed to be an accident. The deceased was known as an alcohol and medicine abuser, and the case could be a combined poisoning. Unfortunately alcohol analyses was not performed due to the lack of blood and vitreous humor. The blood or muscle range of maprotiline concentrations in the toxic cases were from 1.0-1.7 μmol/kg with a mean of 1.4 μmol/kg.

In the last 3 autopsy cases, only therapeutic concentrations of maprotiline were found (table IV). In all 3 cases there were other causes of death. In case no. 16, which most probably was an accident, an ethanol concentration of 4.47 ‰ was found in the vitreous humor. In the last 2 cases there were letters of farewell, and in case no. 17 fatal concentrations of methadone were found, and in case no. 18 the person had hanged himself. The concentration range of maprotiline in blood or muscle in these cases were from 0.2-0.6 μmol/kg with a mean of 0.4 μmol/kg.

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TABLE IV

Therapeutic maprotiline concentrations in post mortem specimens

Case number	*Hours since last seen alive*	*Maprotiline µmol/kg*			*BAC in ‰*	*Other findings in µmol/kg blood (B) or cause of death*
		Blood	*Muscle*	*Liver*		
16	1	n.a.	0.6	5.5	3.60	Ethanol 4.47 ‰ in vitr. hum.
17	6	n.a.	0.4	150	0	Methadone 3.4 (B)
18	28	0.2	n.a.	35	1.05	Death by hanging

n.a. = not analysed.

TABLE V

Maprotiline concentrations in whole blood from persons alive

Case number	*Maprotiline µmol/kg blood*
19	0.02
20	0.05
21	0.08
22	0.20
23	0.20
24	0.21
25	0.40
26	0.60

In table V are shown the maprotiline concentrations found in whole blood from persons alive. In these cases the blood concentrations of maprotiline range from 0.02-0.60 µmol/kg with a mean of 0.2 µmol/kg. The samples are from persons involved in traffic accidents or in acts of violence.

DISCUSSION.

It is not surprising, that intoxications by cyclic antidepressants occur, as they are prescribed to patients suffering from depression, and because of their low therapeutic index. However, though maprotiline has been marketed for about 10 years now, not very many fatal poisonings have been described in the literature. In table VI are listed the fatal cases described by other authors. Only cases, where maprotiline has been determined in whole blood, are included. In these cases, the blood concentrations range from 4.7-160 µmol/kg with a mean of 38 µmol/kg. Interesting is the case described by Okoye and co-

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TABLE VI

Fatal maprotiline cases, reported by other authors

Author(s)	*Estimated amount ingested (mg)*	*Hours since last seen alive*	*Maprotiline μmol/kg*		*Other findings μmol/kg blood*
			Blood	*Liver*	
Clarke D.G. and Roberts D.A. (2)	2100	3 1/2	22	1008	Salicylic acid 4900
Sengupta A. (3)			13	22	
Meinhart K. *et al.* (4)	3000	120	5.8	122	
Rejent T.A. and Doyle R.E. (5)	4500-6000		160	2180	
Okoye M.I. *et al.* (6)	200 mg daily	Short time	4.7		Pentazocine 1.4
Robinson A.E. *et al.* (7)			22* 7.2**	295	

* Axillary.

** Cardiac.

workers (6), where death occurred after a daily dose of 200 mg maprotiline. The patient was found convulsing, was rushed to the hospital, but died soon after. The daily dose according to Clarke (8) is from 30-150 mg maprotiline hydrochloride, so the case described by Okoye (6) together with case no. 9 in our investigation indicates a low therapeutic index, also for maprotiline. On the other hand, according to A.M. Jukes (9), a 58 year old woman is known to have recovered fully after having swallowed 5 g of maprotiline.

After daily oral doses of 50, 100 and 150 mg Riese and coworkers (10) found that the steady state whole blood concentrations were 0.07-0.54 μmol/kg (mean 0.25), 0.25-0.90 μmol/kg (mean 0.507), and 0.50-1.15 μmol/kg (mean 0.79). In our investigation we found a therapeutic range of 0.02-0.60 μmol/kg (mean 0.20).

TABLE VII

Comparison between fatal, toxic and therapeutic maprotiline concentrations in whole blood (or muscle)

		Maprotiline Blood (or muscle) levels μmol/kg	
		Range	*Average*
Fatal	n = 12	3.0 - 53	22
Toxic	n = 3	1.0 - 1.7	1.4
Therapeutic (dead persons)	n = 3	0.2 - 0.6	0.4
Therapeutic (persons alive)	n = 8	0.02 - 0.6	0.2
Fatal (reported by other authors) (2-7)	n = 6	4.7 - 160	38

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but then we didn't know the medicamentation, or if the patients had taken their medicine.

In table VII the fatal, toxic and therapeutic ranges and average blood or muscle maprotiline concentrations of this investigation are listed together with range and average of the 5 cases reported by other authors (2-7). As is seen, our results are in agreement with those of other authors and thus give a further confirmation of the concentration range of maprotiline, which may be considered to be fatal, and also an indication of, which concentration range may be considered toxic.

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Influence of biological matrix on retention behavior and identification possibilities of selected neutral and acidic drugs in thin-layer chromatography. An interlaboratory investigation

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SUMMARY.

Samples of autopsy blood and liver were spiked individually with aminophenazone, p-aminosalicylic acid, chlordiazepoxide, clonazepam, cyclobarbital, furosemide, medazepam, phenacetin, phenazone and phenobarbital and extracted with diethyl ether at pH 5. The extracts, as well as solutions of pure drugs, were developed in three TLC systems : chloroform-acetone (80:20), ethyl acetate-methanol-ammonia (85:10:5) and chloroform-methanol (90:10). The investigations performed in parallel in two laboratories showed that the intra- and interlaboratory variability of Rf values is larger for drugs extracted from liver. The biological matrix affected both precision and accuracy of results. Also the numbers of analysts involved in TLC procedure affected the intralaboratory precision.

Acute poisoning with chlorinated phenoxy herbicides in man

Analytical and clinical aspects

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SUMMARY.

Chlorinated phenoxy herbicides such as 2,4-D, mecoprop and dichlorprop are sold for domestic or commercial use often with other herbicides or pesticides. There are few reports as to the acute toxicity of these compounds in man, although nausea, vomiting, pyrexia, hyperventilation, hypoxaemia, deep coma and renal and muscle damage have been reported following oral ingestion (Berwick, 1970 ; Prescott *et al.*, 1979 ; Wells *et al.*, 1981).

A simple HPLC assay for some of these compounds has been developed. Sample or standard (100 μl) is vortex-mixed (30 sec) with methanol containing an internal standard (200 μl). After centrifugation (9,950 g, 1 min.), 20 μl of the supernatant are analysed using a 125 × 5 mm (i.d.) stainless-steel column containing Zorbax ODS (Du Pont) using methanol:water (1:1) containing sodium acetate (5 g/l, pH 4 or 6) at a flow-rate of 2 ml/min. as eluent. Detection is by UV (240 nm) and the limit of sensitivity is 20 mg/l.

Application of this assay suggests that (1) chlorinated phenoxy herbicides can cause serious poisoning in man which may only become apparent several hr post-ingestion, (2) forced alkaline diuresis may enhance the excretion of poorly metabolised compounds (2,4-D, dichlorprop) and (3) emergency plasma assays may be useful in management.

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Analysis of primary bioactive amines by positive and negative ion mass spectrometry of their isothiocyanates

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INTRODUCTION.

The so-called mustard oil reaction is a well-known analytical test for the recognition of primary amines (1). They are converted by carbon disulfide to dithiocarbamic acids, which decompose to the mustard oils on heating in the presence of a heavy metal ion, producing metal sulfide (Scheme A). Already in 1967, we have shown that amphetamine is easily and quantitatively converted to its isothiocyanate derivative by just adding carbon disulfide to its organic solution at room temperature (2). We had subsequently established that quite a number of other primary phenylalkylamines behave accordingly (Scheme B) and have used this spontaneous reaction for analysis and trace detection of these compounds in biological fluids (3, 4). Table I lists the compounds which we dealt with in our previous studies. The hydroxyl groups present in the rings and the side chains could be silylated simultaneously with the conversion of the amino to the isothiocyanate group (3).

The resulting mustard oils can be detected and dosed better than their corresponding amines for the following reasons :

1. They are less volatile ; no losses are to be feared on evaporating the extracts.

2. They are less polar ; less tailing and less irreversible adsorption can be observed in gas chromatography.

3. They can be detected not only with flame ionization and nitrogen detectors, but also by semi-specific detection systems such as electron capture or sulfur detectors.

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TABLE I

Compounds derivatized previously

Amphetamine	Norephedrine
Mescaline	Dopamine
STP	Noradrenaline

4. They show higher stability in electron impact mass spectrometry ; no excessive degradation occurs ; the few intense ions formed consist mainly of the molecular ion and the fragments resulting from β-degradation of the side chain. This is a prerequisite for trace analysis by selected ion monitoring (SIM).

In this report, we describe that the analysis for primary phenylalkylamines can be further perfected by incorporating negative ion mass spectrometry into the analytical scheme. We have also extended the method to the detection of phenoxyalkylamines, which undergo the same spontaneous conversion to the corresponding mustard oils as the phenylalkylamines.

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EXPERIMENTAL.

The free bases of phenethylamine, amphetamine, mexiletine, mexiletine p-methyl analogue, STP (DOM) or mescaline were dissolved in methyl t-butyl ether and a few ml of carbon disulfide added. After standing at room temperature for at least fifteen minutes, the solution was evaporated and the residue re-dissolved into ether.

Gas chromatography — mass spectrometry was carried out under the following conditions :

a) For positive electron impact mass spectrometry (positive EI-MS), an LKB-9000 equipped with a packed column (2 mm ID × 1.7 m) with 1.5 % SE-30 was used. The flow of the helium carrier gas

was 30 ml/min., to a large part removed by a two-stage jet separator. The spectra were taken at an electron energy of 70 eV. SIM was carried out at 20 eV.

b) For negative ion mass spectrometry, a modified LKB-2091 (5) was used, with a Megabore capillary (0.53 mm ID × 15 m) coated with DB-17 and coupled to the mass spectrometer by a one-stage jet separator. As carrier gas, helium was used with a flow rate of 7.5 ml/min. The box current and box potential were 500 uA and 100 eV, respectively. Negative ion mass spectrometry was carried out by chemical ionization (negative CI-MS) with methane at an ion source pressure of 10^{-2} torr. This yields not only quasi-molecular anions, but also some analytically useful anionic fragments (6, 7). Since methane acts mainly as a moderator for the production of low energy electrons, the reaction mechanism consisted mainly of electron attachment (7).

c) For dual mass spectrometry (dual-MS), a Ribermag R 10-10 C was used. The capillary column (0.32 mm ID × 30 m), coated with DB-1, was led directly into the ion source. Helium was used as carrier gas with a flow rate of 1 ml/min. As reagent gas, methane was used at an ion source pressure of 3×10^{-3} torr. As described previously (7-9), electron impact is hardly affected by the presence of reagent gases at such a low pressure, since positive CI-spectra can only be obtained at ion source pressures near or over 10^{-1} torr. On the other hand, negative CI-spectra can already be recorded at source pressures of 10^{-3} torr upwards. Their pressure dependence curve reaches a plateau at around 10^{-2} torr. In the pressure range of 10^{-3} to 10^{-2} torr, positive EI and negative CI fragmentation can therefore be obtained simultaneously, and the 2 basically different spectra recorded quasi-simultaneously side by side in a single analytical run.

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RESULTS AND DISCUSSION.

Tables II and III show the absolute and relative retention times of the compounds investigated using two different types of columns.

In table IV, the main anions obtained with negative CI-MS using the LKB-2091 are summarized and compared with the cations obtained with positive EI-MS using the LKB-9000. Positive EI-MS

TABLE II

GC Data : 1.5 % SE-30, Packed, 2 mm ID, 1.7 m, 30 ml He/min

Isothiocyanate of	*RT (min)*			*RRT*
	90° C	*140° C*	*180° C*	
Phenethylamine	14.8			0.15
Amphetamine	17.1	1.8		0.18
Mexiletine		6.5		0.63
p-Methylmexiletine		10.2	3.6	1.00
STP			4.7	1.32
Mescaline			6.9	1.93

TABLE III

GC Data : DB-17, Megabore, 0.53 mm ID, 15 m, 7,5 ml He/min

Isothiocyanate of	*RT (min)*		*RRT*
	140° C	*180° C*	
Phenethylamine	2.1		0.23
Amphetamine	1.9		0.21
Mexiletine	6.1		0.68
p-Methylmexiletine	9.0	1.7	1.00
STP		2.8	1.65
Mescaline		6.4	3.76

TABLE IV

Main Ions of 6 Isothiocyanates

Isothiocyanate of	*Mr*	*EI Pos.*		*CI Neg. (CH_4)*	
		B^+	*M^+*	*B^-*	*$[M-H]^-$*
Phenethylamine	163	91	163	58	162
Amphetamine	177	91	177	58	176
Mescaline	253	181	253	58	252
STP	251	165	251	58	250
Mexiletine	221	122	221	58	220
p-Methylmexiletine	235	136	235	58	234

EI Pos. : LKB-9000, 70eV
CI Neg. : LKB-2091, CH_4 10^{-2} torr, Box current 500. Box eV 100

yielded the substituted tropylium ions for phenylalkyl-isothiocyanates and the substituted phenol residues for phenoxyalkyl-isothiocyanates. These base ions result from β-degradation of the side chains and from normal phenylether degradation, respectively. With both types of isothiocyanates, considerably less intense molecular ions were observed. In all negative ion spectra,

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the isothiocyanate anion with mass 56, resulting from α-degradation of the side chain, is base peak. The other anion of interest is always the rather weak quasi-molecular ion [M-H]⁻.

The dual-MS presentation of the isothiocyanate of p-methylmexiletine, which is a synthetic chemical and can be used as an internal standard, is given in figure 1. The main ions from all compounds are listed in table V. With the conditions we used,

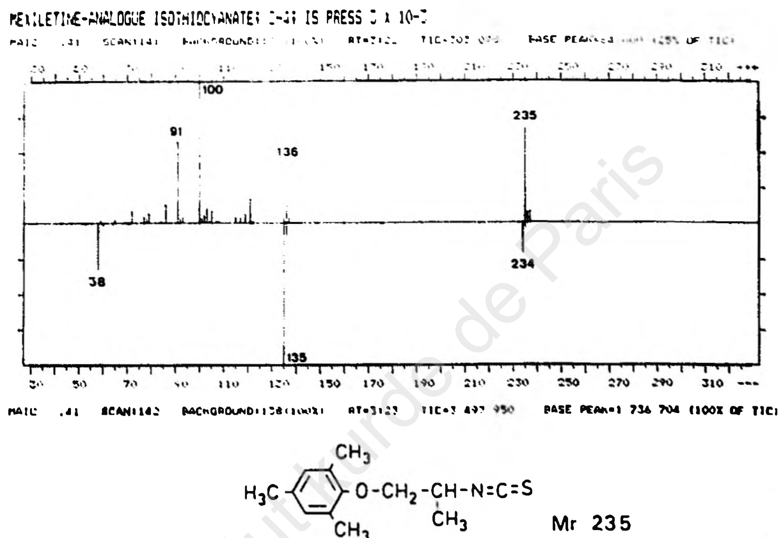

FIG. 1.

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TABLE V

Comparison between EI Pos. and CI Neg. in Dual-MS

Isothiocyanate of	*TIC-Ratio EI Pos. : CI Neg.*	*Main Ions (% I)*					
		EI Pos.			*CI Neg. (CH_4)*		
Phenethylamine	1.0 : 1.1	91 (100)	105 (24)	163 (12)	162 (100)	58 (17)	
Amphetamine	1.0 : 0.5	91 (100)	86 (71)	177 (7)	176 (100)	58 (21)	
Mescaline	1.0 : 2.3	253 (100)	181 (44)		252 (100)	58 (9)	
STP	1.0 : 2.4	251 (100)	165 (52)		250 (100)	58 (4)	
Mexiletine	1.0 : 17.1	100 (100)	221 (52)	105 (53)	121 (100)	58 (28)	230 (2)
p-Methylmexiletine	1.0 : 15.3	100 (100)	235 (84)	91 (53)	135 (100)	58 (39)	234 (21)

Nermag R10-10C, CH_4 3×10^{-3} torr

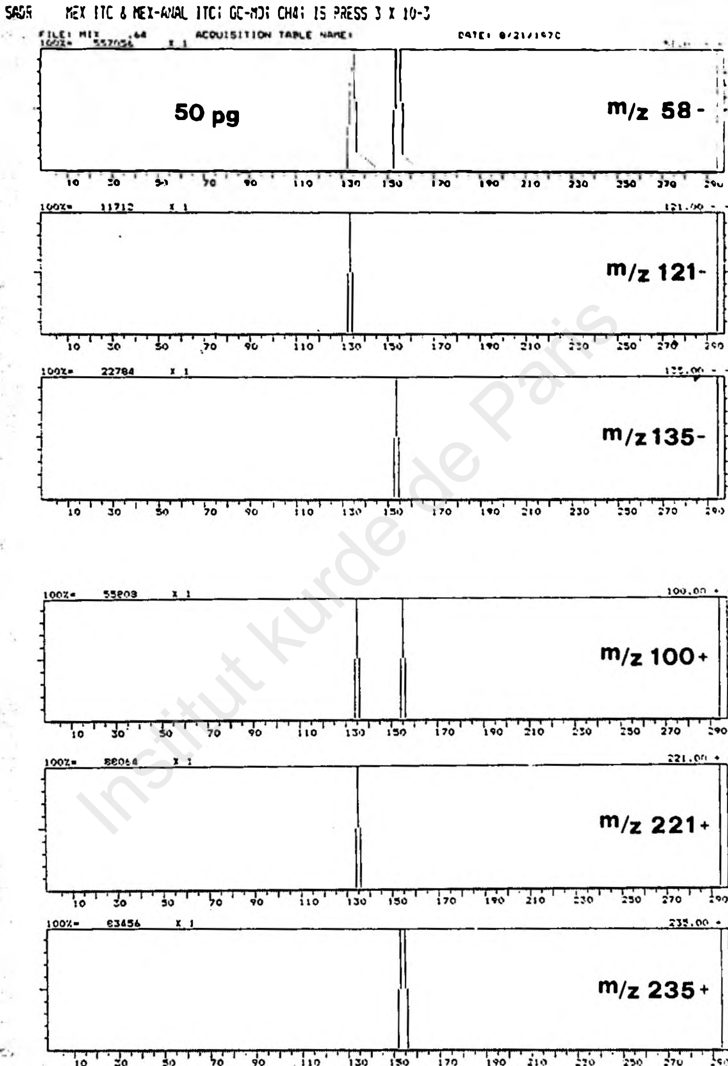

FIG. 2.

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positive EI-MS showed the fragments resulting from β-degradation for phenylalkyl-isothiocyanates and from the normal phenylether degradation for phenoxyalkyl-isothiocyanates. These fragments were base ions except for the isothiocyanates of mescaline and of STP, which showed the molecular ions as base peaks. Negative CI-MS yielded intense isothiocyanate anions for all compounds. The anions $[M\text{-}H]^-$ were base peaks for phenylalkyl-isothiocyanates but only weak for phenoxyalkyl-isothiocyanates, which yielded substituted phenol ions as base peaks, obtained by normal phenylether degradation.

As shown in table V, with phenoxyalkyl-isothiocyanates, the total ion currents in negative CI-MS were more than ten times higher than in positive EI-MS. Phenylalkyl-isothiocyanates showed total cation and total anion currents of similar intensity.

Since every isothiocyanate derivatives investigated yielded a relatively intense anion with mass 58, representing the isothiocyanate group, this anion can be used for group analysis by SIM, while molecular ions in positive EI-MS or the anions $[M\text{-}H]^-$ in negative CI-MS can be used for detecting individual compounds. In addition, the positive ions resulting from β-degradation or normal phenylether degradation should also be helpful for group analysis as well as single compound detection.

An example for the trace analysis by SIM is presented in figure 2. The mixture of 50 pg each of the isothiocyanates of mexiletine and p-methylmexiletine was injected. Depending on the GC-column used, the lowest limit for detection can be 1 to 50 pg.

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Appraisal of wide bore capillary columns for drug analysis in forensic toxicology

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INTRODUCTION.

Gas chromatography (GC) is one of the most widely used techniques in a forensic toxicology laboratory for the qualitative detection of drugs and for their quantification. The identification of an unknown is made by comparing its retention properties (retention index, RI) with those of reference samples recorded in a database. Packed columns have been used by toxicologists in the past but capillary columns are now being increasingly investigated because of their superior resolution and the advantages imparted by fused silica column technology and bonded stationary phases.

Conventional capillary GC has involved the use of narrow bore columns, typically with internal diameters of 0.2 mm and with thin films of stationary phase (about 0.25 microns). More recently wide bore (0.53 mm I.D.) fused silica capillary columns with chemically bonded thick films (typically 1-5 microns) have been introduced and are becoming increasingly popular amongst chromatographers. They offer comparable sample capacities to packed columns, can be installed into a packed column injection port of a gas chromatograph and are often regarded as a « capillary replacement » for packed columns. Other features of practical importance to the toxicologist include the use of direct flash vaporization injection whereby the total sample is passed on to the column, potentially simplifying and improving quantification. This contrasts to the use of narrow bore columns where special injection techniques are required (split and splitless).

The extensive use of packed column GC in the past has resulted in the generation of several databases giving retention index values of compounds of toxicological significance on SE-30 type columns (1-3). Following the introduction of narrow bore chemically bonded capillary columns, some studies have been carried out to compare the RI values of compounds on these narrow bore columns with those on packed columns (4-8). The introduction of wide bore columns and their potential application in forensic toxicology requires that their retention properties also be examined.

In this paper the retention properties of wide bore columns (under isothermal and temperature programmed conditions) are discussed and compared to those of narrow bore capillary and packed columns. An assessment is made of the validity of transferring RI values between wide bore thick film capillary columns, narrow bore thin film capillary columns and packed columns all having an SE-30 type stationary phase. In addition, the resolution given by these three types of column is compared. The installation and operating conditions of wide bore columns have been investigated in some detail and finally the application of both narrow bore and wide bore capillary columns to toxicology casework are discussed.

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EXPERIMENTAL.

Materials.

N-Butyl acetate and pentane were Analar grade from BDH (Poole, Great-Britain). Straight chain alkane hydrocarbons were obtained from SGE (Milton Keynes, Great-Britain) and Sigma (Poole, Great-Britain). All drugs came from the collection of the Central Research Establishment, Home Office Forensic Science Service.

Columns.

The columns used in this study were :

a) BP1 narrow bore, 25 m × 0.22 mm I.D. with 0.25 micron film thickness (SGE, Milton Keynes, Great-Britain).

b) BP1 wide bore, 25 m × 0.53 mm I.D. with 1.0 micron film thickness (SGE, Milton Keynes, Great-Britain).

c) DB1 wide bore, 30 m × 0.53 mm I.D. with 1.5 micron film thickness (J and W Scientific, Jones Chromatography Ltd, Glamorgan, Great-Britain).

d) HP1 wide bore, 30 m × 0.53 mm I.D. with 0.88 micron film thickness (Hewlett Packard, Reading, Great-Britain).

e) 2 m × 4 mm I.D. glass column packed with 3 % (w/w) SE-30 on Chromosorb W HP (80-100 mesh), (Supelco, Radley and Co Ltd, London, Great-Britain).

Gas chromatography.

Chromatography was performed using Perkin Elmer Sigma 3B and 8,500 gas chromatographs with an AS8300 autosampler fitted to the latter. For the narrow bore column (0.22 m I.D.), a capillary injector operated in the split mode at a split ratio of 20:1 was used. For the wide bore columns (0.53 mm I.D.) and the packed column, a 1/4 in. packed column injection port was employed. The carrier gas was nitrogen operated at flow-rates of 1 ml/min., 10 ml/min. and 40 ml/min. for narrow bore, wide bore and packed columns respectively. For some of the studies involving wide bore columns, helium carrier gas was also used. All studies utilized a flame ionization detector (FID).

Chromatography under isothermal oven conditions was performed at 130°C, 200°C and 275°C. For studies using temperature programmed conditions the oven temperature was increased from 100°C to 300°C at rates of 5°C/min., 10°C/min., 15°C/min. and 20°C/min.

Drugs (free acids or free bases) were dissolved in n-butyl acetate while solutions of straight chain hydrocarbons were prepared in pentane for the calculation of retention indices and Trennzahl (TZ) values.

Installation of wide bore columns.

Seven different ways of installing wide bore columns into a 1/4 in. packed column injection port were investigated :

a) a glass injection port liner (for direct flash vaporization injection) with a tapered restriction at both ends, positioning the column in the lower restriction (J and W Scientific, Jones Chromatography Ltd, Glamorgan, Great-Britain).

b) a glass liner (for direct injection) with the inlet end of the liner chamfered to facilitate sample injection (Supelco, Radley and Co Ltd, London, Great-Britain).

c) a glass lined stainless steel adaptor (SGE, Milton Keynes, Great-Britain) for direct flash vaporization injection, with the column positioned at the base of the adaptor.

d) a stainless steel adaptor with a fused silica insert liner (Chrompack UK Ltd, London, Great-Britain) for direct flash vaporization injection. Connection and positioning of the column was made at the base of the adaptor.

e) a glass liner for direct flash vaporization injection (narrow bore, flat end) (Chrompack UK Ltd, London, Great-Britain), with the column positioned in the middle of the liner.

f) a glass liner (narrow bore) and an injection adaptor connecting and positioning the column at the base of the adaptor, for direct flash vaporization injection (Perkin Elmer, Beaconsfield, Great-Britain).

g) a glass injection port liner (for on-column injection) having a tapered restriction at the top (septum end) of the liner with the column positioned into this restriction (J and W Scientific, Jones Chromatography Ltd, Glamorgan, Great-Britain).

Connection of the wide bore columns to the 1/8 in. detector base of the gas chromatographs was made using a 1/8 in. Swagelock nut, backing washer and graphite ferrule, after first removing the detector receiver. Te column was positioned in the jet of the FID. Make-up gas to the detector was not used.

RESULTS AND DISCUSSION.

The correlations observed for the isothermal retention indices of drugs on narrow bore and wide bore capillary and packed columns are shown graphically in figures 1, 2 and 3, and the correlation coefficients for the pairs of columns examined are presented in table I. The results clearly demonstrate that all pairs are highly correlated. Compounds known to produce poor peak shapes on SE-30 packed columns did not chromatograph well on the wide bore and narrow bore capillary columns (i.e. nicotinic acid, acetylsalicylic acid, physostigmine, tolazoline, warfarin and atropine) and gave RI values which differed by more

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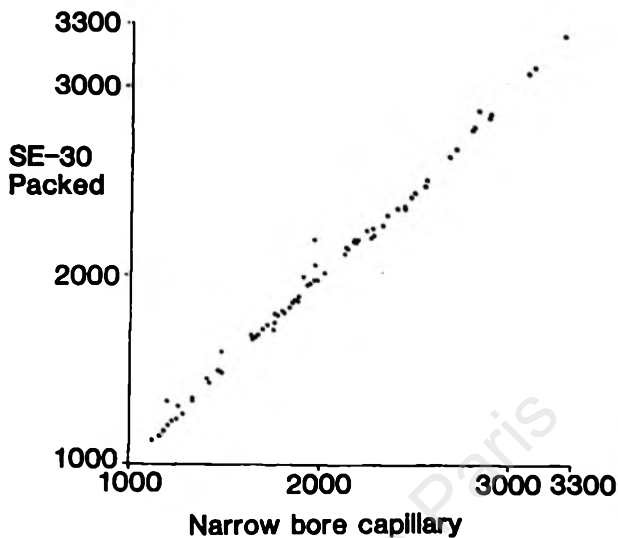

FIG. 1. — Correlation of the retention indices of drugs and other compounds of toxicological interest on narrow bore capillary and packed columns (r = 0.9965).

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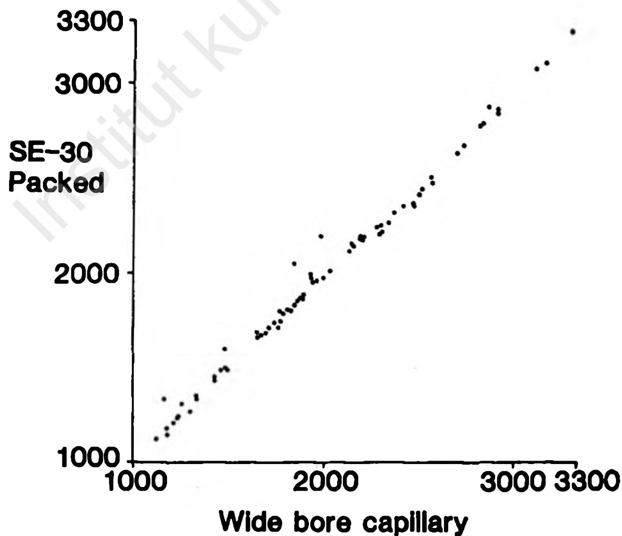

FIG. 2. — Correlation of the retention indices of drugs and other compounds of toxicological interest on wide bore capillary and packed columns (r = 0.9950).

than ± 50 from the packed column data of Ardrey and Moffat (2). Otherwise all other RI values on the capillary columns were within the range ± 50 RI units from the mean data for packed columns (2). In contrast the variation in RI values between narrow bore and wide bore capillary columns was within

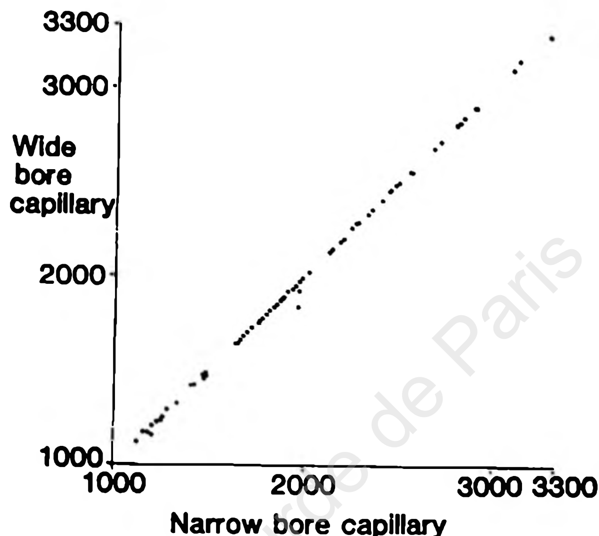

FIG. 3. — Correlation of the retention indices of drugs and other compounds of toxicological interest on SGE wide bore and narrow bore capillary columns (r = 0.9994).

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TABLE I

Correlation coefficients between GC columns
(NB = narrow bore ; WB = wide bore)

Column	*Column*		
	SGE, BP1 (WB)	*J and W, DB1 (WB)*	*SE-30 (Packed)*
SGE, BP1 (NB)	0.9994	0.9975	0.9965
SGE, BP1 (WB)		0.9967	0.9950
J and W, DB1 (WB)			0.9979

± 20 from the mean values. The correlation coefficients of the isothermal retention properties for each of the three types of column (table I) indicate that databases of retention indices for drugs and poisons generated on packed columns are applicable to both wide bore and narrow bore capillary columns.

A comparison of drug retention indices determined on a BP1 wide bore column under isothermal and four different temperature

programmed conditions is given in figure 4. The histograms show the differences (ΔRI) between temperature programmed RI values and isothermal RI values for a group of drugs chosen to chromatograph totally under programmed conditions and not during any isothermal phase following the program. Although a general increase in RI was observed with an increasing temperature programming rate (from 5°C/min. to 20°C/min.) the data show that ΔRI is small for most drugs and within the normal search windows applied when using RI data bases for the identification of unknown compounds. Consequently the use of wide

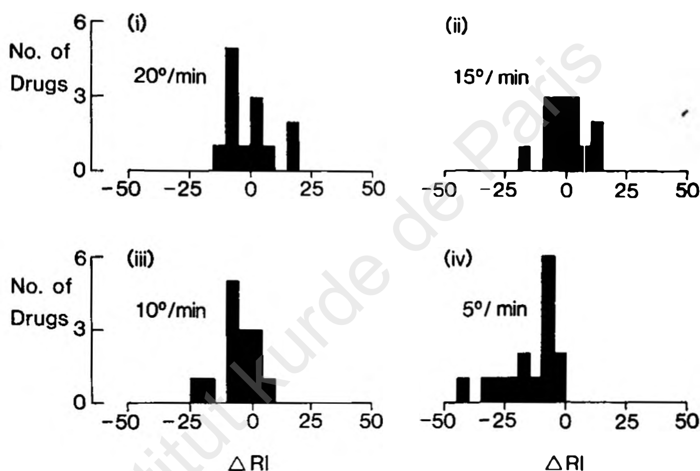

FIG. 4. — Histograms showing the differences between temperature programmed and isothermal retention index values for a selection of drugs under different temperature programmed conditions on a wide bore BP1 column. Δ RI = RI (temperature programmed — RI (isothermal). (i) 20°C/min, (ii) 15°C/min, (iii) 10°/Cmin, (iv) 5°C/min.

bore columns in forensic toxicology under isothermal and temperature programmed conditions may utilize the same data bases.

The chromatography of a mixture of eight barbiturate drugs on a wide bore capillary column operated over a range of carrier gas flow rates (3-40 ml/min.) is shown in figure 5. Wide bore columns can be operated at high carrier gas flow rates, figure 5 *(a)* (i.e. in the packed column mode) or at low carrier gas flow rates, figure 5 *(e)* (i.e. in the capillary mode approaching the optimum carrier gas velocity for the column). Operation at high flow rates gives very short analysis times but resolution is lost. However, to maximize resolution, analysis times become inconveniently long and are impractical. A convenient nitro-

gen flow rate for operation of wide bore columns is therefore 10 ml/min. when a compromise between resolution and analysis time is obtained. Consequently, all subsequent studies have used a nitrogen carrier gas flow rate of 10 ml/min.

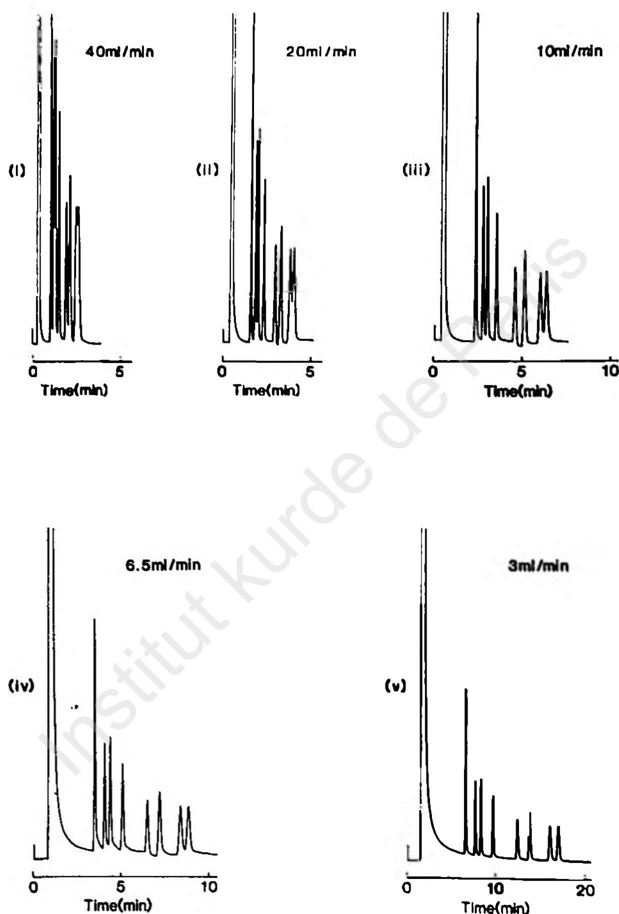

FIG. 5. — Chromatography of barbiturates using nitrogen carrier gas flow rates of (i) 40 ml/min, (ii) 20 ml/min, (iii) 10 ml/min, (iv) 6.5 ml/min, (v) 3 ml/min. DB1 wide bore column.

Comparisons of the resolution given by packed, wide bore capillary and narrow bore capillary columns, under isothermal conditions, for four drug groups commonly encountered in forensic toxicology (barbiturates, opiates, benzodiazepines and antidepressants) are shown in figures 6 to 9 respectively. The chromatograms clearly show the superior resolution of the narrow bore

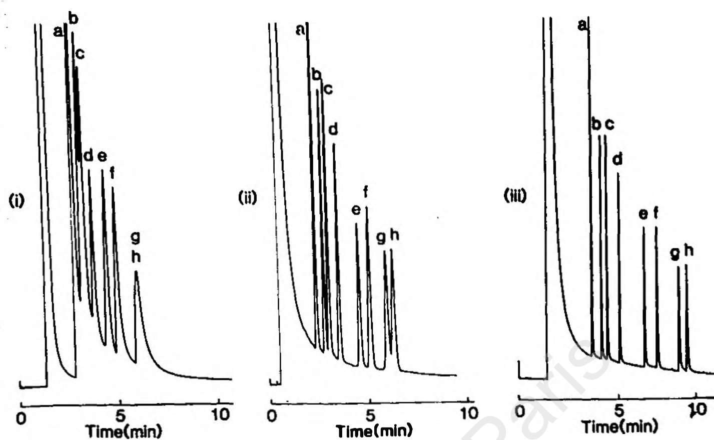

FIG. 6. — Chromatography of barbiturates on (I) SE130 packed column, (II) DB1 wide bore column, (III) BP1 narrow bore column. Peaks : a = butobarbitone ; b = amylobarbitone ; c = pentobarbitone ; d = quinalbarbitone ; e = thiopentone ; f = methylphenobarbitone ; g = phenobarbitone ; h = cyclobarbitone.

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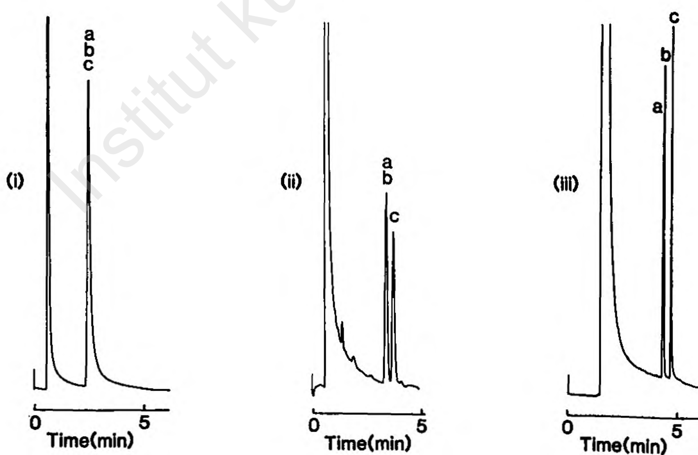

FIG. 7. — Chromatography of opiates on (I) SE-30 packed column, (II) DB1 wide bore column, (III) BP1 narrow bore column, Peaks: a = codeine ; b = dihydrocodeine ; c = morphine.

column and that the resolution of the wide bore is closer to that of the packed column. This is further illustrated by the Trennzahl (TZ) values calculated for the different columns and shown in table II. The TZ value is a measure of the separation efficiency of a column and gives the number of peaks which can

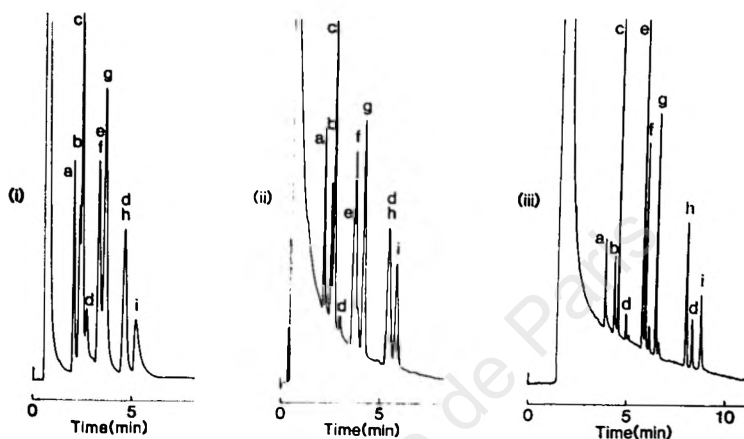

FIG. 8. — Chromatography of benzodiazepines on (I) SE-30 packed column, (II) DB1 wide bore column, (III) BP1 narrow bore column. Peaks : a = oxazepam ; b = lorazepam ; c = diazepam ; d = chlordiazepoxide ; e = flunitrazepam ; f = nitrazepam ; g = prazepam ; h = flurazepam ; i = clonazepam.

TABLE II

Separation efficiencies in terms of trennzahl (TZ) values for SE-30 packed, DB1 wide bore capillary and BP1 narrow bore capillary columns

The TZ values are the averages of those calculated for pairs of alkanes in the range C-18 to C-22 at an isothermal oven temperature of 200° C. Nitrogen carrier gas flow rates were 40 ml/min (packed column), 10 ml/min (wide bore column) and 1 ml/min (narrow bore column)

Column	*TZ value*
SE-30 packed	2.4
DB1 wide bore capillary	3.9
BP1 narrow bore capillary	11.2

be located with baseline separation between two consecutive members of an homologous series differing by one methylene unit.

It must be emphasized that the chromatographic conditions chosen for the comparisons in figures 6 to 9 may not be optimised for particular separations and improved separations might be obtained by, for example, changing the oven temperature. For the purposes of these studies, convenient chromatographic conditions

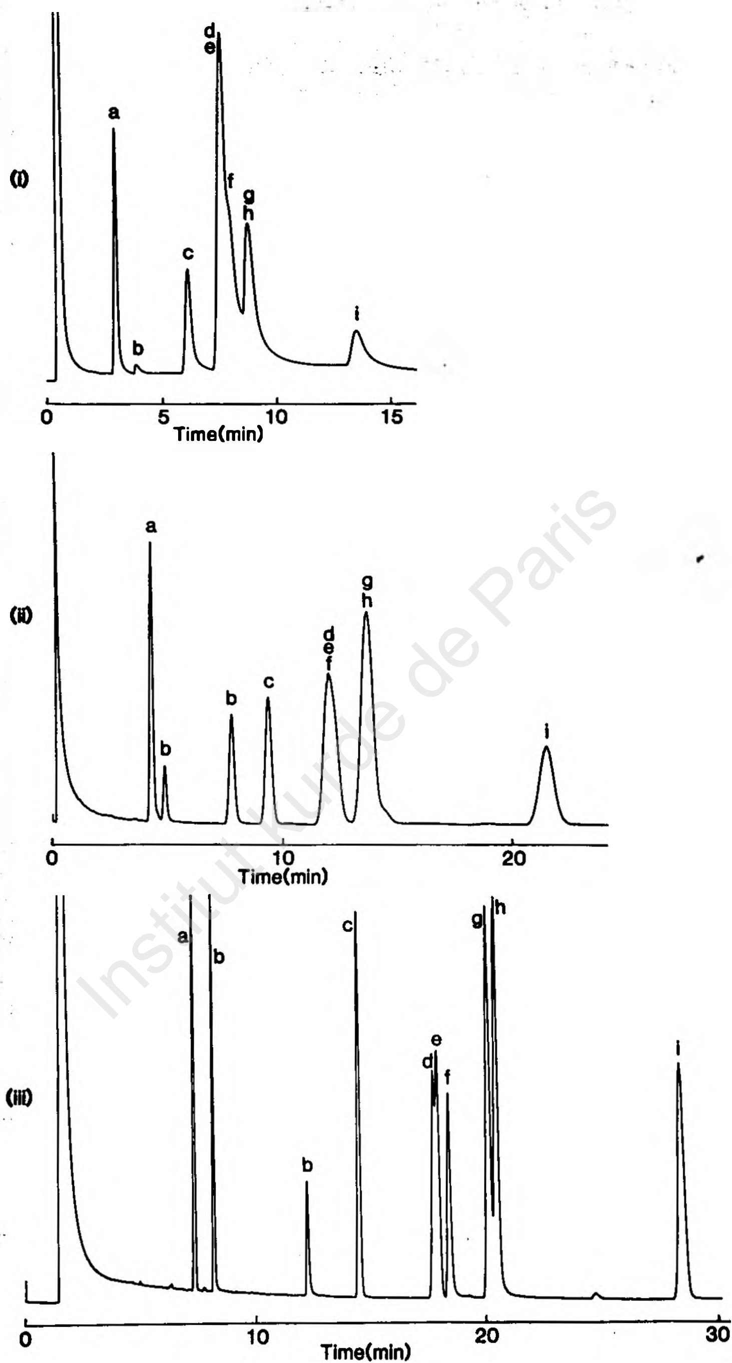

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FIG. 9. — Chromatography of antidepressants on (I) SE-30 packed column, (II) DB1 wide bore column, (III) BP1 narrow bore column. Peaks : a = clorgyline ; b = isocarboxazid ; c = nomifensine ; d = amitriptyline ; e = butitriptyline ; f = nortriptyline ; g = desipramine ; h = protriptyline ; i = dothiepin.

were chosen for use by all three types of column so that direct comparisons could be made.

Figures 6 to 9 also illustrate the well documented advantages of using fused silica bonded phase columns, namely enhanced sensitivity because of the increased sharpness of peaks and an inert system leading to a reduction in peak tailing.

There are two basic designs of injection port liner currently commercially available for direct flash vaporization injection with wide bore columns and these are shown schematically in figure 10 *(i)*

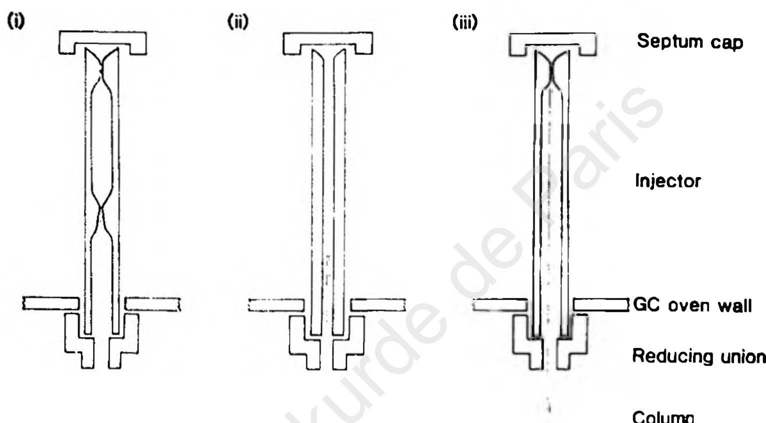

FIG. 10. — Schematic diagrams showing the different types of injection port liners for installing wide bore columns into a 1/4 in. packed column injection port (i) direct flash vaporisation injection liner (J. and W. Scientific). (ii) narrow bore injection port liner similar to those supplied by Supelco, SGE, Chrompack and Perkin Elmer (dashed line indicate possible positioning of the column inside the liner for direct flash vaprisation injection). (iii) on-column injection liner (J. and W. Scientific).

and *(ii)*. The injection port liner supplied by most manufacturers is a narrow bore glass tube of constant internal diameter throughout its length [fig. 10 *(ii)*], where the column is mounted with the end either at the bottom of the liner (recommended by several manufacturers) or further into the liner. The second type of liner is that supplied by J and W, having a wider diameter and a narrow restriction at both ends, the column being positioned at the lower restriction [fig. 10 *(i)*].

Figure 11 shows the isothermal chromatograms obtained for identical injections of the barbiturate test mixture (fig. 6) on a DB1 wide bore column mounted into the injection port liners from different manufacturers. Different results were obtained from the various liners with respect to the size and degree of

tailing of the solvent peak, with the J and W liner [fig. 11 *(i)*] showing better performance characteristics than the other liners [fig. 11 *(ii)* to *(VI)*].

The requirements of any GC injection process are to place a sharp initial band of sample on to the top of the column, since the final peak width at the detector is influenced by contributions to broadening made by the injector. Injection of a liquid into a

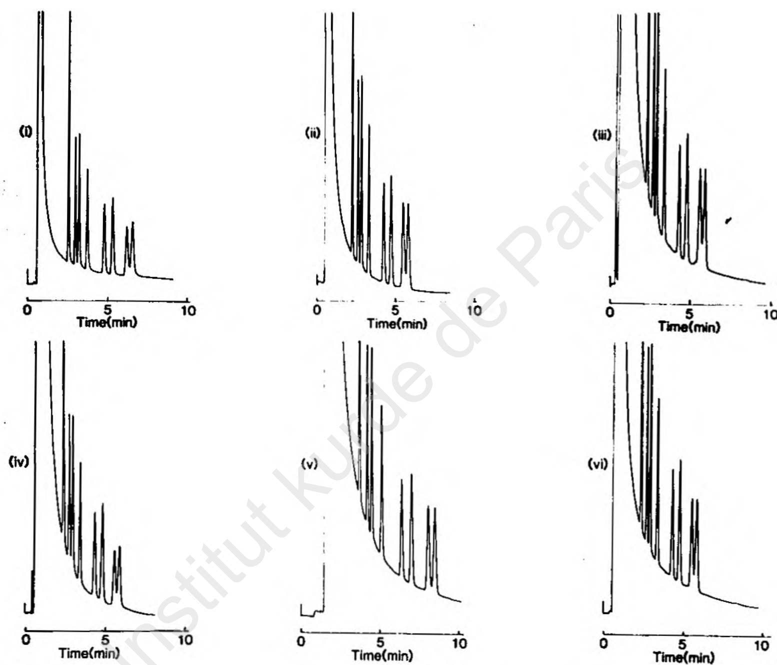

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FIG. 11. — Chromatography of barbiturates using direct flash vaporisation injection port liners (i) J. and W. Scientific, (ii) Supelco, (iii) SGE, (iv) Chrompack fused silica lined stainless steel adaptor, (v) Chrompack glass liner, (vi) Perkin Elmer. DB1 wide bore column.

hot injection port gives rise to a pressure increase and as vapour passes into the column the pressure surge subsides.

The differing results for the various injection liners (fig. 11) can be explained by a consideration of the different geometries of the liners and the positioning of the column in the liners.

For the J and W liner [fig. 10 *(i)*] the sample vapour has no option but to be swept directly on to the column, whereas for the other type of liner [fig. 10 *(ii)*], the bulk of the sample will be directed on to the column but some may pass beyond the column entrance to the unswept area between the column and

the bottom of the injection liner. As the pressure surge subsides the remaining sample diffuses upwards and can then be swept on to the column giving rise to a tailing solvent peak as shown in figure 11. Flashback against the septum, also arising from the pressure increase on injection, can further contribute to solvent tailing : this is prevented in the J and W liner by the restriction at the top but may occur with other liners.

The pressure increase on injection is lowered by the use of a larger injector volume, however, increasing this volume must be balanced against the need to minimize dilution of the sample with carrier gas. The injection liners of the type shown in figure 10 *(ii)* were all calculated to have smaller internal volumes (100-300 µl) than the J and W liner (500 µl) and so the overriding factor contributing to the tailing solvent peak obtained for the former is likely to be the area of unswept dead volume below the column entrance.

The distance between the end of the syringe needle and the column entrance was also found to be important and for optimum performance should be approximately 1 cm-2 cm. The influence of this factor on the overall performance of the injector is illustrated in figure 11 *(ii)* for the Supelco liner. In this case the column entrance was positioned 1 cm from the end of the syringe needle and although the unswept dead volume was consequently increased the overall performance was satisfactory.

The performance of each injection liner is thus governed by several inter-related factors, with the J and W liner combining the most positive features which contribute to a successful injection. Using this liner, injections were possible at lower attenuation settings while still avoiding interference from the solvent peak. It is important to emphasize a point made by the manufacturer's of this liner that the optimum performance requires the top of the wide bore column to be cut « cleanly » and square so that a snug fit is obtained between the column and the restriction, otherwise solvent tailing was found to occur.

Figure 12 shows the chromatography obtained for the barbiturate test mixture using on-column injection on to a wide bore column with a J and W on column liner [fig. 10 *(iii)*]. Injections can be made using a standard 26 gauge syringe needle because of the increased diameter of these columns, thereby simplifying on-column injection. The chromatogram in figure 12 shows solvent tailing to be reduced, apparently because contributions to broadening of the initial band of sample by the injector are

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diminished. It is assumed that on-column injections using other types of liner such as those in figure 10 *(ii)* are possible by positioning the column close to the septum although this was not investigated here.

Figure 13 shows the analysis of the same barbiturate test mixture on three manufacturer's columns (DB1, HP1 and BP1), all

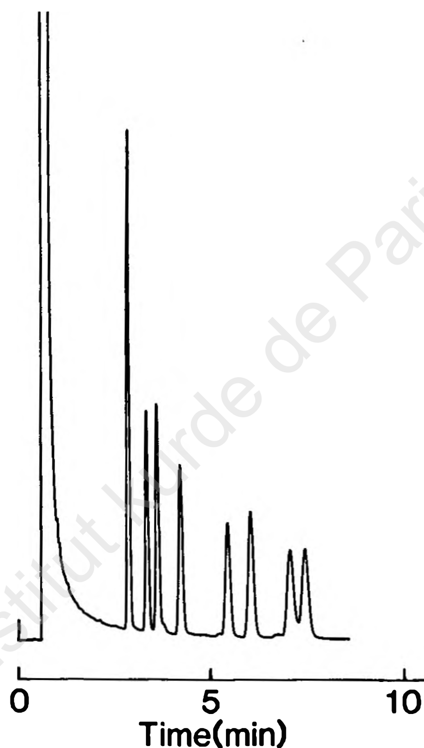

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FIG. 12. — Chromatography of barbiturates using a J. and W. on-column injection port liner. DB1 wide bore column.

having an SE-30 type stationary phase and showing the same retention properties. Slight differences in resolution and analysis times were observed due to the differing lengths and film thicknesses of the columns but overall the columns were found to be very similar.

The performance of wide bore columns can be improved by the optimization of certain operating conditions. Figure 14 shows the analysis of the benzodiazepine test mixture at injector temperatures of 300°C [fig. 14 *(i)*] and 285°C [fig. 14 *(ii)*] with the

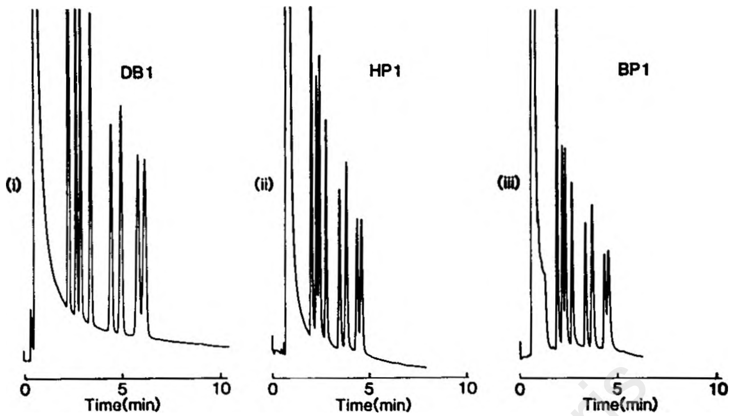

FIG. 13. — Chromatography of barbiturates on (I) DB1 wide bore capillary column, 30 m × 0.53 mm ID, 1.5 µm film thickness (II) .HP1 wide bore capillary column 30 m×0.53 mm ID, 0.88 µm film thickness, (III) BP1 wide bore capillary column, 25 m×0.53 mm ID, 1.0 µm film thickness.

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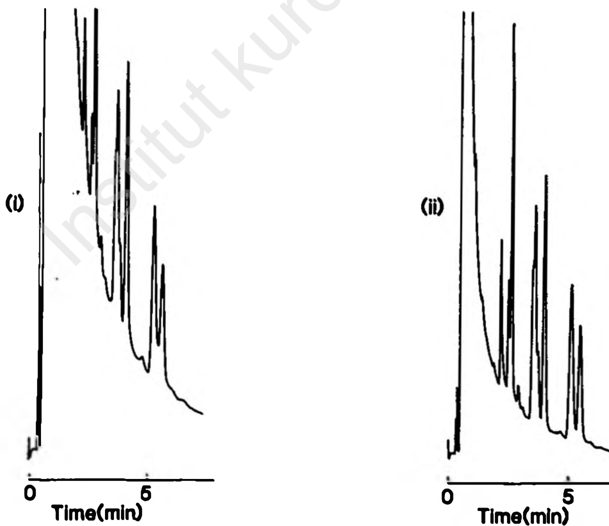

FIG. 14. — Chromatography of benzodiazepines with injector temperatures of (I) 300°C, (II) 285°C. DB1 wide bore column with SGE injection port adaptor.

SGE injection port adaptor. At 300°C the vaporization of the sample is more violent giving rise to an increased pressure surge and flashback against the septum. It is therefore advisable to use the lowest injector temperature compatible with the boiling point of the solvent and the required oven temperature.

The choice of carrier gas also affects the performance of wide bore columns. The chromatograms obtained for the analysis of barbiturates using nitrogen and helium are shown in figure 15,

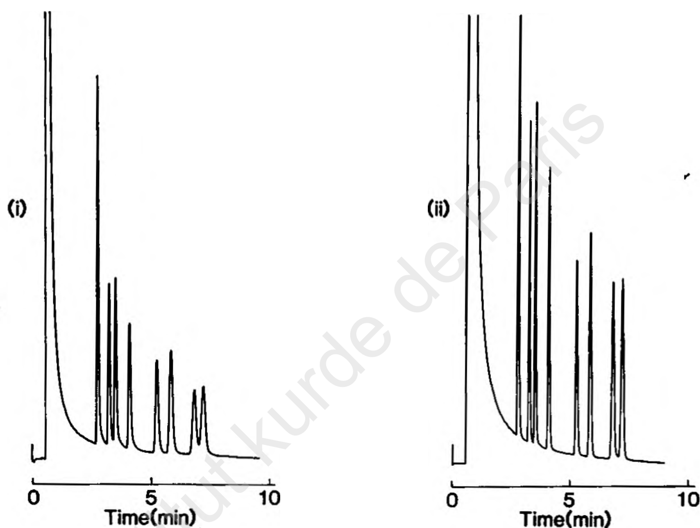

FIG. 15. — Chromatography of barbiturates using :
(i) nitrogen carrier gas at a flow rate 10 ml/min.
(ii) helium carrier gas at a flow rate 5 ml/min. DB1 wide bore column.

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illustrating that helium gives superior resolution to nitrogen for the same analysis times.

For the qualitative detection and quantification of drugs in forensic toxicology by GC, a column of high separation efficiency and high capacity, which permits fast analysis and allows simple injection techniques to be used is highly desirable. However, these chromatographic parameters are inter-related and consequently the requirements cannot be met by just one type of GC column. The comparison of packed, wide bore and narrow bore capillary columns has demonstrated the advantages of replacing packed columns by wide bore capillary columns for toxicological analysis. Long columns ($\simeq$ 30 m) of film thickness $\simeq$ 1 μm are preferable to obtain maximum separation efficiency while pro-

viding sample capacity. These columns are easily installed into a packed column injection port for direct flash vaporization injection. The use of selective detectors rather than FID may reduce problems of solvent interference on chromatographic peaks.

Although quantification using wide bore columns with direct flash vaporization injection has not been studied here, it is reported to yield good results because all of the sample is directed on to the column. This contrasts to split and splitless injection on to narrow bore columns where part of the sample is purged from the injector. Problems of adsorption and degradation in the injector liner will occur for all injection techniques involving sample vaporization (direct and split/splitless). On-column injection using wide bore columns is simplified by the use of standard 5 µl syringes with 26 gauge needles unlike on-column injection on to narrow bore columns where fused silica needles are necessary.

Despite the advantages of wide bore columns applicable to toxicological analyses, the separation efficiency of these columns is very much inferior to that of narrow bore columns and so the latter must be the column of choice for analyses of complex samples containing components with similar retention properties.

The correlation of the retention properties of wide bore and narrow bore capillary columns and the comparison of retention indices determined under both isothermal and temperature programmed conditions has demonstrated that the transfer of retention data between columns is possible.

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CONCLUSION.

This paper has compared the properties of packed, wide bore capillary and narrow bore capillary columns assessing their relative merits with a view to analytical applications in a forensic toxicology laboratory. The work has illustrated the value of replacing packed columns with wide bore fused silica capillary columns having chemically bonded stationary phases. Installation into a packed column injection port is relatively straightforward and best carried out using a J and W liner for direct flash vaporization injection. Operation of these columns using helium carrier gas (flow rate 5-10 ml/min.) is advisable to achieve the best performance. Both wide bore capillary and conventional narrow bore capillary columns will find applications in the

toxicology laboratory dictated, in the main, by the separation efficiency required for a particular analysis because the two columns differ markedly in resolving power.

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How to interpret the unexpected analytical results of a « common » euthanasia case

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SUMMARY.

Today the possibilities of a forensic toxicological laboratory increases enormously. Unfortunately the knowledge how to interpret the magnificent analytical results grows only slowly. Therefore the difficulties in a forensic toxicological laboratory shift from the analysis to the interpretation and elucidation of the case.

In this paper an euthanasia case will be discussed, in which the analytical results did apparently not correspond with the given drugs.

A physician declared to the police to have administered several drugs for euthanasia to a very ill man. The 74 years old patient drank a glass of water with phenobarbital sodium and hydroxyzine hydrochloride. Because he did not die the next hours the doctor injected him with midazolam and pancuronium. Some minutes later the patient died. Post-mortem we found in the blood 25 mg l^{-1} phenobarbital, 2,000 $\mu g\ l^{-1}$ hydroxyzine, 516 $\mu g\ l^{-1}$ imipramine, 53 $\mu g\ l^{-1}$ desipramine, 614 $\mu g\ l^{-1}$ morphine and 15 $\mu g\ l^{-1}$ midazolam. No ethanol and pancuronium were detectable.

By means of clinical toxicological experience and knowledge about pharmacokinetics the difference between the doctor's first information and the analytical results has been elucidated.

INTRODUCTION.

A forensic toxicological investigation consists of at least six parts :

1. Collection of all available information.
2. Taking samples.
3. To set up an analytical scenario.
4. The analysis.
5. The interpretation of the results.
6. The report of the conclusions.

The quality and quantity of the samples often depends on the situation of the victim. Unfortunately, samples are usually not taken by the toxicologist (1).

Today the analytical possibilities of a forensic toxicological laboratory have increased enormously. New techniques make it possible to detect and measure many compounds in body samples very sensitively and reliably.

On the other hand the knowledge how to interpret their magnificent analytical results grow only slowly. Therefore the difficulties in a forensic toxicological investigation shift from the analysis to the interpretation and elucidation of the case. Sometimes clinical experience and knowledge about pharmacokinetics, clinical toxicology and pharmacology can help us. In this paper we will present and discuss an euthanasia case, in which the analytical results did not apparently correspond with the given information.

CASE REPORT.

The doctor of a 74 years old man declared to the police investigator the following story :

An old man was suffering from melanosarcoma with diaspora in an endstage. He was in great pain, could hardly eat and all treatment failed. So the patient asked his doctor for euthanasia. The doctor discussed this request with him and his family and it was decided that the patient should drink a suitable euthanatic mixture. The doctor ordered a mixture of 6 grams of phenobarbital sodium and 1 gram of hydroxyzine hydrochloride from the local pharmacist. The pharmacist prepared this mixture. At 10.30 a.m. the patient drank a glass of water in which the phenobarbital and the hydroxyzine were dissolved. Fifteen minutes

later the patient was comateus. Two hours later a cheyne-stokes respiration was noticed. Because the patient did not die, the doctor injected him one ampoule of midazolam and two ampoules of pancuronium, 6 hours after the first attempt. Some minutes later the patient died. The doctor declared as cause of death : « not natural ». Therefore the public prosecutor ordered a port-mortem.

During the post-mortem an old yellow and very lean man was seen, who's liver was seriously affected by melanosarcoma with diaspora all over the body. It was clear that the patient was in a very bad clinical condition before he died.

The toxicological analysis.

Our laboratory was requested to do those analyses, that are necessary to elucidate the cause of death.

In these cases we use the following analytical scheme :

1. Alcohol screening on methanol, ethanol, trichloroethanol and acetone (2).

2. EMIT® tests on barbiturates, benzodiazepines and antidepressants.

3. If there is urine the EMIT® tests on drugs of abuse (opiates, amphetamines, cocaine, cannabinoides, methadon and dextropropoxyphen) are carried out as well.

4. TLC screening on hypnotics, neuroleptics and analgesics (3).

5. General capillary gaschromatografic screening on two different columns : one column of C.P.Sil-5, and the other with C.P.Sil-19. Both are 0.12 mm capillary columns, 10 m long, with a film thickness of 0.2 μm. The C.P.Sil-5 is connected with both a N.P.FID and an electron capture detector ; the C.P.Sil-19 only with N.P.FID. The GLC is connected on a Nelson data system with an I.B.M. Personal Computer with a 20 M.Byte hard disk.

On the disk the retention indices of about 1500 drugs and metabolites have been stored.

6. The drugs found in the screeningsprocedures and the drugs mentioned by the police or other persons are confirmed and quantitated by means of one or more HPLC systems (4, 5, 6).

Depending on the case, special analyses are carried out. In this case pancuronium was determined by a very sensitive chromatographic and fluorimetric assay (7).

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RESULTS.

We found in the blood taken from the subclavian vein, the following compounds :

Bloodconcentrations			*Reference values (8)*		
Drug	*Units 1⁻¹*	*Found*	*Therapeutic*	*Toxic*	*Lethal*
Phenobarbital	mg	25	20 - 40	60 - 80	120
Hydroxyzine	µg	2000	50 - 90	100 - 500	—
Imipramine	µg	516	45 - 150		
Desipramine	µg	53		sum : 400 - 600	2000
Morphine	µg	614	10 - 70	100 - 500	600
Midazolam	µg	15	80 - 250	—	—
Ethanol	g	< 0,1	—	1 - 2	5
Pancuronium	µg	< 50	100 - 750	—	500

Especially for morphine and ethanol the toxic level is very dependent on the habituation of the patient. For a muscle relaxant such as pancuronium it is hardly possible to speak about therapeutic, toxic or lethal levels, because the use of these compounds requires always artificial ventilation. Therefore therapeutic levels can be lethal.

DISCUSSION.

In the Netherlands euthanasia is not legal, although in special cases, in which it is committed very carefully and on request of the patient the charge can be dismissed.

The physician has to declare if the patient died natural course or not. Only in the last case, on request of the family or after notification by somebody, such as a nurse, inquiries into the cause of death have to be set up.

There seems to be a contradiction between our analytical results and the story of the doctor. Let me discuss the drug concentrations we found and try to elucidate the differences.

Phenobarbital and hydroxyzine.

In several countries there are tablets available, called Vesparax®, with a mixture of two barbiturates and hydroxyzine. This combination is rather dangerous and very effective for suicide. Therefore in our country a combination of a high concentration of one

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of two barbiturates and hydroxyzine is often given as an euthanatic drug. In this case 6 grams of phenobarbital and 1 gram of hydroxyzine were prescribed.

The pharmacist or his assistent must deliver the drugs which are prescribed by a doctor. But when he or she presumes that a mistake has been made or an overdose is ordered the doctor has to be phoned and the request will not be fulfilled. For euthanatic drugs the doctor has to contact the pharmacist personally. If the pharmacist accepts this request he, and not his assistent, prepares the drug and gives it to the doctor. If we look at the measured serumconcentrations we see a therapeutic phenobarbital level and a very high hydroxyzine level. So we assumed that the doctor told the truth when he told the police what he had prescribed. But the pharmacist might have made a mistake by mixing up the amounts of phenobarbital and hydroxyzine. This might also be the reason why the patient did not die after taking this mixture.

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Imipramine and desipramine.

The toxic concentration of imipramine and the very low metabolite level can be caused by : *a)* an overdose on the day of death, or *b)* a longterm treatment with imipramine and a bad metabolic function.

Mostly we found these concentrations after an overdose. But in this case the physician told us afterwards that the patient had taken imipramine three times 50 mg daily during many weeks. So a higher metabolite concentration could be expected. As forensic toxicologist I am mostly present at the post-mortem. There I had noticed the very bad condition of the liver of this patient, so a metabolic disfunction was explicable (9).

Morphine.

Several times we have found in post-mortem blood, morphine levels of about 650 µg per liter both after euthanasia with morphine and after heroine overdose by addicts.

Therefore in the beginning we concluded in this case « euthanasia by morphine in combination with other drugs ».

Then the doctor declared that this patient received four times 10 mg of nicomorphine subcutanously during several days. This

normal dose could not explain this toxic morphine level. But then it is important to know that imipramine and morphine both are metabolized in the liver by dealkylation and glucuronidation. Taken this in mind the statement of the doctor and our analytical results could be in conformity.

Midazolam.

Till today I have not a good explanation for the subtherapeutic level of midazolam. Midazolam was determined on a 15 cm, 3 mm I.D., 5 C18 HPLC-column, with UV 254 nm detection (Eluens : 60 ml methanol and 40 ml buffer pH 5.9 ; flow 0.8 ml.min^{-1}). The level of 15 µg per litre is about our detection level, so we were not able to confirm the identity of this compound.

There are some hypotheses for this low level :

1. The drug is not intravenously but intramuscularly injected.
2. The midazolam ampoules had been switched to other ones.
3. The midazolam had not yet reached the sampling place, due to the very bad circulation at that moment. Anyhow, midazolam is hardly toxic, and therefore not very important in this case.

Ethanol.

The patient did not take alcohol and we did not find alcohol. For a strong effect it should be better to take the phenobarbital with alcohol.

Pancuronium.

After intravenous administration of two ampoules of the muscle relaxant pancuronium it was very remarkable that no pancuronium could be found in the sample.

Because our HPLC method (9) for the bioanalysis of this kind of quarternary ammonium compounds is not sensitive enough to measure pancuronium in blood, we waited several weeks before we send a sample to the laboratory of Dr. Agoston in Groningen which is specialized in the analyses of muscles relaxants. During the time of storage the sample could be defrosted several times, which might be disastrous for the stability of pancuronium. Till today we have no other explanation, in spite of the fact that pancuronium is rather stable in serum.

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The interpretation of the toxicologist.

To the public prosecutor we declared that we have no indication to counter the statement of the doctor. But that on the other hand it could also not be excluded that the patient died by the high morphine and imipramine level as a result of accumulation of the treatment during the days before. The doctor was acquited of the charge.

IN CONCLUSION.

What lessons can we learn from this case ?

1. It is not sufficient to determine only those drugs which are mentioned in the report.

2. The conclusions can be wrong if these are based on an interpretation of the analytical results without sufficient knowledge about :

a) the background of the case ;

b) the post-mortem findings (liver status) ;

c) pharmacokinetical, pharmacodynamical and toxicological properties of the compounds.

If this had not been taken into account in our case the doctor might be under suspicion of not telling the truth and be convicted for murder.

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Signification of kerosene components detected in two burned bodies from criminological aspects

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ABSTRACT.

The chemical analysis of volatile hydrocarbons was made on 2 burned bodies, a 85-year-old man with the vital signs such as soot in the airway and 60 % of Hb-CO; while his 35-year-old grandson failed to reveal these findings. The hydrocarbons with carbon number 7 to 9 were found in both victims, who were considered to be exposed to kerosene vapor before death. The aliphatic hydrocarbons with carbon number 9 to 12, which are seldom observed in vapor phase of kerosene, were detected in the blood of the young victim. This fact strongly suggested that the victim was directly exposed to kerosene before vaporization. The conclusion drawn from the results of chemical analysis and anatomical findings was that the old man must have killed himself by setting fire to kerosene sprinkled, while the grandson was killed by fire after the direct exposure to kerosene.

INTRODUCTION.

The authors have so far been engaged in the detection of volatile hydrocarbons such as fuel components including gasoline and/or kerosene from medico-legal aspects, so that not only the cause of death in fires or explosions could be elucidated, but also how they originated in the first place.

This paper deals with a case of hydrocarbon analysis on 2 victims killed by fire, which led to the solution of the problems

regarding not only the cause of death, but also the process of the accident.

OUTLINE OF THE CASE.

The charred body of a 85-year-old man, K., was found upstairs in a house destroyed by fire, and the victim's grandson T., age 35, also dead, was discovered at the foot of the stairs together with a burned fuel container.

The old man's body in a supine position was entirely charred and there were many spots found emitting fuel odor. The autopsy revealed vital reactions such as soot in the airway and 60 % of Hb-CO. On the other hand, no soot was observed in T.'s body and the blood level of Hb-CO was 17 % which is often seen in case of post-mortem exposure. In contrast with the widely charred body surface, the damages caused to the surrounding walls and floor were relatively slight.

The difference in anatomical findings seen in the 2 bodies came into question and thus elucidation was required. The blood of the 2 victims were submitted to GC/MS analysis for volatile hydrocarbon.

MATERIALS AND METHODS.

1. Analytical procedure.

The partly improved method presented at the 21st TIAFT was employed (1).

The collected blood sample is divided into 2 portions. About 0.5 to 1 g of each portion is mixed with 1.0 μg of toluene $D_8(C_6D_5CD_3)$ as internal standard. In the first proces for detection of lower boiling points group such as gasoline, the sample is placed into an airtight glass tube and warmed at 40°C for 20min., and then 0.5 ml of the head space phase is analyzed by GC/MS. In the second process for higher boiling points group such as kerosene, the sample is extracted with 10 ml of carefully refined n-pentane and concentrated with nitrogen stream. Washing process of solvent phase with acid and alkali was omitted from this procedure because the water remaining prevents the recovery of components. As the final step, 1 μl of solvent phase is submitted to GC/MS analysis.

2. GC/MS conditions.

The apparatus was a computer-controlled quadrupole type of Shimadzu QP-1000 (EI mode). Different column systems and temperature conditions were employed according to the processes. Table I shows the conditions.

TABLE I

GC/MS conditions for analyzing hydrocarbons

Method	*Process 1 (MC)*	*Process 1 (MF)*	*Process 2 (MC, MF)*
Column	1.1 m × 3 mm Porapak P 80-100 mesh	2.1 m × 3 mm 10 % OV-17 on Chromosorb W HP*	2.1 m × 2.6 mm Chemlpak CN*
Temperature (°C)			
column	180	140	180
injection port	220	150	190
separator	250	220	220
ion source	270	270	270
Ion. energy (eV)	20	70	20
Carrier gas (He) (ml/min)	30	30	30

* Connected with a precolumn, Chromosorb G AW, 3 cm in length. MC : mass chromatography. MF : mass fragmentography.

RESULTS AND DISCUSSION.

In the first process of head space method, no aliphatic hydrocarbons with carbon number 5 to 6, indicating gasoline components, were detected. In the second process of solvent extraction method, the hydrocarbons with carbon number 7 to 9 and aromatic group including benzene, toluene and xylenes were detected in both samples. These data indicate that the detected hydrocarbons were the components of kerosene, and the fuel vapor was inhaled by both victims before death. The estimated blood levels based on the indicator, trimethylbenzenes, were 0.017 μg/g in the old man, and 0.059 μg/g in the younger victim. Besides the above data, the aliphatic hydrocarbons with carbon number 9 to 12 including nonane, decane, undecane and dodecane were found in the young man's blood. The analytical results are summarized in table II.

Based on the high concentration of Hb-CO and the large amount of soot in the airway, there was no problem in considering that K. was burned to death. K. was presumably exposed to vaporized kerosene, because no hydrocarbons with high boiling points were

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TABLE II

Blood level of hydrocarbons (μg/g)

	K (85 y)	*M (35 y)*	*Control**
Hydrocarbon (C 5-7)	—	—	—
Benzene	0.138	0.075	— or trace
Toluene	0.064	0.034	— or trace
m, p-Xylene	0.163	0.145	—
o-Xylene	—	—	—
Stylene	+ +	+	—
Indene	+	Trace	—
Naphthalene	+ +	+	—
Trimethylbenzene**	0.017	0.059	0.003
Hydrocarbon (C 9-12)	—	+ +	—
Hb-CO (%)	60	17	— or trace
Alcohol	—	—	—

* Normal persons (n = 10) and burned bodies (n = 24) not exposed to fuel.
** Total concentration of pseudocumene, mesitylene and 1, 2, 3-trimethylbenzene.

found. On the other hand, the aliphatic hydrocarbons with carbon number 9 to 12 detected in T.'s blood have never been observed so far in kerosene vapor exposures, either in animal experiments or in previous autopsies. This fact strongly suggests that the younger victim was directly exposed to kerosene before vaporization, as seen in « human torch » fatalities. The relatively high blood level compared with that of the grandfather also supports the speculation. In spite of the lack of significant vital signs such as soot in the airway and the low level of Hb-CO formation, the cause of T.'s death could be diagnosed as an atypical burning.

In regard to the difference of anatomical and chemical findings between the 2 victims, the following question arose as to who should be held responsible for the fire. Two possibilities were discussed. One was that the grandfather splashed kerosene around his immediate area upstairs to kill himself and his grandson must have tried to rescue him, but fell down with the kerosene container to the foot of stairs in a ball of flames. The second speculation was that the young man doused himself with kerosene at first, then ignited and fell down to the foot of stairs, and the grandfather was exposed to vaporized kerosene and died.

The police investigation team learned from the bereaved family's statements that the fuel container was always kept downstairs and that the grandson seldom went up because he had trouble

in the lower extremities. Taking the police investigation record into consideration, the conclusion was that the old man carried the fuel container upstairs and killed himself by setting fire to the sprinkled kerosene, and the grandson must have tried to extinguish the fire and fell down the stairs together with the kerosene container.

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Forensic chemistry on « post-mortem » material from fire victims

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SUMMARY.

Toxicological results obtained on post mortem material from fire victims are presented. In particular, the finding of carbon monoxide and cyanide in organs and organ juice are discussed. It seems as these autopsy materials can be useful for toxicological evaluations of fire cases.

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INTRODUCTION.

In the last decade there has been an increasing demand for investigating fire victims for toxic substances other than ethanol, drugs and narcotics. A number of toxic gases, which can be generated and inhaled during a fire is now of special analytical interest, since detection of some of these substances can elucidate the circumstances about the fire and possibly the cause of death too.

MATERIALS AND METHODS.

The present material consist of 26 fatal cases received at the institute in an one year period from July 1985. All cases were, according to police documents, reported as dead from fire.

Table I shows the total number of fires and fire victims and sex and age distribution of these victims. Only one person was brought to hospital, but death had taken place before arrival to the hospital.

Most fire fatalities were single cases, except two who died from a fire in a summerhouse and three who died from a fire initiated by a car accident.

TABLE I

Data on the total investigated material

Number of fires	23	
Number of victims	26	
Number of females	8	(age : 20-93)
Number of males	18	(age : 24-71)

All the toxicological examinations were performed on extremity blood (normally stabilized with 1 % NaF), extremity muscle, muscle juice, liver, liver juice, stomach contents and/or urine. None of these autopsy materials were isolated from the body before 6 hours after death, according to danish law. — In fact the isolation took place 48 hours or later after death, implying that some instable compounds could have disappeared or been decomposed before the autopsy material was secured. — Further, some putrefactions, involving origin of other substances could have taken place in the dead body. Ethanol estimation was carried out on blood from all the cases, whereas investigations for drugs and narcotics only were performed in certain cases. — Investigations for the fire gases carbon monoxide (CO) and hydrogen cyanide (HCN) were performed on each case as far as sufficient autopsy materials were available. The gas analyses (for CO and HCN) were conducted as soon as possible — otherwise the autopsy material was frozen at —20°C.

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Method used for Carbon monoxide estimation.

A spectrophotometric method ad modem Maehly (1) was used for the determination of the carboxyhemoglobin (CO-Hb) content in blood and liver juice. Absorbance measurements were made in the visible region from 400-690 nm on sample diluted with a solution containing 2 % saponine in 0.02M sodium tetrahydroborate at pH 9.0. Both whole blood and liver juice were diluted about twenty times. Liver juice was best obtained from a thawn liver. Cuvettes with an one mm path-length were used to get the necessary absorption curves on diluted whole blood, whereas cuvettes with ten mm's path-length were used to get suitable absorption curves on diluted liver juice.

Figure 1 shows the typical absorption curves for a sample with and without CO content. Curve 1 shows the absorbance curve for diluted sample containing different amounts of oxyhemoglobin (O_2-Hb) and CO-Hb. Curve 2 shows the absorbance curve for the same diluted sample reduced by sodium dithionite involving that O_2-Hb, but not CO-Hb has been reduced to hemoglobin (Rec-Hb), causing characteristic changes in the absorption spectrum. Curve 3 shows the absorbance difference between curve 2

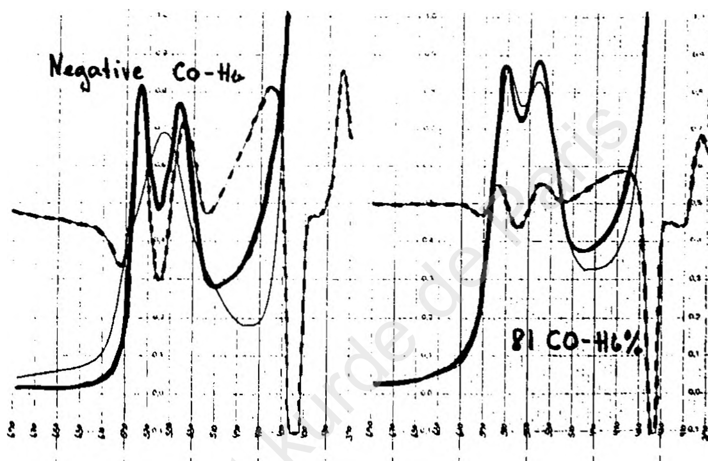

FIG. 1. — Absorption curves on two blood samples.
Curve 1 = —, Curve 2 = —, Curve 3 = — — —.

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and 1. The last curve is, together with curve 1 and an experimental calibration curve, used for calculation of the percentage amount of CO bound to hemoglobin in whole blood or liver juice. It should be pointed out that in post-mortem material qualitative inspection of the spectra details is very important before a CO-Hb result is accepted.

Method used for Hydrogen cyanide estimation.

A colorimetric method was used for the determination of HCN content in the samples. The used method release HCN from the sample and afterwards the gas is converted, caused by chloramine T, to the gas cyanogen chloride which reacts with pyridine and barbituric acid and form a dyestuff the colour intensity of which is proportional with the present amount of cyanide. The

intensity was measured at 580 nm on an UV-spectrophotometer. The method is rather specific for cyanide, and thus thiocyanate, the major metabolite of cyanide, is not interfering with the method. When measuring submicrogram quantities of cyanide our ordinary method, used in cases suspected for serious cyanide intoxications, was given some modifications : about 5-10 g of sample or sample homogenate (homogenized 1+1 with water) was used. For precipitating the proteins and releasing the HCN was an acid saturated lead acetate used.

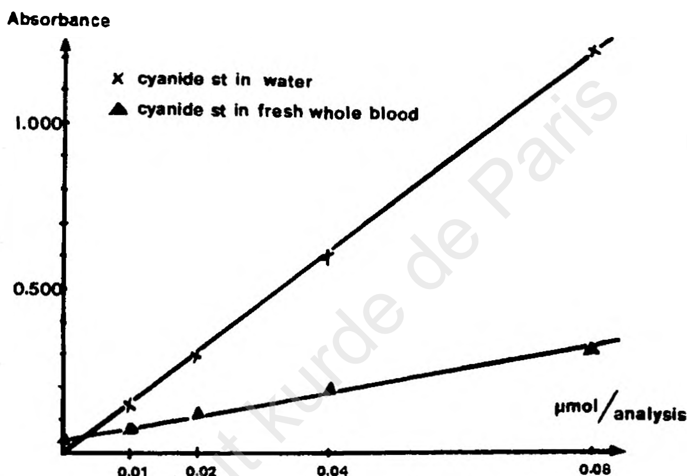

FIG. 2. — Standard curves for cyanide obtained on water and fresh whole blood.

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Unfortunately, the procedure used for HCN estimation was not very reproducible and the recovery was only 30-35 % in fresh whole blood. Figure 2 shows the typical standard curves obtained after cyanide has been added to water and fresh whole blood. As just mentioned the recovery is rather low for which reason all cyanide results in this presentation should be taken as an approximation. — Further, no correction for recovery has been made in the given results.

RESULTS AND DISCUSSION.

As already shown the total material consist of 26 fatal cases. Only in few of these cases analyses for drugs were performed. In one case propanolol and in another case metaqualone were

detected, but none of these concentrations exceeded therapeutic levels.

Ten victims showed no ethanol, whereas 19 % of the cases showed an ethanol concentration below 1 ‰ and 42 % of the cases showed an ethanol concentration bigger than 1 ‰.

The distribution of fatal fires according to cause of fire is shown in table II. Not astonishing smoking is the typical cause. The distribution pattern of fatal fires according to initial item ignited is shown in table III. The typical items are bedding or matresses.

TABLE II

Distribution of fires and victims according to cause of fire

Cause of fire	Number of	
	fires	victims
Smoking	1	1
Smoking ?	10	10
Petrol (car collision)	2	4
Other	1	1
Unknown	9	10
Total	23	26

TABLE III

Distribution of fires and victims according to initial item ignited

Initial item ignited	Number of	
	fires	victims
Bedding/matress	8	8
Chair/sofa	2	2
Other	1	1
Unknown	12	15
Total	23	26

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It is well known that CO is a gas which often developes during a fire. HCN has been shown to arise when plastics are heated to 500°C or more in a stream of nitrogen or when polymers containing nitrogen are combustered. Both compounds are often developed during fire, especially when artificial products such as bedding or matresses are burned. Therefore it was logical to analyse for CO as well as for HCN, both of which compounds can be lethal when inhaled during a fire.

Table IV shows the relations between the ignited matter and the measured CO-Hb %. It is our experience that the concentration of CO-Hb in whole blood and liver juice — obtained from the same body — are of the same order. Therefore, discrimination between these two materials has not been made in this table and the results are thus average values of concentra-

TABLE IV

CO-Hb % in whole blood / liverjuice related to the ignited matter

Number of cases and ignited matter	*CO-Hb %*
3 × bedding/matress 1 × chair/sofa 1 × kitchen 2 × room 2 × house/summerhouse 4 × car 1 × straw	< 10 % (n = 14)
1 × shed 2 × house/summerhouse	10 - 40 % (n = 3)
3 × bedding/matress 1 × kitchen	40 - 60 % (n = 4)
3 × bedding/matress 1 × chair/sofa 1 × house	60 - 80 % (n = 5)

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tions measured in whole blood and liver juice. There was no obvious relationship between the ignited matter and the concentration of CO-Hb measured in these cases.

In autopsy material it has always been complicated, to measure truly inhaled hydrogen cyanide developed from fire — in this presentation mentioned as « fire-cyanide » —, even when a specific and sensitive method is used. The reason is that there are several possibilities for measuring false negative and positive « fire-cyanide » reactions. In table V are some of the most important reasons for false « fire-cyanide » shown. Of these reasons, post-mortem changes in the cyanide content is the most annoying for the analyst. In particular this is a problem in post-mortem blood samples, where the cyanide results varied considerably.

In the light of this knowledge we have tried to analyse HCN in different organs and organ juices. Table VI shows cyanide results obtained on non-fire autopsy cases. Except in whole blood

TABLE V

Reasons for false « fire-cyanide » reactions

False negative :

- A Metabolic changes
- B Diffusion
- C Post mortem decomposition

False positive :

- A Post mortem formation
- B Cyanide originating from non fire (drugs, glycosides, etc.)

TABLE VI

Cyanide concentrations (µmol/kg) in autopsy material from cases where no fire has taken place

Whole blood	*Muscle*	*Muscle juice*	*Liver*	*Liver juice*	*Stomach contents*	*Urine*
0.2 — 3.2			≤ 0.2		≤ 0.7	≤ 0.2
n = 6	n = 8	n = 8	n = 15	n = 15	n = 7	n = 8

TABLE VII

Cyanide concentrations (µmol/kg) in autopsy material from 11 cases where fire has taken place, and where cyanide apparently was not inhaled

Whole blood	*Muscle*	*Muscle juice*	*Liver*	*Liver juice*	*Stomach contents*	*Urine*
≤ 5.6		≤ 0.2			≤ 1.9	≤ 0.2
n = 4	n = 11	n = 7	n = 11	n = 9	n = 10	n = 4

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and perhaps in stomach contents, cyanide could hardly be demonstrated in any of the cases.

Cyanide estimation was further carried out in 17 of the 26 fire cases where relevant autopsy material was available in sufficient amount. Table VII shows 11 cases where it was not possible to demonstrate cyanide in the organs and in the organ juice like in the non fire cases.

Whereas table VIII shows 6 cyanide positive fire cases, where it was possible to demonstrate cyanide not only in whole blood and stomach contents but also in muscle and liver.

Only cases where CO has also been demonstrated either in whole blood or in liver juice, indicating that the victims were alive during the fire have been shown in this table.

TABLE VIII

Cyanide concentrations (μmol/kg) in autopsy material from 6 cases where fire has taken place and carbon monoxide has been demonstrated

Case number	*Whole blood*	*Muscle*	*Muscle juice*	*Liver*	*Liver juice*	*Stomach contents*	*Urine*
1	—	1.0	0.9	0.7	0.8	—	—
2	—	2.6	6.8	1.1	1.1	—	—
3	—	—	1.8	0.4	1.0	—	0.2
4	60	1.2	—	0.6	0.4	20	—
5	13	1.3	—	0.2	0.9	1.7	—
6	0.6	2.2	0.5	0.6	0.8	—	20.0

— = not analysed.

TABLE IX

CO-Hb % in whole blood and liver juice in 6 cyanide positive cases

Case number	*Ignited matter*	*CO-Hb % in*	
		Whole blood	*Liver juice*
1	House	—	22
2	Summerhouse	26	25
3	Shed	—	26
4	Matress	38	49
5	Kitchen	57	53
6	House	74	—

— = not analysed.

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Table IX demonstrates the estimated CO-Hb % in the just mentioned 6 fire cases. As pointed out previously the cyanide concentrations should only be taken as an approximation, but even then there does not seem to be any relationship between the concentration of HCN and the concentration of CO-Hb in these cases.

CONCLUSION.

Despite the lack of sufficient method for quantitative estimation of small amounts of cyanide in autopsy material these preliminary results may give support to the following conclusion :

— Organs and organ juice seems to be relevant materials, when presence of « fire-cyanide » has to be established in dead bodies.

— Endogenous formation of cyanide in organs and organ juice involving false positive « fire-cyanide » in dead bodies does not seem to take place.

This means that organs and organ juice are more suitable as a screening material for « fire-cyanide » than whole blood for detection of « fire-cyanide ». — Further investigations with a sufficient cyanide method are necessary to evaluate whether organs and organ juice can be used for quantitative cyanide estimation and thus be useful for toxicological evaluations of fire cases.

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Extractions with liquid carbon dioxide

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INTRODUCTION.

The most recent trend in chromatography is the use of supercritical fluids, respectively pressure-liquified gases, as mobile phases. Most papers which have appeared so far on this subject deal with liquid carbon dioxide as chromatographic eluent, with or without addition of modifiers for increasing its polarity. The replacement of the chromatographic eluant by a liquified inorganic gas has certainly many advantages. However, in order to be consequent, we should already follow this principle for the extraction step.

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METHOD.

The extraction of solids with liquid carbon dioxide does not pose too many technical problems. It is already being used on a factory scale, i.e. for the decaffeination of coffee. For laboratory trials, a small device is on the market (1). It consists essentially of an ordinary glass Soxleth extractor housed in a steel pressure vessel. Solid carbon dioxide (about 180 ml) is added to the steel vessel (between glass extractor and steel wall), the vessel immersed 6 to 7 cm into a water bath heated to 45°C and the cooling finger on top of the steel vessel fed with ice water or a cooling liquid with a temperature of 1 to 4°C. The solid carbon dioxide evaporates, is condensed to liquid carbon dioxide on the cooling finger, drops into the Soxleth extractor and re-evaporates after reaching the flask of the extractor in the warmer zone of the extraction system. The bath temperature and the cooling finger temperature must be strictly controlled, as well as the pressure in the steel vessel, which is furnished for this purpose with a pressure gauge. We usually worked at 850 to 900 psi.

RESULTS AND DISCUSSION.

Our pilot study deals exclusively with the possibility for using the available pressure Soxleth system for the extraction of biological specimens with liquid carbon dioxide. Replacement of the organic solvent could bring several advantages :

1. Extractions are carried out in the cold, preventing thermal decomposition.

2. No contamination with by-products of organic solvents, especially the omni-present plasticizers such as phosphate and phthalate esters, is possible.

3. This could facilitate an evaluation of the concentration of the different plasticizers in biological fluids and tissues.

In order to extract body fluids or aqueous tissue-suspensions in a Soxleth, they have to be first solidified. We have tried several methods :

1. Lyophilisation of the aqueous solution or suspension.

2. Lyophilisation after addition of a porous solid such as cellulose.

3. Mixing the biological sample with an excess anhydrous sodium sulfate.

4. Solid phase pre-extraction, respectively binding of the compound of interest on a solid adsorbent.

Lyophilisation did not yield consistent results. There were a few satisfactory trials, such as the extraction of benzodiazepines from the stomach content of a poisoned person. In table I, the extraction yields are compared to the yields obtained by liquid-liquid extraction with hexane containing 5 % methanol. Liquid carbon dioxide gave better yields for diazepam, almost equal yields for flunitrazepam, but failed to extract nitrazepam. The inconsistency of the results from parallel extractions of lyophilised biological specimens suggests that our freeze-dried samples did not have the proper texture for Soxleth extraction by liquid carbon dioxide. Addition of cellulose did not improve the situation, and we decided to abandon this approach. The few trials we have carried out by drying the biological samples with anhydrous sodium sulfate gave disappointing extraction yields.

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TABLE I

Case 300/85 stomach content

Extraction yields in mg / 100 ml

Hexane with 5 % methanol		Liquid carbon dioxide
5.9	Diazepam	9.8
2.4	Flunitrazepam	2.1
2.6	Nitrazepam	n.d.

A more promising way of extracting without organic solvents seems to be a combination of solid phase adsorption with subsequent elution by liquid carbon dioxide. Our first trials were carried out with reversed octadecyl-silane extraction columns (Baker). Table II compares the results obtained for 7 different

TABLE II

Separation on C-18 Silica Gel (6 ml column)

Compound	mg	% recovery with ether (1) ethanol (2)	% recovery with liquid CO_2
Phthalate esters	1.8	65 (1)	80
	9.0	—	90
Propyphenazone	1.2	95 (1)	9
Methadone	2.7	76 (1)	0
Flunitrazepam	1.0	68 (2)	19
2-NH_2-5-NO_2-2'-F-benzophenone	2.0	62 (2)	25
Pentachlorophenol	2.3	97 (1)	29
	1.2	—	35
DDT	0.4	95 (1)	58

compounds (or mixtures) with the yields obtained by extraction with organic solvents. From the compounds tried, only the phthalate esters gave good recoveries. RC-18 silica gel is too strong an adsorbent for the other compounds tested. Liquid carbon dioxide is a solvent with too low polarity for breaking the strong adsorption bonds.

Considerably better results could be obtained with other adsorbents. Table III shows the recovery rates for 4 different substances after solid phase adsorption on 5 column types possessing different polarity. We also have chosen substances with different polarity and different acidity. Baker columns were used for the trials with modified silica materials. The carbon column was prepared from Carbopack (Supelco) and the cross-linked

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TABLE III

% recovery

Adsorbent	*(C)*	*Modified silica gels*			*Polystyrene resin*
		C-18	*Phenyl*	*Cyano*	
Pentachlorophenol	16	35	63	96	70
Phthalic acid esters	77	85	35	62	85
Propyphenazone	93	9	38	72	85
Flunitrazepam	82	19	36	60	79

polystyrene resin was Chromosorb-102. Not included in table II are our newest results with Tenax GC, a polymeric column packing based on 2.6-diphenyl-p-phenylene oxide, which gave recovery rates similar to the trials with the polystyrene resin.

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CONCLUSIONS.

1. Extractions with liquified inorganic gases may yield less contaminated extracts and should therefore be investigated.

2. So far we have only used liquid carbon dioxide as a pressurized solvent. It possesses a surprisingly low polarity. The work should be extended to other gases such as nitrous oxide.

3. The Soxleth technique we have used requires that the investigated material must first be solidified. The best way to accomplish this has so far been solid phase adsorption.

4. Up to now, organic resins proved to be best suited for binding exogenous impurities in body fluids and other solutions prior to extraction with liquid carbon dioxide.

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Acute oral poisoning and death from antimony potassium tartrate

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INTRODUCTION.

Antimony belongs to the same periodic group VA as arsenic to which it resembles chemically, biologically and toxicologically. It was known to man at an earlier date than arsenic, perhaps as early as 4,000 BC. It was used by the ancients as both a medicine and a cosmetic. Dioscorides discussed the use of antimony as a medicinal agent and references to its cosmetic use ; it is mentioned also in the Bible.

Since the inorganic compounds are more toxic than the organic ones, the latter are more frequently used in therapy. In the early twentieth century organic antimony compounds were introduced as parasiticides, and tartar emetic for the treatment of schistosomiasis and leishmaniasis. Because of their efficiency in this regard and the absence of less toxic drugs they remain useful in tropical medicine and pharmacies (table I).

The structure for antimony potassium tartrate is variously represented.

The British Pharmacopeia 1973 gave m.w. 324.92 and the US Pharmacopeia XVIII m.w. 333.94 as can be seen in figure 1.

The first reported accidental poisoning with tartar emetic was by Christison, 1836 (1). It was ingested in error for cream of tartar ; the victim was ill for 6 hours, but he recovered.

In the *Journal of British Medicine*, 1858 (2) an outbreak of poisoning occurring in Paris, when seventeen persons ate some barley sugar is reported. The manufacturer had used tartar emetic in mistake for cream of tartar to preserve the transparency of the sweets.

A youth, aged sixteen, interested in the action of poisons until it became an obsession, purchased 25 g of tartar emetic and

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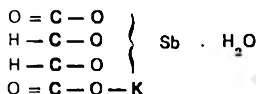
administered the poison to members of his family in biscuits or cake. Fortunately, the victims recovered but he was charged with administering a noxious compound to his father (the *Times,* 7th June, 1962).

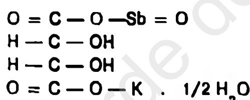
TABLE I

Medical use of tartar emetic

1. Expectorans			2-10 mg P.O.
2. Emetic			40-50 mg P.O.
3. Schistosoma japonicum	—	Total	1.8 g / 25-33 days I.V.
4. S. Mansoni		Total	1.8 g / 25-33 days I.V.
5. S. Haematobium		Total	1.5-1.7 g / 25-33 days I.V.
6. Leishmaniasis			



mw. 324.92



mw. 333.94

6 g accidental ingestion
4 patients

1 g 1 g 2 g 2 g

3 patients survived,
1 patient died on the 3th day.

FIG. 1. — *Antimony potassium tartrate (APT).*

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In Belgium there was a similar case some years ago in the town of Eeklo, where a pharmacist gave by mistake tartar emetic instead of tartaric acid to a teacher of a school. The result was that the students were intoxicated severely, and the pharmacist was punished.

We report here an acute oral intoxication in four patients.

CASE REPORT.

Four adult patients, two male and two female, were admitted to the Emergency Department of the University Hospitals, with continuous vomiting, severe abdominal cramps, nausea and watery

diarrhoea. In one of the patients, a 93-year old man, marked cyanosis and haematemesis were present.

The intoxication started immediately after the ingestion of a self-made cake prepared according to an old English recipe.

One of the ingredients was « cream of tartar », often used in baking ; obtained by purification of crude cream of tartar or argol, which is deposited from grape juice during fermentation, and is also found in the lees of wine.

One of the patients however, confused cream of tartar with the toxic « tartar emetic » which stands for antimony potassium tartrate. About 6 grams of the latter was used in preparing the cake.

TABLE II

Symptomatology in antimony intoxication

Acute intoxication	*Litterature*		*Our patients P.O.*	
	I.V.	*P.O.*	*Survivors*	*Non-survivor*
Gastrointestinal				
— abdominal pain	+	+	+	
— vomiting	+	+	+	
— diarrhea		+	+	
— nausea	+	+	+	
— hematemesis/melena		+		+
Renal				
— oliguria			+	
Cardiac				
— bradycardia	+	+		(+)
— cardiomegalia	+	+	+	
Pulmonary				
— cough	+	+	+	
— pneumonitis	+			+
— hypoxia			+	
Hepatic				
— hepatomegaly	+	+		+
— acute hepatic failure	+	+		
Neurologic				
EEG disturbances	+		+	
asthenia	+	+	+	
Hematologic				
hemolysis } eosinophilia }	+			+
Circulatory collaps	+	+		+
Thrombophlebitis	+		+	
Fluid, electrolyte disturbance		+	+	

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In table II we have the severe clinical symptoms which our patients developed, compared with those given in the literature. Confirmation of the nature of the poison was done from the history of the patients, and by analysing the antimony content in the misused white powder.

Fifteen minutes after arrival at the emergency department, specific therapy was initiated.

SPECIFIC THERAPY.

A. *Traditional supportive measures.*

1. Evacuation of gastric contents by stomach lavage with tap water.
2. Instillation of *activated charcoal* (20 g).
3. *Fluid and electrolytes* replacement.
4. Supply of *furosemide* to increase diuresis.
5. *Oxygen* therapy because of low arterial pO_2.

B. *Chelation therapy.*

BAL, im. for 10 days in decreasing doses from 600-200 mg/day. It could be better with Penicillamine-HCl, 250 mg-2g orally daily.

Repeated biochemical analyses, including ionograms, renal and liver function tests, always remained within normal ranges.

BIOCHEMICAL ANALYSES.

— Serum glutamic pyruvic transaminase (SGPT).
— Serum glutamic oxaloacetic transaminase (SGOT).
— Lactate dehydrogenase (LDH).
— Creatine kinase (CPK).
— Creatine kinase isoenzyme MB.
— γ-Glutamyl transferase (γ-GT).
— Amylase.
— Lipase.
— Alkaline phosphatase.
— Glucose-6-phosphate dehydrogenase (G-6-PD).
— Glutathion reductase.
— Adenosine-5'-Triphosphate (ATP in RBC).
— Bilirubin Direct.

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— Bilirubin total.
— Plasma hemoglobin.
— Sulfhemoglobin.
— Methemoglobin.

Three of the patients were discharged from the hospital on day 12th after admission. The fourth patient was extremely ill, with multiple organ failure and state of shock, which could not be reversed with any therapeutic intervention. This patient died on the third day.

TOXICOLOGY OF TARTAR EMETIC.

In spite of the long standing therapeutic use of antimony compounds, little has been studied in detail about their biochemistry, metabolism and toxicology.

TABLE III

Toxicity of tartar emetic in man (P.O.)

Dose		Effect	Source	Ref.
0.75	g	has caused *death in child*	(Taylor)	(3)
1	g	temporary *sickness*	(Christison)	(1)
2	g	has caused *death* in man	(Modi)	(4)
6	g	severe *poisoning*	(Christison)	(1)
10- 15	g	usual *lethal* dose	(Modi)	(4)
40	g	*death* after 5 days	(Christison)	(1)
60-400	g	immediate *vomiting* (recovery)	(Witthaus)	(5)

TABLE IV

Toxicity of tartar emetic in animals

Animal	*Route*	*Toxic dose*	*Comp. mg/kg*	*Metal mg/kg*		
Mouse	oral	LD	600	225	(Luckley)	(6)
Mouse	s.c.	LD_{50}	55	20	(Ercoli)	(7)
Mouse	i.m.	LD_{50}	52	19.5	(Ron Pedrique)	(8)
Mouse	i.p.	LD	52	19.5	(Luckley)	(6)
Mouse	i.v.	LD	42	15.7	(Luckley)	(6)
Rat	oral	LD	800	300	(Bradley)	(9)
Rat	i.m.	LD	33	12.4	(Luckley)	(6)
Rat	i.p.	LD	11	4.1	(Bradley)	(9)
Guinea pig	i.m.	LD	55	20.6	(Luckley)	(6)
Guinea pig	i.p.	LD_{50}	15	5.6	(Luckley)	(6)
Rabbit	oral	LD_{100}	125	46.9	(Oelkers)	(10)
Rabbit	oral	LD_{50}	115	43.1	(Oelkers)	(10)
Rabbit	oral	LD	58	21.7	(Luckley)	(6)
Rabbit	i.v.	LD	15	5.6	(Luckley)	(6)
Dog	i.v.	LD	10-15	3.8-5.6	(Bromberger)	(11)

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The toxicological data of tartar emetic in man, and in different animals, taken from literature are given in tables III and IV.

TOXICOLOGICAL ANALYSIS.

Until today most determinations of antimony are performed with colorimetric methods and by neutron activation analysis (NAA). Because of the absence of a sensitive and inexpensive method suitable for long term studies, very little information can be found in the literature about the normal values of antimony in biological samples (table V).

TABLE V

Antimony normal values in man

Urine :	traces — 28.5 µg/24 h	COLORIM.		(Hirayama) (12)
	6.2 ± 3 µg/l	NAA		(Mansour) (13)
	109.4 ± 51 µg/l	NAA	(6 months after treatment)	(Mansour) (13)
	27.6 ± 8 µg/l	NAA	(1 year after treatment)	(Mansour) (13)
	14.5 ± 12 µg/l	NAA	(2 years after treatment)	(Mansour) (13)
Blood :	0-135 µg/l	COLORIM.		(Hirayama) (12)
	3.4 ± 2 µg/l	NAA		(Mansour) (13)
	23.0 ± 13 µg/l	NAA	(6 months after treatment)	(Mansour) (13)
	6.7 ± 3 µg/l	NAA	(1 year after treatment)	(Mansour) (13)
	6.9 ± 2 µg/l	NAA	(2 years after treatment)	(Mansour) (13)
Lung :	15.9-69.8 ng/g (wet)	NAA	(Vanoeteren) (14)	

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Antimony determinations in our patients were performed by a hydride atomic absorption spectrophotometric method, which was developed in our laboratory.

The method of determination is based on :

1. *The theory of hydrogen radicals formation* by chemical reaction with oxygen as first proposed by Dedina and Rubeska, 1980 (15).

2. The conclusion that the *atomization* of volatile hydride forming elements in a heated quartz cell must be *due to collisions with free hydrogen radicals*.

3. The introduction of a *glass tube, silanized* with dimethyldichlorosilane, to conduct the gases from the reaction vessel to the quartz cell.

4. The use of *argon, containing 1 % of oxygen,* as a carrier gas of the stibine and to pure the system.

5. The use of *2 N nitric acid* or 4 N hydrochloric acid solution to rinse the reaction vessel in order *to eliminate memory effects* and stabilize the baseline.

Parisis *et al., Analyst,* March 86 (16).

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 spectrophotometer, 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.

A.A.S. 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 antimony electrodeless discharge lamp was used with a Perkin Elmer EDL power supply.

Destruction procedure.

The destruction apparatus used to pretreat the biological samples consisted of a glass tube covered with a layer of aluminium foil, a vapour condenser for an efficient reflux and an aluminium block placed on a hot plate. A scheme of the apparatus with the dimensions is given in figure 2.

The details of the wet destruction procedure which we developed and used in routine analyses during the last two years, are given in figure 3.

The use of concentrated nitric and sulfuric acids in combination with the gradual increasing temperature under efficient reflux conditions, assures the complete decomposition of the samples and the convertion of the organic bound antimony to the ionic state. The use of perchloric acid was avoided because of the high volatility of antimony (III) chloride and antimony (V) chloride leading to low recoveries of the analyte, as was reported by the Analytical methods Committee 1980 (17).

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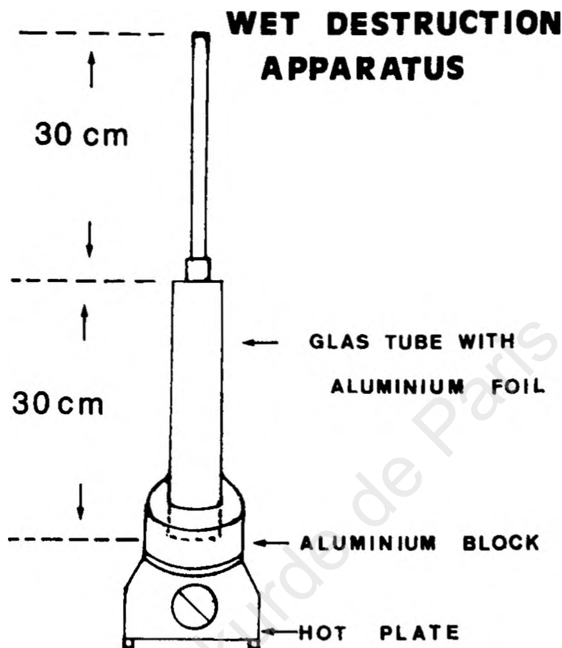

FIG. 2.

2 g sample
or 2 ml blood
or 4 ml urine

+ *20 ml* HNO_3, at 80° C for 2 h and fit air-condenser
— then at 125-135° C, for 2 h
— then at 175-185° C, for 1-2 h
+ *1 ml* H_2SO_4, at 210° C, for 30 min and remove air-condenser
(after 45 min, brown NO_2 vapours appear)

STOP digestion when vapour colour becomes lighter
— after 5 min add *5 ml* H_2O *and mix.*
— after cooling add *1 ml 15 % Hydroxylammonium chloride* and transfer content to 50 ml volumetric flask

During determination : add in reaction flask 10 ml sample + *2 ml 20 % KI.*

FIG. 3. — Wet destruction procedure.

Hydroxylammonium chloride was used in order to eliminate the nitrogen dioxide vapours produced, which could interfere with the hydride method. The reduction of Sb^{5+} to Sb^{3+} was efficiently accomplished by the use of potassium iodide solution. All acids used were suprapure quality (Merck) and the rest of the reagents were analytical grade quality (Merck).

RESULTS AND DISCUSSION.

Initially, blood samples were taken every 12 hours. Peak levels correlated well with the clinical signs and the subjective feelings of illness during the first days.

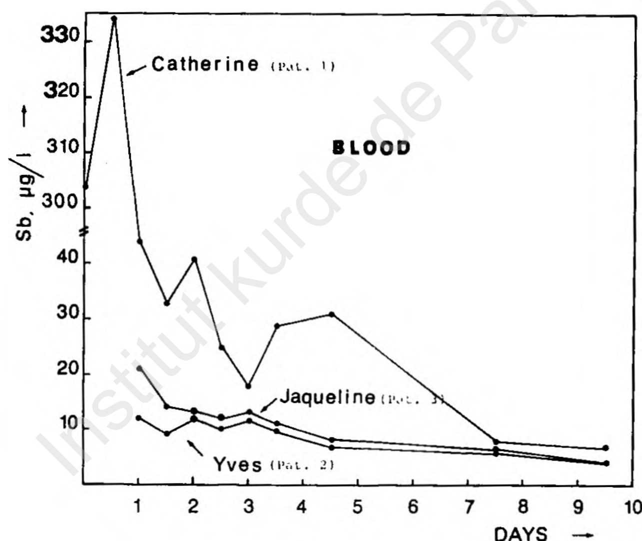

FIG. 4.

Figures 4, 5 and 6 give the antimony values in blood, red cells and serum, respectively for the 3 survived patients. It can be noted that for the patient with the highest antimony level in blood, the value in red blood cells is double than in serum. For the same patient the values returned back to normal levels after 6-7 days and for the other two patients after 4-5 days.

It indicates that tartar emetic is rapidly taken up by the red cells ; this is in agreement with A. Davis, 1968 (18).

The antimony content in the biological fluids from the non-survivor are given in table VI. Also here can be noticed the specific

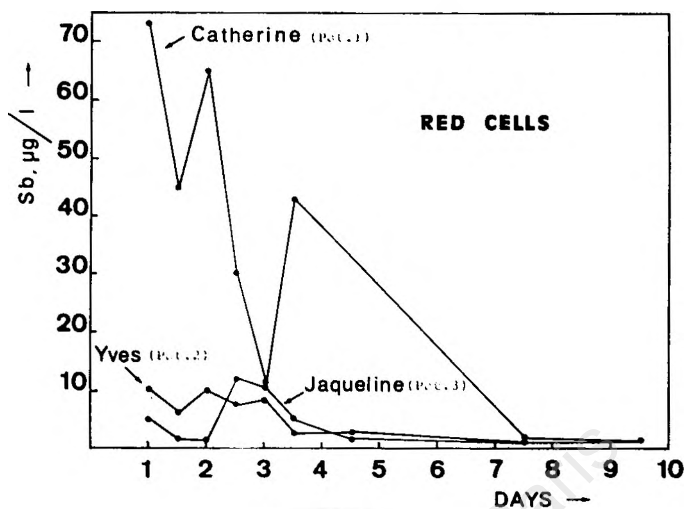

FIG. 5.

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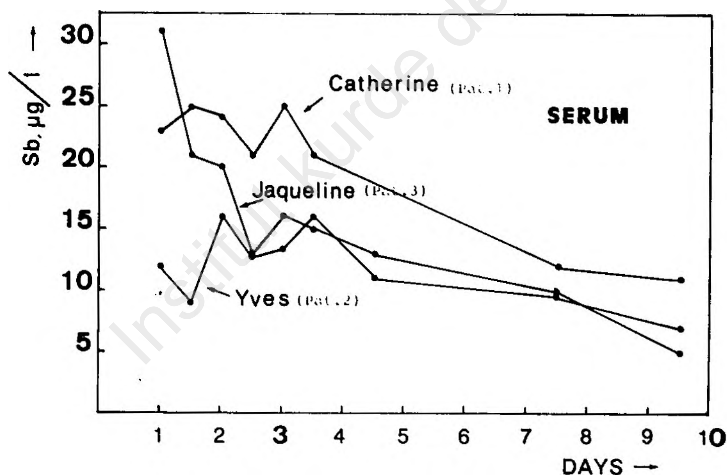

FIG. 6.

TABLE VI

Antimony results in biological fluids from non survivor

Day	*Blood µg/l*	*Serum µg/l*	*Red cells µg/l*	*Hemat.*	*Urine µg/l*	*Urine µg/24 h*	*Urine ml/24 h*
0	66.23	—	—	—	—	—	—
1/2	64.00	—	—	—	1057.50	2115.00*	2000*
1	137.50	95.59	208.86	0.37	344.42	1730.35	2140
1.5	75.44	48.50	109.73	0.44	1272.73		
2	—	128.69	—	—	447.50	89.50*	200*

* µg/12 h.

distribution of tartar emetic in the red cells and that the ratio of antimony concentrations in red cells and serum is 2.

The following figures 7, 8, 9 and 10 show the daily urinary excretion of antimony in our patients. The total urinary excre-

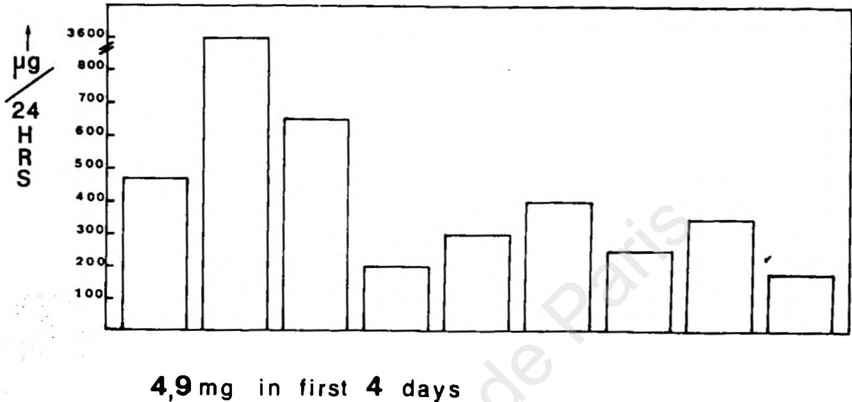

FIG. 7.

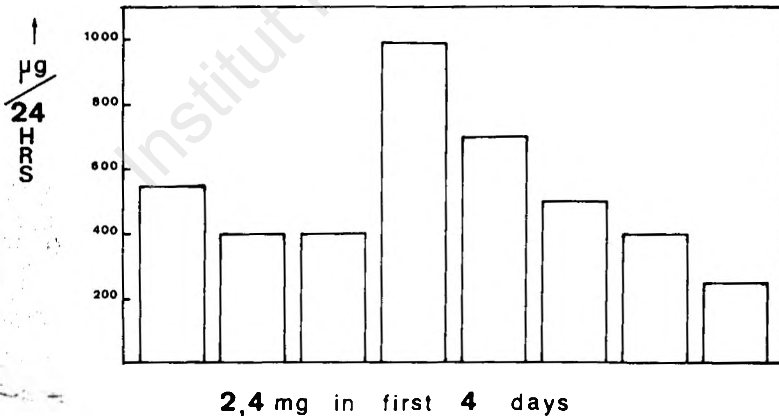

FIG. 8.

tion (4.9 mg) in the first 4 days for the patient with the highest blood values, is double the total urinary excretion values from the second, survived patient (2.4 mg).

It must be noted that all patients received the same therapy at the same time. The urinary antimony values did not return to

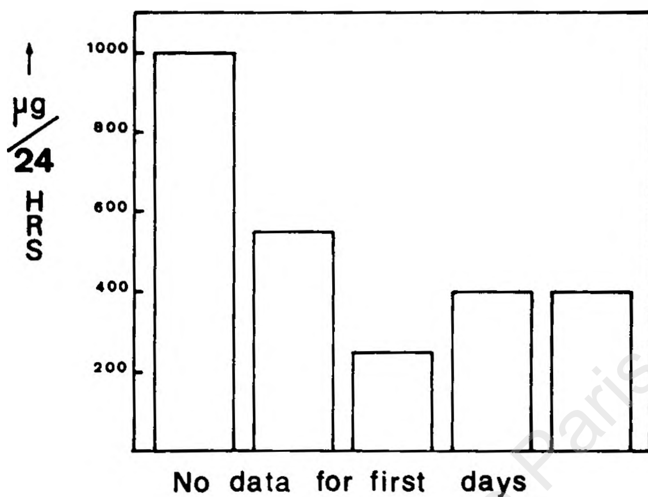

FIG. 9.

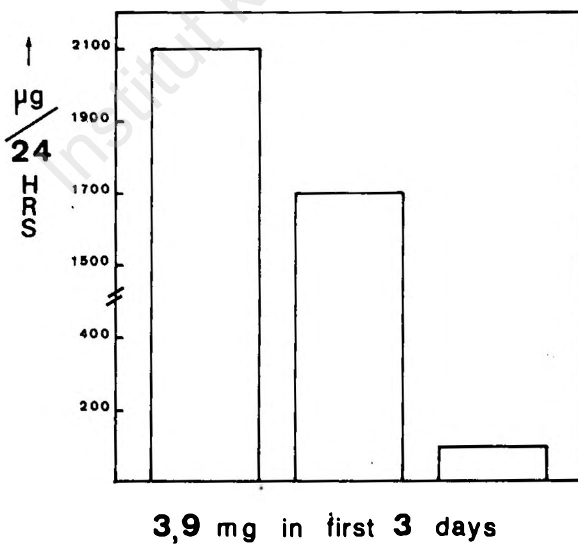

FIG. 10.

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normal levels after 10 days, indicating the big half-life of antimony in the human body, which is in agreement with Mansour *et al.*, 1967 (13), who found that in individuals who received their last antimonial drug for the treatment of bilharziasis 6 months before the analysis, the urinary antimony levels did not return to normal.

A post-mortem examination was carried out in the single fatality. The results of the tissues analysis are given in table VII.

TABLE VII

Antimony results in postmortem tissues from non survivor

Prelevement	*Weight g (wet)*	*Sb ng/g (wet)*	*Mean Sb ng/g (wet)*	*Distribution ratio*
Bile liquid	2.88	15116.32	15116.32	84
Bile tissue	2.47	10587.04	11471.21	64
Bile tissue	1.21	12355.37		
Liver	2.44	8319.67	10424.99	58
Liver	1.32	12530.30		
Kidney	2.35	2478.72	2378.36	13
Kidney	2.41	2278.01		
Intestine (big)	2.87	1169.60	1169.60	7
Intestine (small)	1.49	842.28	1079.52	6
Intestine (small)	2.06	1316.75		
Stomach	2.66	928.57	928.57	5
Lung	1.06	778.30	815.68	5
Lung	2.45	853.06		
Muscle	0.41	545.94	545.94	3
Skin + hair	0.20	403.75	403.75	2
Heart	1.99	269.35	238.03	1
Heart	1.71	206.72		
Testicle (left)	2.25	192.89	192.89	1
Vertebra	2.16	179.40	179.40	1

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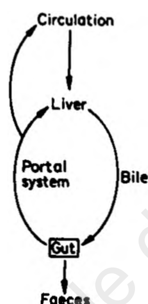
The distribution lays mainly in the bile (liquid and tissue), liver, kidney and secondly in the gastrointestinal tract (intestine big and small, stomach) lung, muscle and heart, which are the highly vascularized organs.

The antimony value in the heart is about 3 times the value in blood, 12 hours before death. The lung value compared with the maximum normal value from table V, is more than 10 times higher.

By recalculation, we could estimate the total body pool between 40-50 mg of antimony or about 0.1 g of the compound itself, which is only 10 % of the ingested dose. Sixty-five-seventy percent of the absorbed antimony was found in the bile, liver and gastrointestinal tract. It is well known that the extent of biliary excretion of a compound is influenced by physicochemical (of the compound

itself) and biological factors. From the physicochemical caracteristics, the most important are the molecular weight and the polarity of the compound. The requirement for extensive biliary excretion to occur is firstly, the compound has a molecular weight 300 and upwards and secondly, the presence of a strongly polar group in the molecular, R.L. Smith, 1973 (19).

Total body pool = 40-50 mg antimony or 107-133 mg compound.
1/3 in liver.
1/3 in GI-tract.
(Enterohepatic cycle ?)



Physico-chemical factors in biliary excretion and enterohepatic circulation of a compound.

— Molecular weight : 300 and upwards.
— Polarity : strongly polar.

FIG. 11. — *Postmortem analysis.*

From figure 1 ,it can be seen that tartar emetic has the above requirements for extensive biliary excretion, and consequently there is a big possibility that the enterohepatic cycle takes place (fig. 11). This also explains the big half-life of the compound in the human body.

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BIBLIOTHEQUE

The combination of on-line gas chromatography with Fourier transform infrared spectrophotometry for chemical toxicology

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INTRODUCTION.

At the International TIAFT Meeting 1985 in Rigi Kaltbad, Switzerland, 2 colleagues (1) lectured about the on-line combination of gas chromatography with Fourier Transform infrared spectrophotometry (GC-FTIR). In the discussion it was stated that the method had not yet been used to solve toxicological problems. We thought that this should be changed, since we did not see why a method so useful in other fields should remain excluded from toxicological laboratories. We therefore supplemented our FTIR with the equipment required for on-line GC work and applied the instrumental combination to analyze :

- test mixtures of different classes of pharmaceuticals, pesticides and other toxicologically important compounds, as well as
- organic solvent extracts from body fluids obtained in the search for unknown poisons.

EXPERIMENTAL.

Our infrared spectrophotometer was a Nicolet 20 SX-B, to which a separate optical unit with the light pipe, and an insulated tube for the gas transfer lines from and to the GC, were added (Nicolet). Light pipe and transfer lines can be heated separately from ambient temperature to 350°C. The light pipe was a gold plated pyrex tube with 1 mm inner diameter and 15 cm length,

corresponding to a volume of about 0.15 ml. A Carlo Erba GC 4100, equipped with Megabore capillary columns (0.53 mm inner diameter, 15 m length) was used. The solutions, respectively extracts, were injected directly into the columns without inlet-split by means of injection needles with 0.3 mm diameter. The column end was directly coupled to the glass-coated steel transfer line possessing an inner diameter of approximately 0.5 mm. No make-up gas was added between column and light pipe.

Most of the chromatograms were run with helium as carrier gas, but argon and nitrogen gave identical results. The flow rate was held within the range of 4 to 8 ml/min. For most of the trials, we used 6 ml/min. The liquid phases were Durabond DB-1 and DB-17 (chemically bonded) with a film thickness of 1 μ.

For column temperatures of 230°C, we chose a transfer line temperature of 240°C and a light pipe temperature of 250°C. At lower column temperatures, we worked with 230 and 240°C in the transfer line tube and the light pipe respectively.

A medium band MCT detector, cooled with liquid nitrogen, was used. It covered the spectral range from 4000 to 750 cm^{-1}. We usually worked with a resolution of 8 cm^{-1}. The chromatograph elution was followed.

1. By checking the 3-dimensional spectral plot (spectra versus elution time) presented on the screen, since every 2 seconds a new infrared spectrum (averaged from 8 scans) is displayed and stored in the computer.

2. With the help of the so-called chemigrams, that is by recording, as a function of time, up to 5 absorption intensities of preselected spectral windows, corresponding to specific absorption bands of the compounds expected.

RESULTS AND DISCUSSION.

In table I, different aspects of GC with packed columns and GC with wide bore and narrow bore capillaries are compared. In the field of applied toxicology, where sample material is often limited, we cannot afford an injection split. For the combination with IR, make-up gas should be avoided, since it raises the detection limits which may be critical. Wide bore capillaries seem to be the best choice. They give a good resolution, permit direct

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TABLE I

Properties of the different types of GC-columns

Column type	Packed column	Wide bore capillary	Small bore capillary
Inner typical diameter	2 mm	0.5 mm	0.2 mm
Injection split	Without	Without	Needed
Make-up gas	Without	Without	Needed
Resolution	Fair	Good	Very good
Capacity	High	High	Low
Substance economy	Good	Good	Poor
Suitable for analytical toxicology	Always	Always	Seldom

TABLE II

Region of IR-bands, in cm^{-1}

Origin of band	General lit. assignment	Barbiturates solid phase	Barbiturates gas phase
Literature	(2) and (3)	(4)	This paper
N-H stretch	3500 - 3300 solids lower	3300 - 3100	3440 - 3400
C-H stretch	3000 - 2800	3000 - 2850	3000 - 2900
Amide I	1680 - 1630	1750 - 1680	1770 - 1720
C = S stretch	1500 - 1470	1540	1520 - 1480

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splitless injection like packed columns and require a carrier gas flow which is compatible with our light pipe volume.

Table II shows that infrared absorption in the gas phase can vary considerably from that in the condensed phases. This holds especially for functional groups undergoing intermolecular interactions such as hydrogen bonding. In the case of barbiturates, for example, we have found a shift to higher wave numbers for the N-H stretching vibration and the amide I band, but not for the C-H stretch. A knowledge of the gas phase infrared spectra of the expected components is therefore helpful for an optimal setting of the optical windows in the chemigrams. The wave length ranges covering the absorption bands of specific functional groups are often narrower in the gas phase than in the condensed phases. Chemigrams with correctly adjusted narrow spectral windows yield better sensitivities than chemigrams with more generously set wider optical windows.

Figure 1 illustrates a 3-dimensional spectral plot recorded during the elution of 5 μg each of dimethyl and diethyl phthalate from a Megabore capillary column with DB-1 at 180°C. The compounds eluted well-separated after approx. 2 and 3 minutes, respectively, each within about 10 seconds. Their IR-spectra are

characterized by strong, sharp bands at 1,740 cm^{-1} (ester C=O stretch) and 1,270 cm^{-1} (ester C-O-stretch), as well as considerably weaker absorptions between 3,000 and 2,950 cm^{-1} (C-H stretch for $-CH_2$ and $-CH_3$), at 1,420 cm^{-1} (probably C-H deformation for $-CH_3$), at 1,100 cm^{-1} (typical for phthalates) and 1,050 cm^{-1}. In order to obtain the exact band positions, it is, of course, necessary to record the spectra of the different peaks in the normal 2-dimensional presentation ; the 3-dimensional plots only permit a rough assignment.

Figure 2 shows a series of 5 chemigrams recorded during a chromatographic separation of 7 barbiturates (between 1 and 2 μg

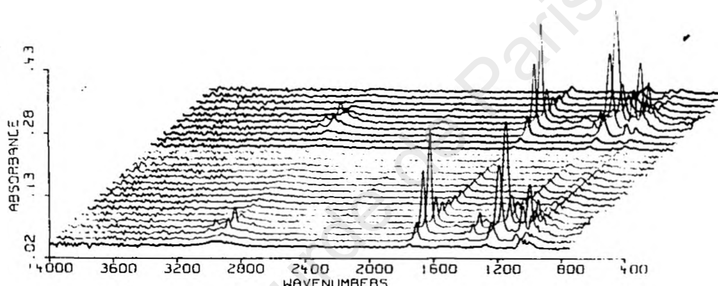

FIG. 3. — 3-dimensional spectral plot recorded during elution of 5 μg each of dimethyl and diethyl phthalate.

each) on a capillary with DB-17, namely — in the order of elution — allobarbital (5,5-diallyl-barbituric acid), butobarbital (5-butyl-5-ethyl-barbituric acid), amobarbital (5-ethyl-5-isopentyl-barbituric acid), pentobarbital (5-ethyl-5-(1-methylbutyl)-barbituric acid), thiopental (5-ethyl-5-(1-methylbutyl)-2-thio-barbituric acid), methylphenobarbital (N-methyl-5-ethyl-5-phenyl-barbituric acid) and phenobarbital (5-ethyl-5-phenyl-barbituric acid). Such chemigrams (up to 5) can be recorded simultaneously with the 3-dimensional plot. In our example, the optical windows have been set to record (from the bottom to the top) the amide I band, the N-H and the C-H stretch, the C=S absorption of the thiobarbiturate and a composite of several functional absorption bands (larger window on the top). As expected, the chemigram based on the strong amide I band gives the best sensitivity. The N-H and C-H stretching vibrations are much less satisfactory for trace analysis. The aliphatic C-H absorption is not visible in allobarbital, eluting first, nor in methylphenobarbital and phenobarbital, eluting last. Chemi-

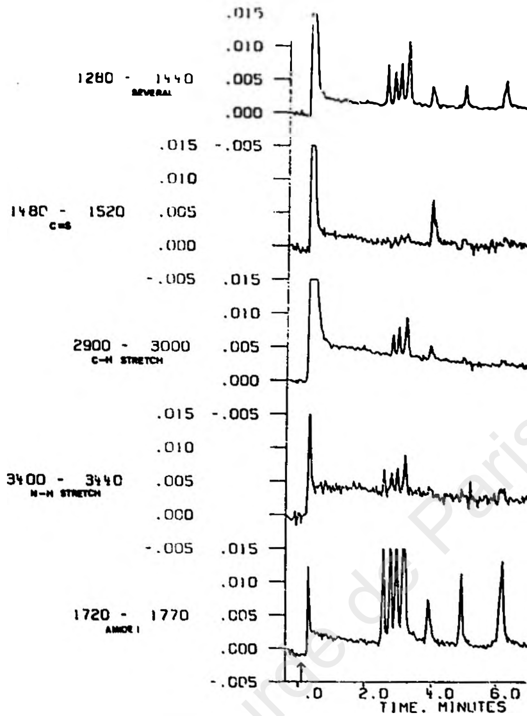

FIG. 2. — Chemigrams recorded during a chromatographic separation of 7 barbiturates.

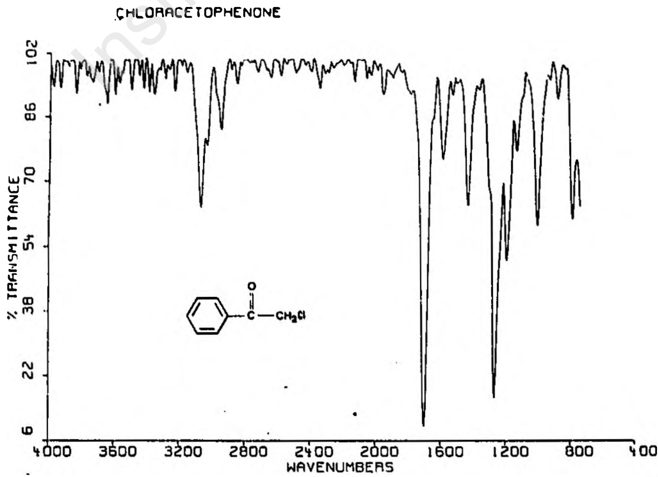

FIG. 3. — IR-spectrum of the tear gas chloroacetophenone, recorded during the chromatographic elution.

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gram 2 (from the top) shows that thiopental elutes in fifth **position** (RT 3.9 minutes).

Based on such a series of chemigrams, it is usually possible to judge whether certain functional groups are present or absent in the eluting compounds. However, the complete spectra which are stored in the computer should always be reproduced and evaluated. The purity of the separated components may be checked by comparing several spectra taken during the elution of a single peak.

We have applied our GC-FTIR combination to analyze quite a number of synthetic mixtures belonging to different classes of drugs (benzodiazepines and their hydrolysis products, the benzophenones, barbiturates and other hypnotics, opiates, cocaine and its metabolites, derivatives of phenethylamine), to pesticides (phosphoric and thiophosphoric acid esters) and to different tear gases. It is not possible to reproduce all the gas phase spectra in this short paper ; this will be done in another context. We will limit ourselves to 1 example, the spectrum of the tear gas CA, respectively CS (chloroacetophenone, figure 3). Its strongest absorption band is, of course, the C=O stretching vibration, situated, as could be predicted for an aryl-alkyl-ketone substituted with a chlorine atom in α-position, at 1,700 cm^{-1}. The single bond C-O-stretch, on the other hand, occurs at a higher wave number than expected, around 1,280 cm^{-1}. The aryl and the alkyl C-H stretching bands are clearly visible in the region 3,100 to 2,950 cm^{-1}, but not the C-Cl stretching band, since this absorption lies beyond the range of our medium band MCT detector.

Compounds possessing strongly absorbing functional groups can be detected and characterized by GC-FTIR if present in the 100 ng range. Detection limits for known compounds may be lower, as long as chemigrams with well-adjusted optical windows are used. However, GC-FTIR cannot yet compete (with respect to sensitivity) with GC-MS. On the other hand, it is often able to supplement the information obtained by MS. For structural elucidation, the two techniques are complementary ; they can and probably will be integrated soon into a GC-FTIR-MS combination.

We have also used our GC-FTIR combination in case studies. As an example, we will describe its role in the investigation of Case 356/85. A driver involved in a minor car accident appeared intoxicated, but breath alcohol analysis was negative. We were asked to analyze his urine for drugs. The tests for narcotic drugs

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were negative and we proceeded with the analysis for pharmaceuticals.

The ether extract containing the weakly acidic components was investigated by combined GC-FTIR and by GC-MS. With both methods, we could identify the presence of the barbiturate butalbital (5-allyl-5-isobutyl-barbituric acid) and of a metabolite of the pyrazolone propyphenazone (2,3-dimethyl-4-isopropyl-1-phenyl-pyrazolone respectively 4-isopropyl-antipyrine or isopropyl-phenazone). The separation was carried out with the DB-17 capillary at 210°C. Butalbital eluted after approx. 2 and the propyphenazone me-

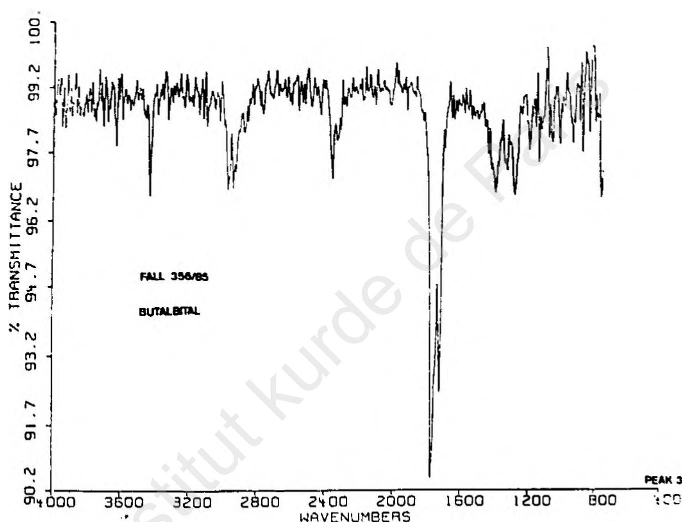

FIG. 4. — IR-identification of a GC-peak from an acid urine extract as 5-allyl 5-isobutyl-barbituric acid.

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tabolite after approx. 4 minutes. The IR-spectrum of the butalbital peak is presented in figure 4. It proved to be identical to the gas phase infrared spectrum of the pure compound. The infrared spectrum and the formula of the propyphenazone metabolite extracted from the urine are presented in figure 5. The mass spectrum of the peak indicated the presence of a compound with the molecular mass 232, corresponding to the monohydroxy derivative of N-demethylated propyphenazone. It excludes a hydroxyl on the aromatic ring and shows that the isopropyl group is intact and not hydroxylated. This leaves the methyl group as position for the hydroxyl. The deduction is confirmed by the infrared spectrum. The OH-stretch just below 3,600 cm^{-1} is only weak, but this is usually the case in gas phase spectra. The bands

around 1,350 and 1,050 cm^{-1} are compatible with the presence of a primary hydroxyl. The amide I band appears slightly above 1,700 cm^{-1}, as can be expected for an amide function in a 5-membered ring. Two smaller peaks in the GC of the extract with the weakly acid components could only be identified by GC-MS. One was hydroxy-butalbital, the other a propyphenazone metabolite with a molecular mass of 248.

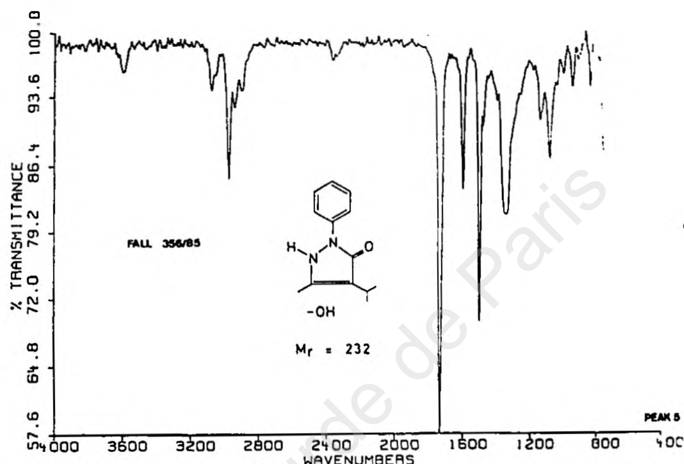

FIG. 5. — IR-identification of a GC-peak from an acid urine extract as N-demethylated and hydroxylated propyphenazone, a metabolite of the pyrazolone.

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In the ether extract containing the neutral components, non-metabolized propyphenazone could be detected by GC-MS. The accused had taken Optalidon, a preparation containing the active ingredients butalbital and propyphenazone. The quantitative data, obtained (for the barbiturate) by UV-spectrophotometry, indicated a massive overdose.

CONCLUSIONS AND OUTLOOK.

1. The instrumental combination of GC with FTIR is a very useful tool in the field of analytical toxicology. It supplements the information obtained by GC-MS and is of special value for structural elucidations. Smaller laboratories without MS-facilities may use GC-FTIR as a substitute, since it is somewhat less expensive and much simpler to run than GC-MS.

2. For toxicological problems, large bore capillaries (or narrow packed columns) and splitless direct injection should be used and

the addition of make-up gas between column and detector (light pipe) avoided. Carrier gas flow and light pipe volume must be compatible.

3. The chromatograms can be recorded simultaneously by 2 different methods, as 3-dimensional spectral plot (IR-spectra recorded as a function of elution time) and with a set of chemigrams (absorption intensities of pre-selected spectral windows corresponding to specific absorption bands, respectively to specific functional groups, as a function of time). While the first method permits a general search for IR-absorbing compounds, the second is better suited for the trace detection of known compounds.

4. Gas phase IR-spectra are often considerably different from the spectra in the condensed phases. More knowledge about gas phase absorption of larger molecules, especially compounds of toxicological interest, is needed in order to take optimal advantage of the combination technique. The gas phase libraries presently available are of little help to the analytical toxicologist. It may be advisable to establish experimental standard conditions prior to the accumulation of large data banks.

5. In contrast to GC-MS, detection limits can be critical in GC-FTIR. They should and probably will be improved, i.e. by lowering the noise level. In order to increase the sensitivity for a given compound, conversion to a derivative, which is a very strong infrared absorber, is possible. Very recently, we have tried such an approach for phenethylamines. By adding carbon disulfide to an organic solution of the primary amines, they are converted (just by standing at room temperature) to isothiocyanates. This functional group shows an absorption band around 2,050 cm^{-1} with an intensity more than ten times stronger than the bands of the corresponding primary amines. We will describe this possibility for improving the sensitivity in the next TIAFT meeting in Banff (July, 1987).

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A fatal poisoning by digitalis

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ABSTRACT.

An 11-month-old baby was brought to the University, Hospital with symptoms of cardiac failure. The diagnose was Digitalis Poisoning : the blood level of digitoxin was 305 ng/ml, while therapeutic levels in adults go up to 25 ng/ml. Despite the reanimation efforts, the baby died. The mother, who is a nurse, added 6 leaves of Foxglove (Digitalis Purpurea) to the baby's lunch. She thought it was Ispaghulla (Plantago), a plant which is known to purify the blood. The determination of the digitalisglycosides on the autopsy samples was done by a ^{125}I Radio-Immunoassay technique.

The total amount of Digitoxin ingestion was estimated.

CASE HISTORY.

A baby of eleven months old suffered from eczema. The mother, who is a nurse, treated the illness by giving macrobiotic food and herbs she found in the garden. On Sunday, the 20th of April 1986 she cooked rice and added six leaves of Foxglove (Digitalis Purpurea) to the baby's lunch. She thought it was Plantago, a plant which is known to purify the blood. During the afternoon, the baby had vomited and slept for the rest of the time.

The following morning, after the baby had drunk its milk, he vomited again. In the afternoon, the baby gets worse : he was weak, the breathing was difficult and he was sleepy.

The mother thought the baby suffered from pneumonia and took him to the hospital with following symptoms : vomiting, and cardiac and respiratory failure.

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After a while, the mother told also the doctor she had put six leaves in the baby's lunch the day before.

She had brought a leaf of the plant with her. After examination, the leaf was recognized as Digitalis Purpurea. Determination of the digitoxin serum level gave proof of an acute digitalis intoxication.

Thirty-seven hours after the intake, the baby unfortunately died, despite reanimation efforts.

TOXICOLOGY.

In literature, a lot of Digoxin intoxications are studied and described, but only few cases of fatal digitoxin intoxications.

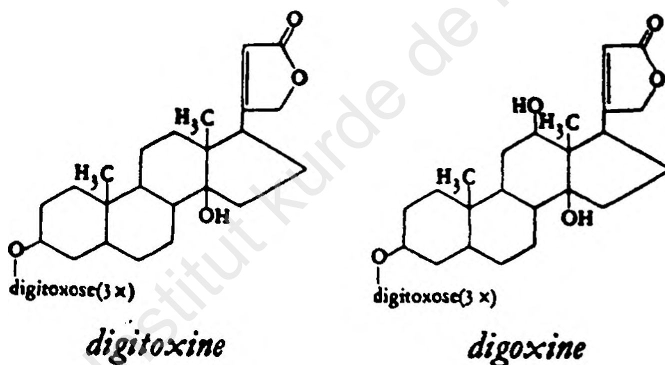

FIG. 1.

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So, it is interesting to compare first the distribution of digitoxin and digoxin in human tissues (1, 2, 3, 4). Digitoxin is rapidly and completely absorbed following oral ingestion, its full effect appears as rapidly as by intravenous injection.

By oral intake, the stomach, but more important the small intestines are places of resorption.

Digoxin however is only for 60 % absorbed. The gastrointestinal absorption is usually completed within 2 to 4 hours.

Digitoxin has a relatively small volume of distribution, when compared with digoxin, but it is distributed similarly in the body. Digitoxin is bound to plasma albumin to the extent of about 97 %, while digoxin is only for 23 % bound to plasma proteins.

This explains the therapeutic plasma concentrations for digitoxin, 15-25 ng/ml, and for digoxin, 1-2 ng/ml.

Digitoxin is very slowly eliminated from the body. The liver is the main site of detoxification of the drug, metabolism occurring rapidly. The unchanged drug and metabolites are passed into the gastrointestinal tract and reabsorbed.

From the total body amount of digoxin, 1/3 disappears daily : 10 % with faeces, 90 % with urine. Urinary and faecale excretion are respectively 95 % and 50 % unchanged digoxin. The renal clearance of digoxin is 140 ml/min. 7 % of the digoxin body amount is recycled in the enterohepatic circulation.

Digitoxin however undergoes an extensive biotransformation to pharmacologically little and even unactive metabolites. From the total digitoxin body amount 10 to 15 % is daily excreted : 11 % with faeces, 89 % with urine.

It concerns as well in urine as in faeces, for 90 % unactive metabolites.

This means that the renal clearance of unchanged digitoxin is to neglect. The enterohepatic recirculation of digoxin is 26 %. Those processes of biotransformation and excretion determine the elimination half-life in plasma. This is for digoxin 36 hours, for digitoxin 5 to 7 days.

Digitalis Purpurea leaf contains a number of glycosides, including digitoxin, gitoxin and gitaloxin. These glycosides have a specific and powerfull action on the myocardium and the cardiovascular system.

Symptoms of digitalis poisoning are : gastro-intestinal effects as anorexia, nausea and vomiting. This results from a central effect (excitation of a chemoreceptor trigger zone).

The alterations in cardiac rate and rhythm occurring in Digitalis poisoning may simulate almost every known type of arrhythmia. Probably the most frequent cardiac effect of Digitalis intoxication is the assurance of extrasystoles. Ventricular fibrillation is the most common cause of death (1).

The autopsy from the 1 year old baby gave no specific cause of death.

A toxicological investigation was indicated the more that the information pointed to a digitoxin intoxication that clinically already was established.

METHODS AND RESULTS.

For the toxicological examination, digitoxin levels were confirmed by analysis according to the method of Selesky (5).

Tissue samples were weighed and homogenized with 5 % methanol distilled water solution in a ratio of approximately 1 ml/g tissue.

An aliquot of 50 μl of the homogenate was used for the assay.

The Coat-a-count ^{125}I radio-immunoassay has been used with an LKB 12-channel γ-counter (table I).

TABLE I

Tissue concentrations are found as follows

Whole blood	305.0 ng/ml digitoxin
Liver	92.9 ng/ml
Bile	368.8 ng/ml
Kidney	369.4 ng/ml
Small intestine contents	955.8 ng/ml
Muscle	124.1 ng/ml

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Digoxin concentrations were also measured. These were for blood as well as for tissues negative.

DISCUSSION.

Blood was analysed directly.

The whole blood digitoxin amount is measured instead of the serum concentrations, since the post-mortem haemolysis invalidated the serum digitoxin measurement. We found 305 ng digitoxin per ml blood. Therapeutic blood values range from 8-14 ng/ml, toxic values are seen from 15 ng/ml, lethal values are registrated from 300 ng/ml.

Experiments confirm that haemolyses do not interfere with the coat-a-count digitoxin procedure (6).

It is known (7) that only 5 % of digitoxin present in the blood, is recoverable from the red blood cells, while digoxin is concentrated more or less equally between erythrocytes and serum ; namely : the erythrocytes/plasma ratio for digitoxin and digoxin are 0.09 and 0.90 respectively (8). Thus plasma, serum and whole blood levels of digoxin are practically the same ; the levels of digitoxin in serum are higher than those in whole blood.

Care must be taken in interpreting post-mortem levels if extensive haemolysis has occurred (9).

The highest concentrations were found in the small intestine contents.

The high renal concentration is understandable since urine is the major route for digitoxin excretion. Maybe also accumulation in the kidney takes place.

Bile contains relatively high amounts of digitoxin.

The relative tissue distribution of digitoxin we found in our case, is in agreement with those observed by Doherty and Okita, who studied the distribution of radiocarbon digitoxin in human tissues (7, 10).

TABLE II

Total tissue concentrations

	Concentrations of Digitalis glycoside calculated as digitoxin µg/g (ml)	*Total amount of Digitalis glycoside calculated as digitoxin* µg
Blood	0.30	204
Liver	0.09	33.5
Kidney	0.37	27.8
Muscle	0.12	263
Small intestine	0.96	101.3
	Total :	629.6 µg

The weight of the tissues counted together gives 3,500 g or 38 % of the total body weight.

In general leaves of Digitalis Purpurea contain 0.3 % of digitoxin (12).

One leaf, as piece of conviction, has been analysed in the department by radio-immunoassay ; one leaf contained about 1 mg digitoxin. The total amount of digitoxin is about 6 mg.

When we take into account the daily excretion, and the elimination by the enforced diuresis performed in the intensive care unit, and also the extensive metabolisation, we can conclude that at least 2 mg is taken, what already can be lethal, as mentioned in literature (11).

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The measurement of glucose levels in biological specimens and its application in forensic toxicology

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1. INTRODUCTION.

Diabetes mellitus is a metabolic disorder which occurs due to faulty pancreatic activity and leads to an accumulation of glucose in the blood and exceeds the normal level of 65-120 mg % (1). This condition can prove fatal if the problem remains untreated. Normally depending upon the type of Diabetes mellitus (i.e. whether juvenile onset or adult onset) the methods of therapy involve injections of insulin or oral hypoglycaemic drugs coupled with a low carbohydrate diet.

A lowering of blood glucose levels below normal values, a condition known as hypoglycaemia, can arise if diabetics suffer an overdose of insulin (or oral hypoglycaemic drugs) and/or they fail to maintain a sufficient body carbohydrate level by means of dietary control. Symptoms of hypoglycaemia resemble those of alcohol intoxication, and a person attempting to drive in such a condition whilst impaired are liable to prosecution under English law.

Since relatively few cases require quantitative blood glucose measurements in this laboratory it was uneconomic to purchase a commercial glucose analyser. Enzyme assay kits, specifically those using hexokinase and glucose oxidase were designed mainly for use with plasma samples, and have proved to be too expensive due to the short shelf-life of the enzymes.

Dns hydrazine (N,N-dimethylamino-naphthalene-5-sulphonic acid) has been used as a fluorescence labelling reagent for the analysis of reducing sugars by thin-layer chromatography (2). Pre-column high-performance liquid chromatographic (HPLC) methods utilizing this derivatisation procedure have been developed (3).

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In this paper we describe an HPLC method using Dns hydrazine for the analysis of glucose in small volumes of blood, cerebrospinal fluid (CSF) and vitreous humour. Full details of the development of the method have been described in a previous paper (4).

2. EXPERIMENTAL.

Materials Dns hydrazine (Grade II), D-(+)glucose and D-(+)-xylose were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of the highest grade commercially available.

Reagent solutions Dns hydrazine solution (1 % w/v), a 50 mg sample of Dns hydrazine was dissolved in 5 ml of ethanol. The solution was stored at —10°C until required for use.

Trichloroacetic acid (TCA) solution (3 % w/v) : a 750 mg sample of trichloroacetic acid was dissolved in 25 ml of distilled water.

Sugar solutions Freshly prepared standards of glucose (0.1 % w/v) and xylose (0.2 % w/v) were prepared in distilled water when required.

Chromatographic conditions.

Analyses were performed on a 25 cm × 4.9 mm I.D. stainless-steel column slurry packed with ODS Hypersil (5 μm particle size, Shandon Southern Products, Cheshire U.K.). An ACS pump (Model 400, Applied Chromatography Systems, Luton, U.K.) was used to deliver the eluant, acetonitrile-water (22:78), at a flow rate of 1.7 ml/min. The eluate was monitored with a fluorimeter (fluoromonitor III, Laboratory Data Control, Riviera Beach FL, USA) that was fitted with a zinc lamp. An excitation wavelength of 214 nm and emission wavelength of 418-700 nm were selected with the appropriate filters. Injections were made under continuous flow conditions via an injection valve (Negretti and Zambra, Southampton U.K.), fitted with a 25 μl volume injection loop.

Derivatization procedure.

Solutions of the internal standard, xylose (60 μl), and trichloroacetic acid (900 μl) were added to the sample (100 μl of aqueous glucose standard or body fluid) and the mixture was vortex-mixed and allowed to stand for 5 min. to precipitate blood proteins. After centrifuging at 2,000 g for 7 min. an aliquot of the clear supernatant (100 μl) was transferred to a screw cap

tube together with distilled water (50 µl) and Dns hydrazine solution (200 µl). Following incubation of the sealed tube at 50°C for 1 hour the reaction mixture was washed with toluene (2 ml twice) by vortex-mixing for 30 sec. An aliquot of the remaining aqueous phase (50 µl) was diluted with HPLC eluant (200 µl) and the aliquots analysed by HPLC.

3. RESULTS AND DISCUSSION.

A typical chromatogram is shown in figure 1.

The method has been shown to be linear up to a blood glucose concentration of at least 500 mg % with a correlation coefficient of 0.99. A blood sample with a blood glucose concentration of 105 mg % was analysed ten times with a resulting coefficient of variation of 2.79 %.

Clinical glucose analysers are required to meet agreed specifications for the detection and measurement of glucose, and to check to validity of this HPLC method a number of samples were analysed by both techniques. A batch of 39 blood and CSF samples previously analysed in a clinical laboratory with a glucose analyser (YSI Model 23A, Yellow Springs Instrument, OH, USA) were analysed within seven days by HPLC.

A scatter diagram showing the correlation between results obtained from the two methods is shown in figure 2. A linear least squares fit for the two sets of data gave a line with a slope of 0.98. The correlation coefficient was calculated as 0.99.

Whole blood samples are submitted to this laboratory generally in vials which contain an anticoagulant (potassium oxalate) and a bacteriostat (sodium fluoride). The amount of fluoride added to these vials will produce a concentration of greater than 1 % (w/v) and therefore glycolysis of glucose to lactic acid is arrested. Blood samples taken by venepuncture from a healthy subject, and aliquots of 2-2.5 ml were placed and sealed in three vials that contained anticoagulant/preservative. The glucose of one sample was determined immediately after which the vials were stored at 4°C, 22°C and 37°C. Glucose levels in each vial were measured periodically thereafter. No discernable changes in the glucose concentrations of the samples stored at 4°C or room temperature were detected over a period of a month, but at 37°C the concentration decreased by approximately 20 %.

The two main applications of the method within our laboratory have been in the determination of the glucose levels in blood

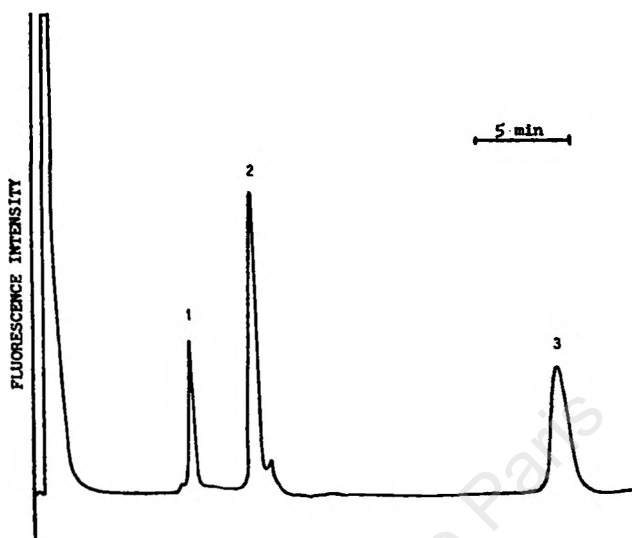

FIG. 1. — Typical result obtained from the analysis of a blood sample after derivatization and clean up procedure. Peak 1 = glucose ; 2 = xylose (internal standard) ; 3 = Dns sulphonic acid, a biproduct formed due to the hydrolysis of dansyl hydrazine under acidic conditions.

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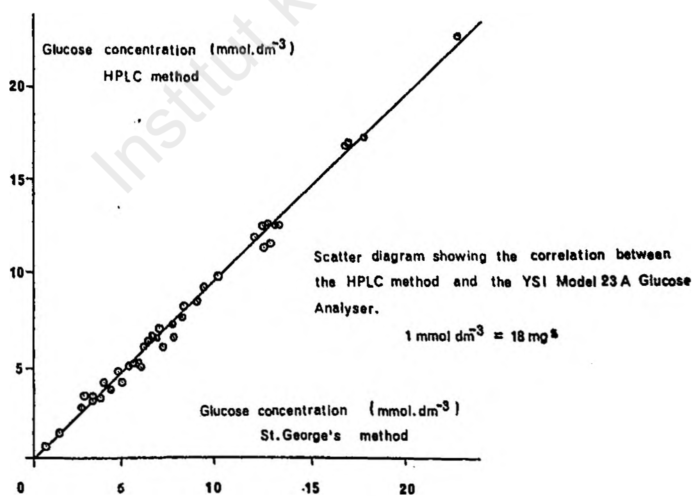

FIG. 2. — Scatter diagram showing the correlation between HPLC method and YSI Model 23A analyse.

samples taken from motorists suspected of driving in a hypoglycaemic condition and the measurement of glucose concentrations in post-mortem vitreous humour samples.

Vitreous humour constitutes an isolated pool of material suitable for many analyses which is far less susceptible to rapid post-mortem chemical changes or contamination than the blood of the CSF. However, it has still been demonstrated that glycolysis still occurs after death in this medium, it is necessary therefore to measure both glucose and lactate levels in order to provide an estimation of the blood glucose level of the person at the time of death.

Sippel and Mottenen (5) measured the combined glucose plus lactate levels in 52 vitreous humour specimens from persons without any history of diabetes and in 10 specimens from persons whose death was due at least in part to diabetes mellitus. The conclusions from this paper are summarized in table I.

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TABLE I

Combined glucose and lactate values in vitreous humour for post-mortem diagnosis of fatal diabetes, lactic acidosis or hypoglycaemia

Glucose plus lactate (mg/100 ml)	*Diagnosis*
< 160	Did not rule out hypoglycaemia
160-374	Not diagnostic (normal value)
375-410	Did not rule out decompensated fatal diabetes mellitus or lactic acidosis
> 410	Decompensated fatal diabetes mellitus

This information together with the HPLC method described has been used in the toxicological examination of two sudden deaths investigated at our laboratory. The lactate levels being measured by isotachophoresis (6).

Case history 1.

The victim was a 39 year old woman lived with her 49 year old common-law husband. Over a period of several months, it was known that the husband had severely beat the victim ; this story was backed up by entries in her diary and the evidence of neighbours. Both the woman and her common-law husband were heavy drinkers, normally of sherry and vodka. The victim was found early one morning dead in her bed ; her death having been

reported to the police by her husband. The husband stated that she had consumed sherry and larger the previous evening.

Medical history revealed that she had been taking the analgesic preparation « Nurofen » for some time and that she had undergone an operation for an abcess on her pancras approximately 3 years prior to her death.

No cause of death could be ascertained from an initial post-mortem examination, the cause of death may be due to either alcohol poisoning or a drugs overdose ; toxicological examination eliminated both of these theories. Examination of the vitreous humour for glucose and lactate gave a combined level of 780 mg %, a level consistent with the person having died due to untreated diabetes mellitus.

TABLE II

Summary of toxicology results for case 1

Specimen	*Results*
Blood	Acetone 46 mg % Ibuprofen 2.7 μg/ml (therapeutic) Trace ethanol and isopropanol
Stomach content . .	150 mls brown liquid containing 1.5 mg ibuprofen
Urine	Acetone 52 mg % Negative ethanol Positive ibuprofen
Vitreous humour . . .	Glucose : 400 mg % Lactate : 380 mg % Combined level : 780 mg % (consistent with fatal diabetes mellitus)

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Case history 2.

The victim was a 27 year old single man who lived alone in his flat. He had not reported for work for several days and his employers informed the police who entered his flat and found the victim lying face down on the bathroom floor. Enquiries revealed he had not been seen for several days. He was fully clothed and appeared to have been involved in home repairs in his bathroom at the time of his death. An electrical drill being found near the body. It was also known that he had just installed central heating into his flat.

A post-mortem examination failed to establish a cause of death — but there was no sign of physical injury to the body and death by electrocution was eliminated.

Initial toxicological examination for alcohol, drugs and carbon monoxide all proved negative, however, acetone was detected in the post-mortem blood and urine specimens at 12 and 13 mg % respectively.

Subsequent examination of the vitreous humour gave a combined glucose phis lactate level of 1,211 mg %, a level consistent with fatal diabetes mellitus. Subsequent investigations by the police showed that there had been a history of diabetes in his family, however, the victim had never received treatment for the condition.

Another case situation in which it is necessary to determine a persons blood glucose level is when an insulin dependent diabetic driver drives whilst impaired in a hypoglycaemic condition ; an offence under English law. This situation normally arises due to the person not maintaining a sufficient body carbohydrate level through the eating of regular meals.

A summary of the case histories and analytical results are given in table IV.

TABLE III

Summary of toxicology results for case 2

Specimen	*Results*
Blood	Negative alcohol and carbon monoxide Acetone 12 mg %
Urine	Acetone 13 mg % Glucose > 20 mg %
Vitreous humour . .	Glucose : 635 mg % Lactate : 576 mg %
	Combined level : 1211 mg % (consistent with fatal diabetes mellitus)

TABLE IV

Summary of case histories and blood glucose levels from hypoglycaemic drivers

Subject (age)	*Case history and blood glucose level*
PJS (48)	Prescribed insulin 60 units per day. Ate only one sandwich all day. Stopped due to erratic driving. Specimen taken 17.52 hour blood glucose level : 30 mg %.
RS (46)	Insulin dependant diabetic for many years. Involved in accident following late night working. Travelled down the wrong side of the road. Specimen taken 01.17 hour. Blood glucose level : 23 mg %.
CW (43)	Found slumped over steering wheel of car by passerby, engine still running with emergency indicators flashing. Insulin dependant diabetic working as taxi driver. Specimen taken 01.35 hour blood glucose level : 30 mg %.

CONCLUSIONS.

A pre-column derivatisation with Dns hydrazine and subsequent analysis by HPLC offers a method for the determination of glucose in ante-mortem blood and post-mortem vitreous humour specimens.

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Toxicological analysis of organochlorine phytopharmaceuticals and PCBs in commercial milk products

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INTRODUCTION.

Besides human milk, cow's milk was also reported to contain some organochlorine insecticides and PCBs as residues (1, 2, 3). From our previous work (4, 5) we could separate organochlorine insecticides, PCBs, PCDFs and TCDD ; we therefore applied the method to the analysis of some commercial milk products.

MATERIAL AND METHODS.

Equipment.

A Sigma 2 B Perkin-Elmer gas chromatograph, equipped with a ^{63}Ni electron capture detector was used. The chromatographic column was a 25 m × 0.32 mm i.d., CP tm Sil 5 WCOT fused silica column.

Injection and detection temperatures were 230° and 280°, respectively.

Column temperatures used were the following : for the insecticides, initiated at 160°C for 5 minutes, increased to 180°C with the rate of 5°C/minute, held at 180°C for 1 minute, then increased to 210°C with the rate of 3°C/minute, as described earlier (4, 5) ; PCBs, the same as those described for the insecticides, but the time post at 210°C was extended to 12 minutes ; PCDFs and TCDD, initiated at 180°C for 5 minutes, increased to 250°C with the rate of 7°C/minute and held at 250°C for 10 minutes.

Purified argon/methane (90/10) was used as the carrier gas at the pressure of about 0.8 bar. ECD electronics and recorder from Perkin-Elmer were used.

Reagents.

Hexane for residue analysis was obtained from Carlo Erba (Italy).

Analytical grade : ethanol, diethyl ether, petroleum ether (b.p. 40°-60°C), dimethylformamide (DMF), methylene chloride, sodium oxalate, sodium chloride, anhydrous sodium sulfate, antimony pentachloride ($SbCl_5$) for chromatography, aluminum oxide 90 and silica gel (0.05-0.20 mm) were obtained from Merck (Germany).

Hydrochloric acid was obtained from UCB (Belgium). PCBs (Aroclor 1260) were obtained from Alltech Associate (U.S.A.). TCDD was obtained from the Dow Company (U.S.A.). Dichlorodibenzofuran (DCDF), octachlorodibenzofuran (OCDF) and a mixture of polychlorinated dibenzofurans (PCDFs) were gifts.

A solution of PCBs as well as PCDFs was prepared at a concentration of 2 ppm in hexane. Solutions of TCDD, DCDF and OCDF were prepared at individual concentrations of 1 ppm in hexane. Saturated NaCl solution in water, 5 % w/v Na_2SO_4 solution in water and a DMF/water (92/8 by volume) mixture were prepared and washed with hexane before use.

Glass wool was silanized with 5 % HMDS (hexamethyldisilazane) in benzene. The insecticide mixture containing α-BHC, β-BHC, lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, p,p'-DDE, o,p'-DDD, endrin, p,p'-DDD, o,p'-DDT and p,p'-DDT was prepared as recommended by Thompson *et al.* (6) ; α-endosulfan which was used as the internal standard was also added at a concentration of 40 ng/ml. Methoxychlor at a concentration of 1 μg/ml in hexane was prepared for the use as the internal standard for quantitation of PCBs by perchlorination.

ANALYSIS OF SOME COMMERCIAL MILK PRODUCTS.

Three different brand names of whole milk, concentrated whole milk, cream and butter (and cheese), totally 12 samples were obtained from neighbouring supermarkets (Ghent, Belgium). The following amounts of samples were used : whole milk, 10 ml ; concentrated whole milk, 5 ml ; cream, 3 g ; butter 0,5 g ; and

cheese 1 g. Water was added to concentrated whole milk and cream to approximately 10 ml. Butter and cheese were melted with 7-10 ml warm water. Extraction for fat and partitioning process were done as described earlier (4, 5). Ethanol and sodium oxalate were added to the diluted commercial milk samples. Fat was extracted with a mixture of diethyl ether/petroleum ether (1/1). The organic layer was washed with water in the presence of saturated NaCl solution to reduce the emulsion.

The organic layer was dried over anhydrous Na_2SO_4, concentrated by a K-D concentrator and evaporated under nitrogen to obtain fat. The hexane solution of fat was extracted with a DMF/water (92/8 by volume) mixture. The chlorinated compounds in the aqueous layer were partitioned back into the hexane layer in the presence of 5 % w/v Na_2SO_4 solution and the residues of the insecticides, PCBs, PCDFs and TCDD after partitioning were separated as described earlier.

A recheck of the positive peaks of the insecticides by using a CP tm Sil 8 fused silica capillary column, PCB measurement by perchlorination using $SbCl_5$ as a perchlorinating agent, and the presence of PCDFs and TCDD by conc. H_2SO_4 clean up, were also done in a similar manner to those described earlier (4, 5).

RESULTS AND DISCUSSION.

The results from the analysis of some commercial milk products are indicated in table I.

Chromatograms of standard insecticide mixture, PCBs, PCDFs and TCDD and examples of chromatograms from a commercial milk product are illustrated in figures 1, 2 and 3. The average concentrations of the insecticides and PCBs as indicated in table I were performed only on a fat basis (not on a whole amount basis), because different kinds of milk products contained a great difference in % fat content which could effect the concentrations if expressed as a whole amount basis. Different studies on dairy products since many years ago till recently, revealed that various organochlorine insecticides were detected, namely : BHC isomers, heptachlor epoxide, dieldrin, DDE and DDT with the amounts in the range of 1 to 100 ppb on a fat basis (1, 2, 3). The insecticides found in the present work were in the range of 4 to 16 ppb on a fat basis. Nevertheless, no detectable amounts of heptachlor expoxide could be found. When individual con-

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TABLE I

Organochlorine insecticides and PCBs (ppb) in commercial milk products

Compound	*Number of positive samples (N = 12)*	*Minimum value*	*Maximum value*	*Mean ± SD*
α-BHC	7 (58 %)	W 0	19 (26)	F 6 ± 7 (8)
		F 0	24 (33)	
β-BHC	3 (25 %)	W 0	4 (5)	F 4 ± 9 (5)
		F 0	29 (36)	
Lindane	11 (92 %)	W 0	31 (37)	F 12 ± 10 (14)
		F 0	40 (48)	
Total BHC	11 (92 %)	W 0	55 (68)	F 21 ± 21 (27)
		F 0	70 (88)	
Dieldrin	8 (67 %)	W 0	5 (6)	F 7 ± 6 (9)
		F 0	17 (22)	
p,p'-DDE	11 (92 %)	W 0	11 (13)	F 16 ± 15 (19)
		F 0	53 (64)	
PCBs*	11 (92 %)	W 0	31 (41)	F 25 ± 16 (33)
		F 0	65 (87)	
PCBs**	11 (92 %)	W 0	31 (41)	F 35 ± 20 (47)
		F 0	78(104)	

* Calculated from pattern comparison (using total height of peaks appearing after p,p'-DDE).
** Calculated from perchlorination.
W Whole amount basis.
F Fat basis.
Values in parentheses were corrected for recovery.

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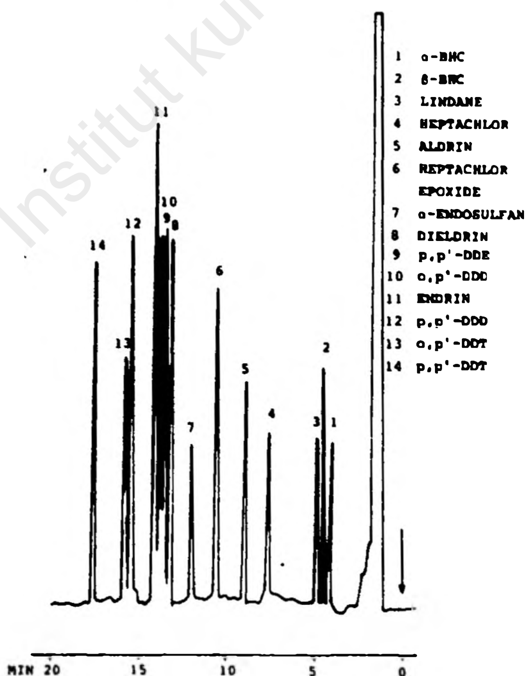

FIG. 1. — Chromatogram of the insecticide mixture
(from a 25 m × 0.32 mm i.d., CP tm Sil 5 WCOT fused silica capillary column).

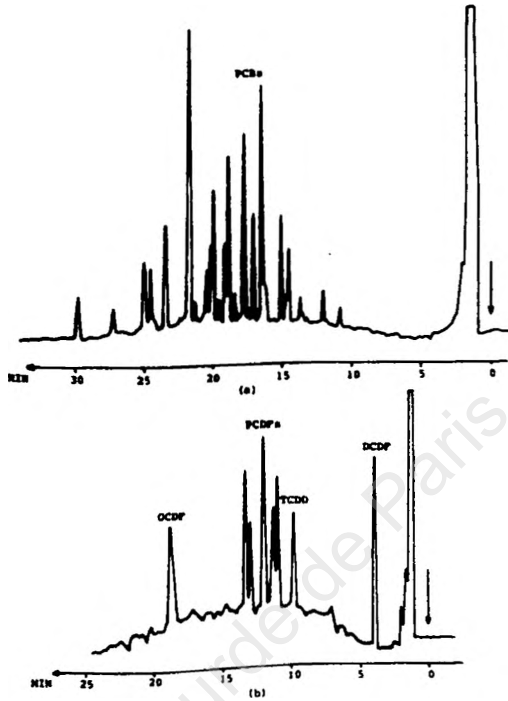

FIG. 2. — Chromatograms of standard PCBs (a), PCDFs and TCDD (b).

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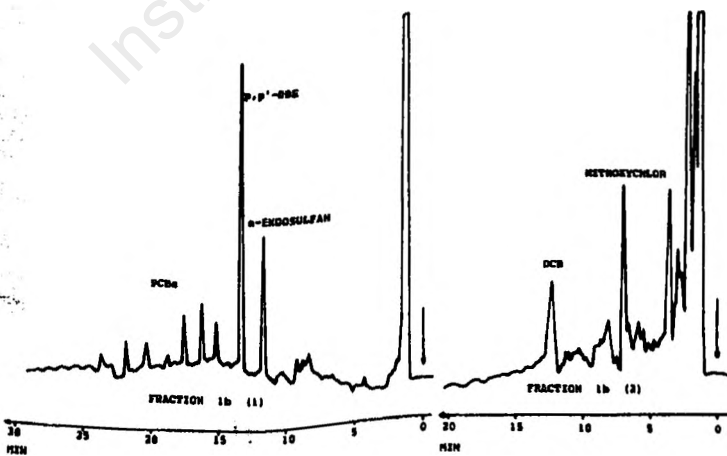

FIG. 3. — Chromatograms from a commercial milk product. (1) Pattern comparison — (2) Perchlorination (3-time dilution).

centrations of the insecticides as well as total BHC were compared to the residue limits under FAO/WHO recommendation (7), none of these samples contained any residues over the limits.

Moreover when compared, the insecticide concentrations found in cow milk products in the present study to the concentrations detected in human milk (4, 5, 7, 8) the concentrations found in the former were much lower. In favour of this finding, it was mentioned that human milk seemed to contain organochlorine insecticides in greater amounts than cow's milk did (9). The reason was that human beings are placed at the top of most food chains and therefore human beings could collect more residues from their food.

Analyzing these samples for PCBs, we found again a lower mean level, compared to that of human milk samples (4, 5).

PCDFs and TCDD were not detected in any commercial milk products in the present work ; therefore the analysis results of the insecticides and PCBs were not interfered by these compounds.

In this work we also analyzed a sample of milk powder, 2 samples of commercial vegetable oil and a sample of margarine. Low levels of p,p'-DDE, p,p'-DDD and p,p'-DDT were found in milk powder which contained fat mostly from vegetable sources, and only a low level of p,p'-DDD was found in the margarine sample. No organochlorine insecticides were found in samples of vegetable oil. None of these samples contained any PCBs, PCDFs and TCDD.

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Use of fused silica capillary columns for the separation of organochlorine insecticides

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ABSTRACT.

Gas chromatography with electron capture detection is a method of much concern for the separation and quantitation of the organochlorine insecticides. Using an SE-52 glass capillary column, the separation of some compounds could not be obtained. A CP ᵗᵐ Sil 5 fused silica capillary column with suitable chromatographic conditions offers a better separation of the mixture containing 14 organochlorine insecticides.

INTRODUCTION.

Organochlorine insecticides are still found prevalent as residues in the environment. Gas chromatography is a very sensitive method for the detection of such residues in various samples. However, for a multi-insecticide residue analysis the separation of different insecticides from each other in a one step gas chromatographic procedure is difficult.

Using an SE-52 WCOT glass capillary column for the separation of the insecticide mixture as described earlier (1), peaks of β-BHC and lindane as well as peaks of p,p'-DDD and o,p'-DDT were not well separated. In this work we developed a method for the separation of 14 organochlorine insecticides using a fused silica capillary column.

EXPERIMENTAL.

Equipment.

A Sigma 2 B Perkin-Elmer gas chromatograph, equipped with a ^{63}Ni electron capture detector was used. The chromatographic column was a 25 m × 0.32 mm i.d., CP ^{tm} Sil 5 WCOT fused silica capillary column. Injection and detection temperatures were 230° and 280°C, respectively. Different column temperatures were applied.

Purified argon/methane (90/10) was used as the carrier gas at a pressure of about 0.8 bar. ECD electronics and recorder from Perkin-Elmer were used.

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REAGENTS.

Hexane for residue analysis was obtained from Carlo Erba (Italy).

The following insecticide solutions were prepared in hexane : α-BHC, β-BHC, lindane, p,p'-DDD and o,p'-DDT at concentrations of 25, 100, 125, 190 and 225 ng/ml, respectively.

The following mixtures containing : β-BHC and lindane ; α-BHC, β-BHC and lindane ; and p,p'-DDD and o,p'-DDT were prepared in hexane at individual concentrations as mentioned. The insecticide mixture of α-BHC, β-BHC, lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, p,p'-DDE, o,p'-DDD, endrin, p,p'-DDD, o,p'-DDT and p,p'-DDT was prepared as recommended by Thompson *et al.* (2) ; α-endosulfan which was usually used as the internal standard at a concentration of 40 ng/ml was also added.

PROCEDURE.

Retention times of different insecticides were determined by injecting individual compounds into the gas chromatograph with different column temperatures used. The separation of individual insecticides from the mixture was also obtained by injecting each mixture into the gas chromatograph, with different column temperatures applied.

RESULTS AND DISCUSSION.

From the previous work (1) using an SE-52 glass capillary column with the chromatographic conditions as described, the separation

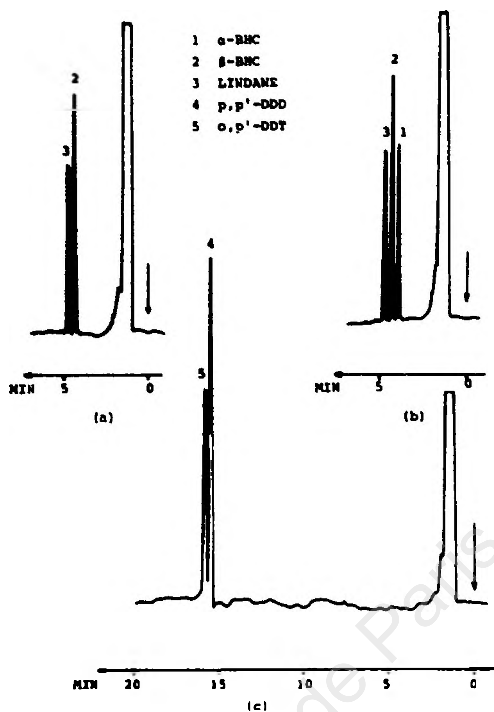

FIG. 1. — Separation of :
(a) β-BHC and lindane ; (b) α-BHC, β-BHC and lindane ; (c) p,p'-DDD and o,p'-DDT.

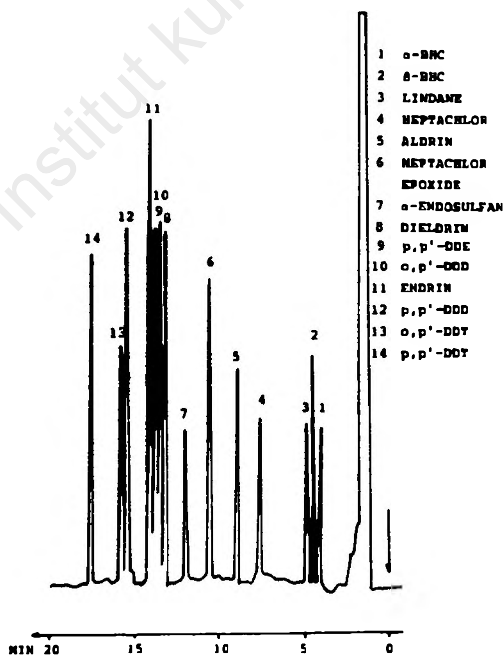

FIG. 2. — Chromatogram of the insecticide mixture
(from a 25 m × 0.32 mm i.d., CP tm Sil 5 WCOT fused silica capillary column).

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of β-BHC from lindane and p,p'-DDD from o,p'-DDT could not be obtained. We could not obtain a good separation of these compounds on that column, although different chromatographic conditions were applied. A longer chromatographic, 60 m × 0.25 mm i.d., SP-2330 Supelco fused silica capillary column which is more polar was used.

This column offered much less satisfaction due to the long analysis time and unability to separate these insecticides.

Another column, a CP ^{tm} Sil 5 fused silica capillary column which has a higher resolution than a glass capillary column was then applied. All column temperatures used, e.g. : 150°, 160°, 170° and 180°C isothermally offered a good separation of β-BHC from lindane, and also a separation of α-BHC, β-BHC and lindane (fig. 1).

Nevertheless, a column temperature of 160°C was considered to be the most suitable condition when applied to the separation of all insecticides in the mixture.

For the separation of p,p'-DDD from o,p'-DDT, various column temperatures used offered a good separation of these two compounds. The column conditions of : temperatures initiated at 160°C for 5 minutes, increased to 180°C with the rate of 5°C/minute, held at 180°C for 1 minute, then increased to 210°C with the rate of 3°C/minute was considered to be the most favourable technique (fig. 1), since it gave the best chromatograms of the standard mixture (fig. 2), and was also time-saving.

It was evident that a CP ^{tm} Sil 8 WCOT fused silica capillary column could also offer a good separation of all insecticides in the mixture at suitable chromatographic conditions ; it can be used also to check the retention times of the positive peaks of insecticides in different samples. However, to have a good separation of all insecticides in the mixture, it requires a longer analysis time than a CP ^{tm} Sil 5 column does.

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Separation of organochlorine insecticides, PCBs, PCDFs and TCDD

Applicability to the ecotoxicological analysis of human milk samples

by N. SOOKVANICHSILP*, B. HEYNDRICKX*, A. HEYNDRICKX* and M. THIERY**

INTRODUCTION.

Besides the organochlorine insecticides, PCBs (polychlorinated biphenyls) were also found prevalent as residues in the environment. PCDFs (polychlorinated dibenzofurans) and TCDD (2, 3, 7, 8-tetrachlorodibenzo-p-dioxin), one of the most toxic isomers of of PCDDs (polychlorinated dibenzodioxins), which were found as contaminant in PCBs (1-4) and other chlorinated products (1, 5-9) were also detected as residues in the environment. They are similar in residue methodology. In addition, the GLC retention times of these compounds are in range of one another. The analysis is therefore difficult when they are present as a complex mixture in some samples. Several methods have described the separation of these compounds. However, these methods have mentioned either the separation of PCBs from organochlorine insecticides with (10, 11) and without (12-14) destruction of the insecticides ; or the separation of PCDFs (or TCDD) from PCBs after destruction or digestion of organochlorine insecticides (4, 15-17) or have described only the separation of PCDFs (or TCDD) from PCBs without mentioning of the insecticides (in cases that the samples were free from an insecticide contamination) (2, 3, 6, 18-22). Therefore the present work is to separate organochlorine insecticides, PCBs, PCDFs and TCDD with a single analysis, and apply to the analysis of human milk samples.

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EXPERIMENTAL.

Equipment.

A Sigma 2B Perkin-Elmer gas chromatograph, equipped with a ^{63}Ni electron capture detector was used. The chromatographic column was a 25 m × 0.32 mm i.d., CP tm Sil 5 WCOT fused silica capillary column. Injection and detection temperatures were 230° and 280°, respectively. Column temperatures were used as the following : for the insecticides, initiated at 160°C for 5 minutes, increased to 180°C with the rate of 5°C/minute, held at 180°C for 1 minute, then increased to 210°C with the rate of 3°C/minute, as described earlier (23) ; PCBs, the same as those described for the insecticides but the time post at 210°C was extended to 12 minutes ; PCDFs and TCDD, initiated at 180°C for 5 minutes, increased to 250°C with the rate of 7°C/minute and held at 250°C for 10 minutes. Purified argon/methane (90/10) was used as the carrier gas at a pressure about 0.8 bar. ECD electronics and recorder from Perkin-Elmer were used.

Reagents.

Hexane for residue analysis was obtained from Carlo Erba (Italy). Analytical grade : ethanol, diethyl ether, petroleum ether (b.p. 40°-60°C), dimethylformamide (DMF), methylene chloride, sodium oxalate, sodium chloride, anhydrous sodium sulfate, antimony pentachloride ($SbCl_5$) for chromatography, aluminum oxide 90 and silica gel (0.05-0.20 mm) were obtained from Merck (Germany). Hydrochloric acid was obtained from UCB (Belgium). PCBs (Aroclor 1260) were obtained from Alltech Associate (U.S.A.). TCDD was obtained from the Dow Company (U.S.A.). Dichlorodibenzofuran (DCDF), octachlorodibenzofuran (OCDF) and a mixture of polychlorinated dibenzofurans (PCDFs) were gifts. A solution of PCBs as well as PCDFs was prepared at a concentration of 2 ppm in hexane. Solutions of TCDD, DCDF and OCDF were prepared at individual concentrations of 1 ppm in hexane. Saturated NaCl solution in water, 5 % w/v Na_2SO_4 solution in water and DMF/water (92/8 by volume) mixtures were prepared and washed with hexane before use. Glass wool was silanized with 5 % HMDS (hexamethyldisilazane) in benzene. The insecticide mixture containing : α-BHC, β-BHC, lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, p,p'-DDE, o,p'-DDD, endrin, p,p'-DDD,

o,p'-DDT and p,p'DDT was prepared as recommended by Thompson *et al.* (24) ; α-endosulfan which was used as the internal standard was also added at a concentration of 40 ng/mL. Methoxychlor at a concentration of 1 μg/mL in hexane was prepared for the use as the internal standard for quantitation of PCBs by perchlorination.

Separation of organochlorine insecticides, PCBs, PCDFs and TCDD.

A mixture of organochlorine insecticides, PCBs, PCDFs and TCDD was prepared by mixing 0.5 mL of each and concentrated to 0.5 mL. A silica gel column was prepared by placing a silanized glass wool plug into the conical end of a chromatographic column

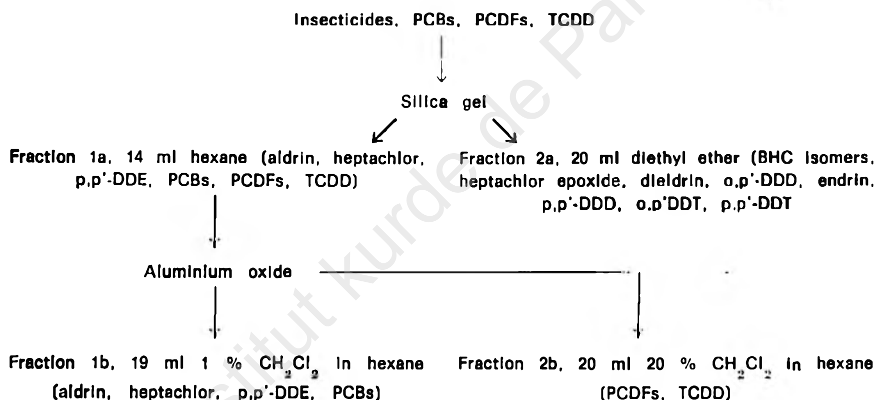

FIG. 1. — Separation of organochlorine insecticides, PCBs, PCDFs and TCDD.

(30 cm × 0.7 cm i.d., with Teflon stopcocks), adding approximately 500 mg anhydrous Na_2SO_4, filling the column with hexane and slowly adding 2 g of activated silica gel ; furthermore topping with anhydrous Na_2SO_4 to prevent surface of the gel from being disturbed when solutions were added. The column was washed with 20 mL diethylether and then 20 ml hexane. The concentrated mixture was added onto the column. The tube was rinsed with two 0.5 mL portions of hexane. The compounds were eluted first with 14 mL hexane (fraction 1a, fig. 1), then 20 ml diethyl ether (fraction 2a, fig. 1). Fraction 2a was evaporated to dryness and the residues were dissolved in 0.5 mL α-endosulfan, the internal standard solution. Fraction 1a was concentrated to about 0.5 mL for a further separation on an aluminum oxide column.

An aluminum oxide column was prepared by placing a silanized glass wool plug into the conical end of the chromatographic column, filling the column with 2 g activated aluminum oxide under continuous vibration and topping with about 200 mg anhydrous Na_2SO_4. The column was washed with hexane. The concentrated solution of fraction 1a was transferred onto the column. The tube was rinsed with two 0.5 mL portions of hexane. The column was eluted with 19 mL 1 % methylene chloride in hexane (fraction 1b, fig. 1) followed by 20 mL 20 % methylene chloride in hexane (fraction 2b, fig. 1). Both fractions were evaporated to dryness and the residues were dissolved in 0.5 mL α-endosulfan solution. Individual fractions (fractions 2a, 1b and 2b) were injected into the gas chromatograph. Column temperatures used for fraction 2a were those described for the insecticides, for fraction 1b were described for PCBs, and for fraction 2b were those described for PCDFs and TCDD.

Analysis of human milk samples.

Human milk samples were taken on the third to the seventh day of lactation from 35 Belgian nursing women admitted at the University Hospitals in Ghent. The extraction of fat and the separation of chlorinated compounds from fat by partitioning were performed in accordance to the methods proposed by the IDF/ISO/AOAC Group of Experts (Group E12) (25).

Ten mL of milk were added to 10 mL ethanol to rupture the fat globule membrane. One hundred mg sodium oxalate were added to soften the curd formed after adding ethanol. Five mL diethyl ether were added and shaken vigorously for 1 minute ; 5 mL petroleum ether was added and shaken again vigorously. The solvent layer was transferred into a separator containing 100 mL water and 6 mL saturated NaCl solution. The aqueous portion was extracted again three times with 5 mL portions of diethyl ether/petroleum ether (1/1 by volume) mixture. The combined solvent layers in the separator were mixed cautiously with the water phase and the aqueous phase was discarded. The solvent phase was washed again twice with a 10 mL portion of water, dried over anhydrous Na_2SO_4, concentrated by using a K-D concentrator and evaporated under nitrogen to obtain fat.

Twenty-five ml hexane solution of fat were extracted three times with a 10 mL portion of DMF/water (92/8 by volume) mixture. The combined DMF layers were added to 200 ml 5 %

w/v Na_2SO_4 solution and 20 ml hexane, and shaken vigorously for 2 minutes. The layers were allowed to separate overnight. The solvent layer was dried over Na_2SO_4, concentrated by using a K-D concentrator, and evaporated to 0.5 mL under nitrogen. The insecticides, PCBs, PCDFs and TCDD were separated as described earlier by first using a silica gel column, followed by an aluminum oxide column. Fraction 1b was divided into 2 portions.

Evaporate the first portion to dryness and dissolve the residues in a 100 µl α-endosulfan solution before injecting into the gas chromatograph ; quantitation of PCBs were done by pattern comparison utilizing total height of peaks appearing after p,p'-DDE.

The second portion was kept for confirmation of the residue measurement of PCBs by perchlorination.

Positive peaks of the insecticides were checked by reinjecting the solutions into a gas chromatograph (Varian — 1740), equipped with a ^{63}Ni electron capture detector and a CP ^{tm} Sil 8 WCOT fused silica capillary column ; column temperatures, increased from 170°C to 215°C with the increasing rate of 4°C/minute and held at 215°C for 25 minutes.

Confirmation of residue measurement of PCBs by perchlorination.

Halves of the eluates from fraction 1b were transferred to Pyrex tubes (N° 13, 8 mL-volume), these are partially submerged in a ± 35°C water bath under a gently stream of nitrogen, concentrated to approximately 100 µL volumes.

The tubes were removed from the water bath, and furthermore evaporated until just dry (prolonged evaporation causes losses of lower chlorinated biphenyl isomers, but not very critical for Aroclor 1260). One hundred µL of chloroform were added to dissolve the residues. Working in a well ventilated hood, 100 µl $SbCl_5$ were added to the tube and immediately sealed with a Teflon-lined cap. The tubes were immersed in a sand bath heated to 160°-170°C ; the reaction was proceeded overnight (± 15 hours). The tubes were removed from the sand bath to room temperature and put in a refrigerator for a few minutes. One mL 6 N hydrochloric acid was added and mixed to inactivate the excess $SbCl_5$ and to prevent a precipitate formation. Decachlorobiphenyls (DCB), were extracted 5 times with hexane portions of 1 mL. The hexane layers were drawn off with a disposable pipet and placed on the top of an anhydrous Na_2SO_4 column using a Pasteur disposable pipet, filled with anhydrous

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Na_2SO_4 till ± 6 cm height, and prewashed with hexane. The eluates were evaporated to just dry, and the residues dissolved in 1-5 mL methoxychlor (1 μg/mL) ; the internal standard, before injecting into the gas chromatograph. Column temperatures used were initiated at 200°C ; increased to 250° with the rate of 5°C/minute, and held at 250°C for 10 minutes. Check the retention time with standard DCB.

Recheck of PCDFs and TCDD by using conc. H_2SO_4 for the clean-up of fat.

Fifteen mL human milk were extracted for fat in the same manner as described earlier. Fat was dissolved in 10 mL hexane and transferred to a separator. Five mL conc. H_2SO_4 was added and mixed gently. The acid layer was discarded. The hexane layer was washed again with two 5 mL portions of conc. H_2SO_4, and afterwards with two 10 mL portions of water. The hexane layer was dried over anhydrous Na_2SO_4 and concentrated to 0.5 mL. The organochlorine insecticides : PCBs, PCDFs and TCDD were separated as described earlier.

RESULTS AND DISCUSSION.

Separation of the organochlorine insecticides, PCBs, PCDFs and TCDD.

The organochlorine insecticides, PCBs, PCDFs and TCDD are all eluted from a 10 % water deactivated alumina using 13 mL hexane as the eluent. Five percent water deactivated alumina with 10 mL hexane as the eluent, Florisil with hexane and 20 % diethyl ether in hexane as the eluents (26), and activated charcoal with 25 % acetone in diethyl ether and benzene as the eluents (27), were tested. None offered a good separation of some compounds from the others ; all gave a partial separation from compounds to compounds, although varying amounts of the eluents were applied. Silica gel deactivated with 3 % water as proposed by some authors (12-14, 28) as well as 1 % water deactivated silica gel was tested with petroleum ether as the eluent. PCBs and some insecticides could be completely eluted with 15 mL of the eluent ; nevertheless, some other insecticides were also partially eluted in spite of varying amounts of the eluent. Activated silica gel (2 g) was then tried with petroleum ether or hexane as the eluent.

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When petroleum ether was used as the eluent, PCBs could be completely eluted from the column with 15 mL of the eluent, but DDT derivatives were partially eluted. Various eluent volumes were applied but similar results of a partial elution of some compounds was still obtained. Hexane at a volume of 14 mL was found to be a suitable eluent to elute : PCBs, PCDFs, TCDD, heptachlor, aldrin and p,p'-DDE from other insecticides. A further elution of other insecticides was accomplished by using 20 mL diethyl ether.

A further separation of : PCBs, PCDFs, TCDD, heptachlor, aldrin and p,p'-DDE was tried on activated aluminum oxide as proposed by many authors for the separation of PCBs from PCDFs and/or TCDD (2, 4, 16, 19-21). A variety of solvents as well as solvent mixtures were applied as the eluents, e.g. : hexane, 20 % benzene in hexane, 20 % carbon tetrachloride in hexane, 50 % methylene chloride in hexane, 20 % methylene chloride in hexane, 1 % methylene chloride in hexane, etc. with different elution volumes. However, 19 mL 1 % methylene chloride in hexane was considered to be the most suitable eluent for the elution of PCBs and the insecticides (heptachlor, aldrin and p,p'-DDE) from PCDFs and TCDD, whereas 20 mL 20 % methylene chloride in hexane were suitable for a further elution of PCDFs and TCDD. The separation of PCBs from heptachlor, aldrin and p,p'DDE as well as the separation of PCDFs from TCDD could be achieved by capillary gas chromatography with chromatographic conditions as mentioned earlier.

Perchlorination of PCBs.

A Pyrex tube N° 13 with a Teflon-lined cap was found to be a suitable reaction tube for perchlorination. In other studies, $SbCl_5$ at a volume of 200 μL was used. In this study, it was found that 100 μL were sufficient for the perchlorination of PCBs (Aroclor 1260), under the conditions described in a wide concentration range (up to 5 ppm tested). The incubation condition was 15 hours (overnight) at 165° $\pm$ 10°C ; however, 7 hours was tested and considered to be sufficient. Besides the sand bath as used in the present work, an aluminum block heater (29) as well as silicone oil bath (30) can be applied. In addition to hexane, benzene was also used to extract DCB (27, 31).

Some authors washed the hexane or benzene extracts of DCB with an aqueous bicarbonate solution (27, 30, 32). How-

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ever, no observable differences in the analysis results between washing and unwashing the hexane phases with the aqueous bicarbonate solution prior to applying onto anhydrous Na_2SO_4 columns, could be detected in the present work. Similar to those mentioned by other authors (29, 32, 33) that DCB was found in the perchlorination reaction blank when $SbCl_5$ from various companies were used, in this work the detectable amount of DCB was evident in the perchlorination reaction blank, when 100 μL $SbCl_5$ were added, and the final solution diluted to less than 500 μL. Moreover, some investigations revealed that certain samples of commercial $SbCl_5$ contained bromine as a contaminant (31, 33) this resulted in the formation of bromononachlorobiphenyl (BNCB) after the perchlorination of PCBs ; BNCB appeared next to DCB (31-33). In the present investigation, no observable peaks next to DCB could be found in any perchlorinated solutions. Perchlorination products of the insecticides, PCDFs and TCDD did not interfere with the peak of DCB.

Recheck of PCDFs and TCDD by using conc. H_2SO_4 for the clean-up of fat.

Since conc. H_2SO_4 is able to destroy the substances that are easily oxidizable it was used for the clean-up of fat. Clean-up of fat with conc. H_2SO_4, with in the detection limit of 0.5-1 ppb for PCDFs and TCDD, was chosen to recheck the presence of PCDFs and TCDD in human milk, in order to confirm that these compounds did not interfere with the analysis results of the insecticides and PCBs. Clean-up by conc. H_2SO_4, higher % recoveries of PCDFs and TCDD were found, but % recoveries of the insecticides were very low. It should be therefore noted that clean-up of fat by a partition method is still preferable in cases of a limited amount of samples, and the need of a single procedure for the analysis of all compounds (insecticides, PCBs, PCDFs and TCDD) ; although it gives lower recoveries of some compounds, it offers recoverable amounts of all compounds analyzed.

Organochlorine insecticides : PCBs, PCDFs and TCDD in Belgian human milk (1984).

The results from the analysis of Belgian human milk samples of 1984 were indicated in table I. Chromatograms of standard insecticide mixture : PCBs, PCDFs and TCDD, and examples of

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TABLE I

Organochlorine insecticides, PCBs, PCDFs, and TCDD (ppm) in human milk from Belgium (1984)

Compound	*Number of positive samples (N = 35)*		*Minimum value*	*Maximum value*	*Mean ± SD*
β-BHC	30 (86 %)	F	0	0.09 (0.11)	0.04 ± 0.02 (0.05)
		W	0	0.002 (0.002)	< 0.001 (0.001)
Lindane	5 (14 %)	F	0	0.04 (0.05)	< 0.01 (< 0.01)
		W	0	< 0.001 (< 0.001)	< 0.001 (< 0.001)
Heptachlor	21 (60 %)	F	0	0.06 (0.11)	0.02 ± 0.02 (0.04)
		W	0	0.001 (0.002)	< 0.001 (< 0.001)
Heptachlor epoxide	33 (94 %)	F	0	0.37 (0.51)	0.14 ± 0.12 (0.19)
		W	0	0.011 (0.015)	0.003 ± 0.003 (0.004)
Dieldrin	6 (17 %)	F	0	0.09 (0.12)	0.01 ± 0.02 (0.01)
		W	0	0.001 (0.001)	< 0.001 (< 0.001)
p.p'DDE	35 (100 %)	F	0.41 (0.50)	2.39 (2.91)	1.21 ± 0.54 (1.48)
		W	0.008 (0.010)	0.055 (0.067)	0.029 ± 0.012 (0.035)
p,p'-DDT	29 (83 %)	F	0	0.41 (0.45)	0.19 ± 0.13 (0.20)
		W	0	0.013 (0.014)	0.005 ± 0.004 (0.005)
Total BHC	30 (86 %)	F	0	0.11 (0.14)	0.04 ± 0.02 (0.05)
		W	0	0.002 (0.002)	0.001 ± 0.001 (0.001)
Heptachlor and its epoxide	33 (94 %)	F	0	0.43 (0.62)	0.16 ± 0.13 (0.24)
		W	0	0.011 (0.015)	0.004 ± 0.003 (0.007)
Total DDT [a]	35 (100 %)	F	0.41 (0.50)	2.76 (3.32)	1.40 ± 0.63 (1.69)
		W	0.008 (0.010)	0.067 (0.080)	0.033 ± 0.015 (0.040)
Total equivalent DDT [b]	35 (100 %)	F	0.46 (0.56)	3.03 (3.66)	1.54 ± 0.69 (1.86)
		W	0.009 (0.011)	0.076 (0.082)	0.037 ± 0.017 (0.044)
PCB [c]	35 (100 %)	F	0.41 (0.55)	1.78 (2.37)	0.93 ± 0.38 (1.24)
		W	0.007 (0.009)	0.064 (0.085)	0.024 ± 0.014 (0.032)
PCBs [d]	35 (100 %)	F	0.55 (0.73)	2.34 (3.12)	1.13 ± 0.45 (1.51)
		W	0.009 (0.012)	0.084 (0.112)	0.028 ± 0.018 (0.037)
PCDFs [e]	0		—	—	—
TCDD [f]	0		—	—	—

F fat basis, W whole milk basis.
a summation of p,p'-DDE and p,p'-DDT.
b total equivalent DDT = DDT + 1.115 DDE.
c calculated from pattern comparison (using total height of peaks appearing after p,p'-DDE).
d calculated from perchlorination.
e, f detection limit of 0.5-1 ppb whole milk (clean-up by conc. H_2SO_4).
Values in parentheses were corrected for % recovery.

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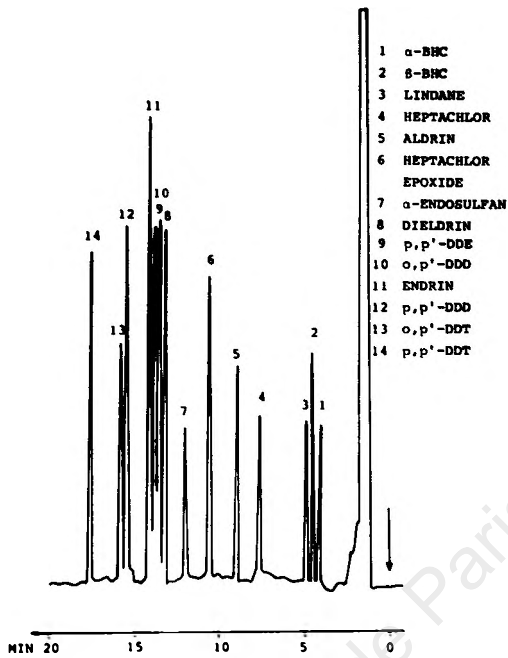

FIG. 2. — Chromatogram of the insecticide mixture (from a 25 m × 0.32 mm i.d., CP tm Sil 5 WCOT fused silica capillary column).

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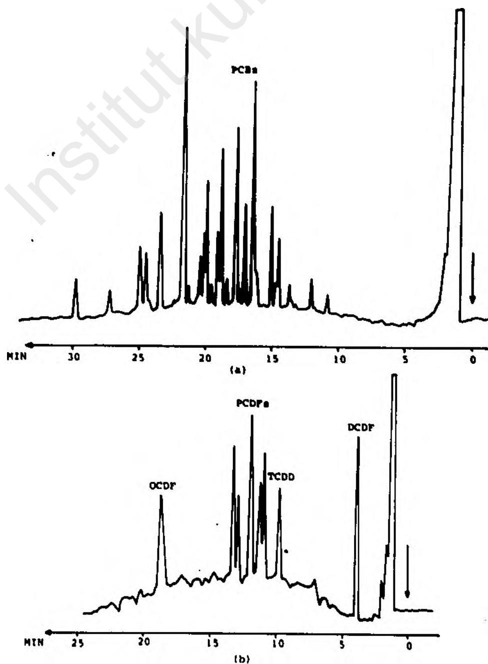
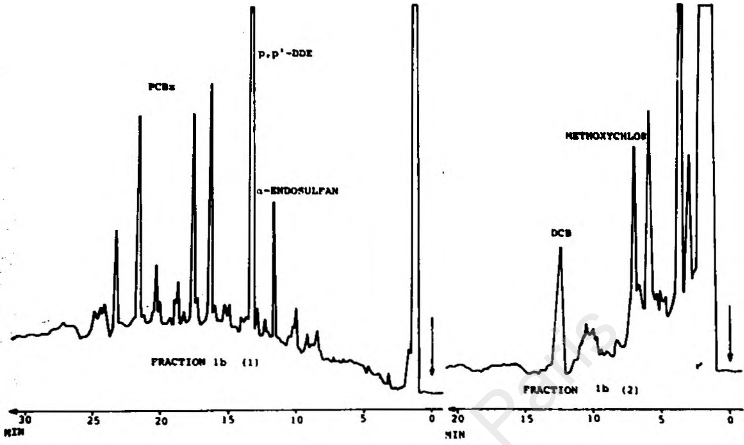


FIG. 3. — Chromatograms of standard PCBs (a), PCDFs and TCDD (b).



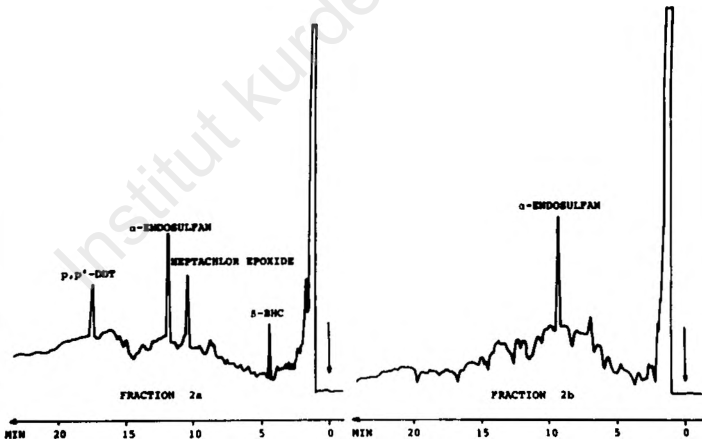


FIG. 4. — Chromatograms from a Belgian human milk sample.
(1) Pattern comparison — (2) Perchlorination (10-time dilution).

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chromatograms from a human milk sample are illustrated in figures 2, 3 and 4. Percent positive samples and mean levels of most insecticides were found lower in samples of 1984 compared to those of 1983 (34), although some differences were not significant (at $p = 0.05$). Aldrin which was found in very few samples of 1983 was not detected in samples of 1984, while dieldrin which is a metabolite of aldrin and was not detected in samples of 1983, was found in samples of 1984.

All these samples analyzed contained PCBs. Quantitation by perchlorination gave higher values than quantitation by pattern comparison ; this might be due to some impurities which might be present in the samples ; they could be perchlorinated in a similar manner to PCBs and gave the perchlorinated compounds interfering with DCB, or the interfering DCB came from the perchlorinating reagent. Although DCB from the reagent blank in the present work could not be detected after making a final dilution to more than 500 µL, it might give a synergistic concentration. On the other hand, an error could come from difficulties in quantitation by pattern comparison. None of these samples contained any measurable amounts of PCDFs or TCDD ; therefore these compounds would not interfere with the quantitation of the insecticides and PCBs. A similar study in human milk samples collected from 103 nursing mothers indicated non-detectable amounts of TCDD found even at the detection limit of 3 ppt (35).

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Chronic intoxication by man due to Thallium sulfate with recovery

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ABSTRACT.

The chronic intoxication due to the absorption of Thallium sulfate (raticide) in a solution of 1 % originated in a very sick condition of a patient. He was brought twice to a hospital. Every time after 2 weeks he was recovering, coming back home the intoxication symptoms started again. This 42 years old man was living with his girlfriend, 54 years old. The last month they were quarreling quite a lot. The physician could not diagnose any specific illness. The last time he was sent over to the University Hospitals for observation and control.

In those urine samples, by screening the general toxicological analyses, we found high amounts of Thallium. There was no doubt that he was intoxicated. The treatment for the Thallium intoxication using Prussian Blue was introduced with good results and recovery.

1. INTRODUCTION.

The gentleman having suffered many times, every time recovering after 2 to 3 weeks of hospital treatment with no specific pharmaceuticals, became worse and in a critical state after 4 months back at home. Finally at the University Hospitals, and by the toxicological screening, we found high amounts of Thallium in urine and blood and concluded to a chronic intoxication by Thallium over different periods of time. After 4 weeks of hospitalization he received at intervals the visit of his girlfriend. Once after those visits he became very ill again. His girlfriend was bringing him some food, in order to take good care of him, because she said the meals of the hospitals were not sufficient. Finally

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she was arrested. He did not believe that she could be the one who gave him the poison, because he was so in love with her, and she was taking so good care of him, that it was impossible. After a very thoroughly police investigation, finally she confessed that she was giving him Thallium sulfate 1 % solution, a table-spoon full at different intervals mixing it with green vegetable soup. His hair fell out and his general condition became very critical.

2. TOXICOLOGICAL ANALYSIS.

Using the different methods as described (1-23) applying the atomic absorption method, we found very high amounts of Thallium in the urine, serum and hair (table I). Also we could find

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TABLE I

Thallium content

Sample arrived on	*Urine*	*Serum/Blood*	*Hair*
11-05-84 17 h 35	1,6 mg/l	Blood : 19.2 µg %	—*
18-05-84 14 h 30	4.17 mg/l	Serum : 13.9 µg %	—
21-05-84 14 h 00	333 µg/l	Serum : 3 µg %	—
23-05 84 14 h 20	300 µg/l	Blood : 7 µg %	(strawberry : neg.)
26-05-84	491 µg/l	RBC : 2.02 µg % (zinc in RBC : 2.24 µg %)	—
27-05-84	176 µg/l	—	—
28 05-84 14 h 15	104 µg/l	Serum : 1.45 µg %	—
30-05 84 14 h 15	56 µg/l	Serum : 1.43 µg %	576 µg %
07-08-84 15 h 30	Negative	—	—

* Sample of 11-05-84 also arsenic determination : urine : 45 µg/l
hair : 42 µg %
blood : 2,3 µg %

in some periods, mainly May 26th and May 28th, much more Thallium in the urine after the visit of his girlfriend. Using 30 g of prussian blue, colloidal form (in ± 100 ml of water) a day, during 3 days, an intermission of 4 days, starting again for 3 days with prussian blue, 30 g a day, same period, we could eliminate the Thallium quite well, the patient recovered very fast.

This treatment was saving him from death with good prospect for the future. Years ago when we did not apply the prussian blue but different methods of detoxification, we were not able in such cases to save the patient ; he died.

This example shows again that the diagnosis in hospitals by many physicians is not so easy, and that one does not think about the possibilities of criminal intoxication. Only by screening and the toxicological analysis we could prove the poisoning. After 2 months the recovery was quite complete (tables I and II). His wife was condemned to life sentence of forced labour.

TABLE II

Thallium content

Sample arrived	*Serum*	*Urine*	*Faeces*	*Hair*
30-05-84	1.43 µg %	56 µg/l	—	576 µg %
06-06-84	Neg.	30 µg/l	—	Top : 92.8 µg % (1 = 2 cm)
				Middle : 156.2 µg % (1 = 2 cm)
				Basis : 213.6 µg % (1 = 2 cm)
				Total length : 6 cm
08-06-84	Neg.	24 µg/l	—	—
12-06-84	Neg.	12 µg/l	Neg.	—
18-06-84	Neg.	10.5 µg/l	Neg.	—
22-06-84	Neg.	8 µg/l	Neg.	Top : 22 µg % (1 = 2 cm)
				Middle : 38 µg % (1 = 2 cm)
				Basis : 48 µg % (1 = 1.5 cm)
				Total length : 5.5 cm

3. CONCLUSION.

The chronic intoxication and the treatment of the patient by complexing Thallium and eliminating the raticide is described with good results. From the history and the surroundings we are quite sure that many intoxications with fatal end are occurring and not appearing before court of justice, due to a lack of medical diagnosis. It is only by chance that those cases came on the surface and that prove was given of a murder attempt. Thallium and parathion are in Belgium still the two most important poisons that are used in criminal offenses. Other European countries have other ways of using poison to kill, e.g. in France still arsenic, etc.

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Toxicology of an acute Thallium intoxication by man ten years after death

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ABSTRACT.

Thallium has been used in many cases of criminal intoxication by man in the last years in Belgium. One of the last was a very important criminal case by which DJ, 53 years old was poisoned by his wife and died after 3 months. The police investigation came after 9 years and 2 months, just before the case would be dismissed (10 years for police investigation), after a long story of intoxicating boyfriends and many attempts to eliminate them. The Ministry of Justice investigated the case further and an exhumation was ordered. The cemetery was located high, sandy, with practically no water content even in winter. By the autopsy even after so many years the dead body was still well preserved, different organs were taken. There was also still urine in the bladder. We could also take the necessary hair, of course due to the intoxication not much hair was left on the skull or other parts of the body ; there was sufficient to run an analysis.

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1. INTRODUCTION.

The husband was already suffering many weeks and months about the so called polyarthritis, had mental disturbances and chronic intoxication symptoms with chronic constipation etc. He was hospitalized twice during 3 weeks, his general physical condition became better, he could go home again. After a few days, the symptoms reappeared, the general condition became worse, he suffered a lot, could not walk anymore or even

reach the first floor of his house, or lay down in his bed. During that time he was living on the main floor, sleeping on the sofa, his wife was taking care of him with lots of love.

During that period the general situation worsened again so that finally the family decided to bring him over for a fortnight to the hospital where an operation would take place on Monday. The Saturday the general condition improved, but in the evening everything worsened again, he could not get up the stairs anymore, and passed the night with lots of suffering on the sofa. During that time he got many wishes by his wife ; they all hoped that next Monday it would be better.

Unfortunately, the general condition was so bad that, the Sunday morning, he was urgently moved to the Hospital, to take care of him ; 4 hours later the condition worsened and during the night from Sunday on Monday he died. His wife was in a terrible condition, was crying a lot and was laying many flowers on his coffin on the funeral day. Nine years and 2 months later he was exhumed. Finally his wife confessed that she was preparing regularly green vegetable soup pouring a spoonful of Thallium sulfate of 1 %, a raticide solution that they had at home, to poison her husband.

So regularly he got his green vegetable soup, that he was warming up by himself, because he was working in night shift, his wife

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TABLE I

	Thallium	*Arsenic*
Stomach content	1.133 µg %	
Kidney	10.504 µg %	1.01 µg %
Muscle	1.597 µg %	
Liver	10.708 µg %	1.56 µg %
Brain	3.722 µg %	
Urine	62.5 µg %	
Hair skull (total)	2.771 µg % (1 = 10 cm)	
Hair skull (basis)	3.677 µg % (1 = 3 cm)	37.1 µg %
Hair skull (middle)	1.781 µg % (1 = 3 cm)	70.5 µg %
Hair skull (top)	2.343 µg % (1 = 4 cm)	95.6 µg %
Hair (pubis)	2.104 µg %	
Teeth	685 µg %	
Skull	1.042 µg %	
Nails (hand)	797 µg %	12.7 µg %
Nails (feet)	185 µg %	7.2 µg %
Ground (coffin)	25 µg %	
Wood (coffin)	84 µg %	
Water (coffin)	Negative	
Coffin (plastic inside).	63 µg %	
Ground (under coffin)	Negative	
Ground water around coffin	Negative	

was in day shift. During all that sick period she went to holy places to pray for his recovery, and brought home holy water.

2. TOXICOLOGICAL ANALYSIS.

We described already many times and years ago Thallium intoxication cases using different methods : polarographic, colorimetric and spectrographic analysis methods. The last years we improved those analytical techniques using atomic absorption where we analysed the different samples. Some biological ones as blood and urine, could be analysed directly by injecting them into the furnace (table I).

3. CONCLUSION.

The very high amounts of Thallium found, compared to environmental samples as mentioned in table I, conclude to a chronic Thallium intoxication with fatal end after 3 months of regularly absorption, with intervals. of the poison Thallium sulfate solution. The arsenic contents which were analysed according to the hydride system (1-23) were practically normal for the different organs, also in the hair. There is no doubt that the Thallium sulfate was the origin of the intoxication. All other poisons, analysed by the general toxicological analysis procedure, were negative, also the determination of alcohol.

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Toxicology and fatal intoxication due to dextromoramide (Palfium®)

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ABSTRACT.

A fatal intoxication due to the absorption of Dextromoramide describes a drug addict who was found dead alone, being known as using many formulations.

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TOXICOLOGICAL ANALYSIS.

Detection of Palfium® (Dextromoramide).

25 g gastric content, 25 g liver and 10 ml urine are acidified with 5 ml HCl 2 N and extracted with 2 × 100 ml ether. The ether layers were rejected.

The acid aqueous layers were alkalized to pH 8-9 with K_2CO_3, and extracted with 100 ml ether. The ether layers were furthermore alkalized with NaOH 10 N, and extracted again with 100 ml ether. The collected ether layers were washed with 2 × 5 ml NaOH 0.001 N, and dried over anhydrous Na_2SO_4.

They were extracted twice with 10 ml HCl 0.1 N. The acid layers were heated to eliminate the ether.

UV-spectra were recorded from 210-350 nm with a Unicam SP 800.

The UV-spectra were not specific and could not be identified.

The HCl layers were quantitatively brought into a separatory funnel, and made alkaline to pH 8-9 with K_2CO_3. The aqueous layers were extracted with 50 ml ether. The aqueous layers were alkalized with NaOH 10 N, and again extracted with 50 ml ether.

The ether layers were collected and dried over anhydrous Na_2SO_4, filtered and evaporated to dryness.

The residues are diluted in 0.1 ml ethanol. A 20 μl aliquot of the alkaline extracts, and the reference solutions of Tryptamine, β-phenylethylamine, Dextromoramide (Palfium®), Methaqualone, Mephenon and Heroin were spot.

The eluent was chloroform, aceton, ammonia (50/50/1).

After drying, the plates are visualized using a short wavelength UV lamp at 254 nm and sprayed with Dragendorff and Iodoplatinate reagent.

The R_F values of the reference solutions were :

Tryptamine	: spot with R_F : 0.56
β-phenylethylamine	: spot with R_F : 0.50
Dextromoramide (Palfium®)	: spot with R_F : 0.72
Methaqualone	: spot with R_F : 0.85
Mephenon	: spot with R_F : 0.61
Heroin	: spot with R_F : 0.33

By comparing the surface and the density of the spots, a semi-quantitative determination is obtained.

The extracts of the gastric content and the liver present a spot with R_F 0.72, which indicates the presence of Dextromoramide (Palfium®).

- — gastric content : 0.08 mg % Dextromoramide
- — liver : 0.04 mg % Dextromoramide
- — urine : 0.02 mg % Dextromoramide
- — blood : 0.01 mg % Dextromoramide

Detection of substances belonging to the groups of the Narcotics, the Analeptics and the Hallucinogens.

1. The urine was analyzed on the presence of substances with narcotic, hypnotic, analeptic and hallucinogenic action, with an Enzyme-Immuno-Assay Technique (EMIT) (1).

Results.

Substances	*Urine*
Opiates	negative
Cocaine	negative
Methaqualone	negative

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Amphetamine	negative
Methadone	negative
Cannabinoids (and metabolites)	negative

2. The alkaline extracts of the urine and liver, and the blood were analyzed on a gas chromatograph Perkin Elmer Sigma 2 with NPD detector.

Column	: 25 × 0.22 mm CP ^{tm} Sil 8 fused Silica
Carrier gas	: Helium (1 atm)
Oven temperature	: 200°C to 300°C

Under these conditions also a standard mixture was injected (fig. 1).

Component	Retention time (R_t) (min)	Concentration (ng/μl)
1. Pethidine HC1	1.85	5
2. Coffeine	2.29	5
3. Phencyclidine HC1	2.72	10
4. Metapyrilene HC1	3.38	5
5. Procaine	3.66	20
6 Normethadone	4.40	10
7. Methadone	4.86	10
8. Methaqualone	5.06	10
9 Cocaine	5.54	10
10. Codeine	7.81	10
11. Morphine	8.44	20
12. Hydromorphine	8.88	20
13. Acetylcodeine	9.59	10
14. 6-Mono-acetylmorphine	9.71	10
15. Nalorphine	10.36	20
16. Heroin	11.14	20
17. Diacetylnalorphine	13.25	20
18. Phenoperidine	14.12	20
19. Palfium	15.68	10
20. Strychnine	18.47	20

In the chromatograms of the extracts, peaks were found with the same retention time as this of the Palfium® standard (2, 3) (fig. 2, 3, 4 and 5).

Concentrations were :

gastric content	: 0.08 mg %
blood	: 0.01 mg %
urine	: 0.02 mg %
liver	: 0.04 mg %

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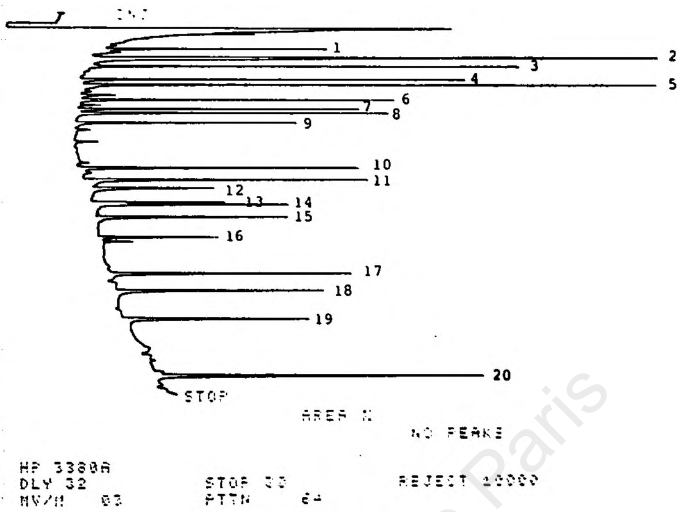

FIG. 1. — Standard mixture.

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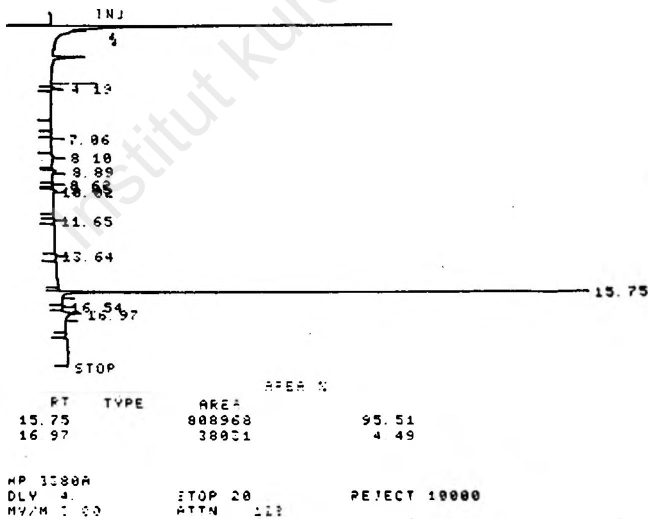

FIG. 2. — Standard Palfium 20 ng.

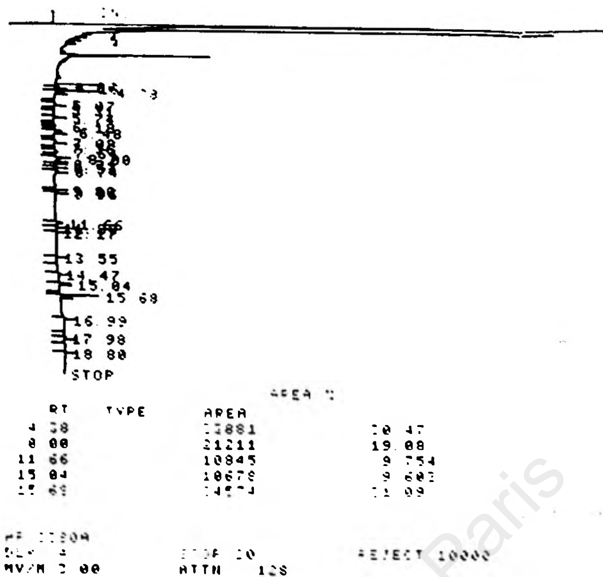

FIG. 3. — 10 ml blood 1/500. Result : 0.01 mg %.

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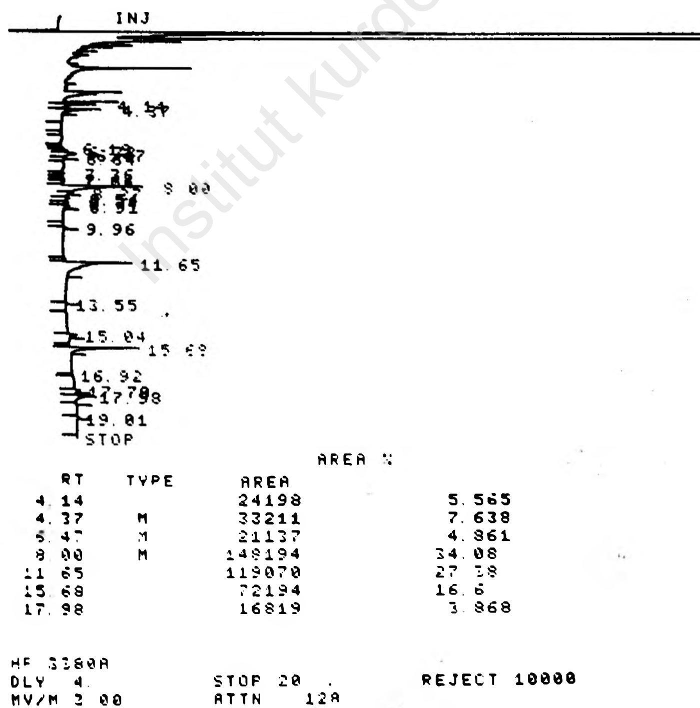

FIG. 4. — 5 ml urine 1/500. Result : 0.02 mg %.

CONCLUSION.

The concentrations found in the given biological samples conclude to a fatal intoxication of Palfium.

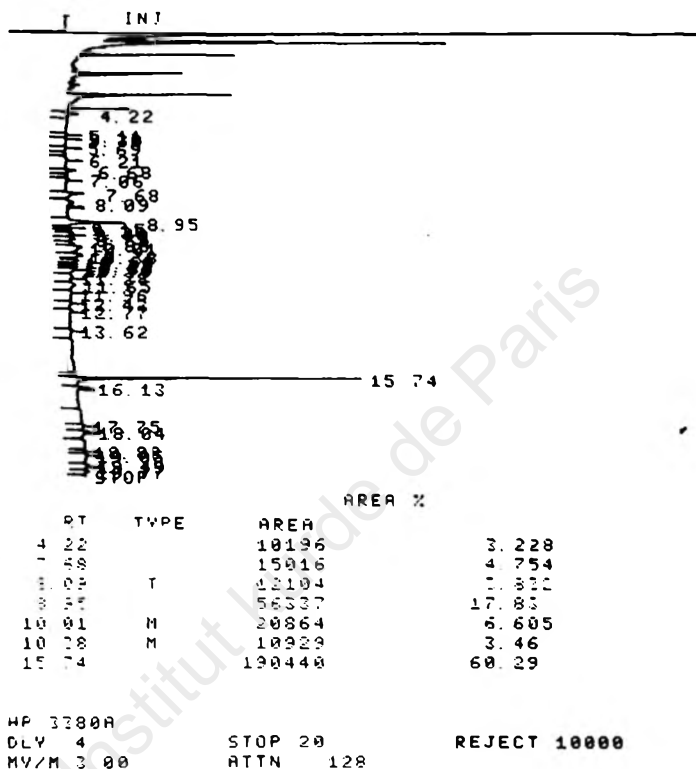

FIG. 5. — 6.25 g liver. Result : 0.04 mg %.

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Toxicology of Undeen. A fatal intoxication in man by carbamate pesticide

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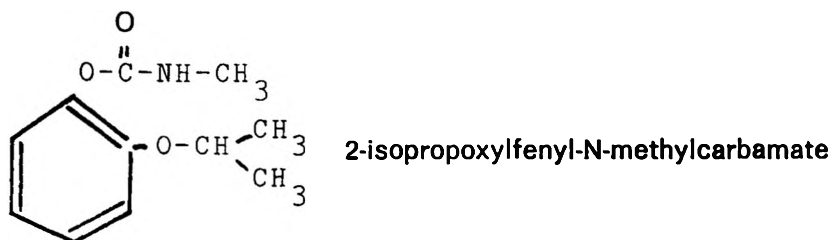
ABSTRACT.

More and more carbamate insecticides are used in agriculture today, in mixtures or alone, in order to control pests. Some of those carbamate pesticides are non cholinesterase inhibitors, other ones are very toxic by inhibiting the cholinesterase activity in plasma. The technique describes the isolation of undeen, its toxicological analysis and the different concentrations found in human organs after a fatal death.

INTRODUCTION.

A man, 40 years of age, was found dead after strange circumstances. The autopsy took place the same day and different organs were analysed in order to find out if there could be poisoning. The anatomic pathological investigation was completely negative for different organs taken after the autopsy. Therefore a further investigation was performed in parallel in order to find out what could be the cause of death. Stomach content, blood and urine were analysed and a general screening was performed.

PHYSICAL PROPERTIES.

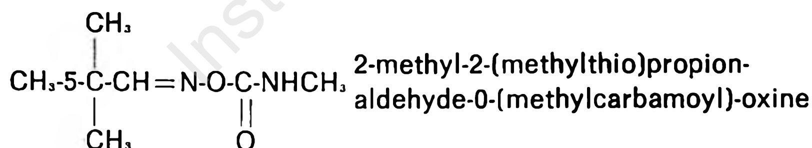


Undeen = propoxur, Bayon.

Molecular weight	: 209.24
Melting point	: 91.5°C
Solubility	: soluble in methanol and acetone and many organic solvents, but only slightly soluble in cold hydrocarbons
Stability	: unstable in alkaline media
Use	: insecticide
LD_{50} rat	: p.o. 100 mg/kg derm. 800-1000 mg/kg

Attention : decomposes at high temperature forming methylisocyanate.

Temik = Aldicarb.



Molecular weight	: 190.25
Melting point	: 100°C
Solubility	: soluble in acetone (35 %), benzene (15 %), xylene (5 %), methylene chloride (30 %)
LD_{50} rat	: p.o. 1 mg/kg derm. 5 mg/kg
Use	: insecticide, acaricide, mematocide

These two insecticidal carbamates are cholinesterase inhibitors, differing from the organophosphorous insecticides because the inhibition they produce is generally more rapidly reversible.

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Solubility	H_2O	C_2H_5OH	$CHCl_3$	$C_2H_5OC_2H_5$
Undeen	—	+ +	+ + +	++
Temik	—	+ +	+ + +	++

TOXICOLOGICAL ANALYSIS.

1. Detection of barbiturates and weak acids.

10 g stomach content, 10 g blood and 10 ml urine were acidified with H_2SO_4 10 % and extracted during 10 minutes with 100 ml of chloroform after intensive shaking (1). The chloroform layers obtained, were filtered, 80 ml of the filtrate was reextracted with 10 ml 0.45 N NaOH. The chloroform layers were retained to identify neutral drugs. The NaOH extract was acidified and re-extracted with 50 ml chloroform.

These 50 ml chloroform were filtered and evaporated to dryness. The residue was redissolved in 0.1 ml ethanol and spot with a known amount on thin layer plates. We used as reference 50 µg of standard solutions of phenobarbital, methylphenobarbital, secobarbital and brallobarbital.

The aliquot for the TLC was a mixture of 9 parts of chloroform and 1 part of acetone. After spraying with the mercury reagent and 5 mg diphenylcarbazone in 50 ml chloroform, we obtained the following standards with R_F-values mentioned :

— Phenobarbital : spot with R_F value 0.38
— Methylphenobarbital : spot with R_F value 0.64
— Secobarbital : spot with R_F value 0.47
— Brallobarbital : spot with R_F value 0.50

In the extracts of the different organs, no spots could be identified of barbiturates or analogues.

2. Identification of neutral drugs.

The $CHCl_3$ layers, separated after the isolation of the barbiturates are washed with water, filtered and evaporated. The residues are taken up again in 0.1 ml ethanol. Of that solution, 50 µl are spot on the TLC plate together with standards containing Meprobamate and Merinax. The TLC plate is furthermore put into an

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eluent solution of equal parts of acetone and chloroform. After evaporation with furfural in acetone and sulfuric acid in acetone, the following standard spots are obtained :

— Meprobamate : spot with R_F value 0.32
— Merinax : spot with R_F value 0.59

In the extracts of the different organs, that were isolated from the dead person, no spots were identified which could be related to meprobamate and analogous drugs.

The remaining parts of the extracts (50 μl) are also spot on the TLC plate, together with the standard solution carbromal. As eluent, we use an equal amount of acetone and chloroform. After the running time (± 15 minutes) the TLC plate is sprayed with a 5 % NaOH solution and heated during 20 minutes at 110°C.

Afterwards the plate is sprayed with the fluoresceine solution. After spraying with a mixture of equal parts of perhydrol and acetic acid, we could visualize the carbromal as a pink spot with R_F value : 0.67 on a yellowish background.

In the extracts of the different organs, no spots could be identified as Carbromal or analogues.

3. **Investigation on alkaline poisons.**

25 g stomach content, 25 g liver and 12 ml urine were acidified with 5 ml 2 N HCl and extracted twice with 200 ml ether. The acid water phases were alkalised to pH 9 with K_2CO_3, and to pH 14 with 10 N NaOH and extracted twice with 200 ml ether. The ether phases are washed with 5 ml fractions of alkaline water (0.001 N NaOH) and furthermore dried over Na_2SO_4. Afterwards the ether phases are twice extracted with 10 ml 4 N HCl. The acid phases are heated so that the ether is evaporated.

1. We were taking the UV spectra from 210 to 350 nm with the Unicam SP 1700. The UV spectra of the extracts of the different organs were not well defined.

2. The HCl phases were quantitatively transferred into a separatory funnel, alcalised to pH 9 with K_2CO_3, and to pH 14 with 10 N NaOH, and twice extracted with 20 ml ether.

The ether phases were dried over Na_2SO_4.

The residues after evaporation are redissolved in 0.1 ml alcohol of which known amounts were spot on the TLC plate together

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with 50 μg of the standards : coffeine, nicotine, tryptamine and β-phenylethylamine.

As eluent we used chloroform/acetone/ammonia : 50/50/1.

To identify, we were using subsequently ferri-, vanadium-, Dragendorff- and iodoplatinate reagent.

The following spots were identified :

— Coffeine : spot with R_F value 0.70
— Nicotine : spot with R_F value 0.67
— Tryptamine : spot with R_F value 0.56
— β-phenylethylamine : spot with R_F value 0.50

In the extracts of the autopsy material, no specific spots were identified which could be related to alkaline poisons.

4. **Investigation on Benzodiazepines which can be hydrolysed (Temesta®, Lexotan®, Mogadon®, Seresta®, Rohypnol®, Valium® and analogues).**

25 g liver, 25 g stomach content and 25 ml urine were alkalised to pH 8-9 with K_2CO_3 and extracted twice with 100 ml ether. The ether phases obtained as mentioned above, were washed with fractions of 5 ml 2 % K_2CO_3 solution and subsequently filtered over anhydrous Na_2SO_4.

The washed ether phases were further extracted twice with 10 ml HCl 4 N. The HCl phases were refluxed during 30 minutes on an oil bath at 125°C.

1. *UV-spectrophotometric investigation.*

After cooling the HCl phases, the UV spectra were registered from 210 to 350 nm with the Unicam SP 1700. The UV spectra of the extracts of different organs are ill defined.

2. *Thin layer chromatography* (TLC).

The HCl phases were quantitatively transferred into a separatory funnel, after which they were extracted with ether. After alkalinisation with 10 N NaOH and cooling under running water, the aqueous phases are again extracted with ether. The collected ether fractions are washed with portions of 5 ml 0.001 N NaOH solution, furthermore filtered over anhydrous Na_2SO_4 and evaporated.

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The residues, after evaporation, are again dissolved in 0.1 ml alcohol of which 50 μl are spot on the TLC plate, together with standard hydrolysis products of Oxazepam (Seresta®), Nitrazepam (Mogadon), Bromazepam (Lexotan®), Lorazepam (Temesta®), Flunitrazepam (Rohypnol®) and Diazepam (Valium®). As eluent we were using 5 % acetonitrile in toluene. After elution, the spots of Diazepam (Valium®) and Flunitrazepam (Rohypnol®) yellow in colour, with R_F-value 0.85 and 0.79 were appearing. In order to identify the other standard compounds, we spray the plate with Ehrlich reagent and dry under a warm airflow (60°C).

The spots of the standard compounds had the following R_F values :

- 10 μg hydrolysed product of Oxazepam (Seresta®) : spot with R_F value 0.60
- 10 μg hydrolysed product of Nitrazepam (Mogadon®) : spot with R_F value 0.42
- 10 μg hydrolysed product of Bromazepam (Lexotan®) : spot with R_F value 0.23
- 10 μg hydrolysed product of Lorazepam (Temesta®) : spot with R_F value 0.63

In the extracts of the autopsy material, no compounds could be identified as benzodiazepine derivatives.

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5. Determination of ethylalcohol.

By gaschromatography, we analysed the different autopsy materials on alcohol :

- blood : negative
- liver : negative
- urine : negative

6. Screening on substances belonging to the group of illicit drugs, weckamines and hallucinogens.

In the urine of the post-mortem sample, substances were analysed using the enzyme-immuno-assay technique (EMIT). The following results were obtained :

Substances	*Urine*
Morphine, Codeine	negative
Cocaine	negative
Methaqualone	negative
Amfetamine	negative
Methadone	negative
Cannabinoids (metabolites)	negative

7. **Screening on carbamate pesticides.**

10 g stomach content, 10 g liver and 10 ml urine were extracted with 100 ml ether, after shaking during 30 minutes mechanically. The ether phases were washed with 10 ml 0.001 N NaOH, dried over Na_2SO_4 and evaporated. The residue was redissolved and spot on a thin layer plate.

1. *Inhibition plate.*

Half of the residue of the extract is spot on the TLC plate, together with standards of Temik, Undeen, Mesurol and Carbaryl. As eluent solvent we used a mixture of acetone/n-hexane/benzene (15/35/90) (2, 3, 4, 5, 6, 7).

After elution the plate is brought in an atmosphere of bromine, and after evaporation sprayed with fresh diluted horse plasma.

After 30 minutes the plate is sprayed with a mixture of naftylacetate in alcohol and Fast Blue as reagent. We obtained white spots on a bluish background.

The spots of the standard solutions had the following R_F values :

— Undeen : spot with R_F value 0.36
— Temik : spot with R_F value 0.13
— Mesurol : spot with R_F value 0.49
— Carbaryl : spot with R_F value 0.32

In the extracts of the autopsy material we notice spots which are identified on the R_F value with Undeen.

2. *Carbamates.*

The other half of the extracts are also spot with the same standards on the TLC plate. After elution in the same eluent, we spray with 1 N KOH in methanol, followed by 0.1 % p-Nitrobenzene-diazoniumfluoroborate in ethanol.

The spots of the standards have the following R_F values :

— Undeen : spot with R_F value 0.36
— Temik : spot with R_F value 0.13
— Mesurol : spot with R_F value 0.49
— Carbaryl : spot with R_F value 0.32

In the extracts of the autopsy material, we noticed an R_F-value 0.36, which corresponds with Undeen.

After comparison semi quantitatively with the standards, we found the following results :

— stomach content : 12 mg % Undeen
— liver : 0.12 mg % Undeen
— urine : 0.18 mg % Undeen

CONCLUSION.

In the autopsy material, we found Undeen (stomach content : 12 mg % ; liver : 0.12 mg % ; urine : 0.18 mg %).

Undeen is a cholinesterase inhibitor of the Carbamate pesticide group. Those concentrations are fatal.

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Toxicology and fatal intoxication by man due to parathion

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ABSTRACT.

A young man of 23 years old was found dead alone in a rural area.

The autopsy and pathology were totally negative. No anatomic pathological source could lead to any intoxication.

He was seen one day before in good health.

No external signs could be found, excluding any traumatology.

The magistrate ordered a toxicological investigation.

TOXICOLOGICAL ANALYSIS.

A. Detection of barbiturates and weak acids.

10 g stomach content, 30 ml blood and 10 ml urine were acidified with H_2SO_4 10 % and shaken during ten minutes.

The $CHCl_3$ layer was filtered. The filtrates were extracted separately with 10 ml 0.45 N NaOH.

The $CHCl_3$ layers were kept for the detection of neutral drugs, the NaOH layers were acidified and extracted with 50 ml $CHCl_3$.

After filtration, the $CHCl_3$ layers were evaporated to dryness. The residues were dissolved in 0.1 ml ethylalcohol. An aliquot of 50 μg of the reference solutions of Phenobarbital, Methylphenobarbital, Secobarbital and Brallobarbital, and a known aliquot of the sample extracts were spot.

The TLC plate was developed in a tank containing 100 ml of a 9:1 mixture of chloroform and acetone.

After elution, the TLC plate was sprayed with mercury reagent and diphenylcarbazone in $CHCl_3$ reagent.

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The R_F values of the reference solutions were :

— Phenobarbital : spot with R_F 0.38.
— Methylphenobarbital : spot with R_F 0.64.
— Secobarbital : spot with R_F 0.50.
— Brallobarbital : spot with R_F 0.47.

In the extracts of the autopsy material no spot appeared, proving the absence of barbiturates or weak acids.

B. **Detection of neutral drugs** (1).

The $CHCl_3$ layers, separated during the detection of the barbiturates, were washed with H_2O, filtered and evaporated to dryness. The residues were dissolved in 0.1 ml ethylalcohol. An aliquot of 50 μl of the sample extracts and the reference solutions Meprobamate and Merinax were applied on the TLC plate.

The TLC plate was developed in an eluent of equal parts of acetone and chloroform. After elution, the plate was sprayed with furfural in acetone and sulfuric acid in acetone.

The R_F values of the reference solutions were :

— Meprobamate : spot with R_F 0.32.
— Merinax : spot with R_F 0.59.

In the extracts of the autopsy material no spot appeared, proving the absence of Meprobamate or analogues.

The other 50 μl aliquot were also spot on a TLC plate, together with the reference solution of Carbromal. Equal parts of acetone and chloroform were used as eluent.

After elution, the TLC plate was sprayed with 5 % NaOH, and heated during 20 minutes at 110°C. The warm plate was then sprayed with a fluoresceine solution.

The visualisation of the pink spot (R_F : 0.67) of the Carbromal reference, was established by spraying with equal parts of perhydrol and acetic acid.

In the extracts of the autopsy material no spot appeared, proving the absence of Carbromal.

C. **Detection of alkaline and amfolytic drugs** (1).

25 ml urine, 25 g stomach content and 25 g kidney were acidified with 5 ml 2 N HCl and extracted with 2 × 100 ml ether.

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The ether layers were removed. The acid aqueous layers were alkalized to pH 8-9 with solid K_2CO_3, and extracted with 100 ml ether. The ether layers were collected and washed with portions of a diluted K_2CO_3 solution, and dried over anhydric Na_2SO_4.
The ether layers were extracted with 2 × 10 ml 0.1 N HCl.

The acid aqueous layers were heated to eliminate the ether fraction.

1. A UV-spectrum was recorded with a Unicam SP 1700 spectrophotometer. The UV-spectra of the extracts of the autopsy material were negative.

2. The HCl layers were quantitatively brought over in a separatory funnel, made alkaline to pH 9 with solid K_2CO_3, and extracted twice with 30 ml ether.

The aqueous layers were alkalized with 10 N NaOH and extracted with 30 ml ether. The ether layers were collected, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residues were dissolved in 0.1 ml ethylalcohol. A 50 μl aliquot of the alcoholic extracts, and of the reference solutions of Tryptamine, β-phenylethylamine, Nicotine and Methaqualone were spot.
The eluent was chloroform/acetone/ammonia : 50/50/1.
The visualization was carried out by spraying with : 1. Ferri-, 2. Vanadium-, 3. Dragendorff- and 4. Iodoplatinate reagent.

The R_F values of the reference solutions were :

— Tryptamine : spot with R_F 0.23.
— β-phenylethylamine : spot with R_F 0.47.
— Nicotine : spot with R_F 0.44.
— Methaqualone : spot with R_F 0.71.

In the extracts of the autopsy material no spot appeared, proving the absence of alkaline or amfolytic drugs.

D. Detection of benzodiazepines (Lexotan®, Mogadon®, Seresta®, Rohypnol®, Valium® and related compounds).

25 ml urine, 25 g stomach content and 25 g kidney were alkalized to pH 8-9 with solid K_2CO_3 and extracted in a separatory funnel with 2 × 100 ml peroxide free ether. The ether layers were collected and washed with 5 ml portions of a saturated K_2CO_3 solution, and filtered over anhydrous Na_2SO_4.

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The ether phase was then extracted with 2 × 10 ml of 4 N HCl ; the assembled acid solutions were heated during 30 minutes under reflux, using an oil bath at 125°C.

1. *UV-spectrophotometry.*

After cooling the solution, the UV-spectrum was recorded with a Unicam SP 1700 spectrophotometer in the wavelength range 210-350 nm.

The UV-spectra of the extracts of the autopsy material were negative.

2. *Thin-layer chromatography* (TLC).

The acid solutions were quantitatively brought over in a 100 ml separatory funnel, made strongly alkaline with 10 ml of 10 N NaOH and extracted with 2 × 50 ml ether. The ether layers were washed with 5 ml portions of 0.001 N NaOH, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residues were dissolved in 0.1 ml ethylalcohol. A 25 µl aliquot of the alcoholic extracts and a 50 µl aliquot of the reference hydrolysed products of Oxazepam (Seresta®), Nitrazepam (Mogadon®), Broomazepam (Lexotan®), Flunitrazepam (Rohypnol®) and Diazepam (Valium®) were spot.

Five percent Acetonitrile in toluene was used as eluent.

After elution, the reference hydrolysed products of Diazepam (Valium®) and Flunitrazepam (Rohypnol®) are seen as yellow spots with respectively : R_F 0.85 and 0.79.

Visualization of the other standards was carried out by spraying with Ehrlich reagent, and drying in a warm air stream.

The R_F values of those reference hydrolysed products are :

— 10 µg hydrolysed product of Oxazepam (Seresta®) : spot with R_F 0.76 (orange-yellow).

— 10 µg hydrolysed product of Nitrazepam (Mogadon®) : spot with R_F 0.65 (red).

— 10 µg hydrolysed product of Broomazepam (Lexotan®) : spot with R_F 0.50 (orange-brown).

In the extracts of the autopsy material no spot appeared, proving the absence of Seresta®, Mogadon®, Lexotan®, Valium®, Rohypnol® or analogues.

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E. **Detection of substances belonging to the group of the narcotics, analeptics and hallucinogens.**

The urine was analyzed on the presence of substances with narcotic, hypnotic, analeptic or hallucinogenic action, with Enzyme-Immuno-Assay Techniques (EMIT) (2).

Results.

Substances	*Result*
Morphine, codeine	negative
Cocaine	negative
Methaqualone	negative
Amphetamine	negative
Methadone	negative
Cannabinoids (metabolites)	negative

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F. **Determination of ethylalcohol** (3).

The ethylalcohol content in the following autopsy material was determined by gas chromatography.

Results

Blood : negative
Liver : negative
Urine : negative

G. **Determination of carbon monoxide in blood** (4).

1. The presence of carbon monoxide was determined by spectrophotometry.
The result was negative.

2. The carbon monoxide content was determined by gas chromatography, using an Aerograph A 90 - P 3 gas chromatograph.
The content was 0.1 % carbon monoxide.

H. **Determination of cholinesterase inhibitor pesticides** (5).

20 g stomach content, 20 g liver and 20 g kidney were acidified with tartaric acid, and extracted twice with 100 ml and 75 ml petroleumether.
The collected petroleumether layers were evaporated to 10 ml.

1. *Diazotation reaction according to Averell and Norris* (6).

2.5 ml of each solution were evaporated. The diazotation reaction according to Averell and Norris (colorimetric procedure) was performed on the residues of the samples.

In the presence of Parathion a red-violet colour appears.

In comparison with the reference solutions of Parathion, the following results were found.

Results

Extract of stomach content	: positive
Extract of liver	: positive
Extract of kidney	: positive

2. *Thin layer chromatography* (7).

2.5 ml of each solution were evaporated. The residues were dissolved in 0.1 ml ethylalcohol. A 50 μl aliquot of the sample extracts, and of the reference solution of Parathion, Methylparathion and Dimethoate were spot.

The eluent was a mixture of hexane-acetone (4:2). After elution, the plate was sprayed with a solution of 0.5 % palladium chloride in 10 % HCl.

The R_F values of the reference solutions were :

— Parathion	: spot with R_F 0.68 (brown)
— Methylparathion	: spot with R_F 0.54 (brown)
— Dimethoate	: spot with R_F 0.32 (yellow)

In the extracts of the liver and the kidney no spot appeared, proving the absence of Parathion (presence of very low concentrations, lower than the detection limit : 10 μg).

3. *Quantitative determination of Parathion by biochemical titration* (8-30).

0.1 ml of the stomach content extract and 5 ml of the liver and kidney extract were evaporated. The residues were incubated with 1 ml of fresh horse plasma during 1 hour (37°C).

An inhibition of the acetylcholinesterase enzyme, present in the horse plasma, occurs in presence of organophosphorous compounds.

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10 ml of 0.01 N acetylcholine (substrate) and 2 drops of cresol red are brought in an Erlenmeyer flask in which 0.5 ml of the plasma, which reacted with the extract, is present.

The amount of acetic acid, expressed during 1 hour of the substrate, represents the enzyme activity, which depends on the amount of cholinesterase inhibition present in the original sample extract.

In comparison with a blank and reference solutions of parathion, we found the following results :

Sample	*% cholinesterase inhibition*	*mg % parathion*
— blank	0 %	—
— 10 μg reference solution	10.0 %	—
— 20 μg reference solution	15.5 %	—
— 50 μg reference solution	20.8 %	—
— 100 μg reference solution	28.2 %	—
— stomach content extract	27.7 %	40.4 mg %
— liver extract	15.8 %	0.24 mg %
— kidney extract	15.2 %	0.21 mg %

I. **Detection of p-nitrofenol (metabolisation product of Parathion and Methylparathion)** (8-30).

Five ml distilled water and 2 ml HCl were added to 5 ml urine and hydrolysed during 2 hours (t° : 100°C).

After hydrolysation the sample was extracted with a mixture of benzene and diethylether (80:20).

The extract was dried over anhydrous Na_2SO_4 and evaporated to dryness.

The residue was dissolved in 0.1 ml of a mixture of benzene and diethylether (80:20). An aliquot of 50 μl of the sample extract and the reference solutions of para-nitrofenol were spot. The eluent was a mixture of petroleumether and acetone (80:20). After elution, the plate was sprayed with 5 % KOH in methanol. In the presence of para-nitrofenol, a yellow spot appears (R_F 0.10).

In the extract of the urine a spot appeared, proving the presence of p-nitrofenol.

Result.

Extract of the urine : 0.6 mg % para-nitrofenol.

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CONCLUSION.

The high amounts of Parathion, found in the stomach content, the liver and the kidney, conclude to an acute fatal poisoning due to Parathion.

The time of survival will be around 10 to 20 minutes after ingestion.

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Hypervitaminosis A, as cause of death by a psoriasis patient

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SUMMARY.

A too intensive treatment of a psoriasis patient caused death about seven years after the beginning of the treatment. The reason was a hypervitaminosis A.

The hypervitaminosis A was confirmed by the clinical symptoms of the patient before death and by the autopsy.

The psoriasis patient took an amount of 100,000 IU vitamin A twice a day during about 6 years, sometimes in combination with vitamin E. During a few periods, the vitamin A treatment was replaced by an Arsenic. The role of Arsenic cannot be evaluated.

Vitamin A was determined in the blood, post-mortem by RP HPLC. It was detected as retinol by UV absorption (330 nm).

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CASE HISTORY.

A few years ago, a psoriasis patient died because of a too intensive treatment of his illness. Local preparations (Dithranol-Dermovate) were used in combination with vitamin A and Arsenic.

First, multi-vitamin preparations were prescribed during two months. The patient took two of these capsules daily, each containing 100,000 IU vitamin A. It must be mentioned that these capsules also contain 50,000 IU vitamin E. During the following three months, an Arsenic preparation with unknown composition was used. After a treatment of four months with the same multivitamin capsules in the same dosage, an interruption of four months occurred.

This interruption was followed by a repetition of the Arsenic treatment during four months. Two months later a vitamin treatment was prescribed for a period of three years and ten months with an interruption of four months after the first ten months. In this period the vitamin treatment was composed of a singular dose of 100,000 IU of vitamin A, twice a day.

After this period of a too intensive administration of vitamin A, the patient showed a chronical liver damage concerning an important portal hypertension and splenomegalia. An anatomo-pathological inquiry confirmed this.

Half a year later, peripherical oedema with distinct ascites was noticed. This confirmed the diagnosis of hepatitis with a secondary splenomegalia and hypersplenism.

Later on, the patient showed a decline of the liver functions with a persisting anaemia.

A few months later, minor bleedings occurred frequently.

About seven years after the beginning of the treatment, the patient died because of a cerebrovascular accident, caused by an intracerebral bleeding.

PSORIASIS (4-8).

Psoriasis is a chronical hyperkeratotic epidermal disorder with unknown aetiology. It concerns typical red bordered patches, often symmetrically spread over the body. The way of spreading shows a strong variation between patients and changes from time to time, so that the illness can be devided into active and subactive periods. Psoriasis doesn't attack the internal organs.

A kind of arthritis, very similar to chronical rheumatism, can develop.

Besides, psoriasis causes psychological problems, because the illness leads to severe social difficulties.

Psoriasis is often treated by an external treatment (Cignoline, Dithranol, tar preparations and sometimes corticosteroids).

In severe cases, a combination of local treatment together with a UV photo-therapy (PUVA) can be used. Methotrexate is rarely employed because of possible liver damage.

In the past, Arsenic was often used as treatment for psoriasis. But because of its toxicity and teratogenicity, it is no longer prescribed.

Also vitamin A was often recommended as a treatment for psoriasis. This similarity between the cutaneous effects of a vitamin A

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deficiency and certain dermatoses, involving hyperkeratosis and hyperplasia of the epidermis, indicated a potential role for the retinoids in treating these conditions.

However, an intermittent application is necessary. Babb and Kieraldo (1) noticed the development of cirrhosis and portal hypertension after a long administration of vitamin A.

Consequently the severe side effects associated with the administration of naturally occurring retinoids, limit the usefulness of these compounds.

By manipulating the vitamin A molecule, analogues were developed that improved the efficacy while minimizing the side effects of these drugs. These new retinoids (mainly etretinate) introduced a significantly broader use.

Nevertheless, the side effects, seen in patients taking etretinate, are closely related to the syndrome of hypervitaminosis A, and include changes in the skin and mucous membranes, as well as systemic effects involving the liver, central nervous and skeletal systems and lipid metabolism.

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INVESTIGATION.

The autopsy confirmed the clinical diagnosis. The structure of the liver made clear the portal hypertension.

Microscopical investigation of the liver showed distinct fibrosis. These symptoms are described in the case of a chronical Arsenic and/or vitamin A intoxication. When examining the post-mortem tissues a low vitamin A blood level was found.

The following concentration of vitamin A, under the form of retinol, was found in the blood post-mortem : 5.1 µg/dl (normal reference value for serum : 20-80 µg/dl).

This can however be explained by the following facts :

— This inquiry took place one year after the end of the vitamin A treatment.

— In consequence of the severe liver damage, a decrease of the retinol binding protein (RBP) could be caused by a reduced production of binding proteins in the liver.

— In the case of a hypervitaminosis A, most of the serum vitamin A is present under the form of retinyl esters. On the other hand, in normal physiological circumstances vitamin A occurs as free retinol. Therefore the concentration of

free retinol in the plasma can be normal in a state of hypervitaminosis.

The daily need of vitamin A stands at 5,000 IU.

A daily intake of 15,000 IU vitamin A is beneath the toxic level.

The toxic levels are given in table I.

TABLE I

Toxic levels for a daily vitamin A intake

Concentration	*Time*
15.000 IU	10 years
41.000 IU	8 years
90.000 IU	4 years
250.000 IU	2 months

When vitamin A is administered in combination with vitamin E, a synergetic effect can be observed. Vitamin E has a restraining influence on the catabolism of vitamin A.

It has to be mentioned that this psoriasis patient took an amount of 100,000 IU vitamin A twice a day during about 6 years.

Also a chronical Arsenic intake can cause a liver damage. The role of Arsenic cannot be evaluated because of the unknown posology.

A determination of Arsenic is done on the following post-mortem tissues : nails, hair, urine, content of the small intestines and liver.

The results, obtained by atomic absorption after destruction, are given in table II.

TABLE II

Concentration of Arsenic in post-mortem tissues

Post-mortem tissue	*Concentration*	*Normal reference value*
Hair	67 ng/g	650 ng/g
Urine	1.5 ng/ml	< 100 ng/ml
Content of the small intestines	1.8 ng/g	—
Liver	4.8 ng/g	5.7 ng/g
Nails	49.8 ng/g	36.2 ng/g

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DETERMINATION OF VITAMIN A BY HPLC (2-3).

The determined vitamin A content doesn't include provitamin A carotenoids. It is given in µg of retinol (vitamin A alcohol) per dl of blood.

One µg of retinol corresponds to 3.33 IU.

Immediate and fast analysis is important.

Because vitamin A is sensitive to UV radiation and oxygen, the work has to be done under absence of UV light. When heating under low pressure, the temperature has to be limited up to 40°C, and the air above the solution has to be replaced by nitrogen.

The following method can be used for the quantitative determination of vitamin A in post-mortem tissues.

The retinol, bound to α1-globulines (RBP) in blood has to be released before extraction. This takes place by a saponification reaction. Ethanol (at least three times the used volume), 50 % potassium hydroxide solution, approximately 100 mg of hydroquinone and 2 ml of sodium sulphide solution (12 %) are added. The reaction mixture is kept boiling under reflux on the water bath at 80°C for 30 minutes, while stirring continuously.

After saponification, the condenser is rinsed with about 20 ml of water and the content of the flask is cooled in running water.

Saponification carried out overnight at room temperature causes losses because the released retinol is exposed to the action of alkali for too long.

The extraction is carried out several times with diethylether or alternatively with a 1:1 mixture of petroleumether and diethylether.

In order to remove alkali and to avoid emulsification, the extract is carefully washed several times, until the washing water remains colourless when phenolphtalein is added.

The extract is dried with anhydrous sodium sulphate and evaporated almost to dryness under vacuum at a maximum temperature of 40°C.

If the concentrate still contains water, a few milliliters of ethanol are added and the water is distilled off azeotropically. Any residual solvent is blown off with nitrogen. The residue is dissolved in methanol.

Because of unavoidable losses during washing, evaporation, etc. a treated sample undergoes the same treatment as the unknown one.

The calculated recovery (about 94 %) is used to correct the content for the unknown sample.

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Separation of the retinol content is done by reversed phase HPLC without separation of isomers.

The chromatographic conditions are the following :

— Stationary phase : C_{18} RP column packing.
— Mobile phase : methanol/water (85/15) or methanol 100 %.
— Loop volume : 50 µl.
— Flow rate : 1 ml/min.
— Detection : 325-330 nm.

The following method is specific for the determination of retinol in serum. Two hundred µl of serum is used. Retinylpropionate, dissolved in methanol, is added as an internal standard. The sample is extracted twice : the first time with 0.6 ml water and 1.0 ml $CHCl_3$, the second time with 1.0 ml water and 1.0 ml $CHCl_3$.
The organic phase is concentrated almost to dryness under vacuum. The extract is redissolved in 100 µl $CH_3OH/CHCl_3$ (4/1). Fifty µl of this solvent is separated by HPLC under the same conditions as mentioned before.
Retinylesters can be determined in exactly the same way.

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Terrorism, Analysis and Detection of Explosives

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Aluminium in organs from person exposed for an aluminium-bomb

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ABSTRACT.

A tragical case of death of a 27 years old man who, 20 days before his death was 85 % burnt by an explosion of an aluminium-containing terror-bomb, is described. The determination of aluminium was performed after digestion of the tissue by graphite furnace atomic absorption spectrometry (GFAAS).

The possibility of errors including interferences and contamination is mentioned. It is concluded that the obtained values for aluminium in the deceased are inside the range of normal values.

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INTRODUCTION.

Aluminium is a constituent of rat-poison (aluminiumphosphide), adstringentia (alum) and antacida (aluminiumhydroxyd). Death by misuse of the rat-poison (1) and of the adstringentia (2) is described. Industrial manufacturing of aluminium is known to cause fatal pulmonaria fibroses (3, 4) and progressive encephalopathy (3). Dementia and death among dialyzed renal patients was seen in Chicago in the period 1972-1976 where the water unfortunately was filtered through aluminium sulphate (5). The values of the aluminium in serum from renal patients are increased by using aluminium containing antacida (6). Though analogous results are found in plasma from healthy persons (7).

Aluminium as a metal is an ingredient of bombs used for terror-activity.

Determination of aluminium in human tissue has by this way been sadly actual.

In the following we describe a method for determination of aluminium in organs from a person exposed for explosion of an aluminium-bomb focusing at errors such as interferences and contamination using graphite furnace atomic absorption spectrometry (GFAAS).

CASE HISTORY.

The 22nd of juli 1985 a bomb exploded outside the North West Orient Airlinies office in Copenhagen. A 27 years old male teacher from Algeria passed the place simultaneously on bicycle and was seriously burnt. The act was supposed to be a part of a larger terror-activity as another bomb was found a little later in the channel by Nyhavn, Copenhagen.

Admitted to a hospital-department for burnt persons, it was ascertained that 85 % of his body was burnt. Only regions corresponding to his pants, both upper arms and a strip on his back were intact.

At once large amount of fouling was removed from his face and pieces of metal from his left thigh.

By tracheoscopi was found an intense red area with pronounced veins near the beginning of the bronchi.

Transfusion was given and on the 2nd and 4th day after the attack skingrafting was instituted. In spite of treatment with antibiotics there was on the 2nd of August demonstrated enterobacter cloacae. The treatment was continued with kolistin (Colimycin®), but sporadical problems with breathing appeared and the blood sugar was elevated. The 10th of August was the situation so critical that the patient was transfered to an intensively therapy department. Beginning renal insufficient (tubulo-interstitial nefropathy) and on the 11th the death occurred under clinical picture of septicemia and cardiac insufficiency. By autopsy was found marks of the above described seriously burning of the body and inflammation of the underlope of the right lung, collapsing of both underlops of the lungs and so called shock kidneys beside a heavy stasis in the lungs.

The investigation of the explosive experts pointed to a swedish bomb from Kiruna.

In secretion collected the 31st of juli was shown 11 μg aluminium.

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MATERIAL AND METHODS.

Material.

At legal autopsy were samples of organs from the deceased removed — unfortunately with a metal knife —. The samples were placed in containers of polyethylene and kept at —20°C until time of analysis.

Digestion.

5.0 g wet weight of the thawed tissue were sliced with a knife of plastic and transfered to a 100 ml kjeldalflask of quartz. All the utensils such as the quartzflask were carefully washed with a 5 % solution of acidum nitricum, prepared from a 65 % suprapure Merck followed by redistillated water.

To the tissue was added 10 ml 65 % acidum nitricum suprapure and 3 quartz pellets of diameter 3 mm. This mixture was digested by slowly rise of temperature on an electrical plate until boiling. Intermittently the solution was cooled and boiled until a few millilitres remained. The process was repeated twice with 10 ml redestillated water.

The evaporation residue was transfered to a 25 ml measuring flask of plast and diluted to 25.0 ml with redistillated water. After centrifuging 1.0 ml of the clear supernatant was diluted with 9.0 ml 0.2 % acidum nitricum in tubes of polyethylene.

Measurement.

25 µl of diluted solution was by use of a Socores pipet [supplied by use of a plast pincet avoiding contamination from sweat from hands (9), with a 2" yellow untreated tip No. 0000-00001 B] injected in a pyrolytically coated graphite tube P.E 109-322 placed in a graphite furnace connected to a Perkin-Elmer atomic absorption spectrometer model 272 equipped with an aluminium-hollow cathode lamp. We used a wavelength at 309.3 nm, a slit of 0.7 mm and a current at nearly 20 mA. The variations in temperature are given in table I.

All samples were determined in duplicate using background correction.

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TABLE I

HCA 500-program (8)

Step	1	2	3	4	5	6
Temp.	110	140	500	20	1400	2600
Ramp (s)	10	65	1	1	1	0
Hold (s)	5	10	5	10	30	8
Ar-Int. flow ml/min.	300	300	0	300	300	10
Int. Alt. ml/min	—	—	AAN*			

* Oxygen 300 ml/min. was used as an alternative internal purge gas (AAN) for optimal oxidation of the biological matrix.

As standard was used a solution of 333.2 mg aluminiumsulphate diluted with 0.2 % acidum nitricum to form a solution of 0.0135 μg aluminium per ml in a plast flask.

For recovery and test of the normal values of aluminium was used tissue from a 60 years old man violently killed.

As reagent blank was used 5.0 ml redistillated water running through the whole process.

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ERRORS IN THE METHOD.

The most seriously source of errors was without doubt interferences and contamination.

Interference.

Matrix interferences can be grouped as spectral, physical and chemical. A survey of interferences by graphite furnace is given by Slavin and Manning (10). Therefore we will only mention that spectral interference is caused of molecular absorption and light scatter.

Physical interferences tend to alter the shape of the absorption peak by changing the appearance time and thus the appearance temperature of the analyte. This results in a change in the signal profile and thus analyte response. Examples include covolatilization of the analyte along with a more volatile matrix crystals.

Chemical interferences can be caused by reaction of the analyte with the hot graphite walls of the furnace to form refractory carbides and formation of stable gaseous molecules which escape without decomposing to atoms. The molecules may be formed as

the analyte is vaporized or they may result from gasphase reactions.

By the use of the background correction system, the spectral interferences will often be reduced significantly especially if two reference measurements bracketing the sample measurement (11). The modern graphite furnace configuration reduces much of the earlier reported interference (10). The use of L'vov platform may reduce both the physical and chemical interference in the pulse type furnace (12). Using pyrolytically coated tubes and 0.2 % acidum nitricum as matrix modifier may reduce the most frequently interference coming from chloride forming a relatively volatile chloride whose dissociation in the gas phase is incomplete. Use of preservatives as sodium fluoride should be avoided as aluminiummonofluoride is too volatile unless nitrates of an earth ion is added as matrix modifier (13). Use of a stronger acidum nitricum than 0.2 % reduces the sensitivity (14). Some recommend the use of the absorptionline for aluminium at 396.2 nm (15). The limit of liniarity for absorption increases from 0.2 to near 1 and the optimum operating current of the hollow-cathode lamp can be 25 mA (15) but Ca ions interfere by the measurement (16).

To be sure of the precision and accuracy by the aluminium-determination or to improve the results gradually one should join an external quality control procedure such as the Guilford projekt (17). In forensic chemistry we only do sporadical aluminium-analyses and often just to prove or exclude aluminium in fatal cases, where the aluminium-concentration is not so critical as in clinical cases, such as Alzheimers dementia.

The most critical source of errors in forensic chemical determination of aluminium is undoubtly contamination.

Contamination.

The results by determination of aluminium in plasma and serum are in the last decade — even by use of the same method (AAS) varying from 2.1 ng ml^{-1} to 38 ng ml^{-1} (18). This clearly shows the presence of seriously contamination, not at least due to airborne dust. If one considers that 1 litre air contains 200 μg dust with 1.5 % aluminium, the possibility of contamination will be more than evident (18).

The equipment used for sampling (knife, containers etc.) must therefore be tested continuously for their aluminiumcontribution. Plast and quartz are more suited than glass which shows high and

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not reproducible releasing of the aluminium from the sample solutions, also after a decontamination treatment. Samples for longer term storage should be kept at —20°C so diffusion phenomena are minimized as well as surface contamination. Aluminium is released at room temperature from polyethylene tubes in µg amounts (18) as plastic pellets in the tube must be avoided in the sample collection (19).

The form of contamination shown in figure 1 is easily discovered by the following injection.

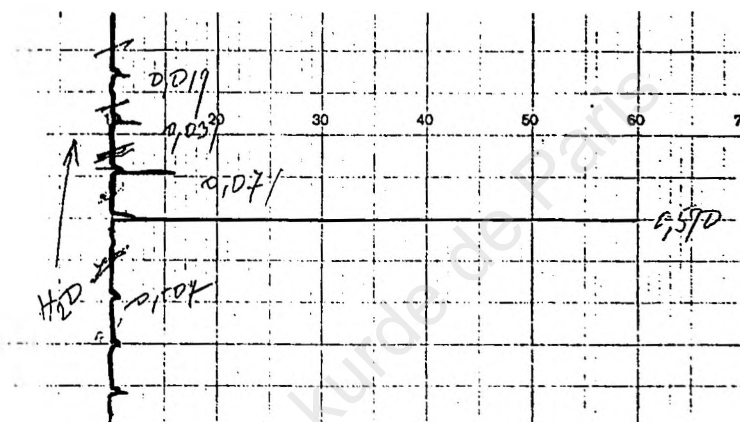

FIG. 1. — Contamination from pipet tip.

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Generally rinse of all utensils with 5 % acidum nitricum followed by redistillated water combined with tests for possible aluminium-contamination in reagent etc. must be recommended (20).

RESULTS AND DISCUSSION.

The results obtained for aluminium content in tissue from the deceased are given in table II.

For comparison are obtained values from a man dying violently and submitted for the same method of analysis. This case is also used for our recovery studies.

Normal values for aluminium in different tissues from populations in USA (21) and UK (22) are also given together with values from a fatal case in Denmark for a 50 years old woman with renal failure eating 1.7 kg aluminium in the period 1979-1984 (23).

TABLE II

Values ($\mu g/g$ wet weight) of aluminium content in tissue from the deceased, from a man dying violently, for populations in USA and UK and from a fatal case

Tissue	*Deceased case*	*Violently death*	*Population*		*Fatal case*
			UK	*USA*	
Blood	0.3		0.2-0.4		
Bone	6.5		68 ash		422
Brain	0.1	0.4	0.5		45.6
Heart	0.1	0.4		0.4	
Kidney	0.5	0.2	0.4	0.5	
Liver	0.5	0.6	2.6	0.8	142
Lung right	6.3	1.0			
Lung left	5.8		18.2	27.5	
Muscle	0.2		0.5		
Skin				3.2	
Spleen	0.3	0.3		1.0	
Subcutis	0.1	0.1			
Testis	0.3		0.4	0.5	

Recovery of 6.75 µg aluminium added to 5 g myocardium, spleen, liver, brain and kidney respectively varied from 97 to 111 %. No correction has been done.

The obtained values for aluminium in tissue from the deceased are as seen in table II inside the expected normal range for aluminium in the two populations and below the value from a fatal case. The death therefore must be related to an infection which might conceivable have been applied in the hospital because of the delaying effect on the healing from aluminium.

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CONCLUSION.

The values of aluminium in tissue from the victim of aluminium-bombattack are normal. The death may be related to infection easily being persistent because of the pollution of the whole body with aluminium.

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Decomposition products of PETN in post-explosion analysis

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ABSTRACT.

TLC of post-explosion debris showed that in some cases PETN was accompanied by other compounds which reacted with Griess reagent. The possibility that these compounds could be lower nitrate esters of pentaerythritol (tri-, di- and mononitrate) was examined. Lower nitrate esters were obtained by hydrolysis of PETN and their structures were determined — following separation — by mass spectrometry and NMR. Chromatographic data of these esters matched data of the compounds which accompanied PETN in certain post-explosion cases. The dinitrate and trinitrate esters of pentaerythritol were isolated from post-explosion extracts and their structures confirmed by mass spectrometry and NMR.

INTRODUCTION.

Post-explosion analysis in the Israel Police Laboratory is usually based on the identification of the original explosive rather than its decomposition products (1). With few exceptions — such as the identification of thiocyanate as an indication to the original presence of black powder (2) — this is the general attitude among forensic explosive analysts. The reason is that many of the decomposition products either escape as gases or are common inorganic salts (e.g. carbonates) from which the type of the original explosive cannot be deduced. In this work we report the appearance of decomposition products which are structurally related to the original explosive — pentaerythritol tetranitrate

(PETN). Being unaware of their existence in post-explosion debris could lead to possible errors.

The appearance of decomposition products of glycerine trinitrate in post-explosion extracts was first noticed by Helie-Calmet and Forestier (3). They found di- and mononitrate esters of glycerine in post-explosion extracts when glycerine trinitrate was involved in the explosion. They prepared the two isomeric glycerine mononitrates and the two isomeric glycerine dinitrates. In all TLC systems tried the R_f of the lower nitrate esters of glycerine corresponded to the R_f of the spots which accompanied glycerine trinitrate in some post-explosion cases. They found similar but less common behaviour in some post-explosion analyses involving PETN.

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EXPERIMENTAL.

Methods.

Thin layer chromatography (TLC).

TLC plates were 10 × 20 cm aluminium plates precoated with silica gel 0.2 mm thick (SI F Riedel de Haen). The developing solvent was (unless otherwise stated) trichloroethylene - acetone (4:1). The developed plates were sprayed with 3 % KOH in ethanol followed by Griess reagent, which was prepared by mixing equal volumes of two solutions : *a)* 5.5 g N-1-naphthylethylene-diamine in 100 ml nitrite-free H_3PO_4, diluted with water to 1 liter ; *b)* 80 g sulfanilamide in 100 ml nitrite-free H_3PO_4, diluted with water to 1 liter.

Liquid chromatography (LC).

The LC system included LKB 2152 HPLC controller, 2140 LKB rapid spectral detector and 2150 HPLC pump. Separations were carried out in a reversed phase mode on a 250 × 4 mm (I.D.) column packed with C_{18} Lichrocart (Merck ; particles size 7 μm). The mobile phase was methanol-water (65:35), operated at a flow rate of 1 ml/min. The solvents were degassed prior to the analysis. UV detection was made at $\lambda = 207$ nm.

Mass spectrometry (MS).

Mass spectrometry was carried out in the chemical ionization (CI) mode on *a)* a Dupont 21490B single focusing magnetic mass spectrometer, using isobutane as reagent gas ; *b)* a Finnigan 4500

quadrupole mass spectrometer, using methane as reagent gas. Samples were introduced directly into the ion source whose temperatures were 200-250°C (in a) and 140°C (in b).

Nuclear magnetic resonance (NMR) spectrometry.

Proton magnetic resonance spectrometry was carried out on a Bruker WM-250 instrument, using 250 MH_z frequency. The solvent was acetone-d_6.

Materials.

Pentaerythritol tetranitrate (PETN) was isolated from a detonating cord. Its purity was determined by TLC and infrared (IR) spectrometry.

Hydrolysis of PETN was based on a work of Dicarlo *et al.* (4). 100 ml of dioxane-water (82:18) and 1 ml conc. HCl were added to 1 g PETN and the mixture was refluxed for 6 hours. Dioxane (50 ml) and ethanol (50 ml) were then added and the mixture was concentrated to 20 ml. Solid $Ca(OH)_2$ and dioxane (50 ml) were added, the mixture was evaporated almost to dryness and 50 ml of ethanol were added. Most unreacted PETN crystallized and was filtered out. The solution was evaporated to dryness and the hydrolysis products were separated on a silica gel chromatographic column. Residual PETN was eluted with CH_2Cl_2; pentaerythritol trinitrate was eluted with CH_2Cl_2 - acetone (96:4) and pentaerythritol dinitrate was eluted with CH_2Cl_2 - acetone (90:10). The purity of the trinitrate and the dinitrate was confirmed by TLC and CIMS. A fraction containing pentaerythritol mononitrate was eluted with CH_2Cl_2 - 2-propanol (90:10) but the pure mononitrate was not isolated.

RESULTS AND DISCUSSION.

Figure 1 shows a TLC plate with an acetone-extract from a post-explosion case. The developing solvent was 1.2-dichloroethane - acetonitrile (9:1) and visualization was made by Griess reagent. The highest spot corresponds to PETN. The lower spots could originate from compounds present in the original explosive (e.g. RDX) or from decomposition products of PETN. The difference is especially important because an original mixture of PETN and RDX, known as « Semtex », is widely used by terrorists.

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Figure 2 shows a TLC plate of the same extract in a different solvent system : trichloroethylene - acetone (4:1). It is evident that the second highest spot in the post-explosion extract does not correspond to RDX.

Figure 3 shows a TLC plate with an acetone-extract from a post-explosion case involving PETN and the hydrolysis products of PETN. The similarity is obvious.

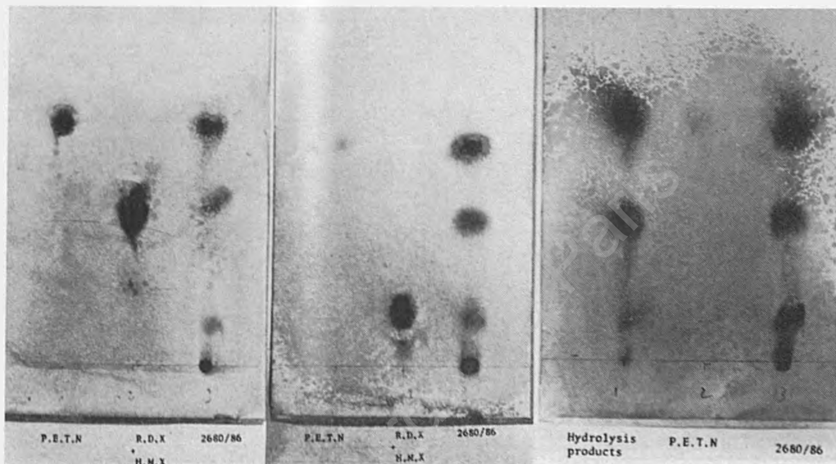


FIG. 1. FIG. 2. FIG. 3.

FIG. 1. — Thin layer chromatogram of an acetone-extract from a post-explosion case (developing solvent : 1,2-dichloroethane - acetonitrile 9 : 1).

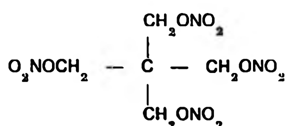
FIG. 2. — Thin layer chromatogram of an acetone-extract from a post-explosion case (developing solvent : trichloroethylene - acetone 4 : 1).

FIG. 3. — Thin layer chromatogram of an acetone-extract from a post-explosion case and of the hydrolysis mixture of PETN (developing solvent : trichloroethylene - acetone 4 : 1).

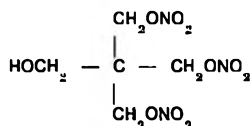
The hydrolysis products of PETN *(1)* are pentaerythritol trinitrate *(2)*, pentaerythritol dinitrate *(3)*, pentaerythritol mononitrate *(4)* and pentaerythritol *(5)*. Their structures are shown in scheme 1.

The next steps were the isolation and identification of the hydrolysis products of PETN. Only the trinitrate *2* and the dinitrate *3* were isolated from the hydrolysis mixture in a relatively pure form.

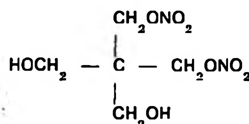
The chemical ionization (CI) mass spectra of *2* and *3* were taken, using isobutane and methane as reagent gases. With isobutane, most of the ion current was concentrated in the $[M+H]^+$ ions, at m/z 272 and 227 in the spectra of *2* and *3*, respectively. The CI-methane spectra, shown in figures 4 and 5, include —



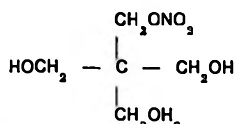
Pentaerythritol tetranitrate (PETN, *1*)



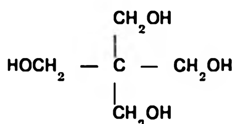
Pentaerythritol trinitrate (*2*)



Pentaerythritol dinitrate (*3*)



Pentaerythritol mononitrate (*4*)



Pentaerythritol (*5*)

SCHEME 1.

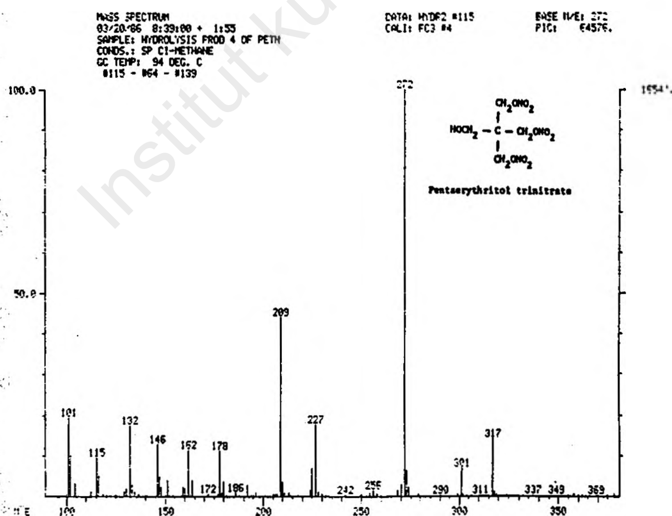


FIG. 4. — CI-methane mass spectrum of pentaerythritol trinitrate (2), isolated from the hydrolysis mixture of PETN.

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beside abundant $[M+H]^+$ ions — also some fragment ions. The ions at m/z 209 (fig. 4) and 164 (fig. 5) result by the loss of nitric acid from the corresponding $[M+H]^+$ ions of *2* and *3*. The process is known to occur in nitrate esters under CI conditions (5). The loss of HNO_3 from the protonated molecule of *2* is shown in scheme 2.

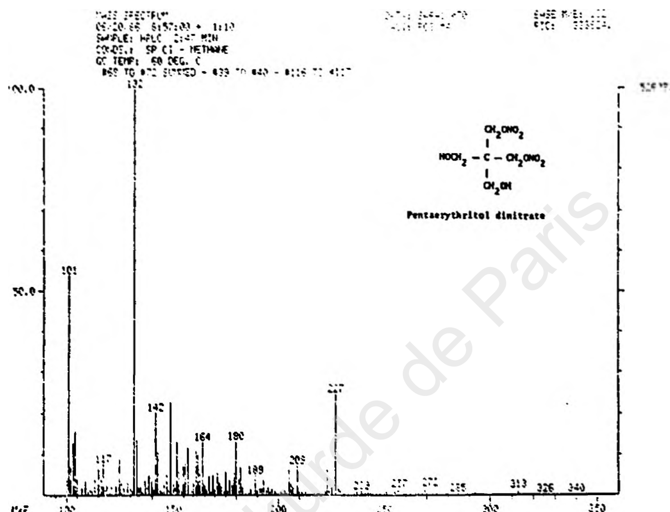
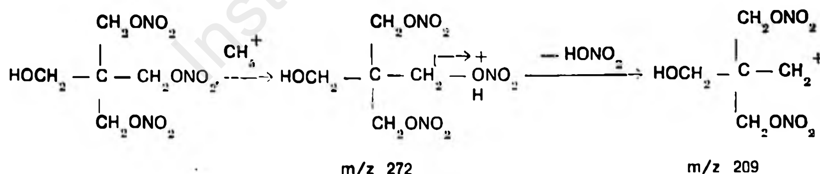


FIG. 5. — CI-methane mass spectrum of pentaerythritol dinitrate (3), isolated from the hydrolysis mixture of PETN.



SCHEME 2.

The CI spectra of the trinitrate *2* (with both isobutane and methane) include an abundant ion at m/z 227, corresponding to the $[M+H]^+$ ion of the dinitrate *3*. A loss of 45 m/z units from the $[M+H]^+$ ion of *2* is highly improbable. The presence of *3* as an impurity in *2* was excluded by TLC of the latter. It seems that the trinitrate ester *2* undergoes hydrolysis in the mass spectrometer to yield the dinitrate *3*, which is then protonated by the CI reagent gas. Other ions which complicate the mass

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spectra of these esters correspond to $[M+NO]^+$ and $[M+NO_2]^+$. They were reported earlier (6) in the mass spectra of nitrate esters and were attributed to ion-molecule reactions favoured by high sample concentration and « tight » ion source. The presence of $[M+NO_2]^+$ ion in the spectrum of the trinitrate *2* (m/z 317 in figure 4) could lead to the conclusion that PETN was present as an impurity. It was shown by TLC that this was not the case.

The structures of the trinitrate *2* and the dinitrate *3* were confirmed by their NMR spectra. The protons adjacent to the nitrate group resonate as singlets at δ = 4.77 ppm and 4.67 ppm

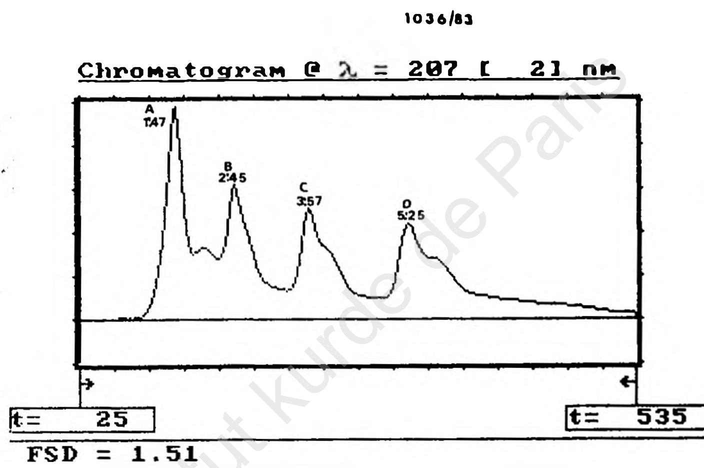

FIG. 6. — Liquid chromatogram of an acetone-extract from a post-explosion case (retention times in minutes are shown under the letters A, B, C, D which mark the peaks).

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in *2* and *3*, respectively. By comparison, the protons of PETN resonate at 4.87 ppm. The protons adjacent to the hydroxyl group resonate as doublets at δ = 3.79 ppm and 3.69 ppm in *2* and *3*, respectively.

Now that we have proven the formation of *2* and *3* during the hydrolysis of PETN, it remained to try and isolate them from actual post-explosion cases.

Figure 6 shows the LC results from an actual post-explosion case involving PETN. The fractions corresponding to the chromatographic peaks B and C were collected and subjected to CIMS and NMR. Their structures fully corresponded to pentaerythritol dinitrate *(3)* and pentaerythritol trinitrate *(2)*, respectively. Peak D corresponded to PETN while peak A remained unidentified. Figures 7 and 8 show the CI-methane mass spectra of fractions C

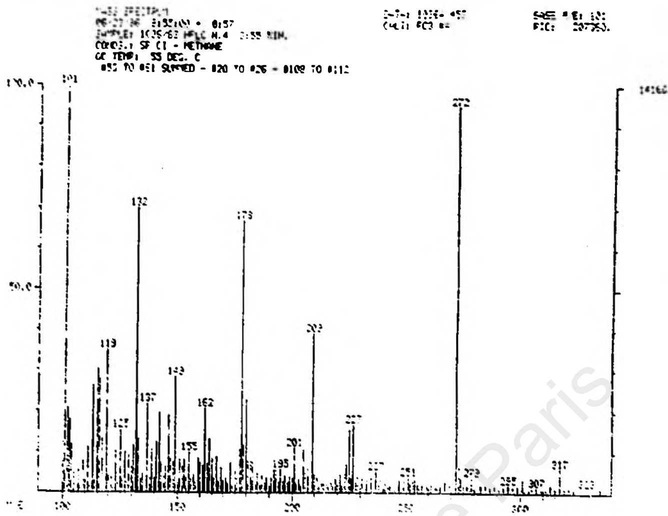

FIG. 7. — CI-methane mass spectrum of pentaerythritol trinitrate (2), isolated by LC (fraction C in fig. 6) from a post-explosion case.

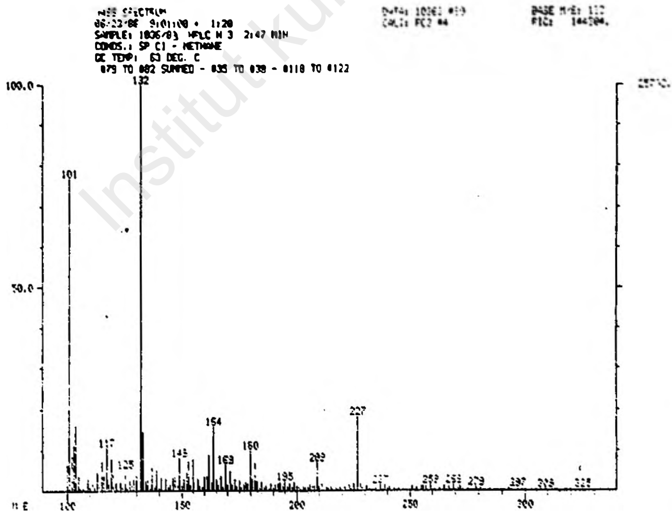

FIG. 8. — CI-methane mass spectrum of pentaerythritol dinitrate (3), isolated by LC (fraction B in fig. 6) from a post-explosion case.

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and B (fig. 6) collected from the LC separation of the post-explosion extract. The similarity to the Cl-methane spectra of *2* (fig. 4) and *3* (fig. 5), respectively, is obvious. The NMR spectra of fractions C and B from the LC separation were also identical with the NMR spectra of *2* and *3*, respectively.

Figure 9 shows the NMR spectrum of an acetone-extract from a post-explosion case. No separation was made prior to the NMR analysis. The sharp singlets at the relatively low field at $\delta = 4.88$,

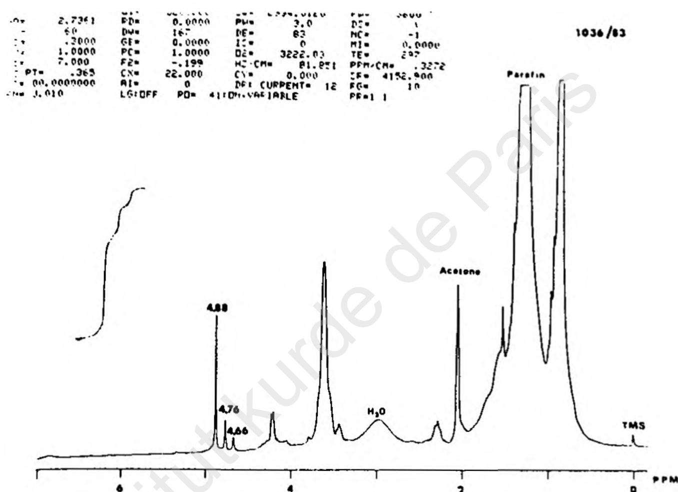

FIG. 9. — NMR spectrum of an acetone-extract from a post-explosion case.

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4.76 and 4.66 ppm originate from the CH_2ONO_2 protons of PETN *(1)*, pentaerythritol trinitrate *(2)* and pentaerythritol dinitrate *(3)*, respectively. Taking into account the different number of these protons in *1*, *2* and *3*, the relative ratio of PETN *(1)* and its decomposition products *2* and *3* can be estimated from the integration.

CONCLUSIONS.

The experimental data discussed above show that lower nitrate esters of pentaerythritol appear in post-explosion debris where PETN was involved in the explosion. We cannot say whether their formation is associated directly with the explosion process or is

due to hydrolytic processes occurring in unexploded PETN at the extreme conditions of the explosion.

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ETK - an operational explosive testing kit

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ABSTRACT.

A chemical kit for field determination of traces of explosives on hands of suspects is described. The system involves three components : dry sampling devices (« detection buttons »), and two tubes of chemical reagents. It detects virtually all the common military explosives, and there is also an option to detect home-made explosives based on inorganic fertilizers. Detection is based on two well known chemical reactions for which optimal conditions have been developed. In stage I polynitro aromatics are detected by the formation of colored Meisenheimer complexes. In stage II organic nitrates and nitramines are consequently detected by modified Griess test.

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THE PROBLEM.

The past several years have seen an alarming increase in the use of explosives in criminal and terrorist activities. In such circumstances many suspects must be screened quickly for recent contact with explosives ; and police, customs and other security agencies must be able to determine rapidly whether a suspected substance is an explosive. The requirement that was presented to us in the late 1970's was for a simple, quick, reliable and inexpensive device for the detection of traces of explosives on hands of suspects and on objects, and to distinguish between explosive and non-explosive material.

WHY A CHEMICAL KIT?

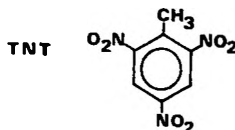
Explosive-vapor detectors are relatively complicated and expensive. Dogs require an elaborate and expensive logistical system of training and are not always reliable. Thus, we decided to study the possibility of utilizing « wet chemistry ».

The idea was to find the shortest sequence of chemical spot-tests which would indicate the presence of minute amounts of explosives by formation of specific colors. Indeed, we found a very short scheme that, by a minimal number of reactions, enables the detection of practically all military explosives. By modifying the chemical composition of some known reagents, we managed to shorten the reaction time and not less important-to carry out the entire process at room temperature. The assembly received the name Explosive Testing Kit (ETK).

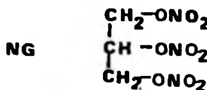
CHEMISTRY OF ETK.

The vast majority of military explosives contain one of the three following chemical groups: polynitro aromatics, organic nitrates (nitrate esters) and nitramines (fig. 1).

1. *Poly-nitro aromatics*. Example : TNT



2. *Organic nitrates*. Example : NG



3. *Nitramines*. Example : RDX

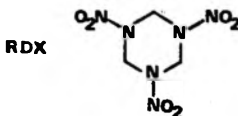


FIG. 1. — Most common chemical groups of military explosives.

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A thorough literature survey (1) as well as numerous laboratory experiments led us to the conclusion that the shortest and most economical sequence for the formation of colored products from all the above military explosives is the following (fig. 2) :

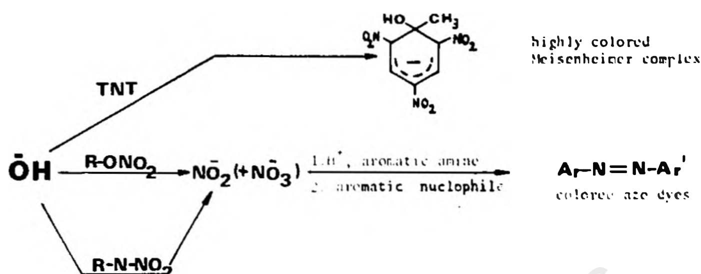

FIG. 2. — Formation of colored products by the reaction of military explosives with alkali.

Polynitro-aromatics, such as TNT, TNB, picric acid and its derivatives, form highly colored compounds (Meisenheimer complexes) upon reaction with alkali. Common alkali (2) formulations for explosives analysis contains 5-10 % KOH or NaOH in ethanol or water (3). Nitrate esters such as NG, EGDN, PETN and NC under similar conditions, undergo alkaline hydrolysis (4) producing nitrite ions (NO_2-) which can be readily detected by the well known Griess reaction (5-7). This includes the action of NO_2- ions on an aromatic amine, usually sulfanilic acid, in an acidic medium. The diazonium ion thus formed is then coupled with an aromatic nucleophile (usually α-naphthyl amine) to produce a colored azo dye. Nitramines such as RDX, HMX and tetryl too, undergo alkaline cleavage to form nitrite ions, NO_2-, which produce the same colored azo compound by the Griess reaction (8, 9). When the above scheme (fig. 2) was applied to actual explosives, it was found that while polynitro-aromatic explosives reacted as expected, certain plastic explosives (and particularly those which are based on nitrate esters) reacted very slowly and required heat for the color formation. This, of course, was a great disadvantage for field work.

An improvement was achieved when dimethyl sulfoxide (DMSO) was used as the major solvent for the alkaline reagent instead of the alcoholic or aqueous solutions that were previously used. This change accelerated the color formation with plastic explosives such as Detasheet (mostly PETN + binder) or Semtex — H (RDX+PETN+Binder).

A second improvement was achieved by the addition of reducing agents such as mercaptanes, ascorbic acid or hydrazine to the alkaline solution. This was effective with explosives containing nitrate esters.

The optimal composition for stage 1 (alkaline reagent) was found to include KOH in EtOH/DMSO with a small amount of reducing agent. A plausible explanation for the advantages of this formulation over the alcoholic-KOH reagent might be the following:

a) DMSO is a better solvent for plastic explosives (particularly for the binder), than water or alcohol.

b) Nitrate esters, under alkaline conditions, produce not only nitrite ions (NO_2-) but also nitrate ions (NO_3-) (10) which reduce the detectability of the parent compound (as the NO_3- ions do not participate in the Griess reaction). The reducing agent in the above composition converts the nitrate ions (NO_3-) to nitrite ions (NO_2-) which increases the sensitivity of the detection (Reduction of nitrate to nitrite by hydrazine in alkaline solution was studied in detail by Sawicki and Scaringelli (11).

A combination of sulfanilamide and N-(1-naphthyl)ethylenediamine for the diazotation and coupling-stage (11) gave more satisfactory results than the more common formulation of sulfanilic acid and α-naphthylamine. The reaction was faster and the color intensity-higher (Also, the formerly used α-naphthyl amine is known to be carcinogenic).

SAMPLING METHOD.

From the very beginning of this project we decided to adopt a « dry sampling » method, the rationale being that if a positive indication for the presence of explosive is obtained, there is still a sufficient amount of material left on the object for laboratory confirmation (The efficiency of « wet sampling » methods such as cotton-wool swabbing or hand-washing with solvents is much higher, thus leaving very little on the object for furthere examination).

The sampling devices that were designed for this purpose are « detection buttons » (fig. 3). They are composed of a circle of the coarse filter paper, compressed between a plastic disc and a plastic ring. They enable the technician to apply pressure

against the fingertips of suspects without contaminating the sampling surface. For sampling surfaces other than fingers, square or round pieces of the same filter paper are used.

FIG. 3. — Components of ETK.
a) Detection buttons. - *b)* Reagent tubes. - *c)* Ampule cracker. - *d)* Box.

SENSITIVITY.

The limits of detection by ETK were determined independently by the Metropolitan Police Laboratories in London and by this group. The human eye was used as detector. It was found to give a visible reaction with amounts as small as 10^{-7} g TNT per button and 10^{-8} g of NG.

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Post explosion analysis of explosives by mass spectrometric methods

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ABSTRACT.

The analysis of trace amounts of explosives from post-explosion debris — one of the most difficult problems in forensic chemistry — is still carried out in many laboratories by chromatographic methods only. In recent years several new methods have been applied to the analysis of explosives. These include mass spectrometric methods (GC/MS, LC/MS and MS/MS) and NMR methods. The possible application of these methods to post-explosion analysis is discussed and various aspects of the techniques are reviewed and compared. The choice of capillary column GC/MS as a routine method in the Israel Police laboratory is explained and examples from actual cases are given.

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INTRODUCTION.

Many forensic laboratories base their post-explosion analysis on chromatographic methods only, particularly thin layer chromatography (TLC) (1) and more recently liquid chromatography (LC) (1). The special implications of a positive identification in forensic analysis led our laboratory to a self-imposed criterion : identification of an organic compound should not rely only on chromatographic methods, even when several combinations of these methods are employed. This criterion is strictly adhered to in the analysis of drugs. If a syringe is suspected to contain traces of heroin, TLC, gas chromatography (GC) or LC are not considered sufficient for positive identification. A mass spectral identification is necessary to confirm the chromatographic results and without it the expert would not give a positive result.

Unfortunately, this criterion cannot always be imposed in the analysis of explosives. There is obviously no problem with unexploded material, where the amount of the sample enables the use of spectrometric methods to confirm the chromatographic results. Infrared (IR) spectrometry, mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectrometry can easily be employed. The problems encountered in applying these methods to post-explosion analysis are described in this paper and the successful use of GC/MS to confirm TLC results from post-explosion exhibits is described.

EXPERIMENTAL.

GC/MS was carried out on a Finnigan 4500 quadrupole mass spectrometer. The GC column was a J and W fused silica capillary column, 15 m $\times$ 0.25 mm (I.D.) with 0.25 μm coating of DB-5. Temperatures were programmed from 70°C to 270°C at 15°C/min. Injector temperature was 180°C. Electron energy was 70eV in both electron impact (EI) and chemical ionization (CI) modes. Helium was the GC carrier gas and methane was the CI reagent gas. More details are reported elsewhere (3).

NMR spectrometry was carried out on a Bruker WM-250 instrument, using 250 MHz frequency. More details are reported elsewhere (4).

RESULTS AND METHODS.

The reason that many forensic laboratories use only chromatographic methods in their post-explosion analysis is probably because the application of spectrometric methods (IR, MS, NMR) to samples taken from the explosion site has often been found impractical. Although exhibits from the post-explosion debris are first extracted with acetone and the extract is cleaned (when necessary) on a chromatographic column, large amounts of impurities often remain. This, combined with the fact that only small amounts of the original explosives are present in the extract, excludes IR from being successful in most cases.

Experiments in the use of NMR for post-explosion analysis gave some unexpected results. A priori it was assumed that when applied to post-explosion residues the method would suffer from similar disadvantages as IR : relatively high detection limits and

interference from impurities. A study designed to test the applicability of NMR to post-explosion analysis showed that the method was successful in several post-explosion cases (4). The proton NMR spectra of certain explosives like RDX and PETN are characterized by one singlet at a relatively low field. Therefore interference from protons of usual organic contaminants is minimal. Figure 1 is an example of the analysis of a post-explosion extract by NMR (4), without any separation prior to the NMR analysis. The protons of RDX and PETN, which resonate as singlets at $\delta = 4.89$ ppm and $\delta = 6.26$ ppm, respectively, are clearly observed. The example in figure 1 proves that NMR could be very valuable in some post-explosion analyses. However, there were cases where spots of nitrate esters and nitramines were detected by Griess reagent on a TLC plate but when the samples were subjected to NMR no explosives were detected. The reason could be the « sensitivity gap » of 1-2 orders of magnitude between the methods [$\sim$ 10 μg for most explosives in NMR (4) vs. 1-0.1 μg in TLC (5)].

Mass spectrometry is probably the best method available for the analysis of sub-microgram amounts of organic compounds, being a sensitive and a reliable method. Direct introduction of post-explosion samples into the mass spectrometer is in many cases impractical. Even after cleaning the extract on a chromatographic column the ions originating from impurities often give rise to a complicated spectrum from which the explosive cannot be identified. There are, however, some characteristic ions like NO^+ and NO_2^+ in the mass spectra of nitrate esters and nitramines which have analytical value even among the many other unrelated ions. Also, the MS analysis usually follows TLC, so there is an indication about the type of explosive. It is then easier to find significant ions related to the suspected explosive. Although this constitutes an improvement over the « TLC only » situation existing in many forensic laboratories, it does not meet our criteria for positive identification.

When a « gentle » ionization method like CI is employed a relatively simple spectrum is produced. It usually contains fewer ions than the corresponding EI spectrum and often includes molecular weight information. These features make CIMS more suitable for a direct MS analysis of post-explosion samples. Figure 2 shows the CI (methane) mass spectrum of a post-explosion extract from the debris of a blown up safe. The sample was introduced to the ion source of the mass spectrometer through the direct

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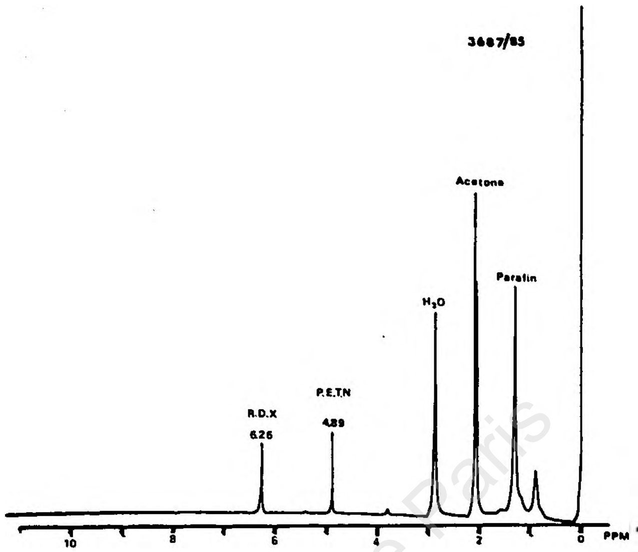

FIG. 1. — NMR spectrum (protons) of a post-explosion extract (case 3687/85). PETN and RDX were identified.

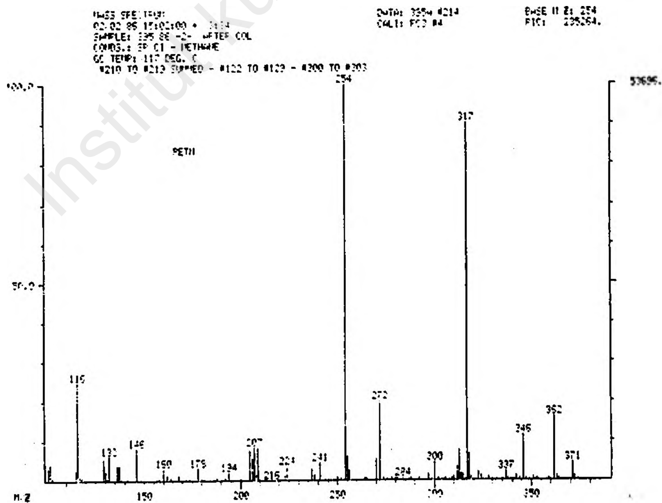

FIG. 2. — CI-methane mass spectrum (using direct probe) of a post-explosion extract (case 395/86). PETN was identified.

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probe inlet, after being cleaned on a florisil column. PETN was identified by its $[M+H]^+$ ions at m/z 317 and by some fragment ions, mainly $[M+H—HNO_3]^+$. The mass spectrum shown in figure 2 seems to be sufficient for positive identification of PETN, especially when an EI mass spectrum is also recorded and the typical low mass ions at m/z 30, 46 and 76 (NO^+, NO_2^+ and $CH_2ONO_2^+$, respectively) are observed. Nevertheless, the method is not general. Some explosives give CI spectra containing only $[M+H]^+$ ions which although indicating molecular weights cannot serve as a basis for positive identification. Moreover, many post-explosion samples will not give interpretable mass spectra even under CI conditions.

The obvious solution seems to use a separation method like LC or GC « on line » with the mass spectrometer. Some explosives are known to be thermally labile under GC conditions. Possible decompositions of RDX (6), PETN (5) and tetryl (3) were reported. Other, like HMX or cellulose nitrate (« nitrocellulose ») are too involatile for GC analysis. It seems that LC/MS is the more suitable method for post-explosion analysis, especially with the introduction of the « thermospray » technique (7, 8). There is, however, a major practical limitation related to the operation of LC/MS in a forensic, service-oriented laboratory. Most mass spectrometers in multi-function forensic laboratories operate in the GC/MS mode, analysing inflammable liquids, drugs and pesticides. It is not practical — although it may be so in the future — to alternate between GC/MS and LC/MS on an instrument which has much workload.

As this is the case in our laboratory we have chosen GC/MS as the method for positive identification in post-explosion analysis. The same column (see EXPERIMENTAL) is used for drugs, pesticides and explosives. We started our experiments in GC/MS of explosives with a 30 m capillary column.

Nitroaromatic compounds like TNT posed no problem but some nitrate esters (e.g. PETN) or nitramines (e.g. RDX) showed decreased sensitivity and possible decompositions. To minimize thermal decomposition we shifted to a 15 m column. Also, the injector was kept at a relatively low temperature (180°C). Under these conditions we succeeded to analyse 10 ng of most common explosives (3), including TNT, glycerine trinitrate (NG), ethylene glycol dinitrate (EGDN) and RDX. PETN was also analysed but with lower sensitivity. Tetryl decomposed thermally but the product — identified as N-methylpicramide (3) — emerged as

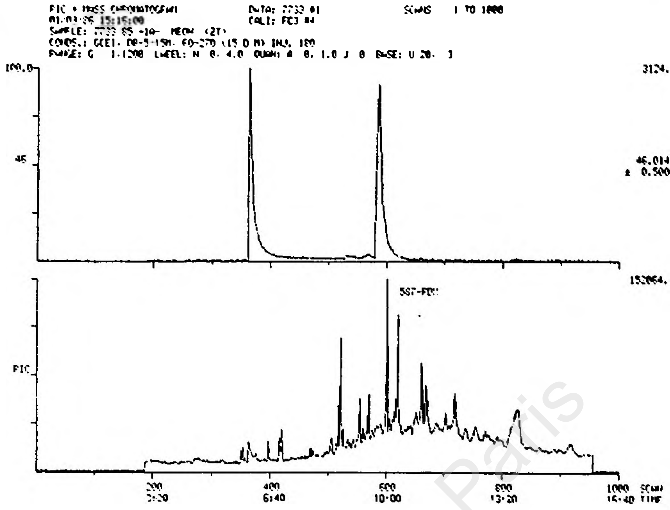

FIG. 3. — Total ion and mass chromatograms of a post-explosion extract (case 7733/85). The peak emerging after 587 seconds was identified as RDX (see figure 4).

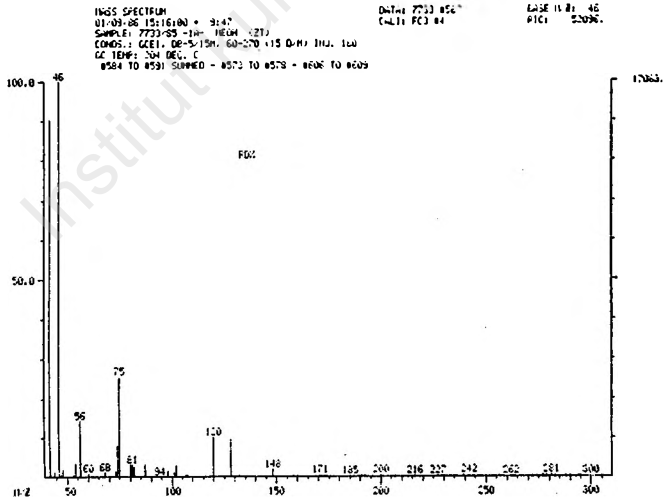

FIG. 4. — EI mass spectrum of the peak emerging after 587 seconds in figure 3 (case 7733/85). RDX was identified.

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a single chromatographic peak from which the original presence of tetryl could be deduced.

The good results obtained with unexploded explosives are hardly surprising. Much more impressive are the results obtained by GC/MS in actual post-explosion samples (3).

Figure 3 shows the total ion and the m/z 46 mass chromatograms from an extract of the debris of a blown up safe. The peak emerging after 587 seconds, located clearly by the m/z 46 mass

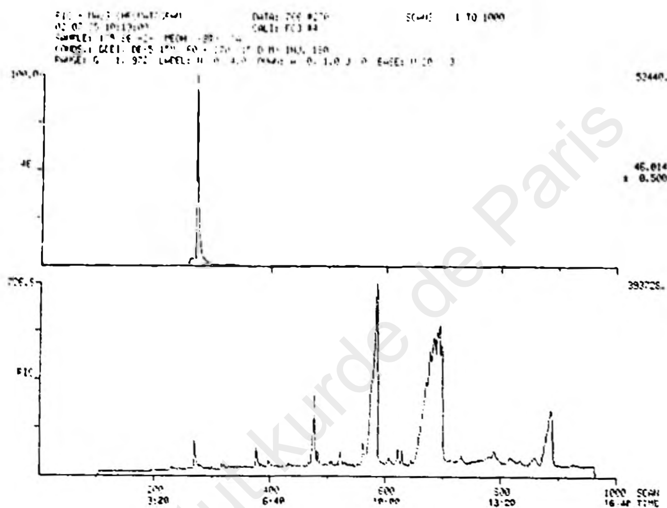

FIG. 5. — Total ion and mass chromatograms of a post-explosion extract (case 135/86). The peak emerging after 270 seconds was identified as NG.

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chromatogram could be attributed to RDX by its EI mass spectrum (fig. 4). The residues from an explosive charge which had been detonated inside a litter can were subjected to GC/EIMS and GC/CIMS. We use GC/CIMS especially when nitrate esters are involved (3). The total ion and mass chromatograms in the GC/EIMS and GC/CIMS analyses are shown in figures 5 and 6, respectively. The resulting EI and CI mass spectra gave a positive identification of NG. Other examples for the successful use of capillary column GC/MS in post-explosion analysis is described elsewhere (3).

In recent years tandem mass spectrometry (MS-MS) was tried on several types of compounds (9), including explosives (10). The equipment is too expensive and too sophisticated for most forensic laboratories so it is not surprising that no reports on analysing

post-explosion samples from actual cases have been published. Yet the method seems potentially suitable for post-explosion analysis. From the ions separated by the first mass spectrometer, which come from both the impurities and the explosive, only pre-selected ions (according to the « suspected » explosive) will go into the collisionally activated decomposition (CAD) chamber. The ions formed by CAD will be separated by the second

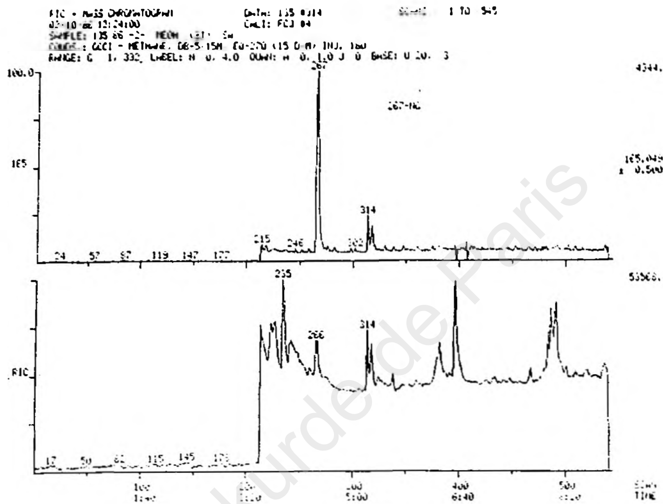

FIG. 6. — Total ion and mass chromatograms (CI-methane) of a post-explosion extract (case 135/86). The peak emerging after 267 seconds was identified as NG.

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mass spectrometer and the resulting CAD spectra will characterize the explosive. Such procedure could save tedious cleaning of the post-explosion extract. But, as stated before, the method has yet to be tested in actual cases.

CONCLUSIONS.

For years forensic laboratories have based their post-explosion analysis only on chromatographic methods, mainly TLC. Although papers about other analytical methods like MS or NMR for the analysis of explosives have appeared, the incorporation of these methods into routine work did not materialize. In the Israel Police Laboratory organic extractions from every post-explosion case are now subjected — following TLC screening — to GC/MS. The examples shown in this paper and elsewhere (3) shown,

demonstrate that GC/MS is a successful method for post-explosion cases involving organic explosives, so the days of « TLC only » should be over.

*
* *

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Capillary column gas chromatography / mass spectrometry of explosives

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ABSTRACT.

Gas chromatography/mass spectrometry (GC/MS) using a 15 m long capillary column has been employed in our laboratory for the analysis of explosives. The technique is suitable for most of the common military explosives. Nitroaromatic compounds like TNT were analysed easily and with high sensitivity and so were the volatile nitrate esters like glycerine trinitrate. Success was also achieved in the analysis of PETN and RDX, which had previously been reported to pose problems under GC conditions. Tetryl was shown to decompose during the analysis but its decomposition product was well defined and could serve as evidence to the presence of tetryl. The method was successfully applied to post-explosion debris from actual cases.

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INTRODUCTION.

Many of the explosives encountered by the Israel Police laboratories are organic military explosives and therefore are analysed by the usual analytical methods for organic compounds. Analysis is performed both on unexploded explosives and on post-explosion debris. It is post-explosion analysis where special difficulties exist. The important steps before the analysis are collecting the right exhibits from the explosion site and using suitable extraction and purification processes. The samples are then subjected to thin layer chromatographic (TLC) analysis (1). Although TLC often gives satisfactory results, it is our policy not to base an identification of an organic compound in a forensic laboratory on chromatographic results only (2). Usually infrared (IR) spec-

trometry or mass spectrometry should be used to confirm the TLC results but this is not always feasible in post-explosion analysis. IR is often impractical : not only that the amount of the explosive is too small but it is often accompanied by large amounts of impurities from the debris, which obscure the IR spectrum. We report here the choice of GC/MS as a reliable method by which TLC results from post-explosion cases could be confirmed.

This choice raises the question of the thermal stability of explosives under GC conditions. Nitroaromatic compounds pose no problems in their GC analysis but possible thermal decompositions were reported for some nitrate esters and nitramines (3). The working conditions described in this paper are suitable for the GC analysis of most common explosives.

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EXPERIMENTAL.

GC/MS was carried out on a Finnigan 4500 quadrupole mass spectrometer. The GC column was a J and W fused silica capillary column, 15 m × 0.25 mm (I.D.) with 0.25 μm coating of DB-5. Temperatures were programmed from 70°C to 270°C at 15°C/min. Injector temperature was 180°C. Ion source temperature was 140°C. Scan range was 30-500 or 40-500 mass units. Scan rate was 1 scan/sec. Electron energy was 70 eV both in electron impact (EI) and chemical ionization (CI) modes. Helium was the GC carrier gas and methane was used as the CI reagent gas.

RESULTS AND DISCUSSION.

Figure 1 shows the total ion chromatogram of the mixture of 6 common explosives : glycerine trinitrate (NG), 2,4-dinitrotoluene (DNT), 2,4,6-trinitrotoluene (TNT), pentaerythritol tetranitrate (PETN), 1,3,5-trinitro - 1,3,5-triazacyclohexane (RDX) and 2,4,6-trinitrophenylmethylnitramine (tetryl). All emerged from the column as well separated sharp chromatographic peaks. The separation was made on a 15 m capillary column (see EXPERIMENTAL). Poorer results were obtained on a 30 m column : PETN and RDX failed sometimes to emerge from the longer column. It seems that the use of shorter columns minimizes the thermal decomposition of explosives with greater thermal instability. The loss of resolution caused by the use of the 15 m column is not a major problem

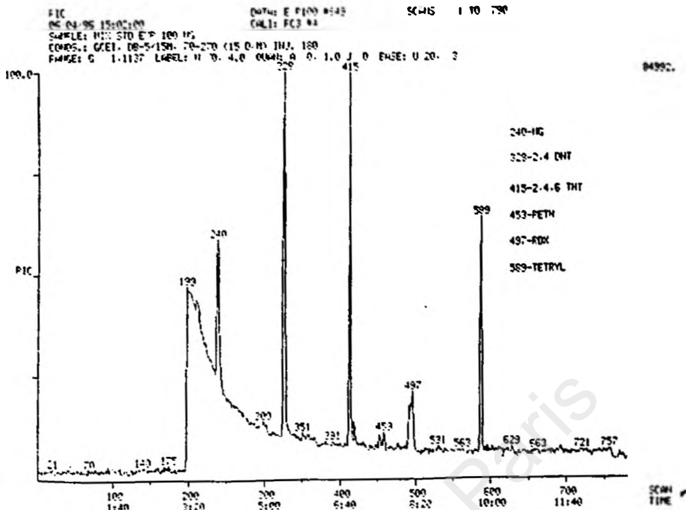

FIG. 1. — Total ion chromatogram of a mixture containing six explosives.

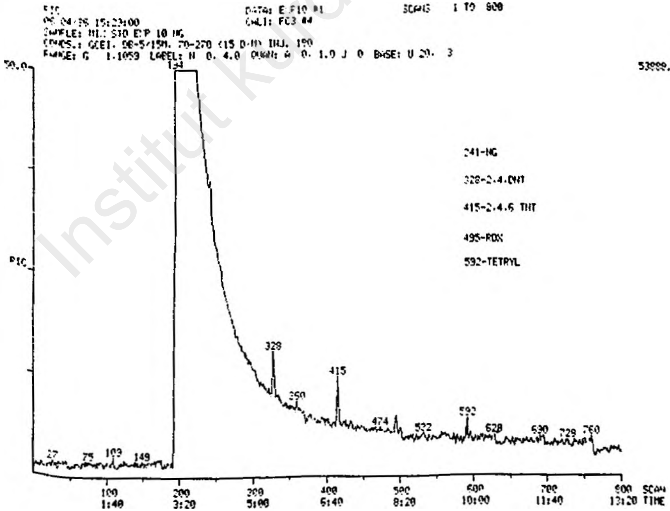

FIG. 2. — Total ion chromatogram of 10 ng of each of the following explosives : NG, 2,4-DNT, TNT, PETN, RDX and tetryl. All but PTN were detected.

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and conditions can be found in which a satisfactory separation is achieved on the short column. In most explosive analyses the GC column serves mainly as a selective entrance to the ion source of the mass spectrometer so that mass spectrometry could be applied to post-explosion extracts.

Figure 2 shows the total ion chromatogram of a mixture containing 10 ng of each of the above listed explosives : all but PETN emerged as distinct chromatographic peaks from which complete EI mass spectra could be recorded.

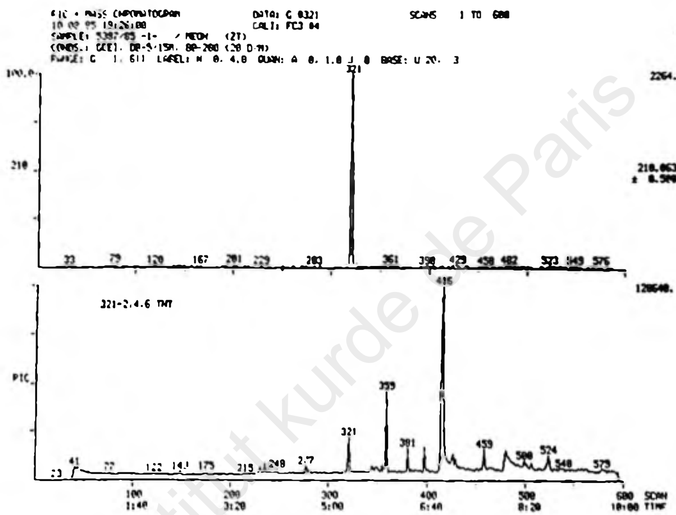

FIG. 3. — Total ion chromatogram of a post-explosion extract (case 5387/85). The peak emerging after 321 seconds was identified as TNT.

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A typical post-explosion total ion chromatogram is shown in the lower part of figure 3. The peak emerging after 321 seconds belongs to TNT, as was proven by its EI mass spectrum. The upper part of figure 3 shows the mass chromatogram for the m/z 210 ions, which are the most abundant ions in the EI mass spectrum of TNT. The mass chromatogram is helpful when there is an a priori indication — often based on TLC results — about the identity of the explosive. In this way the chromatographic peak of the explosive is immediately located and its mass spectrum recorded. The advantage of the mass chromatogram mode is demonstrated in another post-explosion extract (fig. 4), where it is evident that by looking only at the total ion chromatogram the presence of TNT could be missed. Using the m/z 210 mass chromatogram the peak

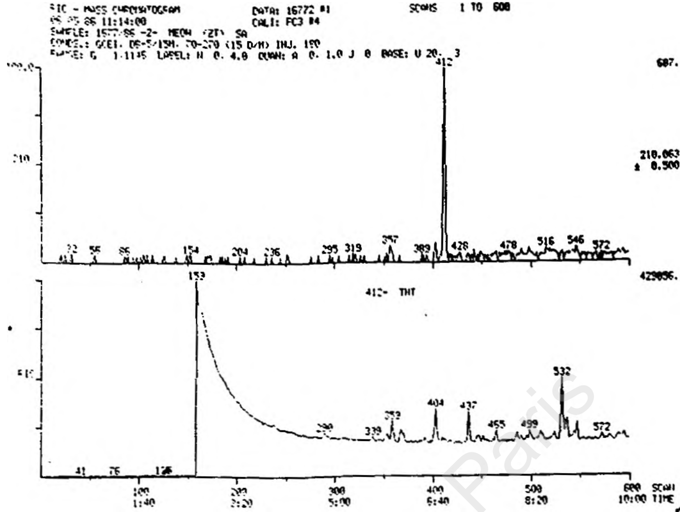

FIG. 4. — Total ion and mass chromatograms of a post-explosion extract (case 1667/86). The peak emerging after 412 seconds was identified as TNT.

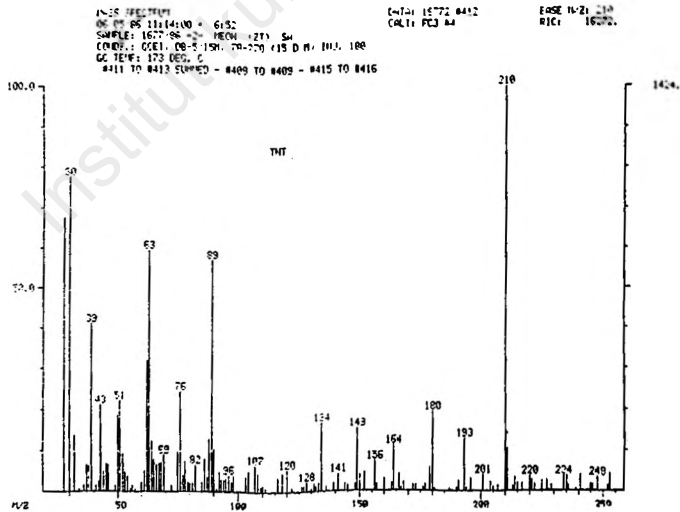

FIG. 5. — EI mass spectrum of the peak emerging after 412 seconds (fig. 4). corresponding to the EI mass spectrum of TNT.

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belonging to TNT was located and its EI mass spectrum recorded (fig. 5). A similar example is taken from a case where a bomb was exploded on the roadside near the city of Nablus. The chromatographic peak of RDX is negligible in the total ion chromatogram (fig. 6 ; marked with an arrow) but it was located by the m/z 30 and 46 mass chromatograms. Its complete mass spectrum is shown in the upper part of figure 6.

Many nitrate esters have very similar EI mass spectra. Thus, the EI mass spectra of NG, ethylene glycol dinitrate (EGDN) and PETN contain characteristic ions at m/z 30 (NO^+), 46 (NO_2^+) and 76 ($CH_2NO_2^+$) but no molecular ions (4). This causes difficulties in the identification of nitrate esters by EIMS. In order not to base their identification on GC retention times only, we use CIMS for the identification of nitrate esters. CI mass spectra of nitrate esters (5) contain molecular weight information and the different esters are easily distinguished. It should be noted, however, that GC/CIMS of explosives on our instrument and under our working conditions (see EXPERIMENTAL) is less sensitive than GC/EIMS.

The similarity between the EI mass spectra of NG and EGDN as well as the differences in their CI-methane mass spectra are demonstrated in figure 7. The major fragment ions in the CI-methane spectra are at m/z 165 and m/z 90 for NG and EGDN, respectively. They result by the loss of nitric acid from the corresponding protonated molecular ions, a well known process in the CIMS of nitrate esters (5, 6). The use of GC/EIMS and GC/CIMS for post-explosion analysis involving a nitrate ester explosive is demonstrated in figures 8 and 9, respectively. The extract was taken from the debris left by a bomb which had been exploded near Tel-Aviv University. Following the TLC results, the presence of NG was first confirmed by its GC/EIMS data, shown in figure 8. However, the EI mass spectrum is not unique enough and other nitrate esters cannot be definitely excluded. The GC/CIMS data, shown in figure 9, give conclusive evidence for the presence of NG.

Another example involving the identification of NG was the post-explosion analysis of an improvised explosive concealed in a litter can near the Government House in the City of Gaza. Figure 10 shows the total ion chromatogram of the extract, using GC/EIMS. NG appeared as a very small chromatographic peak emerging after 297 seconds ; again, it was conclusively iden-

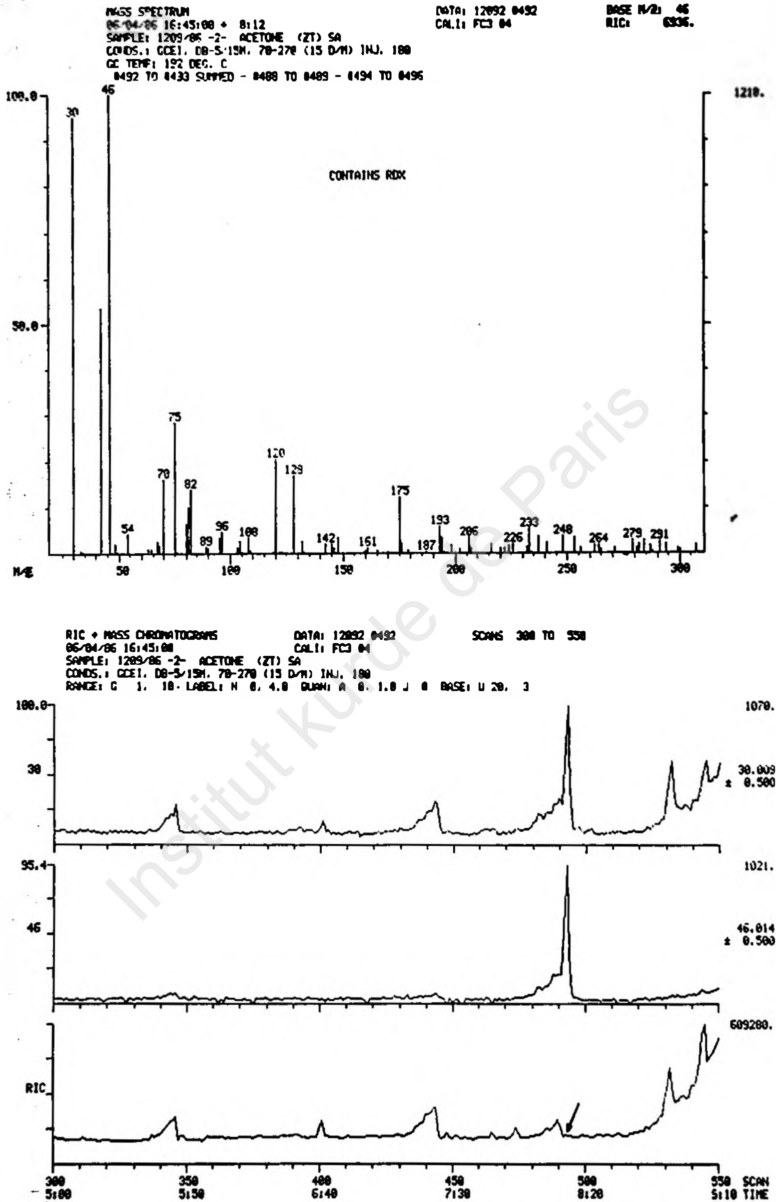

FIG. 6. — Total ion and mass chromatograms of a post-explosion extract (case 1209/86) and EI mass spectrum of the peak emerging after 492 seconds, identified as RDX .

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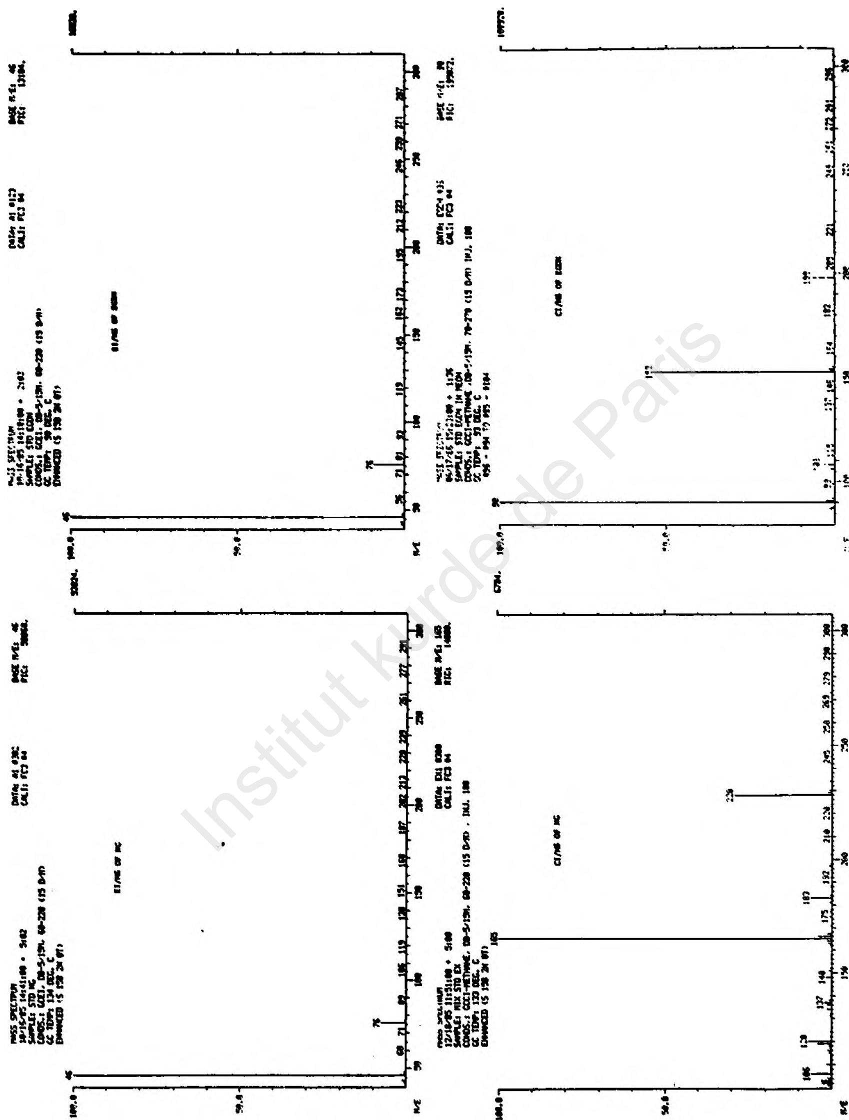

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FIG. 7. — EI and CI-methane mass spectra of NG and EGDN, using capillary GC/MS.

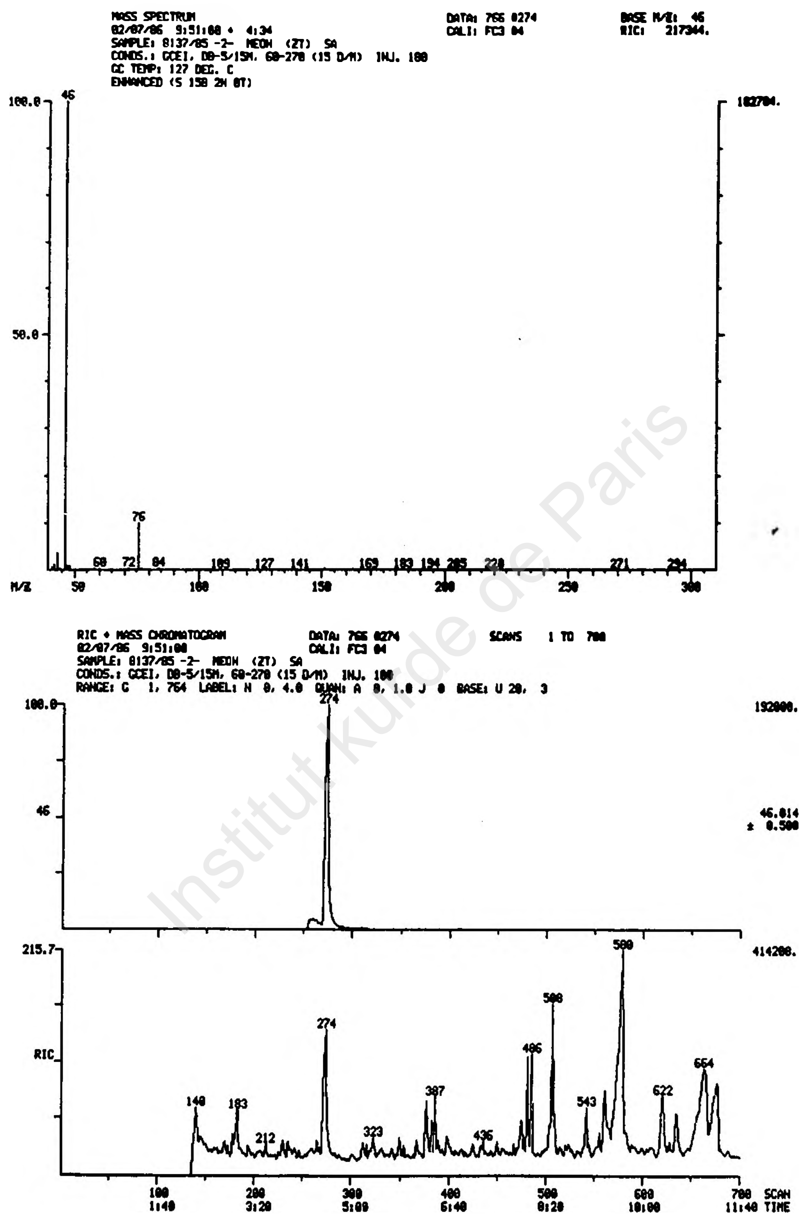

FIG. 8. — Total ion and mass chromatograms of a post-explosion extract (case 8137/85) and EI mass spectrum of the peak emerging after 274 seconds, identified as NG.

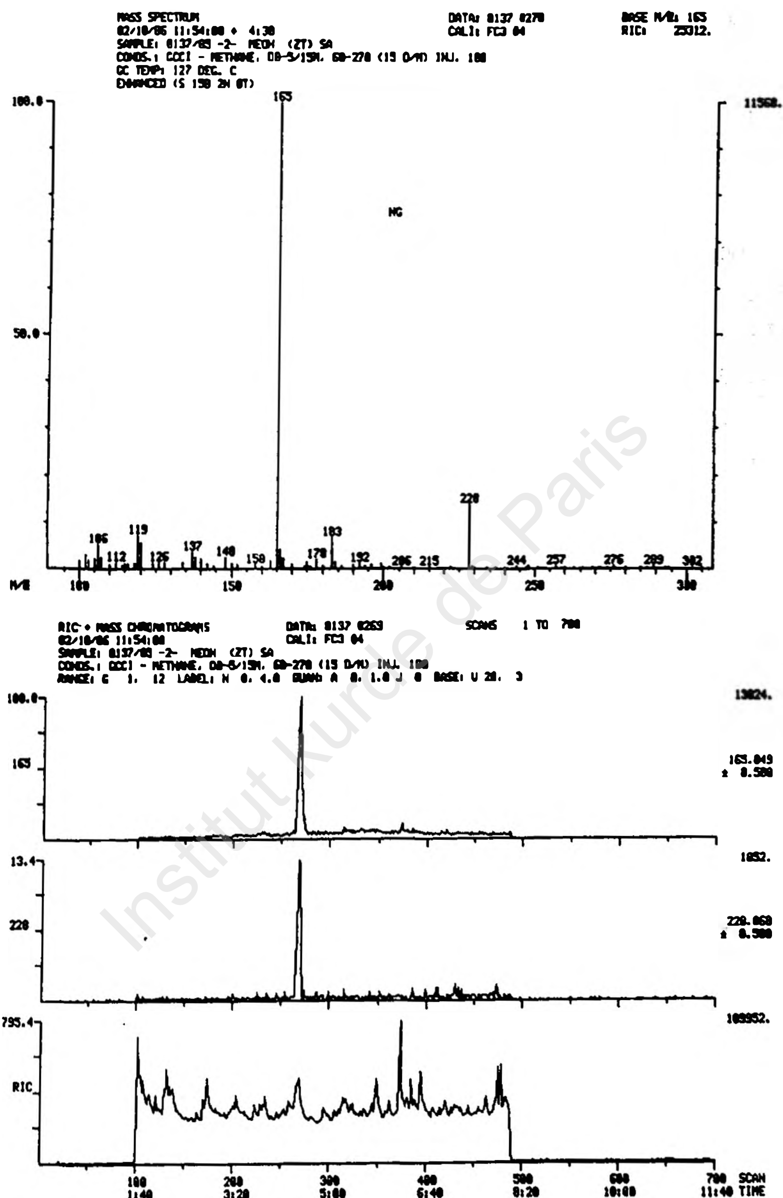

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FIG. 9. — Total ion and mass chromatograms (CI-methane) of a post-explosion extract (case 8137/85)
and CI-methane mass spectrum of the peak emerging after 270 seconds, identified as NG.

tified by GC/CIMS. The main chromatographic peak in figure 10 was identified as ethyl centralite, a common stabilized in smokeless powders. Its presence indicated that the origin of the NG was smokeless powder rather than dynamite.

Smokeless powder is used by terrorists in Israel as the main charge in home-made improvised explosives. It is collected from ammunition and when suitably confined in a pipe it serves as an explosive charge. Compared with the unexploded propellant, the ratio between the amounts of ethyl centralite and NG

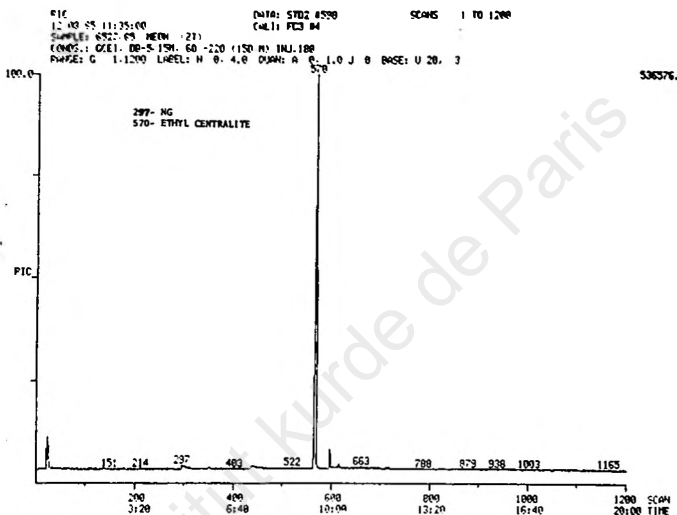

FIG. 10. — Total ion chromatogram of a post-explosion extract (case 6527/86). The peaks emerging after 297 and 570 seconds were identified as NG and ethyl centralite, respectively.

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in figure 10 seems unusually large, even after allowing for possible thermal decomposition of NG and lower sensitivity in its detection by total ion current. A possible explanation could be that NG decomposes during the explosion (or burning) of smokeless powder while ethyl centralite does not.

It is evident from figure 2 that the sensitivity of GC/EIMS for PETN is not as high as for the other explosives studied. As stated above, we use GC/CIMS to confirm the presence of nitrate esters and this — under our working conditions — lowers the sensitivity even more. As a result our record in confirming TLC results for PETN by GC/MS has not been satisfactory, although we have had some success (7). In severel cases where GC/MS failed to confirm TLC results indicating PETN we successfully obtained

its mass spectrum by direct probe insertion. In these cases cleaning the post-explosion extract on a chromatographic column is a necessary prequisite. The CI-methane spectrum of an extract from debris left after a safe in a diamond-polishing factory had been blown up is shown in figure 11. The extract was cleaned on a florisil column, concentrated and then introduced directly into the mass spectrometer via the direct probe. PETN was conclusively identified by the resulting CI-methane spectrum. The ions at m/z 317 and m/z 254 correspond to $[M+H]^+$ and

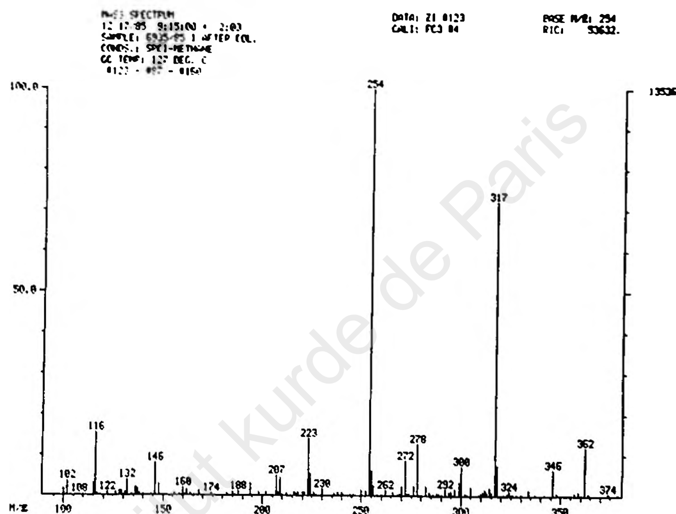

FIG. 11. — CI-methane mass spectrum (using direct probe) of post-explosion extract (case 6935/85). PETN was identified.

$[M+H—HNO_3]^+$, respectively. Two unusual adduct ions are observed at m/z 346 and m/z 362, corresponding to $[M+NO]^+$ and $[M+NO_2]^+$, respectively (5-7).

The chromatographic peak attributed to tetryl in figure 1 gave an EI spectrum (fig. 12) which was different from previously published (6, 8, 9). EI mass spectra of tetryl. The spectrum obtained by GC/CIMS also differed from the previously published (5, 6, 10) spectra and indicated a possible molecular weight of 242. As the previously reported spectra were obtained by using a direct probe, we assumed that tetryl decomposed during its GC analysis. The EI and CI-methane mass spectra of the product emerging from the column during the GC/MS of tetryl could correspond to N-methylpicramide. We prepared N-methylpicramide

by hydrolysing tetryl (11). Its EI and CI mass spectra were similar to those obtained by GC/MS of tetryl, except that the synthesized product contained also some picramide. The hydrolysis of tetryl during the GC analysis could take place at the injector, which

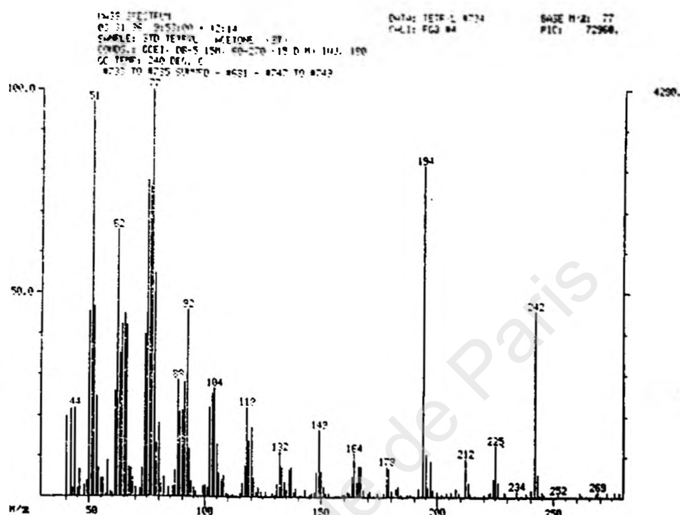

FIG. 12. — EI mass spectrum of the chromatographic peak emerging in GC/MS of tetryl. It was identified as N-methylpicramide.

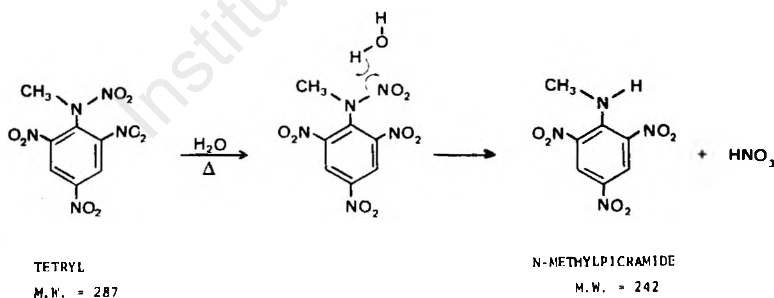

FIG. 13. — The hydrolysis of tetryl to N-methylpicramide.

was held at relatively high temperatures. The hydrolysis scheme is shown in figure 13.

Thus, the identification of N-methylpicramide by GC/MS, combined with TLC results indicating tetryl could serve as evidence for the presence of tetryl. Figure 14 shows the total ion chro-

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matogram of a post-explosion extract from the residues of a bomb which had been detonated on a roadside in Lebanon. The N-methylpicramide, identified by its EI mass spectrum, indicated the original presence of tetryl. RDX and TNT were also identified in this extract.

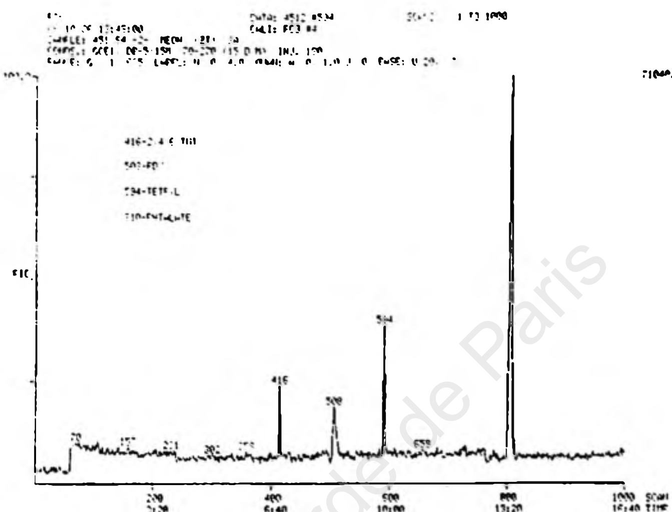

FIG. 14. — Total ion chromatogram of a post-explosion extract (case 451/84). The peak emerging after 594 seconds was identified as N-methylpicramide, indicating the presence of tetryl. The peaks emerging after 416 and 508 seconds were identified as TNT and RDX, respectively.

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CONCLUSIONS.

The « real life » examples discussed above prove that although some explosives are thermally labile, GC/MS is an excellent practical method for a reliable post-explosion identification of explosives.

Acknowledgements. — We would like to thank Dr. A. Basch for his advice concerning the decomposition of tetryl and Ms. S. Abramovich-Bar and Mr. Y. Bamberger for doing TLC analyses.

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Posters

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Epidemiological study of poisoning in the area of Bloemfontein, Republic of South Africa

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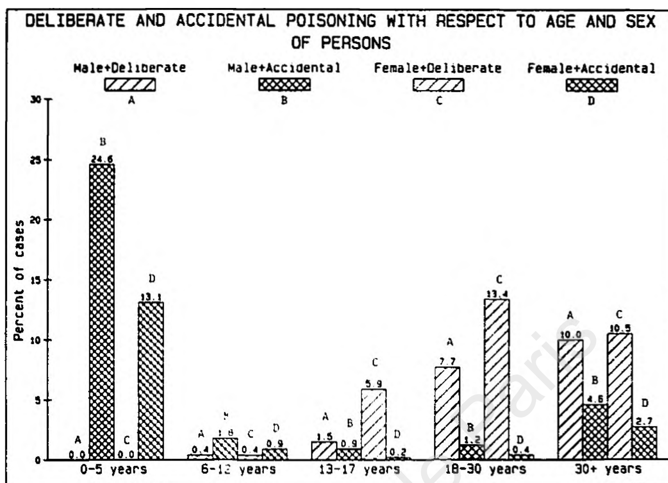
NUMBER OF POISONINGS.

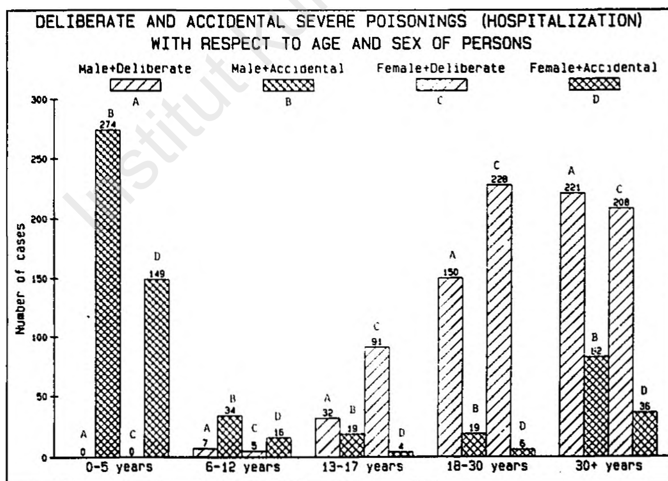
Details of cases of poisoning recorded by the poison control centre at Bloemfontein during the period 1 January 1980-31 December 1985.

	Deliberate poisoning	*Accidental poisoning*	*Total*
Male	688	1141	1829
Female	1047	596	1643
Total	1735	1737	3472

Severe poisonings (persons hospitalized).

	Deliberate poisoning	*Accidental poisoning*	*Total*
Male	410	428	838
Female	532	211	743
Total	942	639	1581

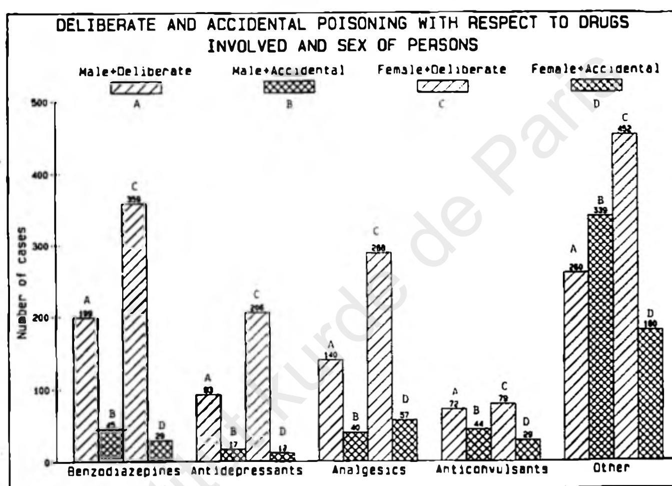

DELIBERATE AND ACCIDENTAL POISONING WITH RESPECT TO AGE AND SEX OF PERSONS
Male+Deliberate
A
Male+Accidental
B
Female+Deliberate
C
Female+Accidental
D
Percent of cases
0-5 years
6-12 years
13-17 years
18-30 years
30+ years

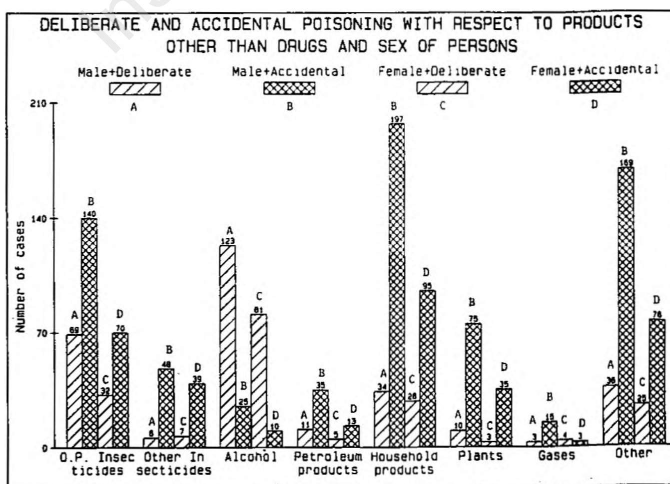

DELIBERATE AND ACCIDENTAL SEVERE POISONINGS (HOSPITALIZATION) WITH RESPECT TO AGE AND SEX OF PERSONS
Male+Deliberate
A
Male+Accidental
B
Female+Deliberate
C
Female+Accidental
D
Number of cases
0-5 years
6-12 years
13-17 years
18-30 years
30+ years

POISONS INVOLVED.

Details of poisons involved :

	Deliberate poisoning	*Accidental poisoning*	*Total*
Drugs	48.2 %	17.8 %	66.0 %
Other than drugs	10.7 %	23.3 %	34.0 %
Total	58.9 %	41.1 %	





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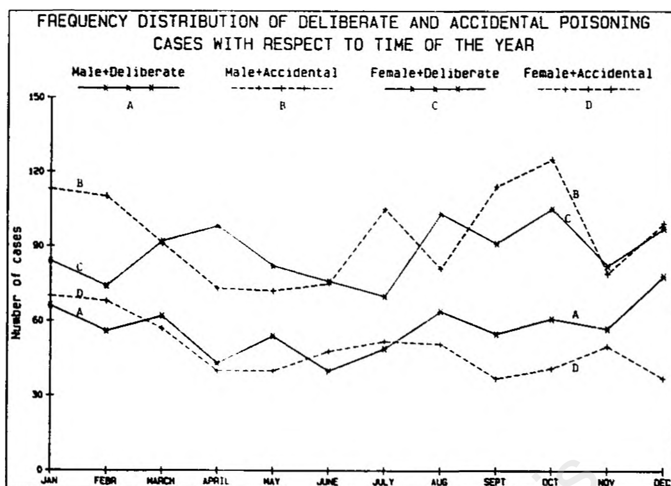

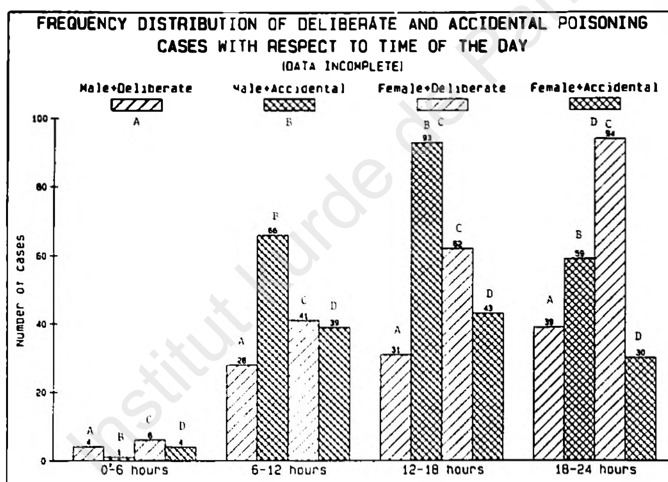

COMPARISON.

Comparison of the incidence of some agents involved in this study with that of two previous studies.

	This study (1980-1985)	Previous studies (1977-1979)	Previous studies (1970-1976)
Drugs	66.0	72.0	48.0
Insecticides (including organophosphates)	9.1	16.0	5.3
Petroleum products	1.4	3.0	11.0
Household products	7.9	1.5	4.7
Gases	0.6	1.7	4.1

Screening of organophosphorous pesticides : A case of fenitrothion and malathion intoxication

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SUMMARY.

A 39 years old male asthma sufferer was found semicomatose in his house, and taken to a hospital. Organophosphorous or carbamate insecticide poisoning was suspected, and these chemicals were screened in the urine samples collected from the patient at 8 days after admission to the first hospital by gas chromatography and gas chromatography-mass spectrometry.

A metabolite of fenitrothion, 3-methyl-4-nitrophenol (MNP), was detected in the urine sample collected from the patient at 8 days after admission, and the concentrations of fenitrothion and malathion were 16 ng/g and 9 ng/g, respectively in the blood sample collected at 17 days after admission. The concentrations of MNP in urine and the serum cholinesterase activities (normal range 175-440 IU) were 22.0 μg/g and 35 IU at 16 days, 12.5 μg/g and 86 IU at 18 days, 5.3 μg/g and 112 IU at 20 days, and 1.0 μg/g and 171 IU at 24 days after admission.

It was suggested that the patient ingested a pesticide containing fenitrothion and malathion. In fact a bottle of Sumison® containing 35 % of fenitrothion and 15 % of malathion was found in the patient's house, and about 30 ml of Sumison® was missing.

INTRODUCTION.

In acute poisoning cases, it is a great help in treatment to know what chemical has entered the patient's body. However, it is often difficult to know in this suicide cases. In the present case,

organophosphorous or carbamate pesticide poisoning was suspected from the symptoms on the eighth day after admission to hospital. Fenitrothion and its metabolite and malathion where detected by our screening method using gas chromatography and gas chromatography-mass spectrometry (1-8).

Fenitrothion [sumithion, o,o-dimethyl-o-(3-methyl-4-nitrophenol) phosphothioate] and malathion [o,o-dimethyl S-(1, 2-dicarbethoxyethyl)dithiophosphate] are low toxic organophosphorous pesticides for mammals (9), and few human poisoning cases by these pesticides have been reported (10-12). The case history, the screening method, and the analytical results are presented .

CASE HISTORY.

A 39 years old office worker, suffering from asthma, was found semicomatose in his house on the night of March 16, 1986, and taken to a nearby hospital. Since the clouding of consciousness did not subside, he was sent to the Intensive Care Unit of our University Hospital four days following admission. Only symptomatical treatment was performed as the cause of the symptoms was not diagnosed. On the eighth day after admission, it was found that the pupil diameter was 1.0-1.5 mm, and that the serum cholinesterase activity was very low, 8 IU (normal range 175-440 IU). At this time, organophosphorous or carbamate insecticide poisoning was suspected, and treated with atropine. However, the patient insisted that he did not ingest any chemicals. The patient recovered after 25 days of admission and treatment, and left the hospital.

EXPERIMENTAL.

Materials and chemicals.

Samples : the patient's blood and urine collected from the eighth day following admission were kept frozen at —20°C until analysis.

Blank : drug free urine was collected from a healthy living man, and drug free blood from a cadaver at autopsy.

Chemicals : Fenitrothion (Sumithion®, Gasukuro, kogyo), malathion (Malason®, Sankyo), 3-methyl-4-nitrophenol (MNP, Aldrich Chemical), p-nitrophenol (Wako Chemical), Trimethylchlorsilane (Pierce Chemical), Hexamethyldisilazane (Wako Chemical), Extre-

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lut® (Merck, Art 11738) and solvents (reagent grade, Wako Chemical) were used.

TMS reagent : Trimethylchlorsilane : Hexamethyldisilazane = 1 : 9.

Extrelut® column : a 10 × 1 cm (i.d.) glass tube packed with 1.0 g of anhydrous sodium sulfate on the bottom layer and 1.2 g of Extrelut® on the top layer was used.

Gas chromatography (GC).

The gas chromatograph used was a Shimadzu GC-R1A equipped with a detector having a two mode, hydrogen flame ionization detector (FID) and a flame photometric detector (FPD). The column was a 1.2 m × 0.26 cm (i.d.) glass tube packed with 3 w/w % SE-30 on Gas Chrom Q (60-80 mesh). The temperatures of the injection port, column and detector were set at 280°C, 170°C and 280°C, respectively. Nitrogen with a flow rate of 40 ml/min. was used as a carrier gas.

Gas chromatography-mass spectrometry (GC/MS).

The instrument used was a Shimadzu GCMS 6020. The column was a 1 m × 0.26 cm (i.d.) glass tube packed with 3 w/w % SE-30 on Gas Chrom Q (60-80 mesh). The column temperature was programmed from 100°C to 280°C with a rate of 8°C/min. The temperatures of the injection port, separator and ion source were set at 280°C, 270°C and 270°C, respectively. The accelerating voltage was 3.5 kV, and ionization voltage was set at 70 eV for electron impact ionization (EI) and 150 eV for chemical ionization (CI). Helium with a flow rate of 20 ml/min. was used as a carrier gas, and isobutane was used as a reagent gas.

Chemical ionization mass fragmentography (CIMF).

The instrument and the conditions were the same as GC/MS except the column temperature.

When MNP was analyzed, the column temperature was set at 150°C. The monitoring ions used for detecting NMP and p-nitrophenol (p-NP), an internal standard, were m/z 226 and m/z 212.

When fenitrothion and malathion were analyzed, the column temperature was set at 200°C. The monitoring ions used for detecting

fenitrothion and malathion were m/z 278 and m/z 331, respectively.

Preparation for analysis of MNP in urine.

The urine sample was diluted from 2 to 50 times with water. The diluted urine (1.5 g), 20 μg of p-nitrophenol as an internal standard and 0.2 ml of concentrated hydrochloride solution were mixed in a test tube, and the test tube was heated for one hour in boiling water. After cooling, the solution was neutralized with sodium hydroxide, and poured into an Extrelut® column. The column was left standing for 20 min. at room temperature, and 10 ml of ethyl acetate was passed through the column. After the eluate was concentrated to about 40 μl, 40 μl of trimethylsilyl deriving reagent was added. One μl of the mixture was analyzed by GC/MS or CIMF.

Preparation for analysis of fenitrothion and malathion in blood.

The blood sample (1.0 g) was diluted with 1.0 g of water and poured into an Extrelut® column. After the column was left standing for 20 min. at room temperature, 10 ml of n-hexane was passed through the column. The eluate was evaporated under reduced pressure, the residue was dissolved in 40 μl of ethyl acetate, and 2 μl of the solution was analyzed by GC/MS.

Determination of the serum cholinesterase activity.

The serum cholinesterase activity was determined by the method developed by Okabe *et al.* (13).

RESULTS AND DISCUSSION.

Screening.

The first sample analyzed was urine collected at the eighth day after admission. As organophosphorous or carbamate insecticide poisoning was suspected, these compounds were screened by FPD-GC and FID-GC, but no organophosphorous and carbamate insecticides were detected. So, the metabolites of these pesticides were screened by FID-GC and FPD-GC after acid hydro-

lysis. A metabolite of fenitrothion, 3-methyl-4-nitrophenol, was detected by FID-GC, and confirmed by GC/MS (fig. 1 and 2).

Since a metabolite of fenitrothion was detected, fenitrothion in the blood sample collected on the 17th day after admission was

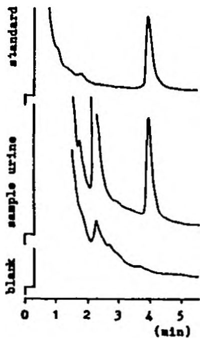

Fig.1: FID-gas chromatograms of MNP

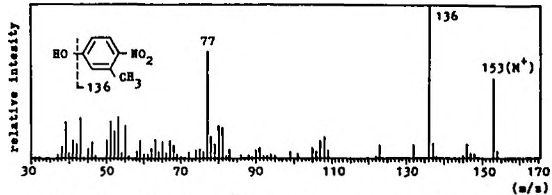

Fig.2: Electron impact mass spectrum of 3-methyl-4-nitrophenol in urine sample

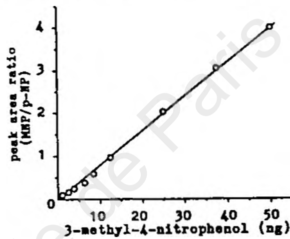

Fig.3: Calibration of MNP-TMS derivative.

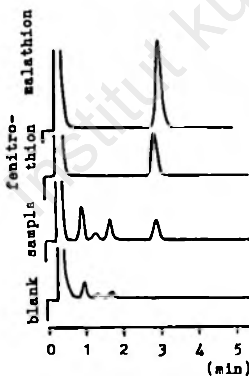

Fig.4: FPD-Gas chromatograms of fenitrothion(2ng) and malathion(5ng),and blood sample at 17 days after ingestion

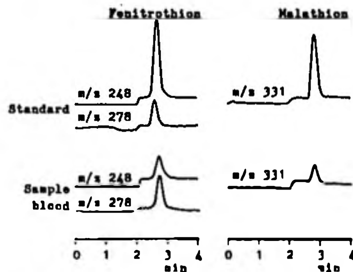

Fig.5: Chemical ionization mass fragmentography of fenitrothion and malathion.

screened by FPD-GC, and fenitrothion and malathion were detected. Since the amount of fenitrothion and malathion were so little, CIMF was used to confirm fenitrothion and malathion. Fenitrothion and malathion were observed as a peak at 3.0 min. and a 3.1 min., respectively (fig. 4).

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Quantitative analysis.

Fenitrothion or malathion concentration was determined by the relation between the peak area and the amount.

Fenitrothion and malathion in the blood sample collected on the 17th day after the admission were quantitatively determined by CIMF, and the concentrations of fenitrothion and malathion were 16 ng/g and 9 ng/g, respectively.

The calibration curve of MNP was obtained by plotting the peak area ratios of m/z 226 (MH^+) of MNP-TMS derivative to m/z 212

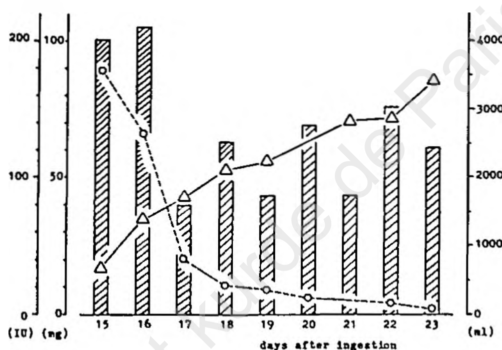

Fig.5: Relation of exction of 3-methyl-4-nitro phenol and cholinesterase activity. [MNP concentration in daily whole urine (mg,--O--), cholinesterase activity(IU,-△-) and total amount of daily urine(ml, ▨)]

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(MH^+) of the internal standard (p-NP-TMS derivative) against the amount of MNP from 1.2 ng to 50 ng. It was a straight line through the origin as shown in figure 3. A correlation coefficient of 0.999 was obtained.

The MNP concentrations (μg/g) and cholinesterase activities (IU) were 22.0 and 35 at 16 days, 15.6 and 69 at 17 days, 12.5 and 86 at 18 days, 4.2 and 106 at 19 days, 5.3 and 112 at 20 days, 1.4 and 145 at 23 days, and 1.0 and 171 at 24 days, respectively after admission. The relation of the MNP amount excreted in urine (mg/day), the cholinesterase activity (IU) and the amount of daily urine (ml) are shown in figure 5. The serum cholinesterase activity increased with the decrease of daily amount of MNP excreted in urine.

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Effect of radiation in a single exposure on rabbits

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INTRODUCTION.

Radiation Toxicology is a specialized area of Toxicology of considerable breadth and depth.

Ionizing radiation constitutes a useful therapeutic and experimental « tool », but at the same time a dangerous one. The increase in availability of sources of radiation with modern advancements in the field of Nuclear energy, makes possible the inadvertent exposure of many more people than ever possible, when X-ray tubes and radium constituted the principal known sources of radiation.

In the event of an Atomic War, entire populations will be subjected to radiation hazards.

Radioactive materials deposited in the bodies of workers in the Atomic energy industry, may cause many disabilities.

The absorption of the energy of ionizing radiation in cells, involves ionization of atoms and the production of ions within the cells.

Although the exact mechanism of action of ionizing radiation is not known, radiation injury is considered to be related in some way to the production of ions within the cell.

AIM OF THE WORK.

The aim of this work is throw a beam of light on the effect of small dose whole body irradiation, which is reflected on the serum level of : Cortisol, Total proteins, Glucose and Insulin.

MATERIALS AND METHODS.

Rabbits were the animals chosen for the study, they were brought from the farm of the Egyptian Organization for Vaccine and Biological products in Helwan city - Cairo.

All the animals were in a good healthy condition and they were looked-after closely for a week before using them for the experiment.

Rabbits were of native breed, nearly same weight about one kilo and 400 grams each.

Sex of choice was « male ».

A total of 60 male rabbits were used for this study, they were divided into 6 groups (10 each).

Animals were kept in iron cages 18 × 24 × 16 cm, 2 animals/cage, in a well ventilated room, they were fed a balanced ration.

1. Radiation exposure.

The first group was used as a control (not exposed to radiation).

The other 5 groups were exposed together to radiation, in a dose of : 500 r/50 cm, dose rate 96.2 rad/minute.

Irradiation of animals were performed at Kasr El Aini Center of radiation, Faculty of Medicine, Cairo University.

The machine used in the study is of the Siemens stabilipan type (300 kv., 14.8 mA. 1 cu. filter and 2 cu. HVT).

Focus treating distance was 50 cm, dose rate 96.2 rad/minute.

All animals, either the irradiated or the control were continuously observed for any change in behaviour or movement.

Blood samples were collected from each ; irradiated and control rabbits as follows :

a) After 2 hours from the second group.

b) After 24 hours from the third group.

c) After 7 days from the fourth group.

d) After 14 days from the fifth group.

e) After 30 days from the sixth group.

2. Serum from blood samples was taken for estimation of cortisol according to Kley and Kruskemper 1975, by RIA technique.

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3. Then, Insulin estimation was done according to Woodhead *et al.* (1974), by RIA technique.

4. Total proteins were estimated according to Puitet method, Reinhold, 1953.

5. Glucose estimation was done according to Torlotin, 1966.

RESULTS

TABLE I

The level of serum glucose in relation to time (mgm / 100 ml serum)

Number	*Control*	*After irradiation*				
		2 hours	*24 hours*	*7 days*	*14 days*	*30 days*
1	100.2	130.3	122.3	120.2	115.1	110.3
2	99.3	122.4	120.3	121.3	117.3	100.3
3	101.6	125.0	121.2	125.6	119.6	100.2
4	100.6	128.5	120.3	128.5	120.1	112.0
5	98.3	132.5	120.5	130.3	114.2	113.6
6	96.5	126.7	126.2	122.2	113.6	110.3
7	97.6	123.2	128.3	126.3	116.5	102.7
8	99.8	132.6	121.5	128.2	115.5	100.2
9	102.6	129.8	122.5	126.5	116.4	105.3
10	103.5	123.9	125.6	124.7	120.3	105.4
M	100.0	127.5	122.9	125.4	116.9	106.0
SE	± 2.08	± 3.77	± 3	± 3.30	± 2.43	± 5.19

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TABLE II

The level of serum cortisol in relation to time (mgm / 100 ml)

Number	*Control*	*After irradiation*				
		2 hours	*24 hours*	*7 days*	*14 days*	*30 days*
1	2.51	7.29	7.23	5.18	4.23	3.12
2	1.38	6.48	6.44	6.10	4.31	2.58
3	1.50	4.36	5.32	4.86	4.60	4.12
4	2.20	5.88	5.20	4.08	3.30	4.02
5	2.32	10.04	8.30	5.04	3.60	2.89
6	1.68	8.45	8.50	7.12	6.20	2.64
7	1.98	9.12	9.30	3.88	5.00	2.50
8	2.02	8.14	7.60	4.44	3.30	3.02
9	1.76	6.98	7.20	5.62	3.20	2.10
10	1.48	8.88	7.90	3.70	3.30	1.98
M	1.88	7.57	7.30	5.00	4.10	2.90
SE	± 0.38	± 1.70	± 1.33	± 1.03	± 0.97	± 0.72

TABLE III

The level of serum insulin in relation to time (mgm / 100 ml)

Number	Control	After Irradiation				
		2 hours	24 hours	7 days	14 days	30 days
1	—	7.3	7.0	6.1	6.1	—
2	5.6	7.0	7.0	6.3	6.1	5.6
3	5.4	8.0	7.2	6.0	6.0	5.2
4	5.2	8.5	7.3	6.2	5.4	5.1
5	5.6	7.9	7.8	6.5	5.5	5.3
6	5.0	8.0	8.0	5.9	5.9	5.2
7	5.1	7.9	8.2	6.3	5.4	5.2
8	5.3	7.3	8.3	6.3	5.0	5.1
9	5.2	7.6	7.0	6.2	5.2	5.0
10	5.6	6.0	6.3	6.3	5.3	5.0
M	5.3	7.6	7.4	6.2	5.5	5.2
SE	± 0.23	± 0.698	± 0.64	± 0.173	± 0.40	± 0.18

TABLE IV

The level of serum total proteins in relation to time (mgm / 100 ml)

Number	Control	After Irradiation				
		2 hours	24 hours	7 days	14 days	30 days
1	5.5	5.8	7.8	7.7	7.2	5.2
2	5.3	5.9	7.9	7.6	7.3	5.3
3	5.4	5.7	7.8	7.3	7.7	5.4
4	5.6	5.4	7.7	7.2	7.4	5.5
5	5.1	5.6	8.2	7.1	7.2	5.6
6	5.4	5.2	7.3	6.9	7.1	5.7
7	5.3	5.1	8.3	7.3	7.9	5.5
8	5.2	5.3	8.9	7.5	7.6	5.3
9	5.0	5.6	8.8	7.6	7.9	5.6
10	5.6	5.6	8.7	7.7	7.3	5.9
M	5.3	5.5	8.1	7.4	7.5	5.5
SE	± 0.20	± 0.26	± 0.53	± 0.27	± 0.30	± 0.20

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DISCUSSION.

The sources of exposure of man and animals to ionizing radiation can be broken down into four major groups :

1. Natural sources of irradiation both external and internal.

2. Medical sources such as diagnostic on therapeutic X-ray irradiation and radiopharmaceuticals.

3. Nuclear reactors and nuclear weapons ; and

4. Other sources, such as industrial X-ray machines.

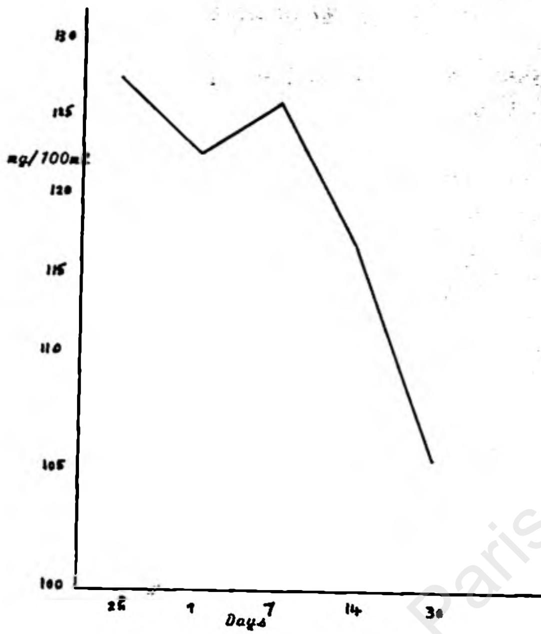

FIG. 1. — Glucose level in relation to time.

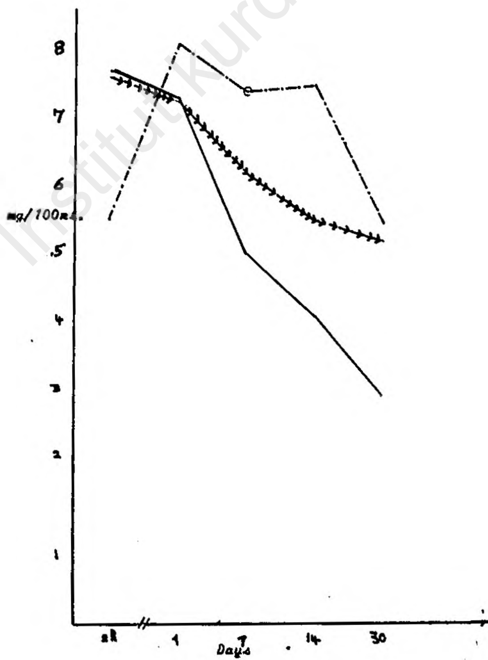

FIG. 2. — Showing the levels of :

Cortisol : ———— Total proteins : — . — . — Insulin : →→→→

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In the present study, the choice of the dose at 500 r/50 cm, dose rate 96.2 rad/minute, for one exposure, agrees with Mclean (1973).

He explained that the body irradiation at doses from 500 to 1000 rads will cause serious effects, not immediately apparent.

In our study we noticed that the levels of Cortisol, Insulin ; and Glucose were highly increased after 2 hours from exposure to irradiation ; and in this finding we agree with the study of many investigators as : Mole (1956), Gould (1959), Langham (1967), Rubin and Casarett (1968) and Berdjis (1971).

In case of the samples collected after 24 hours from irradiation we noticed that the rise in the levels of serum Cortisol, Insulin, and Glucose continued, than the control ; but with as lower rate than the former period, i.e. after 2 hours.

Also in these samples we noticed the start in the rise in the level of Total proteins ; and this finding could be explained by the findings of Shields (1944), who mentioned that the histology of lesions included by radiation is the result of several diverse changes. Some changes were cellular as necrosis alteration of cellular function, some are intercellular ; and some are vascular nuclear chromation in general is affected more than is the cytoplasm ; and due to its relative importance, nuclear injury has profound effect on the cell as a whole, concerning the cytoplasmic alterations. They are primarily due to nuclear injury or direct effects of irradiation on the cytoplasm itself.

In the samples obtained after 7, 14, 30 days the values of insulin cortisol and proteins started to decrease than in the last 2 periods ; but the glucose level increased, relatively, then decreased again to a level lower than the control.

From this study we notice also that all readings were high in the first 2 hours, after irradiation (except that of protein, which started to increase after 24 hours) and continued to increase till 24 hours, then it started to decrease after 7, 14 and 30 days to reach approximately the control. This is explained by the fact that the effect of irradiation stays till 30 days, but in less degree than in the first 2 periods i.e. after 2 and 24 hours.

This finding coincides with the findings of Langham (1967).

This could be explained by the fact that radiation dose is a function of the effective retention half time, the energy released in the tissues, the amount of radioactivity initially introduced ; and the mass of the organ ; and this finding is nearly similar to the studies of the researchers in the field of nuclear energy as (Engelstab *et al.*, 1932 ; Dempsy, 1940 ; Patt *et al.*, 1947 ; Gould *et al.*, 1956 ; Mole, 1956 and Burns *et al.*, 1959).

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Organochlorine pesticides in human adipose tissue collected in Ankara (Turkey) 1984-1985

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The accumulation of organochlorine (OC) pesticide residues in human tissues has been the subject of a number of studies reported during the past two decades. Most interest has centered on DDT and its metabolites and BHC isomers.

The national mean concentrations of OC pesticides which occur at low levels in human adipose tissue have been taken as an index of the average overall degree of exposure of the population to such substances (Abbott *et al.*, 1985).

Usage of OC pesticides started in Turkey in 1945. This paper reports the levels of OC in human adipose tissue in Ankara (1984/1985) and determines the possible trends of the dissappearance of OC whose use were restricted about 8 years ago. The amount of OC pesticides used in Turkey between 1976 to 1983 were 2219, 2947, 2336, 764, 744, 701, 840 and 487 tons respectively. We, herein, compare the levels of some compounds (especially pp'DDE, pp'DDT and Σ DDT) with the previous study done in 1976 (Kayaalp *et al.*, 1979).

MATERIALS AND METHODS.

Human adipose tissue samples were taken during surgical operations in Hacettepe University Hospital from 48 patients. The ages of the sample donors varied from 18 to 72 years, the mean age being 38. The adipose tissue samples were deep-frozen (—20°C) immediately until used in the analysis.

Standard α-BHC, β-BHC, γ-BHC, pp'DDE and pp'DDT were obtained from Environmental Protection Agency (EPA). The pesticide

standard mixture was supplied from Swedish National Food Administration.

100 mg samples were ground with anhydrous sodium sulphate by using a tissue grinder (Sherma *et al.*, 1983) and extracted three times with 4 ml, 2 ml, 2 ml portions of n-hexane (Wickström *et al.*, 1983). The combined n-hexane extract was evaporated to dryness to determine extractable fat content. Using 8 ml n-hexane as the solvent and adding op'DDD as internal standard, the sample was cleaned up with the aid of concentrated sulfuric acid (Murphy, 1972). The extracts were concentrated to appropriate volumes and injected to ECD-GC glass columns packed with 1.5 % OV-17 + 1.95 % OV-202 on Chromosorb WHP (80-100 mesh) for the quantification of the OC levels. The residue levels of OC were calculated by measuring peak heights. Confirmation was routinely carried out using different column (4 % SE-30 + 6 % OV-210 on Chromosorb WHP).

RESULTS AND DISCUSSION.

The means of OC pesticides residues in adipose tissue of Ankara citizens are presented in table I.

Compared to a previous study (Kayaalp *et al.*, 1979) the average levels of pp'DDE, pp'DDT and Σ DDT are lower as indicated in table II.

TABLE I

Organochlorine pesticide residues in human adipose tissue in Ankara 1984-1985 (mean ± SD ppm on extractable fat basis)

Total n : 48	*α-BHC*	*β-BHC*	*Σ BHC*	*pp'DDE*	*pp'DDT*	*Σ DDT*
$\bar{X}$	0.19	1.52	1.72	5.83	0.62	7.12
S.D	0.20	0.79	0.83	3.31	0.50	4.10
Minimum	0.04	0.48	0.54	1.25	n.d.	1.69
Maximum	1.02	5.67	5.78	19.20	3.01	24.42

n.d. = not determined.

TABLE II

Comparison of OC pesticide residues in human adipose tissue in Ankara

Compound	*mg/kg extractable fat*	
	Kayaalp et al. *n : 41*	*Present study n : 48*
pp'DDE	10.2	5.83
pp'DDT	3.2	0.62
Σ DDT	14.6	7.12
DDE/DDT	3.19	9.40

In addition to the reduced mean levels of OC pesticides (during 1976-1985) it should be noted that the DDE/DDT ratio was increased from 3.19 to 9.40. It is interesting that the ratio of DDE/DDT increases in restricted countries and it is low at places, like India where it is still used (table III).

TABLE III

DDE/DDT ratios in human adipose tissues collected in different countries

Country	Year	pp'DDE	pp'DDT	DDE/DDT	Reference
United States	1955	12.5	7.4	1.69	Hayes et al (1956)
	1961-62	8.6	4.0	1.95	Quinby et al (1965)
	1964	5.1	2.5	1.97	Zavon et al (1965)
	1978	5.91	0.81	7.30	Barquet et al (1981)
Canada	1959-60	3.3	1.6	2.07	Read and McKinley (1961)
	1973	3.23	0.90	3.57	Ritcey et al (1973)
	1976	1.72	0.31	5.53	Mes et al (1982)
	1979-81	3.26	0.16	20.3	Williams et al (1984)
India	1964	11.6	13.5	0.85	Dale et al (1965)
	1982	9.39	9.66	0.97	Ramachandran et al (1984)
United Kingdom	1963-64	2.0	1.1	1.82	Egan et al (1965)
	1965-67	2.0	0.78	2.56	Abbott et al (1968)
	1969-71	1.8	0.52	3.46	Abbott et al (1972)
	1976-77	2.1	0.21	10.0	Abbott et al (1981)
	1982-83	1.3	0.11	11.81	Abbott et al (1985)
Yugoslavia	1976	6.02	0.92	6.54	Jan and Zelenko (1978)
Turkey	1976	10.2	3.2	3.19	Kayaalp et al (1979)
	1984-85	5.83	0.62	9.40	Present Study

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TABLE IV

The residue levels of OC pesticides in human adipose tissue by age

Age groups (years)	Residue levels (mg/kg) mean ± S.D					
	α-BHC	β-BHC	Σ BHC	pp'DDE	pp'DDT	Σ DDT
20-30 n : 6 $\bar{x}$	0.08	0.80	0.87	3.26	0.28	3.91
S.D	0.02	0.29	0.30	1.59	0.08	1.82
31-40 n : 19 $\bar{x}$	0.19	1.53	1.71	5.67	0.62	6.94
S.D	0.23	0.57	0.60	2.59	0.79	3.15
41 + n : 17 $\bar{x}$	0.27	1.78	2.05	6.77	0.72	8.27
S.D	0.20	1.06	1.06	4.26	0.71	5.36

The residue levels of OC pesticides in human adipose tissue by age are shown in table IV.

All OC compound concentrations in adipose tissue increased with age. Except α-BHC levels, there were significant differences in the levels of OC pesticide residues between the 20-30 year and 31-40 year age groups respectively ($P < 0.05$). But there were no significant differences in the levels of OC pesticide residues between the 31-40 year age and 41 + year age groups. This

may be explained by the fact that, considering the usage of OC pesticides starting from 1945, there was no significant difference in the exposure period between the latter two age groups.

In conclusion, OC pesticides still have risk potential in terms of environmental pollution although the levels of these compounds have been reduced by restrictions in Turkey.

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Uptake of metals by some selected mushroom species

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SUMMARY.

Nineteen heavy and essential metals were determined in wild growing mushrooms collected in rural areas (Belgium and Luxemburg), supposedly free of industrial air pollution. The analytical methods used were flame atomic absorption (for zinc), Zeeman graphite furnace atomic absorption (for thallium, cadmium, copper, lead, nickel, silver, cobalt, vanadium, manganese, molybdenum, strontium and cesium), hydride atomic absorption (for bismuth, selenium, antimony, arsenic and tin), and cold vapour atomic absorption with amalgamation (for mercury). The values were given in ppb of the dry weight, except for copper, lead, zinc, manganese, strontium and cesium, where the results were given in ppm. For all the elements studied there was a great difference in concentration between the different mushroom species analysed. As compared to the values found in the other mushroom species, some species showed exceptional high concentrations of zinc, manganese, arsenic and vanadium, which were not expectable except for vanadium as it was detected in Amanita muscaria, a mushroom found to contain amavadine, an organic vanadium compound. Distinctly detectable amounts of thallium were also measured in three of the mushroom species studied. The high lead concentration found in one of the mushrooms analysed was likely to be due to lead containing exhaust fumes from motor vehicles, as this mushroom was collected about 30 m away from a secundary road.

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INTRODUCTION.

According to literature heavy metals can be accumulated in the carpophores of mushrooms. However most information available is limited to data about mercury, cadmium, thallium, lead and selenium (1-6). According to Stijve and Besson 1976 (1), mercury values of up to 80 ppm were found in Agaricus macrosporus, while cadmium, lead and selenium concentrations of up to 75, 40 and 7.8 ppm respectively, were detected in other agaricus species.

In this study 19 elements were determined in a single specimen of 14 different mushroom species consisting of toxic, edible and unappetitizing ones. The samples were collected in rural areas, dried and analysed by AAS.

TABLE I

Some specifications about the different mushrooms analysed

Mushroom		Year and place of collection	
Number	Species		
1	Paxillus involutus	1984	Clervaux (L)
2	Laccaria laccata var. amethystina	1984	Nazareth (B)
3	Lepista nuda	1983	Nazareth (B)
4	Collybia butyracea	1984	Clervaux (L)
5	Amanita muscaria	1984	Nazareth (B)
6	Amanita citrina	1984	Nazareth (B)
7	Amanita rubescens	1984	Nazareth (B)
8	Psathyrella candolleana	1985	Ghent (B)
9	Hypholoma fasciculare	1984	Nazareth (B)
10	Gymnopilus spectabilis	1985	Nazareth (B)
11	Gymnopilus penetrans	1984	Nazareth (B)
12	Lactarius camphoratus	1985	Nazareth (B)
13	Lactarius quietus	1985	Nazareth (B)
14	Lactarius hepaticus	1985	Nazareth (B)

(L) Luxemburg - (B) Belgium.

It was not the purpose of this study to make a statistical evaluation of the metal concentrations found. This investigation was just undertaken to obtain an idea about the concentration range of the different elements, with intent to continue the investigation on a broader scale at the same time determining all those elements in samples of the natural substrate collected on the place where the mushrooms were found.

Some specifications about the different mushrooms analysed are given in table I.

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METHODS.

All the reagents were Merck Suprapur. The mushrooms were first dried in a gentle stream of warm air ($< 40°C$) and further dried at 50°C (furnace) until constant weight. They were cooled in a dessicator before being pulverized and weighed.

Sample preparation.

As outlined in figure 1, known amounts of the dried and pulverized mushroom were subjected to both a low temperature wet

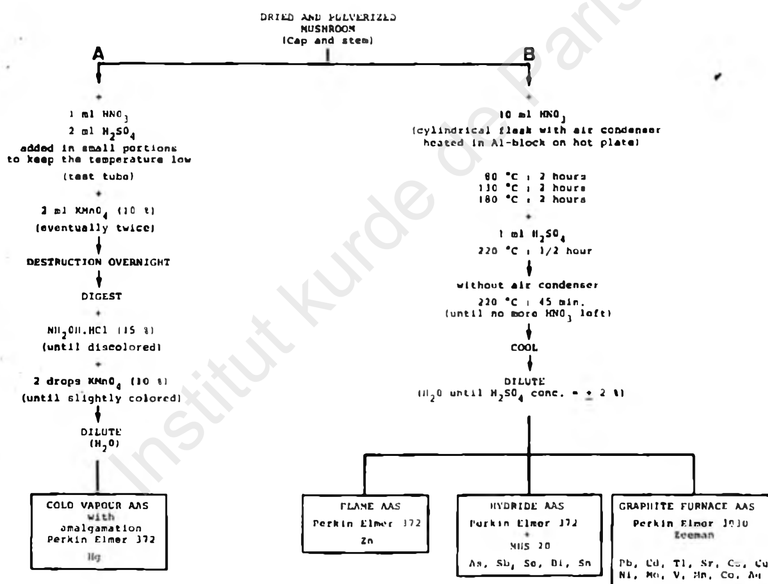

FIG. 1. — Sample preparation by wet oxidation using concentrated nitric and sulfuric acids in combination with potassium permanganate for mercury (A) and a high temperature digestion with concentrated nitric and sulfuric acids for the other elements (B).

oxidation for mercury by using concentrated nitric and sulfuric acids in combination with potassium permanganate as an oxidizing agent, and a high temperature wet digestion for the other elements in a cylindrical flask (pyrex) with air condenser using concentrated nitric and sulfuric acids.

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Atomic absorption spectrometry.

Determination of mercury (Hg).

A fraction (f.e. 1 ml) of the diluted sample digest A was further diluted with H_2O to about 10 ml in the polypropylene reaction flask of a Perkin Elmer MHS-20 Mercury/Hydride System.

The flask was then pushed onto the flange of the Analyser Assembly, which was installed in a Model 372 Perkin Elmer AA Spectro-photometer. After air was purged from the reaction vessel by means of high purity nitrogen, 3 % $NaBH_4$ in 1 % NaOH was dispensed into the flask during 10 seconds at a reduced purge gas flow-rate.

At the end of the reaction the system was purged free of mercury vapour which was transferred to the Amalgam Attachment, where it was quantitatively amalgamated on a gold/platinum gauze. The gauze was then rapidly heated to about 600°C in order to release the mercury, which was transported by the carrier gas to the quartz cell heated to 100°C. Purging and instrumental conditions were set according to the manufacturers recommendations.

Determination of Zinc (Zn).

An aliquot of the diluted sample digest B was directly aspirated in the air acetylene flame of a Model 372 Perkin Elmer AA Spectrophotometer using the instrument and flame settings as recommended by the manufacturer.

Determination of hydride forming elements.

For the determination of arsenic (As), antimony (Sb), selenium (Se), bismuth (Bi) and tin (Sn), a fraction (f.e. 1 or 2 ml) of the diluted sample digest B was further diluted with 1.5 % m/V H_2SO_4 to about 10 ml in the polypropylene flask of a Perkin Elmer MHS-20 Mercury/Hydride system. After the flask had been attached to the system, which was installed in a Model 372 Perkin Elmer AA Spectrophotometer, air was purged from the flask by means of high purity 99 % argon — 1 % oxygen as the purge gas. A solution of 3 % $NaBH_4$ in 1 % NaOH was subsequently allowed to flow into the reaction vessel for 4 seconds at a reduced purge gas flow-rate. At the end of the reaction time the addition of reductant was stopped and the purge gas flow-rate was increased again in order to purge the system free of sample

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vapour, flushing the volatile hydride into the heated quartz cell (900°), where it was decomposed and the absorption of the metal measured. Purging and instrumental conditions were also set according to the recommendations of the manufacturer.

Graphite furnace atomic absorption.

For determining all the other elements 10 to 25 μl of the diluted sample digest B was directly injected into the graphite tube of an HGA-600 Graphite Furnace of a Model Zeeman/3030 Perkin Elmer AA Spectrophotometer, and analysed according to the instrumental conditions recommended by the manufacturer and the temperature settings listed in table II.

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TABLE II

Temperature conditions for the graphite furnace

Element	*Furnace temperature °C*	*Time (sec.)*		*Element*	*Furnace temperature °C*	*Time (sec.)*	
		Ramp	*Hold*			*Ramp*	*Hold*
Co	100	2	20	Pb	110	2	15
	120	10	25		150	10	10
	1200	20	40		700	10	45
	2200	0	3		1700	0	3
	2650	1	6		2650	1	7
Cd	120	2	20	Tl	130	5	15
	160	5	5		170	5	30
	900	30	50		600	60	40
	2000	0	5		1400	0	5
	2650	1	7		2650	1	7
Sr	100	2	25	Cu	120	2	10
	120	10	5		150	5	10
	1300	10	10		600	40	20
	2600	0	5		2300	0	5
	2650	1	6		2650	1	7
V	100	2	20	Mn	120	10	15
	120	20	20		150	10	25
	1400	10	15		1300	20	25
	2700	0	7		2300	0	2
	2650	1	6		2650	1	10
Mo	100	2	20	Ag	120	2	30
	120	20	30		160	10	20
	1400	10	10		650	60	50
	2650	0	3		1600	0	5
	2650	1	5		2650	1	7
Cs	130	2	10	Ni	100	2	25
	160	5	20		120	10	10
	900	5	30		1000	1	50
	1900	0	2		2600	0	3
	2650	1	7		2650	1	7

Calculation.

The concentrations of all elements in the sample digest solutions were determined from standard calibration graphs, prepared for each element by analysing several dilutions of the respective Atomic Spectral Standard stock solutions (J.T. Baker Chemical Co., Phillipsburg, N.J. 08865), and plotting absorbance readings vs. concentrations.

RESULTS AND DISCUSSION.

Data illustrating the reproducibility of the proposed methodology for determining all the elements studied, except mercury, and obtained by analysing five different amounts of the dried and pulverized Amanita muscaria mushroom, are summarized in table III.

Reproducibility tests for mercury were not performed due to the lack of sample available.

For most of the elements the relative standard deviations (CV %) were rather high. For some of the elements they were

TABLE III

Precision of replicate analyses (n = 5) performed on different amounts of the dried and pulverized Amanita muscaria mushroom

	Weight mg (dry)	*Flame AAS*	*Hydride AAS*				
		Zn µg/g	*As* ng/g	*Se* ng/g	*Bi* ng/g	*Sn* ng/g	*Sb* ng/g
Det. 1	288	271	2,458	8,472	13	238	17
2	223	417	1,637	5,969	12	189	13
3	226	279	2,973	7,708	11	206	12
4	330	490	1,576	6,621	11	121	8
5	512	321	3,209	6,943	5	119	11
Mean		356	2,371	7,143	10	175	12
SD		95	749	972	3	53	3
CV %		26.7	32.0	13.6	31.3	30.2	27.2

	Weight mg (dry)	*Graphite furnace / Zeeman AAS*					
		Pb µg/g	*Mn* µg/g	*Cu* µg/g	*Sr* µg/g	*Cs* µg/g	*Cd* ng/g
Det. 1	288	2.15	22.7	49.0	0.46	0.729	20,104
2	223	2.13	18.0	35.2	0.34	0.404	24,798
3	226	1.73	15.8	42.9	0.46	0.633	23,274
4	330	1.87	17.5	36.4	0.38	0.839	16,758
5	512	2.20	21.3	56.4	0.36	0.535	26,826
Mean		2.02	19.1	44.0	0.40	0.628	22,352
SD		0.20	2.8	8.9	0.06	0.168	3,975
CV %		10.1	14.9	20.2	14.1	26.7	17.8

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	Weight mg (dry)	Graphite furnace / Zeeman AAS					
		Co ng/g	Ni ng/g	Ag ng/g	Tl ng/g	Mo ng/g	V ng/g
Det. 1 . .	288	97	326	8,194	97	97	103,646
2 . .	223	184	278	5,919	104	121	100,000
3 . .	226	88	240	7,920	66	125	105,752
4 . .	330	85	270	6,212	61	79	134,545
5 . .	512	176	316	9,443	39	57	122,266
Mean .		126	286	7,538	73	96	113,242
SD . .		50	35	1,465	27	29	14,652
CV % .		39.3	12.3	19.4	36.8	29.8	12.9

even very high. This was supposedly due to the unequal distribution of most of the elements studied throughout the carpophore of a mushroom, the poor homogeneity of most of the samples analysed, as it was very difficult to pulverize a dried mushroom in a mortar without creating metal contamination, and the low absolute concentration of some elements like cobalt (Co), molybdenum (Mo), thallium (Tl), tin (Sn), antimony (Sb) and bismuth (Bi). As a consequence it has to be recommended to perform a wet oxidation on the entire dried mushroom, instead of on a fraction of the pulverized material before analysing the acid digest.

The concentrations of nineteen elements, as determined in a single specimen of fourteen different mushroom species, are given in tables IV to VIII.

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TABLE IV

Mercury concentrations found in a single specimen of fourteen different mushroom species

Cold vapour with amalgamation (Hg)			
Mushroom		Weight mg (dry)	Result ng/g
N°	Species		
1	Paxillus involutus	60	282
2	Laccaria laccata var. amethystina	34	497
3	Lepista nuda	175	1,580
4	Collybia butyracea	93	4,268
5	Amanita muscaria	257	4,247
6	Amanita citrina	216	653
7	Amanita rubescens	98	857
8	Psathyrella candolleana	64	2,138
9	Hypholoma fasciculare	66	308
10	Gymnopilus spectabilis	190	180
11	Gymnopilus penetrans	69	313
12	Lactarius camphoratus	49	186
13	Lactarius quietus	111	125
14	Lactarius hepaticus	73	106

TABLE V

Zinc concentrations found in a single specimen of fourteen different mushroom species

Flame AAS (Zn)			
Mushroom			
N°	Species	Weight mg (dry)	Result µg/g
1	Paxillus involutus	277	2,581
2	Laccaria laccata var. amethystina	220	225
3	Lepista nuda	476	459
4	Collybia butyracea	178	357
5	Amanita muscaria	512	321
6	Amanita citrina	402	347
7	Amanita rubescens	563	310
8	Psathyrella candolleana	333	360
9	Hypholoma fasciculare	116	297
10	Gymnopilus spectabilis	481	192
11	Gymnopilus penetrans	308	148
12	Lactarius camphoratus	132	526
13	Lactarius quietus	280	212
14	Lactarius hepaticus	305	997

TABLE VI

Concentrations of five hydride forming elements in a single specimen of fourteen different mushroom species

Hydride AAS							
Mushroom							
N°	Species	Weight mg (dry)	Sn ng/g	Se ng/g	As ng/g	Sb ng/g	Bi ng/g
1	Paxillus involutus	277	418	117	1,624	185	90
2	Laccaria laccata var. amethystina	220	343	192	172,091	322	86
3	Lepista nuda	476	128	2,225	588	151	1
4	Collybia butyracea	178	160	1,462	3,680	81	25
5	Amanita muscaria	512	119	6,943	3,209	11	5
6	Amanita citrina	402	137	319	535	111	< 0.2
7	Amanita rubescens	563	1,130	424	362	153	< 0.2
8	Psatyrella candolleana	333	203	166	2,363	40	1
9	Hypholoma fasciculare	116	101	103	664	19	14
10	Gymnopilus spectabilis	481	37	350	796	16	5
11	Gymnopilus penetrans	308	80	112	364	93	1
12	Lactarius camphoratus	132	< 1	187	288	31	< 0.8
13	Lactarius quietus	280	71	86	318	74	< 0.4
14	Lactarius hepaticus	305	62	122	272	45	< 0.3

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TABLE VII

Concentrations of several elements determined in a single specimen of fourteen different mushroom species by graphite furnace AAS

	Graphite Furnace / Zeeman AAS						
	Mushroom						
N°	Species	Weight mg (dry)	Mn µg/g	Cu µg/g	Pb µg/g	Sr µg/g	Cs µg/g
1	Paxillus involutus	277	175.8	115.5	7.70	3.24	0.505
2	Laccaria laccata var. amethystina	220	23.3	138.2	139.41	2.54	1.225
3	Lepista nuda	476	30.9	109.9	5.99	1.88	< 0.011
4	Collybia butyracea	178	486.1	144.0	14.74	2.91	< 0.028
5	Amanita muscaria	512	21.3	56.4	2.[illegible]0	0.36	0.535
6	Amanita citrina	402	18.1	17.3	4.31	0.68	5.162
7	Amanita rubescens	563	17.4	215.7	5.38	2.06	< 0.009
8	Psatyrella candolleana	333	5.0	91.0	9.74	1.59	< 0.015
9	Hypholoma fasciculare	116	11.9	32.7	4.31	0.64	< 0.043
10	Gymnopilus spectabilis	481	7.1	36.9	1.72	0.43	< 0.010
11	Gymnopilus penetrans	308	22.4	17.5	3.80	0.79	0.010
12	Lactarius camphoratus	132	11.2	29.8	11.63	0.60	2.106
13	Lactarius quietus	280	17.6	90.9	14.37	1.00	5.795
14	Lactarius hepaticus	305	25.9	222.4	19.59	0.80	0.566

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TABLE VIII

Concentrations of several elements determined in a single specimen of fourteen different mushroom species by graphite furnace AAS (Continued)

	Graphite Furnace / Zeeman AAS								
	Mushroom								
N°	Species	Weight mg (dry)	Co ng/g	Tl ng/g	Cd ng/g	Ag ng/g	Ni ng/g	Mo ng/g	V ng/g
1	Paxillus involutus	277	1,502	< 72	2,635	4,449	2,828	231	13,303
2	Laccaria laccata var. amethystina	220	1,559	< 91	8,432	677	984	473	7,023
3	Lepista nuda	476	105	1,252	20,452	1,954	655	1,037	2,048
4	Collybia butyracea	178	522	174	14,915	5,719	1,413	199	2,837
5	Amanita muscaria	512	176	39	26,826	9,443	316	57	122,266
6	Amanita citrina	402	249	532	4,813	548	371	204	1,667
7	Amanita rubescens	563	60	< 35	25,035	1,925	474	188	1,652
8	Psatyrella candolleana	333	405	321	9,054	2,462	1,081	87	766
9	Hypholoma fasciculare	116	198	< 172	17,371	1,853	1,418	75	603
10	Gymnopilus spectabilis	481	195	< 42	6,268	1,076	772	69	291
11	Gymnopilus penetrans	308	68	< 65	8,425	2,555	568	158	1,396
12	Lactarius camphoratus	132	121	< 151	16,629	2,337	936	151	227
13	Lactarius quietus	280	332	71	19,268	9,037	1,241	134	1,804
14	Lactarius hepaticus	305	298	1,905	16,180	8,711	841	69	1,098

In comparison with the results obtained for some other mushroom species the following conclusions can be drawn:

— *Paxillus involutus*: relative high concentrations of Sr, Co, Ag, Ni, V and Bi and very high concentrations of Mn and Zn (175.8 and 2,581 ppm, respectively).

— *Laccaria laccata (var. amethystina)*: relative high concentration of Sr, Co, V, Sb and Bi and very high concentrations of Pb and As (139.4 and 172 ppm, respectively).

— *Lepista nuda*: relative high concentrations of Hg, Sr, Cd, Mo and Se and a distinctly detectable amount of thallium (1.2 ppm).

— *Collybia butyracea*: relative high concentrations of Hg, Sr, Cd, Ag, Se and As and very high Mn content (486.1 ppm).

— *Amanita muscaria*: relative high concentrations of Hg, Cd, Ag, Se and As and very high V content (122.2 ppm).

— *Amanita citrina*: relative high concentration of Cs and a distinctly detectable amount of Tl (0.5 ppm).

— *Amanita rubescens*: relative high concentrations of Cu, Sr, Cd and Sn.

— *Psathyrella candolleana*: relative high concentrations of Hg, Sr and As.

— *Hypholoma fasciculare*: relative high Cd concentration.

— *Lactarius camphoratus*: relative high concentrations of Cs and Cd.

— *Lactarius quietus*: relative high concentrations of Cs, Cd and Ag.

— *Lactarius hepaticus*: relative high concentrations of Cu, Cd and Ag and a distinctly detectable amount of Tl (1.9 ppm).

As compared to the other mushroom species analysed, Gymnopilus spectabilis and Gymnopilus penetrans showed relative low figures for all elements.

The high lead concentration found in Laccaria laccata (var. amethystina) was likely to be due to lead emissions from gasoline-fueled motor vehicles.

Some mushrooms showed very high levels of zinc (Paxillus involutus), manganese (Paxillus involutus and Collybia butyracea), vanadium (Amanita muscaria) and arsenic (Laccaria laccata, var. amethystina), while distinctly detectable amounts of thallium were

also measured in Lepista nuda, Amanita citrina and Lactarius hepaticus. As all mushrooms were collected in rural areas, out of the influence of industrial air pollution, those high values were not to be expected, with exception of the high vanadium content, as it was measured in Amanita muscaria, a mushroom which is found to contain amavadine, an organic vanadium compound.

It is also generally accepted that the uptake of cesium in mushrooms occurs rather selectively. This is supposed to be due to the pronounced chemical similarity with the alkali metal potassium (K), one of the principal inorganic constituents of mushrooms. As nuclear weapon experiments and nuclear accidents are giving rise to increased atmospheric levels of radioactive fission products like ^{137}Cs and ^{90}Sr, the cesium and strontium concentrations in mushrooms collected after the Tsjernobyl accident, are expected to be much higher then the values found in this study.

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Drug level variation in whole blood samples after storage in different containers

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SUMMARY.

The aim of this study was to test the stability of some drugs in whole blood, stored at room temperature in two different containers : Glass vials (10 ml) with rubber septa and polystyrene tubes (25 ml) with polythene caps. These containers are used in Norway for blood samples submitted to forensic toxicological analyses. Material : Freshly drawn whole blood containing 1 % NaF. The drugs and concentrations used were : Tetrahydrocannabinol (THC) 0.01 μM (μmol/l), flunitrazepam 0.03 and 0.1 μM, nitrazepam 0.1 and 0.5 μM, chlordiazepoxide 1 and 2 μM, diazepam 0.5 and 2 μM, N-desalkylflurazepam 0.3 and 1 μM, morphine 0.3 and 1 μM, codeine 0.5 and 2 μM, ethylmorphine 0.5 and 2 μM, propoxyphene 0.5 and 2 μM, salicylic acid 500 and 1500 μM, paracetamol 20 and 100 μM. The samples were analyzed on the day of preparation and transferred to the different containers. Reanalysed after 7 days storage at room temperature protected from light. Results : THC : 60-100 % loss in plastic tubes, no loss in glass vials. Salicylic acid : About 30 % loss in glass vials, insignificant loss in plastic tubes. Flunitrazepam, nitrazepam and chlordiazepoxide : About 50 % loss, independent of container. Propoxyphene : 20-30 % loss, independent of container. Diazepam, N-desalkylflurazepam, opiates and paracetamol : No loss. This study showed that THC and salicylic acid levels were reduced after storage, depending on containertype, and that flunitrazepam, nitrazepam, chlordiazepoxide and propoxyphene are unstable regardless of container type when stored under conditions resembling mail transport.

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A study on the metabolism of methamphetamine in its abusers

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SUMMARY.

Blood and urine samples were collected everyday from eleven methamphetamine abusers admitted to a mental hospital. The concentrations of methamphetamine and its metabolite, amphetamine, in the samples were determined by chemical ionization mass fragmentography. The detection limit of these chemicals was about 1 nmol/100 g. Daily urine volume and liver function were also examined.

1. The methamphetamine concentration in blood did not correlate with either the presence or the degree of symptoms in some cases.

2. The detection of methamphetamine or amphetamine after the last intravenous injection of methamphetamine lasted from two to eight days in the blood samples and from 17 to 36 days in the urine samples, with the exception of one case, in which urine collection was discontinued on the seventh day following the last intake.

3. The detection period of methamphetamine or amphetamine in blood showed no correlation with that in urine.

4. The concentration ratios of amphetamine to methamphetamine in blood and urine had a tendency to increase with the elapsed time following the last intake. The concentration ratios, however, would seem not to be of use for estimating the elapsed time following the last intake.

5. The methamphetamine concentration in blood decreased exponentially. The urine methamphetamine concentration and daily

amount of methamphetamine and amphetamine excreted in urine also decreased exponentially during the 10 days after the last intake.

6. The half-life period of methamphetamine concentration in blood ranged from 0.41 to 1.38 days (av. 0.76 days).

INTRODUCTION.

Methamphetamine abuse has been one of the serious social problems in Japan. In the past the abuse was limited to organized crime groups called « Yakuza » in Japan and persons concerned with them, but recently it is spreading among the general public.

Methamphetamine and its metabolite, amphetamine, in the biological materials have been analyzed by gas chromatography-mass spectrometry (1-8). The distribution of the chemicals in two cases of fatal poisoning (5, 6), the blood concentration of the drugs in a case of acute poisoning (7), and, the detection of amphetamine in a skeletonized body buried for five years (8) have been reported.

In order to study the metabolism of methamphetamine in it's abusers, blood and urine samples of the abusers admitted to a mental hospital were collected everyday, and the concentrations of methamphetamine and amphetamine were determined by chemical ionization mass fragmentography. Daily volume of urine and liver function were also examined.

MATERIAL.

Venous blood samples were collected from eleven patients everyday for one week after admission. Urine samples were collected from the daily urine stored by the patients during the admission period after measuring its volume. The samples were kept frozen until analysis for amphetamines.

The patients were methamphetamine abusers whose blood collected immediately after admission contained amphetamines. The cases were as follows:

1. A 23-year-old male was a paint thinner sniffer in junior high school. He abused methamphetamine for one year before admis-

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sion, and he was sent to the hospital after intravenously injecting about 0.01 g of the drug three times in two days.

2. A 25-year-old male began to abuse methamphetamine three months before admission. He intravenously injected about 0.05 g of the drug every day for two weeks, and he was admitted two days following the last intake.

3. A 39-year-old male was jailed for eleven months after being convicted of methamphetamine abuse. He again abused the drug everyday for five months after his jail term, and he was taken to the hospital one day following the last intake.

4. A 29-year-old male « Yakuza » gang member had been abusing methamphetamine for about seven years before admission. One of the « Yakuza » bosses sent him to the hospital two days following the last injection. In spite of his long and daily abuse before admission, he had no physical and mental symptoms.

5. A 23-year-old male was a thinner abuser at about fifteen, and began abusing methamphetamine at around eighteen. He was put under police protection because of his abnormal speech and behaviour, and sent to the hospital the same day of his last intake. On admission, he had hand tremors.

6. A 34-year-old male abused methamphetamine for seven months before admission. He intravenously injected the drug everyday, and he was taken to the hospital one day after the last intake.

7. A 33-year-old male began methamphetamine abuse at about 24, and intravenously injected methamphetamine 2-3 times in a month. He was admitted one day after the last intravenous injection.

8. A 31-year-old male abused methamphetamine for two years before admission. One day he used 0.1 g of the drug two times and was admitted to the hospital on the following day.

9. A 33-year-old male had been abusing methamphetamine since he was 26 years old. He was taken to the hospital one day after injecting about 0.03 g of the drug three times in one day. On admission he had a convulsive seizure.

10. A 29-year-old male was arrested on the charge of methamphetamine abuse seven years before admission. He intravenously injected about 0.02 g of the drug once a day for one month be-

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fore admission. He was put under police protection owing to his abnormal behavior, and he was sent to the hospital three days after the last intake.

11. A 38-year-old male had been abusing methamphetamine since he was 33 years old, and he was admitted one day after the last intravenous injection of the drug.

12. The same patient as case number 11. Immediately after the discharge from the hospital, he again abused methamphetamine for three days, and he returned to the hospital two days after the last intake.

Excitement and mental symptoms at admission and liver dysfunction are shown in table I with the estimated methamphetamine concentration immediately after the last intake and the half-life period of methamphetamine concentration in blood.

TABLE I

Symptoms at admission, liver dysfunction, estimated methamphetamine concentration immediately after the last intake and half-life period of methamphetamine concentration in blood

Case number	*1*	*2*	*3*	*4*	*5*	*6*
Excitement	+	—	+	—	—	+
Hallucination or delusion .	+	+	+	—	+	+
Liver dysfunction	—	+	+	—	+	—
Estimated MA (µmol/100 g) .	0.02	0.9	0.1	2.7	0.009	3.0
Half-life (day)	1.38	0.72	0.69	0.56	0.74	0.45
	7	*8*	*9*	*10*	*11**	*12**
Excitement	+	+	+	+	+	+
Hallucination or delusion .	+	+	+	+	+	+
Liver dysfunction	+	—	—	—	+	+
Estimated MA (µmol/100 g) .	0.9	0.2	0.3	2.7	0.7	0.2
	0.51	0.41	0.59	0.67	1.14	1.21

* Same patient.

METHOD.

Methamphetamine and amphetamine were extracted from urine or blood samples by our Extrelut `column extraction method (4) and the concentrations were determined by our chemical ionization mass fragmentography. The detection limit of methamphetamine or amphetamine was around 1 nmol/100 g.

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The amount of methamphetamine or amphetamine excreted in urine was calculated as the weight of methamphetamine hydrochloride or amphetamine 1/2 sulfate. When the daily urine was not more than 500 ml, the excretion amount of methamphetamine or amphetamine was not calculated.

RESULTS.

Summarized results of amphetamines analyses in case numbers 2, 4, 11 and 12 are shown in figure 1. Case number 2 was a typical case. Case number 4 was an abuser with no physical and mental symptoms. Case numbers 11 and 12 were the same patient.

The methamphetamine concentration in blood did not correlate with either the presence of the degree of symptoms in some cases.

In all twelve cases, methamphetamine concentration in blood decreased exponentially. Urine methamphetamine concentration and the daily amount of methamphetamine and amphetamine excreted in urine also decreased exponentially during the 10 days following the last intake of methamphetamine.

The detection of methamphetamine or amphetamine following the last intravenous injection of methamphetamine lasted from two to eight days in the blood samples and from 17 to 36 days in the urine samples, with the exception of one case, where urine collection was discontinued on the seventh day after the last intake.

The detection period of methamphetamine or amphetamine in the blood samples showed no correlation with that in the urine samples.

The concentration ratios of amphetamine to methamphetamine in blood and in urine had a tendency to increase with the elapsed time following the last intake. The individual differences in them, however, were greater than the variations of them in the elapsed time.

The half-life period of methamphetamine in blood ranged from 0.41 to 1.38 days (av. 0.76 days) as shown in table I.

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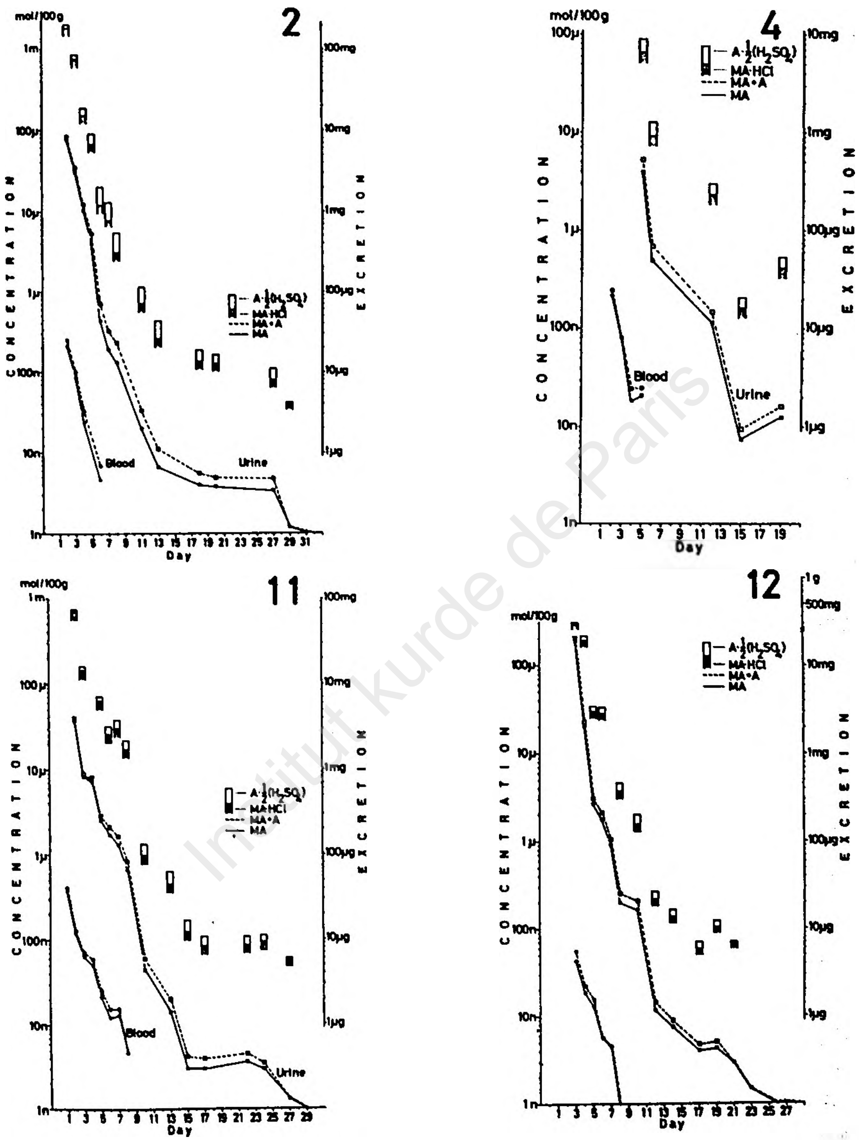

FIG. 1. — Summarized results of amphetamines analyses in case numbers 2, 4, 11 and 12.

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DISCUSSION.

Nagata *et al.* (9) tentatively established the following criteria of amphetamine blood levels (umol/100 g or ml) : fatal level at more than 3-4, high or serious level at more than 2, intermediate with the possibility of mental disorder at more than 0.3-0.4, and low or mild level with little or no remarkable mental disorder detectable at below 0.2. The methamphetamine concentration in blood, however, did not correlate with either the presence or the degree of symptoms in some cases.

The methamphetamine concentration in blood decreases exponentially after reaching the maximum (10-14). As the exponential decrease of the methamphetamine concentration in blood was observed in all cases of the present study, the following equation was used to estimate the blood methamphetamine concentration immediately after the last intravenous injection of the drug. The equation is :

$$\log C = \log C_0 - \frac{kt}{2.303}$$

where C is the concentration present at time t, C_0 is the concentration immediately after the last intake, and k is the rate constant. The estimated concentration ranged from 0.009 to 3.0 umol/100 g (table I).

The half-life period of methamphetamine concentration in blood calculated from the data reported by Bruce *et al.* (9) and by Driscoll *et al.* (11) is 0.06 days and 0.4 days, respectively. In the present study, it ranged from 0.41 to 1.38 days and the average was 0.76 days (table I). There was no correlation of liver dysfunction with the half-life period, and the cause of these differences is unknown.

The concentration ratios of amphetamine to methamphetamine in blood and urine had a tendency to increase with the elapsed time following the last intake, but the individual differences in them were greater than the variation of them in the elapsed time. Therefore, the concentration ratios would seem not to be of use for estimation of elapsed time following the last intake.

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Nicotine and cotinine detection in biological fluids and nicotine detection in filters of smoked cigarettes

A new method

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INTRODUCTION.

Previously published methods for measuring nicotine and its main plasma metabolite cotinine in biological fluids are afflicted with many drawbacks. Sensitivity and specificity of the vast majority of these methods are needlessly reduced by an inadequate choice of work-up procedure, chromatographic column, detector or internal standards. As a consequence, many procedures require bulky samples (e.g. Stehlik *et al.*, 1982), whereas other procedures are suited to only nicotine analysis (Feyerabend and Russell, 1979 ; Dow and Hall, 1978) or to only cotinine analysis (Feyerabend and Russell, 1980), or are applicable to only one kind of biological fluid. We developed a procedure for the simultaneous analysis of nicotine and cotinine in concentrations lower than 0.5 ng/ml, applicable to plasma and urine samples. Moreover a procedure for the analysis of the nicotine content of smoked cigarette filters is outlined.

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PROCEDURE.

To 1 ml plasma or 0.4 ml urine samples nicotyrine and nikethamide were added as internal standards for nicotine and cotinine, respectively. Each sample was diluted with an equal volume of 0.05 M borax buffer (pH = 9.0) and was flushed through a C-2 solid phase extraction column (100 mg sorbent). The column was washed with 2 ml of water and subsequently with 100 μl 80 % methanol. Then it was dried by air suction. Nicotine, cotinine and their internal standards are eluted by a 500 μl

methanol column wash and are caught in a conical vial. Twenty µl of propyl acetate were added to the eluate, which then was concentrated to approximately 15 µl by a very gentle stream of dry nitrogen at 45°.

Half a µl of the concentrate is splitlessly injected into a gas chromatograph equipped with a 0.2 mm × 12 m dimethyl silicone WCOT column and a nitrogen detector. A two-level three-ramp GC oven temperature program was used, the starting level being 60° and the terminal level being 200°.

RESULTS AND DISCUSSION.

Peaks are highly resolved, widths at half-height varying from less than a second (nicotine) to 2-3 seconds (cotinine). Coefficients of variation are 2.7 % for nicotine (5 ng/ml) and 5.0 % for cotinine (25 ng/ml) in plasma. The recoveries are 75-85 % for nicotine and virtually 100 % for cotinine. The chromatogram runtime is 9.0 min. including the cooling time of the GC oven (HP 5890). Specificity, sensitivity and rapidness of work-up and chromatography of this method indeed rival with the, in our view, best methods published so far (e.g. Curval *et al.*, 1982).

To determine the nicotine content of cigarette filters, 10 filters of smoked cigarettes were crushed in a household blender after removal of residual tobacco and ashes. The resulting filter « wool » was quantitatively suspended in 100 ml of methanol, ultrasonically vibrated for 30 min. and filtrated through cellulose acetate filters (pore 0.45 µm. Then the *external* standard nicotyrine was added, the filtrate was diluted 25 times with propyl acetate and 0.5 µl were injected into the GC. For this purpose the earlier mentioned GC oven temperature program was cut after appearance of the nicotyrine peak and lasted 5.5 min. Determination of the filter nicotine content allows one to make an estimation of a smokers nicotine intake, if the ratio of the amounts of nicotine in the mainstream of the smoke/in the cigarette filter is known.

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Ion-pair extraction of basic drugs from enzyme digested livers

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INTRODUCTION.

Ion-pair extraction has been a part of the arsenal of pharmaceutical analysts for about two decades as a method for the selective extraction of certain highly water-soluble compounds from aqueous solutions to organic solvents (1). However, simple matrices and inorganic counter-ions have been commonly used. Only recently Hoogewijs and Massart applied the method for the extraction of basic drugs in general from biological fluids using organic counter-ions and HPLC-detection (2). The present study investigates the possibility of coupling the ion-pair extraction of basic drugs with the enzymic digestion of liver samples to produce extracts suitable for TLC-screening.

A strongly hydrophobic counter-ion, bis(2-ethylhexyl)phosphoric acid (HDEHP) was chosen because of its high extraction power (2, 3). To find the optimal extraction conditions, ten drugs with different polarities were extracted as HDEHP ion-pairs to two potentially effective solvents, dichloromethane and ethyl acetate, at four different pH's. For comparison, the drugs were extracted conventionally from basic solution to dichloromethane. The recoveries were measured by GLC.

The liver samples, spiked with the same drugs, were extracted both conventionally and under optimal conditions, and the extracts were analysed by HPLC. TLC in the normal and reversed phase mode was evaluated as a method for the qualitative analysis of the extracts.

EXPERIMENTAL.

Extraction procedures.

All of the drugs were used as hydrochlorides except for atenolol and salbutamol, which were free bases. The drugs were added to the extraction tubes in ethanolic solutions of 2 mg/ml, and the solvent was evaporated. After adding the aqueous phase, the concentration of all of the drugs was 0.0001 M.

Extracts for GLC : 10 ml of 1 M Tris-buffer (Sigma), with pH 4.2 ; 5.2 ; 6.3 ; 7.4, was extracted with 10 ml of an organic solvent (dichloromethane, ethyl acetate) containing 0.001 M HDEHP (Aldrich) for 30 minutes by shaking the tubes horizontally. CH_2Cl_2 at pH 11 was used for the conventional extraction. After centrifugation, 1 ml of the organic phase was evaporated to dryness in a vial, and the residue was allowed to react with 200 μl of 1.5 M trimethylsilylimidazole in pyridine (Tri-Sil Z, Pierce) for 10 minutes at 70°C. 1 μl was injected to the gas chromatograph.

Extracts for HPLC : about 10 g samples of liver obtained at autopsy were digested by the method described by Osselton (4) and modified by Hammond and Moffat (5), but using 5 mg of trypsin (Type IX, Sigma) and 20 ml of 1 M Tris-buffer, pH 7.4. 350 μl of 5 M HCl was added to 10 ml of the liver digest, it was extracted for 15 minutes with 10 ml of diethyl ether, and centrifuged. To prepare the ion-pair extracts, 350 μl of 5 M NaOH was added to the aqueous phase returning the pH to 7.4, and it was extracted for 30 minutes with 10 ml of dichloromethane containing 0.01 M HDEHP. To prepare the conventional extracts, 2,150 μl of 5 M NaOH was added to the aqueous phase raising the pH to 11, and it was extracted for 30 minutes with 10 ml of dichloromethane. In both cases, after centrifugation, 2 ml of the organic phase was evaporated to dryness, and the residue was mixed with 400 μl of methanol in a vortex-mixer. The mixture was centrifuged, and an aliquot of the supernatant was taken to the HPLC analysis.

Extracts for TLC : the extracts were prepared as for HPLC, but the whole dichloromethane-phase was evaporated to dryness. The residue was shaken with 100 μl of ethanol and centrifuged. Ten μl of the supernatant was applied to the plate.

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Chromatography.

Gas chromatography was performed with a Nordion HRGC 412 equipped with a flame ionization detector. The column was a 15 m × 0.32 mm Nordion SE-30 silica column with a 0.15 μm film thickness. Helium was used as a carrier gas with a split ratio of 1:30. The oven temperature was increased 10°C/min. between 170°C and 250°C with different initial and final temperatures depending on the compound. Both the samples and the standards contained the same amount of the counter-ion and an internal standard, maprotiline.

High performance liquid chromatography was performed with a pump and a 254 nm UV-detector by Waters and an injector by Rheodyne. The column was a 25 cm × 4.6 mm Merck Lichrosorb

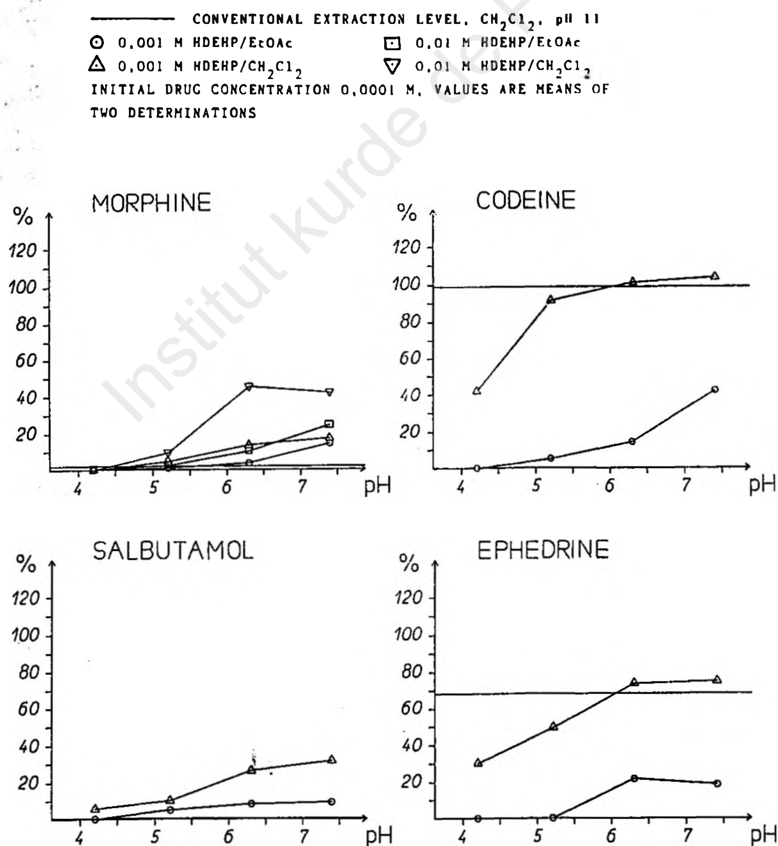

FIG. 1.

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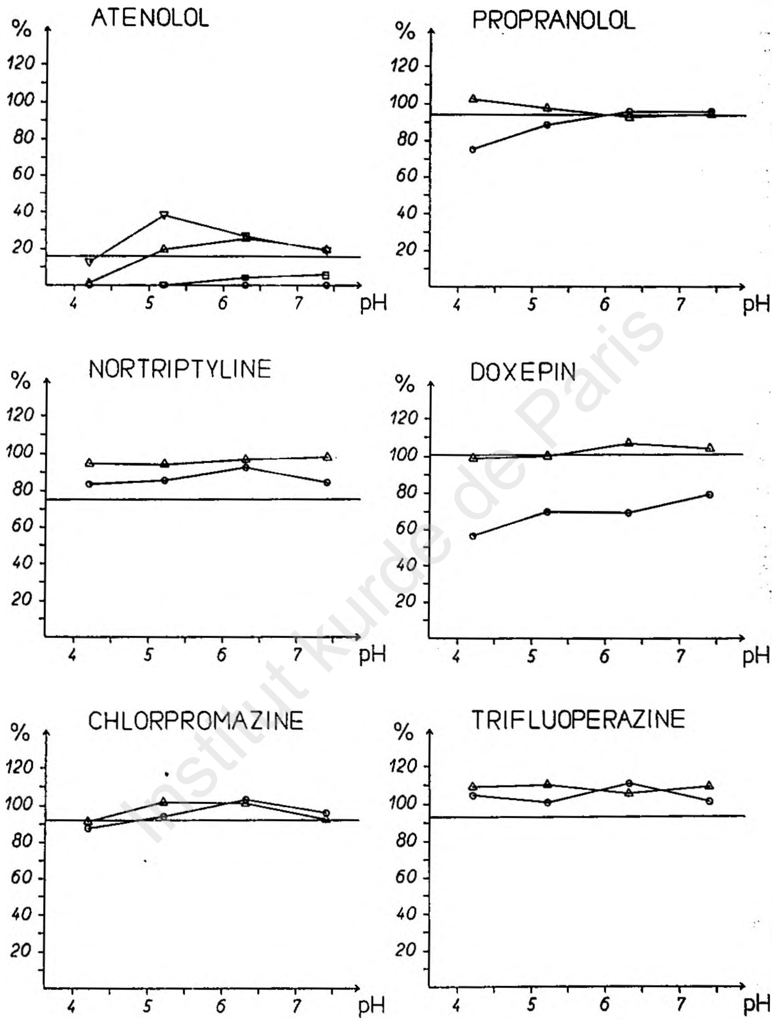

FIG. 1. — Percent recoveries of basic drugs extracted from aqueous solutions at different pH's.

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CN with a particle size of 10 μm. The eluent was acetonitrile : water : diethylamine = 50 : 50 : 0.1, which was neutralized to pH 7 with phosphoric acid. Maprotiline was used as an internal standard.

Thin layer chromatography was performed on Merck Silica Gel 60 F_{254} plates using toluene : acetone : ethanol : NH_3 = 45 : 45 : 6 : 3 and cyclohexane : toluene : diethylamine = 75 : 15 : 10 (6), and on Merck RP-18 F_{254}s plates using methanol : H_2O : HCl = 50 : 50 : 1.

RESULTS AND DISCUSSION.

The extraction behaviour of the ten drugs, representing five therapeutic categories, is shown in figure 1. The drugs belonging to the same category lie adjacent, the more hydrophilic one on the left. It is clear that the ion-pair extraction with dichloromethane is, in most cases, far superior compared to the ion-pair extraction with ethyl acetate. The advantage of the former over the conventional method is apparent in the cases of the most hydrophilic drugs, morphine, atenolol and salbutamol, while in the other cases the difference is smaller.

In the GLC analysis of the ion-pair extracts, it was necessary to make the counter-ion more volatile by derivatization. If injected without derivatization, HDEHP sticks in the injector causing adsorption of the drugs.

The ion-pair extraction with dichloromethane at pH 7.4 was chosen as the method to be tested with the liver samples. The extraction recoveries are shown in table I. Although in most cases the ion-pair method is superior or equal to the conventional extraction, there are also a few opposing cases. This may be due to the diminution of the number of available counter-ions, which is caused by other cations than drugs. An advantage of the ion-pair extraction is that the recoveries should be the higher the lower the drug concentration, if the counter-ion concentration remains the same.

The HPLC analyses of basic drugs extracted as HDEHP ion-pairs have been previously performed at a strongly basic pH (7). It seems that the neutral pH used in this study would also be suitable. This, however, requires further investigations.

TLC was found to be well-suited for the screening of the ion-pair extracts. Fairly apolar basic eluents, such as those described

TABLE I

Percent recoveries of basic drugs extracted from liver samples by ion-pair (A) * and conventional method (B) **

	A	*B*
Morphine	18	1
Codeine	79	88
Salbutamol	27	0
Ephedrine	62	27
Atenolol	16	23
Propranolol	68	78
Nortriptyline	60	33
Doxepin	113	113
Chlorpromazine	77	77
Trifluoperazine	48	49

* 0.01 M HDEHP/CH_2Cl_2, pH 7.4, values are means of two determinations.
** CH_2Cl_2, pH 11, single determinations.
Initial drug concentration 0.0001 M.

in the experimental section, should be used in the normal phase mode to prevent the counter-ion from interfering. In the screening of polar drugs, reversed phase ion-pair TLC was found practical, because the counter-ion remained at the starting point.

We are continuing the research regarding the ion-pair extraction to evaluate the method's usefulness in the routine screening of liver samples for drugs.

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PART II

SECOND WORLD CONGRESS

NEW COMPOUNDS IN BIOLOGICAL AND CHEMICAL WARFARE: TOXICOLOGICAL EVALUATION INDUSTRIAL CHEMICAL DISASTERS CIVIL PROTECTION AND TREATMENT

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Industrial Disasters, Disasters Management

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Chemical accidents

Role and responsibility of a poison centre

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A Poison Centre has an important role in handling chemical accidents in many ways. It has a central position, and can in this way guide and co-ordinate activities within this field.

Poison Centres have existed for several years in many countries. In one country there may be one or several Centres according to the size and population of the country. The fundamental role of a Poison Centre is to give information and advice on a 24 hour basis on the telephone in all situations of acute, in some centres also chronic, poisoning with the help of highly trained and specialized staff. Apart from the telephone service in the acute situation, the Poison Centre also has responsibilities in other activities dealing with poisoning. Systematic dissemination of qualified information is a very important task for the Centre. Prevention, toxicovigilance, education of medical and paramedical personnel are examples of other important activities, and a close collaboration between the Centre and public authorities are important in activities like legislation and regulations.

In the following, different aspects of a Poison Centre's activities in the chemical safety programme dealing with chemical accidents will be given, examplified by some activities of the Swedish Poison Information Centre.

Enormous volumes of hazardous materials are transported every day and several billion tons of hazardous materials are in storage sites throughout the world. Accidents with chemicals do happen and the tragedy in Bhopal in India in December 1984 is a frightful illustration of this. More than 2,000 persons were killed and thousands of people were severely injured. In total 150,000-200,000 persons were to some extent affected by the toxic gas. Since then, there has been a number of accidents, but fortunately

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no one of the same magnitude. In Sweden in January 1985 for example, there was an uncontrolled leak of sulfuric acid for many hours, and vast areas of a town of 35,000 inhabitants were covered by the gas cloud. There were no serious injuries to humans, but if it had been a more reactive or toxic gas, like chlorine, the consequences could have become disasterous. In August 1985, in West Virginia, USA, there was an incident of a leaking irritating gas affecting more than 100 persons in spite of the fact that modern and new security systems had been installed.

Even an accident just affecting one or two persons might be of the utmost interest, serving as an indicator of what could happen in case of a major accident. An example of such a minor accident is one that recently happened at a plant in Sweden. A 38-year-old truck driver was splashed with monochloracetic acid when unloading his truck. Within a couple of minutes he was undressed and was placed in a shower for 20 minutes. Initially he presented just first degree burns over 25 % of his body surface. Within the following hours he developed signs of systemic poisoning including CNS-depression, later on seizure activity, cardiac insufficiency and progressive renal failure. Despite optimum symptomatic and specific antidote treatment he died seven days after the accident. Blood analyses revealed high concentrations of monochloroacetic acid.

The list of examples can be much longer, and the fact that chemical accidents seem to become more common, stresses us to take our responsibility in dealing with chemical accidents in a more sophisticated and organized way.

Apart from giving information on the toxic substance including toxicity data, risks, first aid measures, hospital treatment etc. in the acute situation, other important tasks for the Poison Centre are to taking an active part in the contingency planning for minor as well as for major chemical accidents, educating rescue personnel and disaster medicine planners, giving guidelines on supplying antidote stores, providing rescue personnel with adequate information adapted for these situations. The Centre can act on a centralized basis in this work using the same standards, saving a lot of work for example by creating a uniform strategy and by co-ordinating activities when appropriate.

In the very acute situation, information on risks, first aid measures, etc. must be easily and immediately available so adequate measures can be undertaken, minimizing the damage of the toxic

substance. To meet this demand emergency data sheets or transport cards are available in most plants and transport vehicles. This information is adapted to the very acute situation and therefore short. For more detailed information Poison Centres will provide the rescue personnel, including medical staff and hospitals with necessary, more detailed and advanced information.

Apart from giving information in the acute situation on the telephone to the general public, doctors, hospitals, rescue teams, etc., adequate information must, at an early stage, also be given to the mass media, especially the radio and TV. Information including risks, symptoms, how to protect oneself, where to go, etc., can in that way quickly be spread to the general public.

The Poison Centre has generally detailed an easily accessible information about most household products and chemicals frequently used in industries. However, for less frequently used chemicals information is often scarce or difficult to overview in the acute situation. Therefore it is of vital importance that when an accident has happened, an early measure at the site of accident should be to inform the Poison Centre about the toxic substance, possible synonymes, etc. This measure must be included in the check list at plants and emergency centres. In this way the Poison Centre gets more time to search for relevant information if necessary.

To improve the knowledge of less frequently used chemicals it is of the utmost importance that activities are started for example within the framework of Poison Centres. Some activities within this field have already been undertaken. One example is a pilot activity organized jointly by the World Federation of Clinical Toxicology and Poison Control Centres and the International Programme for Chemical Safety. Six Poison Centres from different parts of the world have participated in an activity preparing one document each of a toxic substance including detailed information on toxicity, risks, symptoms, first aid measures, hospital care etc. in case of acute exposure. Conclusions we have drawn from this pilot activity and from similar activities performed in the Scandinavian countries is that, although valuable, this work cannot be included in the Centres' regular work unless extra resources are placed at disposal. To find out what substances should be included in this work, it is very important to provide the Poison Centres with information about what substances are stored and used at working places and at plants and what substances are transported on roads, railroads or ships.

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Regarding contingency planning for chemical accidents the Poison Centre must take its responsibility and act in several ways to achieve and maintain effective systems. Disaster medicine planning must be extended to include chemical accidents. A close collaboration between the disaster medicine planners and the Poison Centre must be established. For example the Centre has to provide the planners with guidelines on measures for decontamination, first aid, supplying antidote stores, etc. At the request of the Stockholm County Council the Swedish Poison Information Centre has taken part in the working out of an emergency plan for major chemical accidents. Apart from general information about management of poisoning this plan includes detailed information about some selected chemicals including information about main risks and detailed instructions on the management of poisoning. The information has been adapted to be understood by both nonmedical and medical personnel. Guidelines on supplying antidote stores at four of the Stockholm County Council Hospitals have also been given including information regarding recommended package, number of packages, shelf life, etc. Each antidote store is intended to suffice the requirements of several seriously poisoned persons for three days. A list of suitable antidotes to be stored at mobile units that will be brought to the place of accident is also included in the plan. This plan worked out by the Poison Centre has constituted as a model and similar emergency plans now exist in several regions in Sweden. In this context it is also important to stress that a close collaboration between Company Health Services and local hospitals must be established to improve the preparedness in case of a chemical accident.

Education of all members of the rescue team is another very important task. Lectures, training programmes and exercises dealing with chemical accidents have to be arranged and the Poison Centre must take an active part in these activities. The rescue team must be provided with information about main risks, first aid measures, hospital treatment etc. and this information must be adapted to these situations. To meet this demand in Sweden, a handbook has been worked out jointly by the Poison Information Centre, the Organizing Committee for Disaster Medicine, the Fire Fighting Services and the National Board of Health and Welfare. This handbook deals with different aspects on rescue work in case of chemical calamities and is meant to be used by both medical and nonmedical staff.

Another very important activity is to visit sites of accidents as observer in order to gather information and experiences from a present accident. This is important not only in the acute phase but also at a later stage for follow-up studies to reveal any possible late sequelae. Together with the Scientific Secretary of the Organizing Committee for Disaster Medicine in Sweden I had the opportunity to visit Bhopal in India ten days after the gas leak of methylisocyanate. By visiting local hospitals, interviewing doctors, the Mayor of Bhopal, victims etc. we got a good idea of the toxic action of methylisocyanate, the symptomatology, the efficacy of treatment measures, the rescue organization, etc. This information we would never get unless we visited the site of accident. The experiences from this visit to Bhopal has been of great value for our future activities in the field of disaster medicine planning for chemical accidents.

A close collaboration between Poison Centres all over the world and between Poison Centres and other organizations dealing with disaster medicine planning like the World Association of Emergency and Disaster Medicine, the International Program for Chemical Safety, the World Association of Clinical Toxicology and Poison Control Centres is of course of vital importance.

In conclusion, the primary theme of disaster management is information management. Timely information can change the entire character of an accident and the Poison Centre has a central position in this matter.

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Mass poisoning with chlorine in a public swimming hall

Critical evaluation of the disaster management and the medical treatment

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INTRODUCTION.

The clinical symptomatology of acute chlorine poisoning is characterized by coughing attacks, irritation of the mucosa of the upper airways, laryngospasm, bronchospasm, dyspnoea and bronchitis (1-4). Severe intoxication leads to a hemorrhagic toxic pulmonary oedema and subsequent respiratory failure which may be associated with fatal outcome if therapy is not initiated immediately. In general, the onset of symptoms is observed immediately after exposure to chlorine. However, a latency period of up to 12 hours may precede the manifestation of toxic pulmonary oedema (5). Fortunately, the olfactory threshold of chlorine is rather low, thus protecting from unnoticed exposure to toxic concentrations (table I).

TABEL I

Chlorine gas concentration and clinical symptomatology

Concentration [ppm]	*Clinical symptomatology*
0.02 - 0.5	Olfactory threshold (1)
1.0	Little irritation ; no pathological findings in spirometry (1)
2.0 - 3.0	Moderate irritation ; working for a longer period of time causes discomfort
4.0	working not possible (1)
40 - 60	Chlorine intoxication after 30-60 min of exposure (2)
100 - 1000	Lethal intoxication after exposure for 1 - 10 min (2, 3)

Treatment of acute chlorine poisoning should include repeated inhalation of a corticosteroid spray (e.g. dexamethasone), seda-

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tion, and inhalation therapy with broncholytics. If the physical examination and chest X-ray reveal beginning pulmonary oedema, intravenous administration of a corticosteroid and repeated blood gas analysis are necessary. Early intubation and ventilation with a respirator is required in cases of massive pulmonary oedema and respiratory failure. All patients — even those without initial symptoms — should be kept under observation for at least 24 hours. Before discharge, auscultation of the lungs and a control of the chest X-ray are mandatory.

In general, therapeutic management of 1-5 patients with chlorine poisoning leads to no problems for a normally equipped hospital. However, a mass intoxication may be associated with logistic difficulties which are examplified by the following case.

CASE REPORT.

In a public swimming hall, chlorine was generated by reaction of hydrochloric acid and sodium hypochlorite via a dosing pump. On a Saturday afternoon, an employee wanted to refill the container with hydrochloric acid and — by mistake — filled hydrochloric acid into the container for sodium hypochlorite. Large amounts of chlorine developed and were distributed in the showers and the swimming hall by the air conditioning system. At this time, the swimming hall was crowded with about 50 children who were 2-15 years old and their mothers. The rising concentration of chlorine caused coughing attacks and panic in the visitors of the swimming hall. Trying to escape from the chlorine they found all emergency exits of the swimming hall closed. Therefore, they had to pass through the showers in order to leave the building.

Thirteen ambulances, 3 ambulances with an emergency physician, and 11 emergency trucks of the fire brigade were sent to the site of the accident. Nearly all visitors of the swimming hall showed symptoms of acute chlorine poisoning. Forty-three persons, 23 adults, and 20 children between 2 and 15 years were immediately treated with a corticoid spray or intravenous administration of a corticosteroid and then transferred to 6 hospitals. Nineteen of the patients were admitted to our intensive care unit (Reanimationszentrum) which has a capacity of 20 beds. Fourteen of the beds had already been occupied at this time. Seven of the 14 patients were ventilated by respirator, 1 patient was on hemodialysis. The admission of the additional

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19 patients raised some difficulties. However, these problems could be solved by prompt recruitment of additional nurses and physicians. The clinical symptoms of the patients and the therapy are summarized in tables II and III. A chest-X-ray was performed

TABEL II

Frequency of symptoms in 19 patients with acute chlorine intoxication

Symptom	n
Coughing attacks	19
Nausea/vomiting	3
Irritation of nose and throat	11
Rales	17
Dyspnoea	6
Cyanosis and tachypnoea	4
Beginning toxic pulmonary edema (chest-X-ray)	2

TABEL III

Therapy of 19 patients with acute chlorine intoxication

Therapy	n
Repeated application of corticoid spray	19
Intravenous corticoid	7
Inhalation therapy	4
O_2-Insufflation by nasal tube	3
Intubation, *ventilation* by respirator	1

in all patients. One child that had to be intubated and ventilated was transferred to the intensive care unit of the university pediatric clinic.

On the next day, all patients, except the one mentioned before, could be discharged in complete health.

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DISCUSSION.

One aim of medical disaster management is to try to provide medical treatment for a large number of patients at the same standard as for an individual patient. In the case presented here, fortunately, all patients received appropriate medical treatment within a reasonable period of time. Only one case of severe chlorine poisoning which required ventilation by a respirator occurred. One may speculate what might have happened if all 19 patients had had to be ventilated immediately : Ventilation of all patients would have been impossible due to the lack of respirators and trained personnel. Transport of the patients to other hospitals might have led to an unacceptable delay of appropriate treatment.

One should be aware that artificial ventilation of a large number of patients in a case of mass poisoning is not realistic. The medical, surgical, neurosurgical and pediatric intensive care units of the hospitals of the City of Berlin (West) with about 2,000,000 inhabitants provide a total of about 420 beds with the possibility of respirator therapy. Since, on average, 70-90 % of these beds are occupied a « reserve capacity » of a maximum of 40-120 beds may be calculated for mass poisoning. We conclude that respirator therapy for a large number of patients may lead to serious difficulties, even in a large city with a high medical standard.

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Effects of the radioactive cloud of Chernobyl on Italy

The prevention measures applied by the Italian government to avoid an industrial chemical disaster

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SUMMARY.

The authors refer the radioactivity monitoring date in Italy in food after Chernobyl catastrophy and the prevention measures applied by the Italian Government concerning milk and vegetables. No important acute nuclear intoxications have been registered, but only psychic reactions in subjects. However, considering the mild increase of radioactivity in food, only attaining alarm threshold but never the dangerous one, long time damages may be expected.

The follow-up of the tumors incidence, especially on blood and on gastrointestinal tract, will show whether the measures applied by the Italian Government have been effective in this side.

A protocol and a kit for the treatment of acute nuclear intoxication is also referred, nevertheless in our situation their use was not necessary.

Italy after Chernobyl disaster for the first time has risked a nuclear catastrophy. The global radioactive fall-out had an amount of an approximate value of 20 million curie nuclear material. A part of it, on account of the weather conditions of that period, was pushed over North, Central and South Europe. In Italy, northern provinces were more exposed than those in the centre and in the south.

Radioactive fall-out and accumulation on earth ground was favoured by weak precipitation, unable to wash out the radioisotopes from superficial into underlying layers of ground.

One of the consequences was the contamination of the cultivatedlands, attaining an alarm but not toxic threshold (table I).

TABLE I

Monitorized radioactivity in Italy

Day	*Air*			*Vegetables*			*Milk*		
	cm			*kg*			*liter*		
	North	*Centre*	*South*	*North*	*Centre*	*South*	*North*	*Centre*	*South*
2 May . . .	1	0.2	—	100	90	6	1.5	1	—
3 May . . .	0.5	0.1	0.1	70	50	10	2.5	2	0.2
4 May . . .	0.2	0.05	0.05	50	30	15	7	4	1.4
5 May . . .	0.08	0.1	0.13	97	63	24	6	4	4
6 May . . .	0.03	0.08	0.05	98	68	55	4.4	3.6	4.5
7 May . . .	0.03	0.03	0.03	94	61	59	7	4.5	14.1
8 May . . .	0.05	0.02	0.03	94	39	35	8	5	15
9 May . . .	0.03	0.02	0.02	108	32	26	12	6	9
Alarm threshold .	3.5 nano - Curles			15 nano - Curles			15 nano - Curles		
Danger threshold .	35 nano - Curles			150 nano - Curles			150 nano - Curles		

Radioactivity levels in the air, vegetables and milk were monitored (table I).

The following precaution measures were taken in Italy to avoid contamination by I^{131}, an isotope which has a short T/2 (8,624 days).

A. Prohibition to sale large leaf vegetables for a period of fifteen day.

B. Prohibition for pregnant women and children under 10 years to eat any bovine or ovine or goatish fresh milk and their products with a seasoning below of fifteen days : the use of long life milk was permitted if prepared before May 2nd. The administration of it was not recommended.

EEC radioactivity limits for agricultural products are the following ; for vegetables and fruits the limit was 350 becquerrel per kg (1 becquerrel = 37 nanocurie) and for milk 500 becquerrel per kg ; afterwards these limits were changed up to 1000 becquerrel for agricultural products ; for milk the limit remained the same.

Everywhere italian products were over EEC limits ; in northern provinces the agricultural products had the value of 3,589 bec-

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TABLE II

Symptoms, therapy and prognosis after acute global irradiation

		1 - 10 Gy (100 - 1000 rad) Treatment area			*10 Gy (1000 rad)*
Dose interval	0 - 1 Gy	1 - 2 Gy	2 - 6 Gy	6 - 10 Gy	10/15 - 50 Gy
Therapy	Not necessary	Clinical observation	Therapeutic efficiency	Therapy sometimes	Symptomatic treatment
Vomiting	No	1 Gy : 5 % 2 Gy : 50 %	3 Gy : 100 %	100 %	100 %
Lapse of time between irradiation and vomiting	—	3 h	2 h	1 h	30 min.
Mainly interested organs and/or systems	More	Haemopoietic	Haemopoietic	Haemopoietic	GI and CNS
Characteristic symptoms	—	Mild leukopenia	Leukopenia hemorragia	Hemorragy Infection	Diarrea fever Tremor letargy
Critic period after exposition	—	—	4 - 6 weeks	4 - 6 weeks	5 - 14 days 1 - 48 h
Therapy	Psychotherapy	Psychotherapy Hematologic ob.	Blood transfusion Antibiotics	Bone marrow T. Platelets conc.	Symptomatic
Prognosis	Very good	Very good	Reserved	Reserved	Bad / lethal
Recovery period	—	Some weeks	6 - 8 weeks 1 - 2 months	Very long 1 - 2 months	—
Lethal	0	0	0 - 80 %	80 - 100 %	100 %
Death after	—	—	2 months	2 months	2 - 15 days
Possible death cause	—	—	Hemorragy Infection	Hemorragy Infections	Irreversible Shock Edama C.

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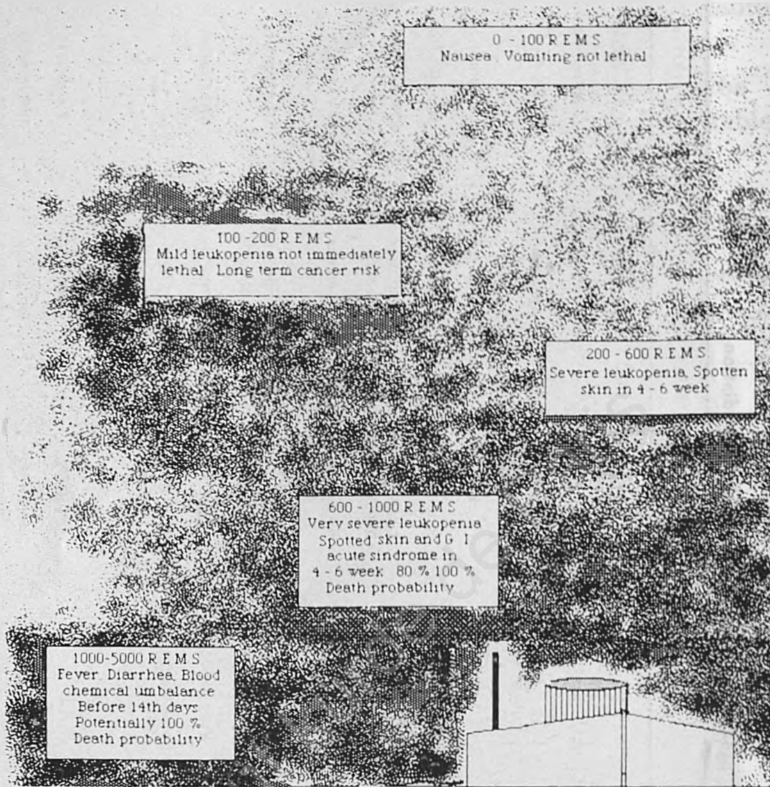

FIG. 1. — Symptoms to radioactivity.

TABLE III

Protocol for DTPA treatment

Administration Times after Incident		*Dose, Administration way and frequency*
As soon as	0.5 - 1 g i.v.	
1st week	0.5 g i.v.	1 inoculation every day or 3 in the week
For the 2 following months .	0.25 - 0.5 g i.v.	2 inoculations every week
3rd month	Treatment stop	
Afterwards	0.25 - 0.5 g i.v. or inhalation	Monthless cycles with 1-2 administrations far week with an interval of 2-3 weeks

The duration will he according to the individual situations.

querrel, in central 2,331 and in Southern 88. On the contrary milk had low standard value.

High radioactivity levels, caused by Cs^{137} contamination causing alarm but not attaining toxic levels were found in some of the northern provinces. This fact induced local authorities to take new security measures (restriction for some kinds of meat over a temporary period). No acute toxic effects have been observed in men. In fact the radioactivity calculated was much more lower than 100 REMS, and therefore no important acute symptoms were produced (fig. 1 and table III).

According by our Poison Control Center has been consulted very frequently but we have never found important symptoms of acute

TABLE IV

Medical kit for acute radiocontamination

Iodine container: 2 bottles of 50 % KI in water + Na thiosulphate 1/1000 with KI; preserve out of light.

Rare earth, plutonium, transplutonics:

— 10 ampoules to 4 ml containing 1 g DTPA (Dietilentriamino pentacetic acid) in saline solution.
— 1 bottle 500 ml DTPA 1 % pH 4 for skin and wounds decontamination.

Cesium container: 21 bottles each containing 1 g of Prussian Blue (1 g for 3 times day).

Strontium container:

— 7 ampoules of Ca or Na arginate (agonist ingestion).
— 7 ampoules of K rodizenate (for wounds).
— 7 service cups of Mg sulphate (to increase gastrointestinal transit speed).

Uranium container: 1 bottle 250 ml Na bicarbonate 14 % in saline solution for fleboclisis.

Polonium container: 12 BAL ampoules.

One sampling box containing:

— 14 rhinopharynx tampons.
— 14 test tubes for blood with 7 heparinized.
— 7 faeces containers.
— 7 urine containers.

nuclear intoxication, only, very rarely conjunctival irritation and/or nausea but not definitely correlated with nuclear action. Most of complaints can be attributed to the fear or to the alarm nevrosis. Many consultations regarded how to protect themselves from nuclear pollution and/or to prevent its effect. Therefore we never suggested our protocol for acute nuclear intoxication and the relative kits (table IV).

However the ingestion or inhalation of increased radioactivity surely occurred in men (fig. 2), suggesting the possibility of

some long term effects, especially increase of tumors, particularly of gastrointestinal tract and leukemia. But only the future follow-up will show definitely how these measures, applied by the Italian Government, did avoid or reduce long term toxic effects of increased nuclear pollution.

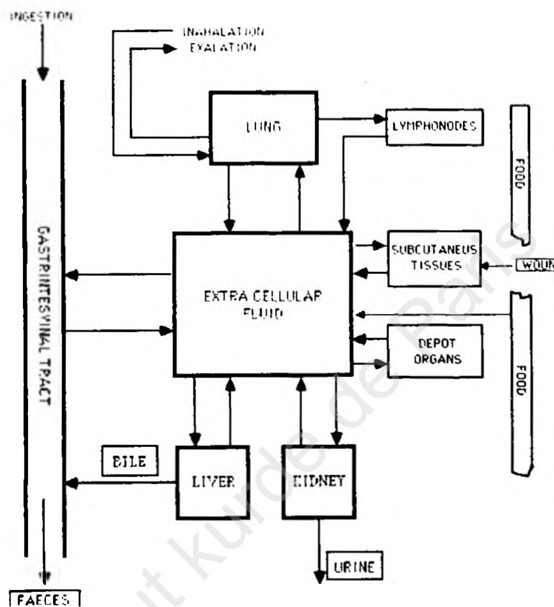

FIG. 2. — Nuclear farmacokinetics in men.

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Many information reached us from the following newspapers:

La Repubblica. Domenica 4 - Lunedì 5 - Martedì 6 - Mercoledì 7 - Giovedì 8 - Venerdì 9 - Sabato 10 maggio 1986.

Corriere della Sera. Domenica 4 - Lunedì 5 - Martedì 6 - Mercoledì 7 - Giovedì 8 - Venerdì 9 - Sabato 10 maggio 1986.

Il Giornale. Domenica 4 - Lunedì 5 - Martedì 6 - Mercoledì 7 - Giovedì 8 - Venerdì 9 - Sabato 10 maggio 1986.

Il Tempo. Domenica 4 - Lunedì 5 - Martedì 6 - Mercoledì 7 - Giovedì 8 - Venerdì 9 - Sabato 10 maggio 1986.

La Stampa. Domenica 4 - Lunedì 5 - Martedì 6 - Mercoledì 7 - Giovedì 8 - Venerdì 9 - Sabato 10 maggio 1986.

L'Umanità. Martedì 13 maggio 1986.

One year after the catastrophy of Bhopal

Toxicology of the environment

by B. HEYNDRICKX* and A. HEYNDRICKX**

ABSTRACT.

Investigating the environment one year after the intoxication by MIC of people and the city, we were able to evaluate the impact of the catastrophy.

The surroundings of the factory of Union Carbide were again populated and we could see many of the survivors.

The general conditions were practically the same as before, the night of December 2nd, 1984 (what the living quarters are concerned, the hygienic conditions and the problems of the slums.

We were also able to evaluate the conditions and many of the patients, where we came to the conclusion that many of the toxic effects of the MIC compound were still noticeable.

Evaluating the conditions of the patients at the University Clinics in the special MIC quarter, we came also to the conclusion that the clinical conditions of many of them were still very serious and that the prognosis was negative.

We discussed the difficult treatment that was given to many of those patients, also according to our suggestions from one year before and the proposals that we made. They were tried by our colleagues professors at the University, Department of Internal Medicine. Unfortunately 6 months later (June 1986) all those suggestions were not helpful.

The paper discusses the evaluation and treatment.

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THE CATASTROPHY.

On the night of December 2nd, 1984 at 10.45 p.m. the first intoxication symptoms appeared in the catastrophy of one of the worst industrial ecotoxicological problems that have ever been occurring in the history of mankind.

At that night in the slum area just across the Union Carbide factory, producing methylisocyanate, called also MIC, the first intoxication symptoms appeared. Speaking with intoxicated persons who survived, they all agree that the first casualties occurred much earlier than it is said officially by Union Carbide and Government Agencies, stating that only at 1 p.m. in the morning of December 3rd, MIC escaped from the factory.

People sleeping on the ground with quite no protection, because no solid buildings in many parts were erected, were surrounded on the floor by a cloud with a special taste and smell which is so well described by the slum dwellers, so that very fast they had difficulties to breath and died. Many of them after 15 to 20 minutes after the intoxication. The cloud was further processing into the slum area. Many tried to escape, running away in the direction of the highway, but fell down due to the fact that the wind direction was coming towards the city, the same direction as they were running. People living opposite the factory of Union Carbide, and so in the opposite wind direction, even only 300 m from there, did not have any big problem.

The cloud processing towards the city and also towards the railroad station invaded those parts before 1 o'clock that night. Practically all people waiting in the station or sleeping there, waiting for a coming train, died an acute death, due to MIC. At the same period, when the massive intoxication occurred, Straw factory ($\pm$ 600 m from Union Carbide, in prevailing wind direction) which employs about 900 labourers, started to be invaded also by the gas cloud. The director of the factory, an old army officer, called directly the army quarters for help. Thanks to the army and the trucks that arrived quite soon, only 33 died in the factory ; all the other ones could be evacuated.

In the meantime, a police officer still on duty, and his superintendent, came back to their office and investigated the matter. With a few colleagues they were the only ones who were on the job or coming back to try to do something about this critical situation. Nobody knew at that time, that MIC was escaping, neither about the toxicity of the gas. At the same period of time

all the government officials, ministers etc., ran away with the official cars with their families and left the city. Parallel in that critical situation, the first patients were brought into the Hamidia University Hospital for treatment. One understands that soon the hospital was overcrowded, some of the university professors had to treat over 800 patients coming in in one hour. At the same time the lack of drugs and all kinds of facilities became a big problem. From the university they called Union Carbide, asking what was going on and what the situation was. The answer was given that everything was under control and that one should not worry about it. Patients coming in further in a very critical state and many already dying or dead, made the colleagues even more suspicious. They called again to Union Carbide but they repeated the same answer. About half an hour later another telephone call was made to Union Carbide ; there was no answer anymore. In that period of time, the general warning in Union Carbide with the sirene was going on twice, only for a short period of time. Nobody knew really what was going on.

Parallel in this critical situation, at about 10.30 p.m. one of the labourers found that pressure was going up and also the temperature in one of the tanks (no. 610). He told this to his superiors but nothing was done. A new shift was coming in at 10.45 p.m., taking up the duties of the other ones. They also found that temperature and pressure were going up and became practically out of control. In that period, the safety valve must have been disrupting, releasing the gas into the atmosphere. Three tanks were used according to the flow sheet production : 610 611 and 619. It was tank 610 which gave all the problems. One of the superintendents tried to obstruct the safety valve, but he did not succeed.

A labourer knowing that three other trains had to arrive in the Bhopal station, called up the railway station and told them that a poisoned gas was escaping from Union Carbide. Thanks to him three trains were stopped, they did not come into Bhopal. He has saved thousands of persons ; this courageous labour man who called, died in the catastrophy in the factory.

In the mean time, nobody in Union Carbide knew to do and how to act. It means their reactions went completely out of sense.

The reason of this exothermic reaction is probably due to cleaning one of the pipes with water, so that water leaked into the tank 610, and resulted in the exothermic reaction, with high tempera-

ture and pressure ; MIC leaving the factory through the vent line or otherwise. At that moment, nothing else could be done anymore.

One of the solutions would have been that a part of the capacity and contents of tank 610 should have been pumped over to tank 619 which was empty, using nitrogen. Tank 611 was partially filled, also with MIC. According to investigations of Indian specialists in the field, about 15 tons escaped. Prof. Dr. S. Varadarajan, Director General of the Council of Scientific and Industrial Research, by recontrolling and investigating, thinks that even from these 15 tons of thank 610, only 13.8 tons escaped from the factory, calculating that 1.2 tons still remained in the plant in thank 610.

The capacity of each tank is 60 tons. Tank 610 held 40 tons, tank 611, 15 tons. Some people say that 40 tons have escaped from the factory. I think that this last investigation is still a question mark. Later on, after the accident, the factory was put into operation again to produce from the remaining MIC the necessary herbicides, so that the MIC left could not be any problem anymore for the future. It means that the 13.8 tons that left the factory produced a gas cloud of about 40 km^2, reaching the grounds of the Hamidia University hospital, it means a very toxic wave, much more toxic than originally thought by industry.

According to the layout of the factory, most of the MIC left the vent line which is 33 m high. It means also that the water curtain would be completely ineffective, if operating, because only 12 to 15 m can be reached, when the MIC vapour reaches out 33 m above ground level. We may not forget that the flair tower was also out of order, as well as the refrigeration system and the scrubber. In these conditions the factory should not have been operating and Union Carbide is completely responsible for what was happening : it is not an accident but negligence ; what we can compare to the Seveso case in Italy where we had the intoxication by dioxines, due to the fact that Hoffmann-La Roche was operating the ICMESA unit. When the safety valve bursted open in Seveso, there was also no confinement and ± 3.5 kg of dioxine caused the damage that we all know.

At 2 a.m. the professors and physicians at the Hamidia University Hospital had the first symptoms of intoxication : eye irritation and irritation of the throat, because the cloud was reaching the emergency unit and the hospital building. During that period, officially between 2000 and 3000 people were already dead, other

ones were still dying, and to some officials, qualified physicians and personnel, around 15,000 to 18,000 people finally died, and between 100,000 and 200,000 intoxicated. One understands that during that apocalyptic night, where in some parts of the slum area there was also no electricity and no water, Bhopal had one of the greatest industrial intoxications that have ever been occurring. When the sun was rising that morning, all around the Union Carbide factory, what was alife was dead : holy cows, goats, dogs. People were gazed as rats. The only animals that escaped were the chicken. We know that they have a special breathing system (air sacks) and that their body temperature is around 40°C. They also have a different physiological air filtering system, which could explain why they survived ; the boiling temperature of MIC is 39°C.

Many scientists say that MIC is hydrolyzed very fast by water and that at that time it becomes inoffensive. This is completely contradictory from what I investigated personally on the spot.

The fish died in Bhopal lake, even two days after the catastrophy, so that the water was also undrinkable. If MIC would hydrolyse, we would not have fish dead in the lake. Also by the autopsies, the professor who performed them told me that after seven autopsies he got intoxicated : his eyes and throat were aching, due to MIC liberated from the corpses.

Another example of stability of MIC in body fluids, or toxic hydrolysis compounds.

A third example of the stability of MIC is that even 2 1/2 months after the catastrophy, stagnating water in pools was still toxic.

The conclusion is that only water curtains and using water to protect us from MIC gases is an illusion. By the autopsy, the colleagues said that the blood was reddish coloured and totally abnormal. There was also necrosis of liver, kidney, toxicity on the lungs, which could be proven by anatomopathological investigation. Many of the patients were blind and had a very irritating effect of the gas on the eyes. The cornea was affected, the retina was normal. In general, after seven or eight days, sight came back and the irritation was less painful.

According to the colleagues ophthalmologists, the best results they obtained was by using atropine sulphate solution the first three days, followed by corticosteroids. If they used corticosteroids from the beginning, the results were worse. The final prognosis, if there would be any influence left after this toxic reaction, is a question mark. From the ones who survived

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in general after 9 to 10 days, no positive X-rays were seen anymore, which was not the case in the beginning of the intoxication, where the X-ray pictures were very impressing about the positive reaction and the high toxicity of MIC.

During this apocalyptic night, the students of the university went to knock at the doors of all chemist shops in Bhopal, to ask the pharmacists to bring all the drugs they had to the university hospitals. They all did and were very cooperative. The greatest help that was given that night, was done by volunteers who did a tremendous job in helping their neighbours as much as they could. Thanks to them and all what they have done, the great suffering of the people was to some part alleviated. There was no help coming from any official organization and Union Carbide did not even tell the university professors what gas was escaping. It was a complete question mark what it was and how the patients should be treated.

The only help from WHO came 48 hours later by a telex telling that the treatment should be sodiumthiosulphate and corticoids, etc. : means symptomatic treatment. The medical team which was coming in from Union Carbide, United States, consisted of the director physician who is an Indian and some colleagues.

They could not suggest any treatment neither help, it means that after 48 hours they went back to their own country. What the laboratory facilities are concerned, only a Hartridge spectroscope was brought to Bhopal, and a colleague brought with him a spectrophotometer. It means that the clinical toxicological investigation was very poor and that we can only have some macroscopic description given by the physicians, and the professor pathologist and his staff who were treating those patients. They have done what they could in the difficult situation where they were working, using all means that they could find. Also volunteers from Calcutta and other cities came over to Bhopal to help in the treatment of all those patients. They have done an outstanding job, using the means they had. From official side there was practically nothing, also the international help was totally neglectable.

The scientific information was nihil and everything had to be done from scratch. According also to some statistics, 2 1/2 months after the catastrophy, there are twice more still born children, means 1 out of 4 from mothers who were probably intoxicated ; before it was : one child out of 8. According to some of the information which still has to be confirmed, one thousand are

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blind. All those statistics are very difficult; there is no civil registration. Practically none of them can write and read, many cadavers were burnt in a hurry after death, according to the Indian tradition, due to the hygienic conditions in the city and the high temperature even in winter (30-32°C at noon — at night they had a temperature of about 6-8°C and the night of the accident), a slow wind blowing from Union Carbide into the direction of the city, it was an open sky, one could see the stars very nicely. More official details about the meteorological conditions are missing.

Officially the sequence of the events is as follows:

Many thermometers and pressure gauges are out of order.

— 11 p.m. (December 2nd, 1984): in tank 610 the pressure rises from the normal 3 pounds per square inch to 10, in neighbouring tank 611 one had increased deliberately the pressure by injecting nitrogen to move the MIC into the pesticide manufacturing unit. The new staff coming in pays little attention to the pressure rise in tank 610 and believes that the pressure has been risen by the earlier shift, to transfer MIC to the pesticide unit.

— 11.30 p.m. the operating staff feels irritation of the eyes due to the MIC leak. They are not upset, because sometimes tiny leaks occur and are not unusual. At midnight the operators around the MIC unit feel the leak and report to the production assistant, Mr. Shakil Ibrahim Qureshi.
Parallel from the MIC control room, the operator reports to Mr. Qureshi that the pressure in tank 610 is high.

— 12 p.m. Mr. Qureshi and an operator check tank 610 and they find that the disc is ruptured. This occurs when the pressure reaches 40 pounds per square inch and the safety valve, the next check point, is opened.

— 0.30 a.m. — December 3rd: the water which washes the tubes is turned off but this is already much too late to bring the situation under control.

— 1 a.m.: MIC vapour untreated is escaping through the 33 m high vent line into the air.

As said earlier this official report does not coincide with the testimonies of escaping people from the slum area, as told to me, where people were already gased at 10.45 p.m. on December 2nd, much earlier than given in the official report.

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EXPERIMENTAL RESEARCH.

Calculating in function of the amount of MIC from 10 to 40 tons in correlation with the vent line and also the possibility that the gas was coming out through the factory itself, what we will never know, directly from the tanks or otherwise, we come to the following calculations (fig. 1, 2, 3 and 4 — tables I, II, III and IV).

Putting those figures down on the map of Bhopal, we can have an idea, what the catastrophy would be around a factory in the Western World and the cities and villages around it, if it would occur.

From recent accidents in Belgium, where a great electrical plant (Ebes, Ghent) delivering electricity to a steel mill had great difficulties with the transformer that exploded and the CO-intoxications which occurred, even three fatal ones by labouring people, where safety devices did not work, we come to the conclusion that even in our industrialized countries catastrophies can happen.

One can imagine, if the electricity would be cut off and the refrigeration units would not operate anymore, what we could do with the MIC storage tanks where we gave officially by the State also the authorization to have twice 50 tons of capacity of storage (Bayer, Antwerp, Belgium). One can claim that the stand by units should directly be operating. The examples of today and what happened on Saturday, March 9th, 1985 in the steel mill of Sidmar in Ghent makes us thinking. Of course the risk is much smaller than in developing countries, where we don't always have the punctual trained staff and all other facilities. Even if we would have had computerized systems in Bhopal, as many say, the catastrophy could not have been avoided. Three units which are vital to the production did not work.

We should have also in our Western countries, more qualified personnel at night and on weekends, where we have at the moment, for financial reasons, a cut down of highly specialized scientists and technicians. When the production is going out of order, no computer will solve the problem. It is only by fast and qualified personnel coming in that those catastrophies can be avoided.

There will always be a calculated risk in operating those huge factories, which are becoming greater and greater compared to 20 years ago. It means also that the risk of big catastrophies becomes much higher than it was in the past.

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GAUSSISCH VERSPREIDINGSMODEL
(STAD ANTWERPEN/CLV)

WS 2.0 [m/s]
Pasq.Klasse: F
RL 1.00 [m]
HB 2 [m]
D 0.11 [m]
Q 0.694 [kg/s]
RF 100 [%]
US 30.0 [m/s]
T 100.0 [0C]
WH 1.0 [m]
Xm= 150 [m]
Cm= 1262 [mg/m3]

ISOLIJNEN BIJ
VOLGENDE
CONCENTRATIES
in [gr/m3]
0.005
0.010
0.050
0.100
0.500

SCHAAL = 1/50000

5000m
10000m

FIG. 1.

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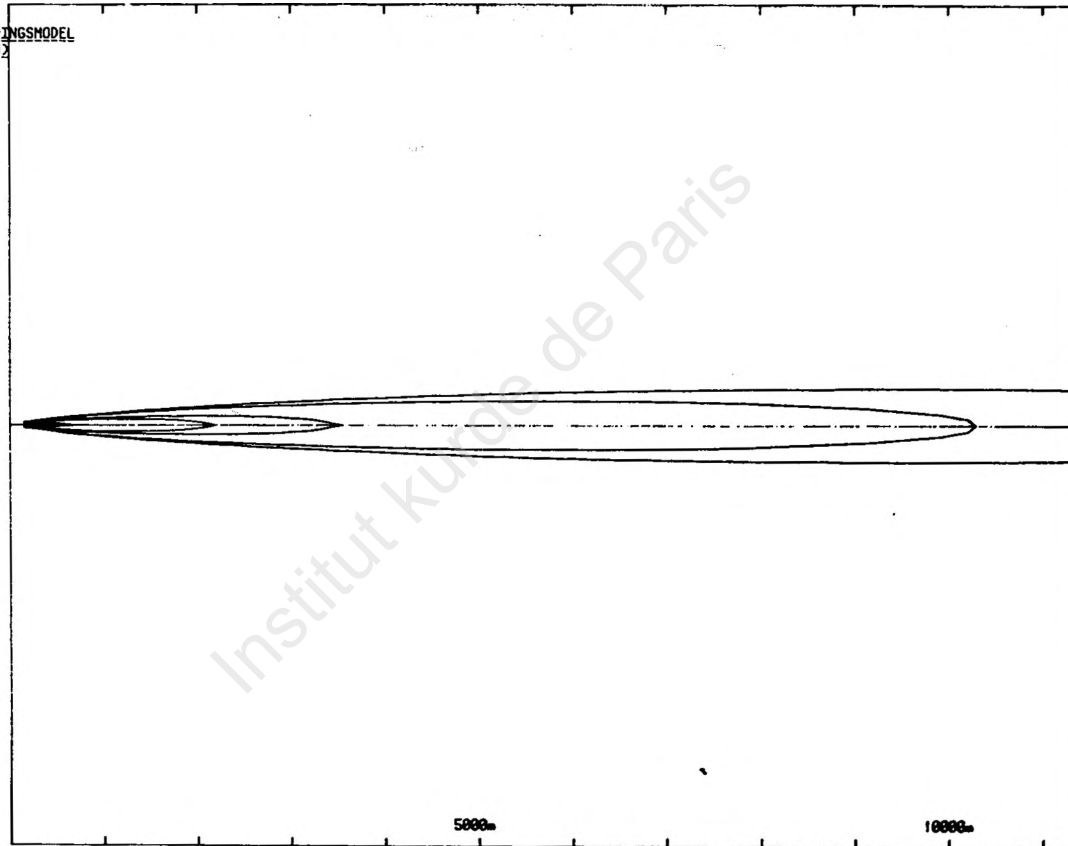

FIG. 2.

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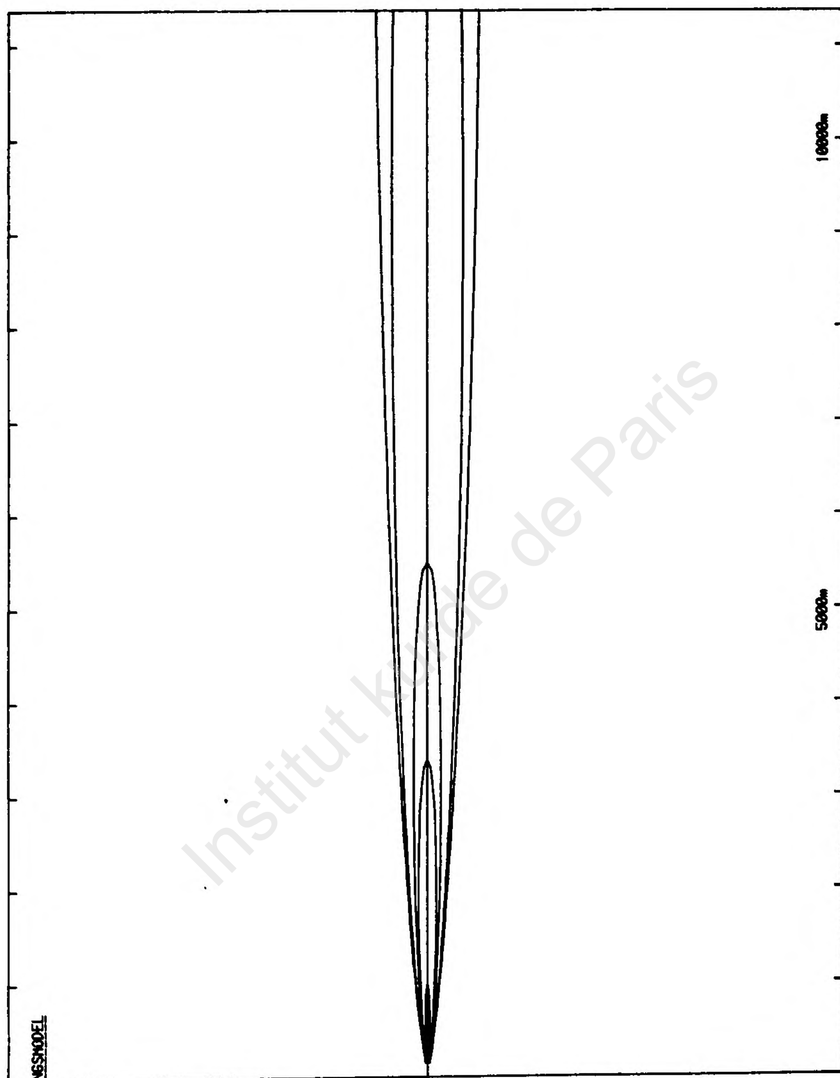

FIG. 3.

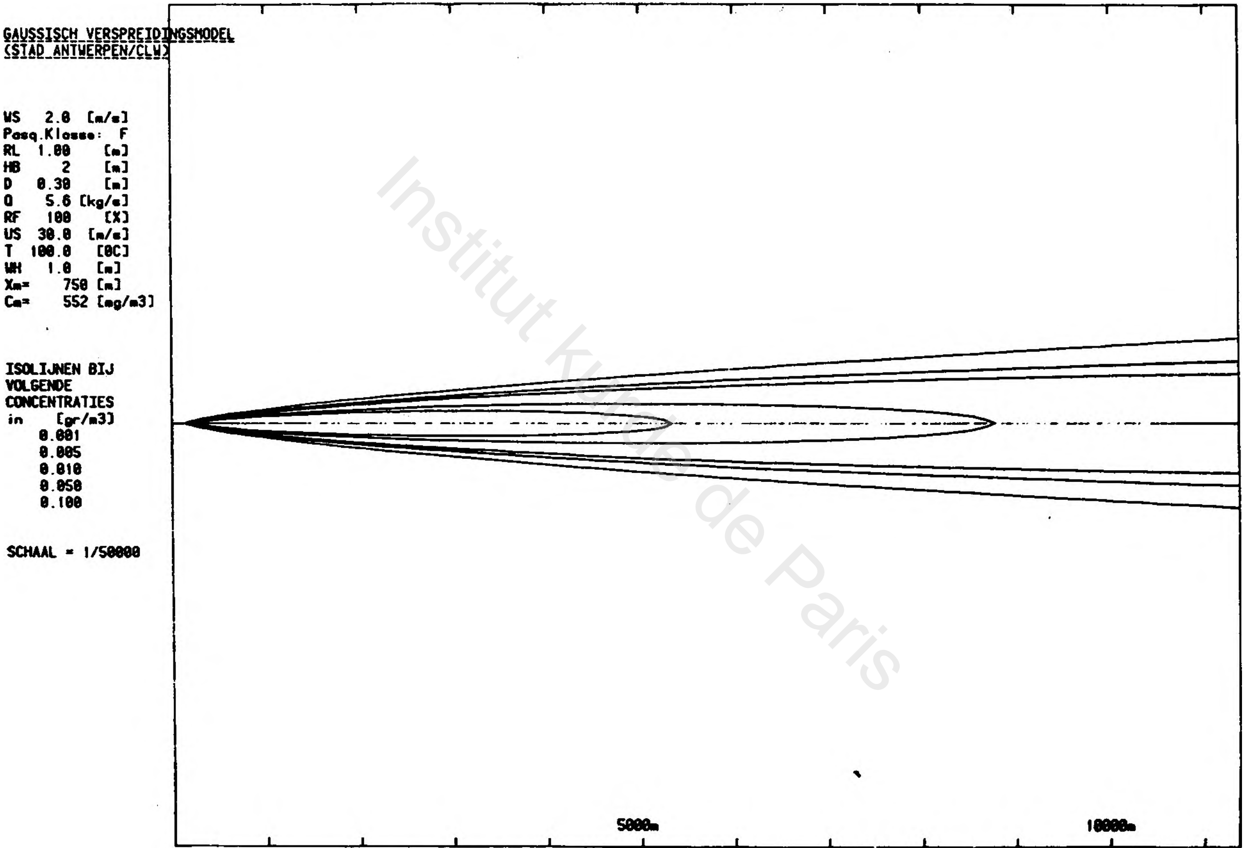

FIG. 4.

TABLE I

GAUSSISCH VERSPREIDINGSMODEL
(STAD ANTWERPEN/CLU)

US 2.0 [m/s]
Pasq.Klasse: F
RL 1.00 [m]
HB 2 [m]
D 0.11 [m]
Q 0.694 [kg/s] 21/2 t/h
RF 100 [%]
US 30.0 [m/s]
T 100.0 [OC]
WH 1.0 [m]
Xm= 150 [m]
Cm= 1262 [mg/m3]

X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)
150	1261.965	300	675.491	450	422.912	600	294.731	750	220.050
900	172.236	1050	139.503	1200	115.953	1350	98.349	1500	84.785
1650	74.072	1800	65.438	1950	58.358	2100	52.468	2250	47.505
2400	43.277	2550	39.641	2700	36.486	2850	33.727	3000	31.299
3150	29.149	3300	27.234	3450	25.519	3600	23.977	3750	22.583
3900	21.320	4050	20.169	4200	19.119	4350	18.156	4500	17.271
4650	16.456	4800	15.702	4950	15.005	5100	14.357	5250	13.755
5400	13.193	5550	12.668	5700	12.178	5850	11.718	6000	11.286
6150	10.880	6300	10.497	6450	10.137	6600	9.797	6750	9.475
6900	9.170	7050	8.881	7200	8.607	7350	8.347	7500	8.100
7650	7.864	7800	7.640	7950	7.426	8100	7.222	8250	7.027
8400	6.841	8550	6.662	8700	6.491	8850	6.328	9000	6.171
9150	6.020	9300	5.876	9450	5.737	9600	5.603	9750	5.475
9900	5.351	10050	5.232	10200	5.118	10350	5.007	10500	4.900
10650	4.797	10800	4.698	10950	4.602	11100	4.509	11250	4.419
11400	4.332	11550	4.248	11700	4.167	11850	4.088	12000	4.012
12150	3.937	12300	3.866	12450	3.796	12600	3.728	12750	3.663
12900	3.599	13050	3.537	13200	3.477	13350	3.418	13500	3.361
13650	3.306	13800	3.252	13950	3.200	14100	3.149	14250	3.099
14400	3.051	14550	3.003	14700	2.958	14850	2.913	15000	2.869

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TABLE II

GAUSSISCH VERSPREIDINGSMODEL
(STAD ANTWERPEN/CLW)

US 2.0 [m/s]
Pasq.Klasse: F
RL 1.00 [m]
HB 2 [m]
D 0.15 [m]
Q 1.4 [kg/s] -- 5t/h
RF 100 [%]
US 30.0 [m/s]
T 100.0 [OC]
WH 1.0 [m]
Xm= 150 [m]
Cm= 1275 [mg/m3]

X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)
150	1275.041	300	992.069	450	698.382	600	514.576	750	396.686
900	316.947	1050	260.395	1200	218.697	1350	186.962	1500	162.172
1650	142.383	1800	126.295	1950	113.010	2100	101.891	2250	92.476
2400	84.421	2550	77.467	2700	71.415	2850	66.109	3000	61.427
3150	57.270	3300	53.561	3450	50.234	3600	47.237	3750	44.525
3900	42.062	4050	39.818	4200	37.765	4350	35.883	4500	34.151
4650	32.554	4800	31.077	4950	29.708	5100	28.436	5250	27.252
5400	26.148	5550	25.116	5700	24.150	5850	23.244	6000	22.393
6150	21.593	6300	20.839	6450	20.128	6600	19.456	6750	18.820
6900	18.219	7050	17.648	7200	17.107	7350	16.592	7500	16.103
7650	15.637	7800	15.193	7950	14.770	8100	14.366	8250	13.980
8400	13.610	8550	13.257	8700	12.919	8850	12.594	9000	12.283
9150	11.985	9300	11.698	9450	11.423	9600	11.158	9750	10.903
9900	10.658	10050	10.422	10200	10.194	10350	9.974	10500	9.762
10650	9.558	10800	9.360	10950	9.170	11100	8.985	11250	8.807
11400	8.634	11550	8.467	11700	8.305	11850	8.149	12000	7.997
12150	7.849	12300	7.707	12450	7.568	12600	7.434	12750	7.303
12900	7.176	13050	7.053	13200	6.933	13350	6.817	13500	6.704
13650	6.594	13800	6.486	13950	6.382	14100	[illegible]	14250	[illegible]
14400	6.085	14550	5.991	14700	5.900	14850	5.811	15000	5.724

TABLE III

GAUSSISCH VERSPREIDINGSMODEL
(STAD ANTWERPEN/CLW)

```
WS   2.0  [m/s]
Pasq.Klasse:  F
RL  1.00    [m]
HB     2    [m]
D   0.21    [m]
Q    2.8 [kg/s] -- 10t/h
RF   100    [%]
US  30.0  [m/s]
T  100.0   [OC]
WH   1.0    [m]
Xm=    300 [m]
Cm=    880 [mg/m3]
```

X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)
150	413.336	300	880.198	450	848.992	600	726.448	750	610.316
900	515.377	1050	439.965	1200	380.000	1350	331.836	1500	292.667
1650	260.326	1800	233.314	1950	210.567	2100	191.217	2250	174.603
2400	160.233	2550	147.699	2700	136.696	2850	126.977	3000	118.344
3150	110.635	3300	103.719	3450	97.488	3600	91.850	3750	86.730
3900	82.064	4050	77.798	4200	73.886	4350	70.288	4500	66.970
4650	63.903	4800	61.061	4950	58.422	5100	55.966	5250	53.676
5400	51.537	5550	49.535	5700	47.658	5850	45.896	6000	44.239
6150	42.679	6300	41.207	6450	39.817	6600	38.504	6750	37.260
6900	36.081	7050	34.963	7200	33.900	7350	32.890	7500	31.929
7650	31.014	7800	30.140	7950	29.307	8100	28.511	8250	27.751
8400	27.023	8550	26.326	8700	25.658	8850	25.018	9000	24.404
9150	23.815	9300	23.248	9450	22.704	9600	22.180	9750	21.676
9900	21.190	10050	20.723	10200	20.272	10350	19.837	10500	19.417
10650	19.012	10800	18.620	10950	18.242	11100	17.876	11250	17.522
11400	17.180	11550	16.848	11700	16.527	11850	16.216	12000	15.914
12150	15.622	12300	15.338	12450	15.063	12600	14.796	12750	14.536
12900	14.284	13050	14.040	13200	13.802	13350	13.570	13500	13.345
13650	13.126	13800	12.913	13950	12.705	14100	12.503	14250	12.307
14400	12.115	14550	11.928	14700	11.746	14850	11.568	15000	11.395

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TABLE IV

GAUSSISCH VERSPREIDINGSMODEL
(STAD ANTWERPEN/CLW)

US 2.0 [m/s]
Pasq.Klasse: F
RL 1.00 [m]
HB 2 [m]
D 0.30 [m]
Q 5.6 [kg/s] -- 20 t/h
RF 100 [%]
US 30.0 [m/s]
T 100.0 [oC]
WH 1.0 [m]
Xm= 750 [m]
Cm= 552 [mg/m3]

X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)	X(m)	CONC(mg/m3)
150	3.469	300	154.287	450	381.427	600	509.325	750	552.296
900	549.370	1050	525.194	1200	492.619	1350	457.945	1500	424.130
1650	392.461	1800	363.399	1950	337.003	2100	313.145	2250	291.620
2400	272.199	2550	254.657	2700	238.784	2850	224.389	3000	211.305
3150	199.382	3300	188.490	3450	178.516	3600	169.185	3750	160.593
3900	152.678	4050	145.370	4200	138.607	4350	132.337	4500	126.511
4650	121.088	4800	116.031	4950	111.307	5100	106.887	5250	102.745
5400	98.856	5550	95.202	5700	91.761	5850	88.518	6000	85.458
6150	82.566	6300	79.830	6450	77.239	6600	74.782	6750	72.450
6900	70.234	7050	68.126	7200	66.120	7350	64.209	7500	62.386
7650	60.646	7800	58.984	7950	57.395	8100	55.874	8250	54.419
8400	53.024	8550	51.687	8700	50.404	8850	49.172	9000	47.989
9150	46.852	9300	45.758	9450	44.705	9600	43.691	9750	42.715
9900	41.773	10050	40.865	10200	39.989	10350	39.143	10500	38.326
10650	37.537	10800	36.774	10950	36.036	11100	35.322	11250	34.630
11400	33.961	11550	33.312	11700	32.684	11850	32.075	12000	31.484
12150	30.910	12300	30.354	12450	29.814	12600	29.289	12750	28.779
12900	28.284	13050	27.803	13200	27.334	13350	26.879	13500	26.436
13650	26.004	13800	25.584	13950	25.175	14100	24.777	14250	24.389
14400	24.011	14550	23.642	14700	23.282	14850	22.931	15000	22.589

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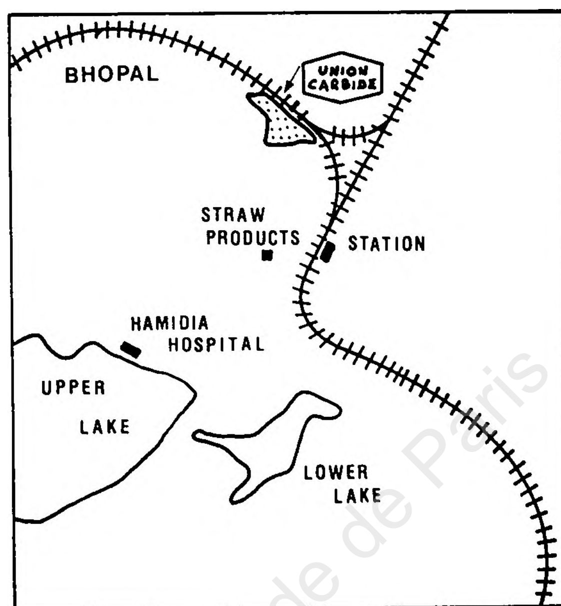

FIG. 5.

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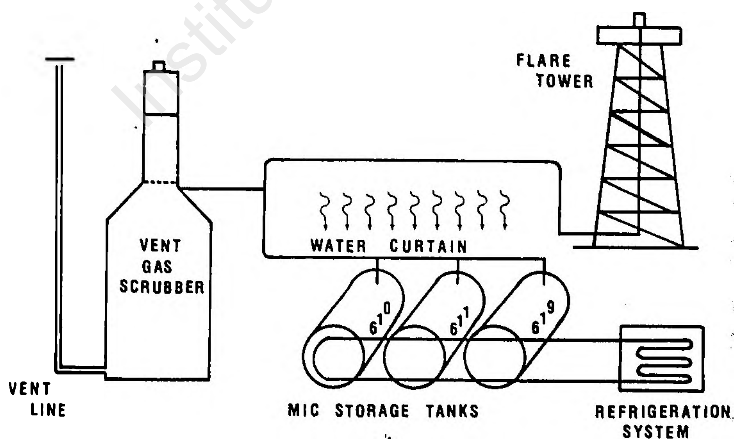

FIG. 6.

TOXICOLOGICAL INVESTIGATION.

Methylisocyanate, brought into contact with human blood, does not produce any carboxyhemoglobin in our experiments. We could not detect either by spectrophotometry or gas chromatography, any quantity. We could however find very high quantities of methemoglobin (only *in vitro*). It is impossible for the pathologist, macroscopically to differentiate. At that moment the possibilities in the laboratories of the Bhopal university hospitals were not such that they could have been differentiated.

For that production 2 patents are used :

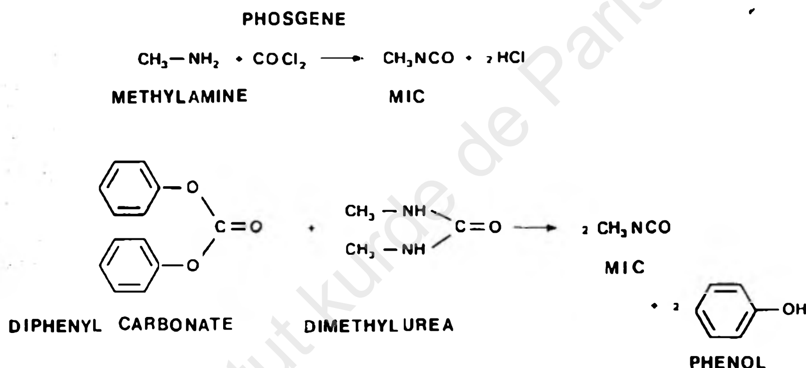

FIG. 7.

Union Carbide is using phosgene in its production scheme. Of course small concentrations of phosgene have also been present the night of the catastrophy, between 0.03 and 0.1 ppm, because phosgene is used as a stabilizer during the production and of course also during the storage in tank 610. These concentrations are of course working synergistically with MIC, but are only of little importance, relatively to the high concentrations of the MIC and the toxicity on the human being. We can neglect that influence. It appears that MIC is much more toxic than many war gases used today in the world, as the ones we have analyzed in Iran, and the ones in Cambodia and Afghanistan. It means also that it is a warning for modern industry and also for civilization that we should be much more strict for industry about the possible catastrophies that can occur. Many of those chemicals are so dangerous with no antidotes for man when the intoxication will occur, only a symptomatic treatment. It means

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also that in our consumption industrial organization we have to be much more careful in setting up those production units. It is not with theoretical safety devices and disaster plans if something would occur, disaster plans which have never been tried, that we can solve the problems of the community.

Safety valves and scrubbers can only be accepted if they are in the confinement.

It is much too dangerous just to use these scrubbers and rupture discs etc. to save the factory from explosion and great financial damage, and not to solve the problem of the human beings working in that factory or in villages nearby. Industry has to understand that we cannot accept that risk anymore and that many safety devices do not protect us.

The patients in Bhopal have to be followed in the future to see if there could be a teratogenic effect or some pathology that we do not know today. By investigating them regularly and bringing them for control, we could perhaps help them much better than it has been done up until now, and see how we can help them in their future lives. The war gases from 1914 and our veterans — many of them suffered all of their lives, because their lungs were burnt — are a warning for what can happen to man if we are not controlling industrial production. In many cases there will be no help but only a symptomatic treatment which is so ineffective.

Six months after the catastrophy people were still suffering from pulmonary complications and inefficiency (in- and outbreathing), no permanent blindness (?). For the teratogenic effects on babies, we still have to wait another three months from today (May 15, 1985).

ONE YEAR AFTER THE CATASTROPHY (January 1986).

When we investigated locally with the professors of the Faculty of Medicine in Bhopal, we could not conclude that there would be any teratogenic effect on the born babies, conceived during the period of the gas intoxication of the pregnant mothers.

Comparing different treatment methods, e.g. high concentrations of Vitamin C intravenously (2 g/day), the sodium thiosulphate therapy as suggested by WHO, acetylcystein intravenously as we do in the case of Mustard Gas intoxication in chemical warfare, as in Iran, there was no improvement at all of those patients

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treated in the MIC quarter of the University Hospitals. These treatments in those cases are worthless.

At the moment we have no specific antidote or pharmaceutical. compound that could be helpful in those conditions.

In rabbit experiments, where we used also the above mentioned compounds, we have seen that sodium thiosulphate, given before the intoxication occurs, yields a good protection. Vitamin C was less efficient.

Munn (1) describes the hazards of isocyanates, the toxicity values and the MAC concentrations.

In general it is accepted by the American Conference of Governmental Industrial Hygienists that 0.02 ppm is the maximum tolerant level. These values are still discussed. According to the Bhopal tragedy, it should be less.

We agree with the colleagues in Bhopal (Prof. Misra — personal communication) : the toxicology is much more complicated than originally thought, the decontamination much more difficult and the treatment unfortunately only symptomatic with very poor results. It is a very dangerous compound with a much higher toxicity than in general found in literature.

From the figures we have calculated, we can also propose some values with concentrations that probably we have had in the air, which were fatal in the slum area, and also values for the subacute toxicity.

Further on, the lesions of the patients (mainly pulmonary) were after 1 year of intoxication irreversible. At the moment, we don't see any specific treatment, what active pharmaceutical compounds are concerned, to help the intoxicated survivors.

REFERENCE.

1. MUNN A. *Ann. Occup. Hyg.*, 1965, *8*, 163.

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Toxicological chemical reactions between MIC (Methylisocyanate) and human blood

by B. HEYNDRICKX* and A. HEYNDRICKX**

ABSTRACT.

In toxicology we still have big problems, trying to evaluate and determine the difficult reaction that human hemoglobin in the living patients yields, according to the intoxications of dangerous industrial chemicals.

Since many years we study this phenomenon, where we faced the very difficult problem, not solved yet to our knowledge, of the chemistry of hemoglobin and the interreaction with those hazardous toxic compounds.

The problem is that all physical chemical methods are so difficult to study in a medium as water, not knowing what the basic hemoglobin as physical properties has.

The linkage that we found between hemoglobin and MIC is very stable, practically not possible to disconnect or reactivate, with very strange spectra or identification patterns that we found by electroforesis or electrofocusing.

Already with the naked eye or by spectroscopy, we can see this strange phenomenon.

The interreaction is discussed in order to try to find a suitable treatment which is not found yet.

INTRODUCTION.

Isocyanates undergo decomposition to isocyanic acid and olefin, which are in general not very polar.

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Around 600°C (Back and Childs) (1) we have the pyrolysis of isocyanic acid, yielding CO, CO_2 and N_2.

In the Bhopal case the temperature was much lower, so that we could not have CO and HCN. Those kinetic studies, what the decomposition is concerned, are not realistic.

Furthermore the symptoms of the patients were completely different from a CO or cyanogen intoxication.

We known that MIC is pyrolyzed in the gas phase in the temperature range of 427-548°C at pressures from 55 to 300 torr.

DETERMINATION OF METHYLISOCYANATE (MIC).

Kimmerle and Eben (2) have investigated the toxicity of MIC and the quantitative determination in the air. A very extensive study has been made and also the method given.

The detection being done by absorption spectroscopy is not very sensitive and specific.

Since then, techniques have been set up, using HPLC, which are much more reliable. Of course the sampling is still a big problem. In their toxicity studies, mainly on rabbits, mice and rats, they found in function of the time, going from 30' to 1 day, even to 18 days, practically identical symptoms on rabbits. Also the edema of the lungs was noticed, as well as toxicity on the skin ; analogous problems that we have seen by the intoxicated men in Bhopal. The toxicity is primarily associated with the respiratory system.

William Rye (3) has also found, much earlier, those symptoms and pathology in workers exposed to those materials and their fumes. Isocyanates can polymerise easily, the reaction kinetics being in many cases unknown and still under investigation.

The final length of the reaction is almost unlimited what the final compounds are concerned. In general, the larger the molecular weight of the isocyanate in question, the lower the toxicity, the lower the volatility and vapour pressure, which is very important in the industrial management, what the flow sheet of the production is concerned.

The low molecular weight ethyl and methyl isocyanates are not only skin irritants but can also cause permanent eye damage. Higher molecular weight compounds as methyl diisocyanate have less skin toxicity and in general no permanent toxic reaction in the eye.

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In the breathing zone, a concentration of 0.5 ppm isocyanate can give an imminent respiratory response.

The symptoms of the subacute intoxication are mainly : increasing secretion, cough, pain of the respiratory tract, edema and dyspnea. From acute intoxication as found in the patients of Bhopal, where some died after 7 to 8 minutes, they died from acute asphyxia.

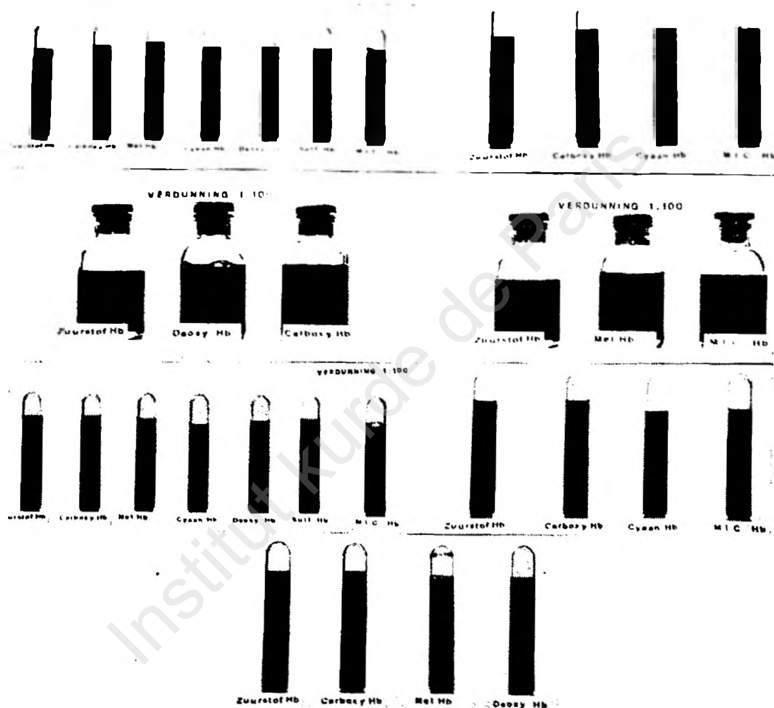

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FIG. 1. — Reaction of human blood and MIC.

The symptoms of death that were occurring, were completely different from a CO or cyanide intoxication.

Looking microscopically to the blood (fig. 1), we can distinguish already by the naked eye, that the blood seen by the autopsy was completely different from a CO, cyanide or chlorate intoxication. The blood has a typical colour, which we have never seen in any post-mortem before.

We analysed the blood spectroscopically and spectrophotometrically, but could not find any wavelength that could be of any value for determining the poison.

FIG. 2.1.

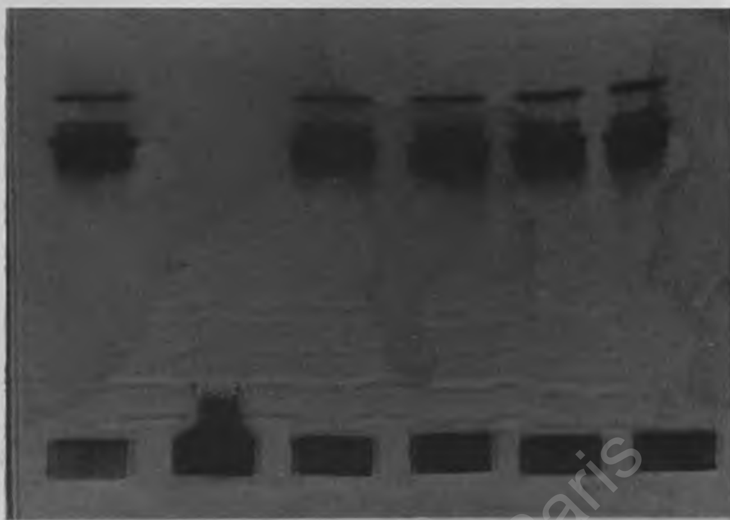

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FIG. 2.2.

FIG. 2.1 and 2.2. — EAP (Erythrocyte Acid Phosphatase).

Parameters : Isoelectrofocusing (IEF) — PAG-plate (LKB), pH 3.5-9.5 — 1500 V — 50 mA — 30 W.

	Person	*Blood grouping*	*EAP*
Band 1	SD	O +	B
Band 2	SD + MIC		Not detected
Band 3	AC	O +	BA
Band 4	AVDB	O +	CA
Band 5	MA	O +	A
Band 6	SD	O +	B

Top : cathode.

FIG. 2.1. — At the end of the IEF-run.

FIG. 2.2. — Visualisation of the EAP enzymes with methyl-umbelli-pheryl-acetate.

We have here a very stable linkage between the hemoglobin and MIC, that could not be further differentiated, using the two mentioned methods.

The electrophoresis and electrofocusing (4) however are very interesting (fig. 2), where we could see specific bands that we don't find in general poisonings as mentioned above. It is that linkage which is so poisonous and which plays a big part in the intoxication syndrome.

The MIC/hemoglobin syndrome ; the linkage that we were unable up to now to split searching for compounds which could be useful in the treatment of men, is so stable that we cannot propose any pharmaceutical compound that could split it in normal human conditions.

Therefore MIC is a very dangerous toxic chemical.

We must be very careful to prescribe all hygienic conditions, in order to prevent the intoxication of men. The ones who recovered the toxic wave can impossibly be treated effectively with the compounds we have at our disposal at the moment in medicine and pharmacy.

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Regulatory approach to safety of industrial installations

The «Seveso» directive

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It may appear rather surprising to associate under the tittle of a Congress, *Chemical Warfare* and *Industrial Chemical Disasters*. Agreed, both terms include the word « chemical », and this morning we were given further reasons by Prof. Heyndrickx, but actually they are quite different in their essence :

— The former is a deliberate destructive action, put to the highest degree of sophistication and efficiency by a small number of persons.

— The latter is an unfortunate deviation of an activity necessary to the society, and where much intellectual and physical resources of a number of people aim at reducing both its probability and consequences.

What I intend to develop today is a few considerations on the way the industry and the authorities manage the risk of such deviations. My colleague, Jos Bormans, will later on present an actual example of part of this management, namely how the emergency planning is performed in this country.

Well, there are several reasons pushing a manufacturer to make sure that his processes, plants or products are safe. Just to list some of them :

— First, his personal ethics : the role of a manager is not to put at danger the life or health of his co-workers, customers or people living around his plant. If in this audience there are persons who like me, are or have been plant managers, they certainly know how strong is this feeling.

— Then, his responsibility towards his shareholders : the money they have lent him must not be wasted in an accident. It is much sounder to use it for creating wealth for the mankind.
— He has to protect the market and the image of the company, which could be seriously jeopardized by certain accidents.
— And finally, his liability, both civil and criminal. We all know the amount of the compensation granted by the courts for some accidents.

To achieve this objective of safety, it is now customary in all reputable companies to establish a *Safety Policy*. There are obviously many possibilities to draft such safety policies, but all of them turn around *five fundamental principles* :

1. At the conceptual stage of an activity, *the risk* should be assessed and minimized. This means that already at the laboratory stage, everything has to be known about the product and the process, that at the design stage, a safety analysis has to be carried out — and a number of highly sophisticated methods have been developed for that — and that all remedial measures have been taken accordingly.

2. During the operation, the *initial level of safety* has to be maintained by the means of organizational and managerial measures : detailed operating manuals and instructions, education and training of the workforce at all levels, safety audits, etc.

3. As a zero-risk situation does not exist, *consequences of possible deviations* from normal situation should be minimized by technical measures (safety values, detectors, fire fighting equipment) as well as by organizational ones : establishment of an emergency plan, both internal to the site, and external, for the surrounding community, with appropriate drills and information to the population.

4. When *an accident did occur,* the analysis of its causes and circumstances gives lessons which have to be given consideration to prevent the further occurrence of similar ones.

5. And finally, and this is the key for such a policy being successful, all the workforce, from the top manager to the shopfloor attendant, should be *motivated and committed to safety*.

This being said, is a legislation on industrial safety necessary ? In many countries, the answer to this question has been no, at

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least partially, and this did not prevent these countries to achieve quite good safety records. But other countries have decided to develop such a legislation, mostly because of cultural or political tradition.

As a matter of fact, there are some advantages in having clear and sensible regulations in that matter :

— they set safeguards against the actions of certain irresponsible managers ;
— they can compensate the lack of experience or training of some managers, especially in the small or medium-size companies ;
— they give the public some sense of protection, meeting its aspiration to the right-to-know, which indeed is no´ longer necessary like it is with the « liberal » approach to document its claims for compensation.

In our European countries, there has been a long history of safety regulations : as far back as the XVth century, the Republic of Venice enacted regulations defining the strength of ship ropes. With the steam engine and the Industrial Revolution, the XIXth century saw a flourishing of *equipment-related* regulations, covering specific technical fields.

It was only recently however that certain European countries began drawing up some safety rules for *industrial establishments* taken as a wholeon a site basis. This trend accelerated in the seventies in the wake of a number of spectacular accidents, Feyzin 1966, Flixborough 1974 and Seveso 1976. Even if these accidents led to relatively minor consequences in terms of human deaths, compared to other natural or industrial catastrophs, they have had a considerable impact on the public opinion.

Both to meet the public concerns and to avoid the possibility of economic distorsions stemming from different national approaches, the Commission of the European Communities started in 1976 the preparation of a Community legislation which resulted in 1982 in the adoption by the Council of the « Directive on Major Accident Hazard of certain Industrial Activities », better known under its nickname of « the Seveso Directive ».

Of course, I do not intend to describe the Directive in full detail, I only want to identify its major features :

1. The *installations* falling under the Directive are defined as those carrying out one of the activities listed in a first annex

and handling one of the 178 substances listed in another annex with individual threshold quantities ranging from 1 kg to 50,000 tons.

One may wonder why the scope of the Directive has been limited by such restrictive conditions. The answer is very simple : for such a regulation to be effective, its compliance should be checked, but this requires a corps of highly competent inspectors. And resources are not infinite, both in terms of money and of expertise.

2. The provisions of the Directive are nothing but the translation into legal terms of the fundamental principles of the safety policy described earlier — with the exception, of course, of the last one : motivation and commitment for safety cannot be imposed by law !

In practical terms, all installations covered by the Directive have to be *notified* to the competent authorities. The dossier of notification has to give information on the chemicals handled, on the process used, including the results of the safety analysis, on the technical measures decided to ensure the safety of operation, and finally all information relevant to the setting up of the emergency plans.

3. The Directive finally defines the role and duties of the *competent authorities*. They have to assess the content of the dossier supplied by the manufacturer. Notification does not mean authorization. As a matter of fact, even in the Member States who have not established, strictly speaking, a licensing system, the powers granted to the competent authorities are such that they are quite able to check the quality of a notification dossier, and therefore to effectively control the safety of the installation. The competent authorities have to make sure that an effective emergency plan, both within the site and for the vicinity community, has been established. Finally, they have to be notified of every major accident undergone and to report them to the European Commission, for the setting up of a register of such accidents for a prevention purpose.

As it is the rule with the EC legislative machinery, the Directive has to be implemented by the Member States after integration into their own national regulation system. Where do we stand in that respect, more than two years after the effective date of enforcement of the Directive ?

Formally, only two Member States have been able to notify to the Commission that they have effectively translated into their national legislation all provisions of the Directive. On the other hand, two Member States have just begun to consider the drafting of a corresponding legislation. But this does not mean that the other countries are in a bad situation, on the contrary, but the adaptation of the legislation existing prior to the release of the Directive implies some delays. This is for example the case with Belgium and France.

It is important to stress that Europe with the Seveso Directive and its translation in the various Member States is in a unique position : nowhere else in the world exist such comprehensive regulations for the Safety of Industrial Installations (with the exception of the nuclear industry). Of course, this unique situation has developed a definite interest from the part of certain countries of international organizations, such as the ILO or the World Bank. It is likely that in the future, the Directive will be taken as a model for some national or international regulations.

However, the success of the Directive, both within the EEC and outside, should not be misleading : no regulation, whatever the powers of the political regime, can stipulate : « it is forbidden to have an accident » !

A regulation is only a part of the safety package, and would never replace or lower the importance of its other components, which are :

— a real motivation and safety commitment of the manager, and through him, of all the workforce,

— the quality of conception of the product and the process, and of the design, construction and maintenance of the installations,

— the level of education and training of the personnel,

— the quality of operational instructions and their strict compliance, in other words, everything which makes a good management.

Negative ion chemical ionization of macrocyclic trichothecene mycotoxins

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SUMMARY.

The macrocyclic trichothecenes are mycotoxins produced by the fungi *Myrothecium, Stachybotrys and Cylindrocarpon.* In general, they are more toxic than the simple trichothecenes that have been implicated in recent chemical warfare allegations. The macrocyclic trichothecenes possess, as part of their macrolide sidechain, a *cis, trans*-dienic ester group. This group confers upon the macrocyclic trichothecenes high sensitivity in the negative chemical ionization mode. This property has been exploited in the development of a sensitive analytical and confirmative GC/MS procedure (NJAES # J-10201-2-86).

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Chemical Warfare,
Human and Medical Findings

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Clinical and paraclinical findings in 233 patients with sulfur mustard poisoning

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SUMMARY.

Clinical and paraclinical features of sulfur mustard (yperite) poisoning was previously reported, but frequency of the manifestations has rarely been described. Therefore, it was decided to record the clinical and laboratory findings in 233 consecutive Iranian male fighters aged 15-60 years ($X = 24$) who were exposed to the chemical warfare agents between 12th February and 20th April 1986.

Diagnosis of yperite poisoning was confirmed by toxicological analyses of urine and blister fluid. A questionnaire and special examination chart were designed. Routine laboratory tests such as CBC, platelet and reticulocyte counts, urine analyses were performed for all patients. Other investigations e.g. Chest X-ray, Pulmonary and liver function tests, ECG and EEG were carried out whenever clinically indicated.

The most common clinical findings (including symptoms) were found on the respiratory tract (95 %), eyes (92 %), skin (83 %), CNS (83 %), digestive (68 %) and cardiovascular (58 %) systems. The subjective findings were recorded more than the objectives. Acute tracheobronchitis was the most common disorder found in 83 % of all patients. On chest X-ray, increased pulmonary vascular patterns particularly on the right lower zone were reported in 19 out of 52 patients. Other respiratory diseases were asthmatic bronchitis (2 %), pneumonia (1.5 %) and ARDS (1 patient). The eye diseases were recorded as conjunctivitis (48 %), keratitis (3 %), and subconjunctival haemorrhage (2 patients).

Dermatic erythema was found in 48 % of all patients. A majority of them (35 % of the total) had either single or multiple blisters. The mean burn area was almost 15 % of the body surface. Neuropsychiatric disorders were recorded as neurosis (24 %), agitation (12 %), mental depression (7 %) and convulsions (2.5 %). Gastroenteritis, anorexia and dysphagia were recorded in 35 %, 43 % and 15 %, respectively. The cardiovascular symptoms were mainly palpitation (42 %) and left anterior chest pain (41 %), but there were no physical and ECG abnormalities except for 4 patients.

Transient hematuria, glycosuria and urobilinogen were found in 11 %, 4.6 % and 2.6 % respectively. Eosinophilia (38 %), reticulocytosis (36 %), neutrophilia (27 %), lymphocytosis (27 %), ESR elevation (8 %) were the common primary findings. Lymphopenia (15 %), neutropenia (5 %) and leukopenia (1.4 %) were found later in severe poisoned patients.

Most of the patients had used protective clothes but only a few of them had completely applied face mask. This could explain the high frequency for respiratory and general toxic effects rather than dermatic features.

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INTRODUCTION.

The first large-scale use of chemicals in warfare occurred on April 22, 1915, when the Germans unleashed clouds of chlorine on French and Canadian troops near Ypres, Belgium. The French and Canadian were taken completely by surprise and were without protection. They suffered 20,000 casualties (1).

For decades, bis 2-chlorethyl sulfide (mustard gas, sulfur mustard, yperite) was regarded as the « King of Chemical Warfare Agents ». Despite the availability of highly toxic organophosphorus and other chemical warfare (CW) agents, this compound can not be omitted any present-day appraisal of CW agents (2).

Mustard is an oily liquid heavier than water. It ranges from colorless to dark brown and sparingly soluble in water ; time for 99 % hydrolysis at 20°C is 110 minutes. Main chemical reaction are nucleophilic reactions (slow in water emulsions, but fast when dissolved) ; react with N-bases, alcoholates, undergoes oxidation, chlorination, forms sulphonium salts (1, 3).

Clinical and paraclinical features of sulfur mustard poisoning was previously reported (4-11), but frequency of the manifestation

has rarely been described (12). Therefore, it was decided to record the clinical and laboratory findings in 233 consecutive Iranian male fighters aged 15-60 years (X = 24) who were exposed to the chemical warfare agents between 12th February and 20th April 1986.

PATIENTS AND METHODS.

In order to study the frequency of clinical and paraclinical findings in Iranian fighters with chemical gas poisoning who were referred to the Poisons Treatment Unit of Ferdowsi University, a questionnaire and special examination chart were designed.

Diagnosis of yperite poisoning was confirmed by toxicological analyses of urine and blister fluid by the method of Heyndricks *et al.*, 1984, in the toxicology laboratory of poisons Unit. Plasma cholinesterase activity was performed by U.V. Spectrophotometry using Merck Kit.

Routine laboratory tests such as CBC, platelet and reticulocyte counts, urine analyses were performed for all patients. Other investigations e.g. Chest X-ray, pulmonary function tests, ECG and EEG were carried out whenever clinically indicated.

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RESULTS.

The most common clinical findings (including symptoms) were found on the respiratory tract (95 %), eyes (92 %), skin (83 %), C.N.S. (83 %), digestive (68 %) and cardiovascular (58 %) systems (fig. 1).

The subjective findings were recorded more than the objectives (tables I-VI).

Acute tracheobronchitis (as judged by persistant coughing) was the most common disorder which was found in 83 % of all patients. Other common respiratory symptoms were dyspnea, burning sensation in the respiratory tract, productive sputum, hoarseness and sore throat which were found in 77, 63, 58, 56 and 54 % of the patients, respectively (table I). Less than 10 % of the patients had obvious signs of respiratory disorders such as pulmonary rale, severe throat erythema and respiratory distress syndrome.

On chest X-ray, increased pulmonary vascular patterns particularly on the right lower zone were reported in 19 out of 52 patients (table VII).

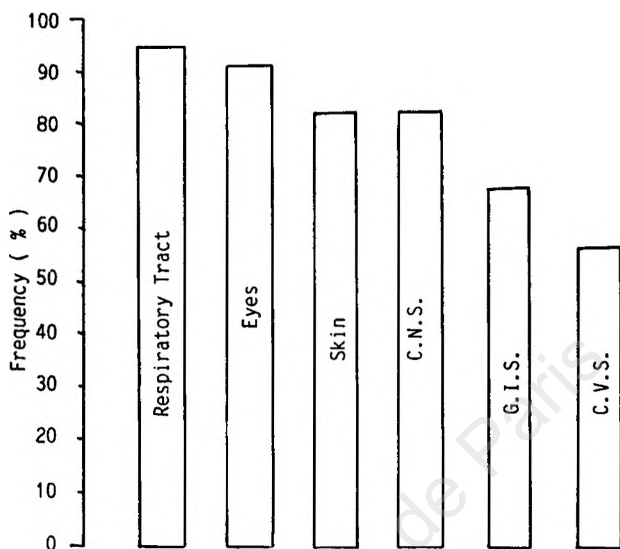

FIG. 1. — Frequency of toxic effects of Yperite on organ's bodies.

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TABLE I

Frequency of toxic effects of Yperite on the respiratory tract of 233 patients

Clinical manifestations	*Number of patients*	%
Cough	193	83
Dyspnea	179	77
Feeling of burning in respiratory tract	147	63
Increased nasal discharge	142	61
Sputum	135	58
Hoarseness	131	56
Sore throat	125	54
Nasal obstruction	115	49
Sneezing	105	45
Nose burning	99	42
Haemoptysis	25	10.7
Throat inflammation	22	9.5
Pulmonary rale	17	7
Sever throat erythema	16	6.8
Bronchopneumonia and pneumonia	2	0.8
ARDS	1	0.4
Acute pulmonary oedema	0	0

TABLE II

Frequency of toxic effects of Yperite on the eyes of 233 patients

Clinical manifestations	*Number of patients*	%
Burning and itching	197	84
Photophobia	179	77
Excessive tearing	174	75
Blurred vision	164	70
Pain	158	68
Decreased visual acuity	152	65
Feeling of foreign body	120	52
Conjunctivitis	112	48
Belpharospasm	23	10
Keratitis	7	3
Subconjunctival haemorrhage	2	0.9
Others	11	4.4

TABLE III

Frequency of toxic effects of Yperite on the skin of 233 patients

Clinical manifestations	*Number of patients*	%
Itching and burning	165	70
Erythema	113	48
Itching and burning of the scrotum	84	36
Blister	82	35
Pain	53	23
Itching and burning of axilla	52	22
Burn	46	20
Single blister	44	19
Inflammation, erythema of scrotum	34	15
Suppurative blister	8	3.2
Inflammation, erythema of axilla	7	3
Others	10	4

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TABLE IV

Frequency of toxic effects of Yperite on the CNS of 233 patients

Clinical manifestations	*Number of patients*	%
Headache	142	61
Insomnia	109	47
General body pains	69	30
Anxiety	63	27
Fear	50	21
Restlessness	31	13
Agitation	29	12
Confusion	19	8
Depression	17	7
Convulsion	6	2.5
Others	19	8

TABLE V

Frequency of toxic effects of Yperite on the GI system of 233 patients

Clinical manifestations	*Number of patients*	%
Nausea	109	47
Vomiting	100	43
Anorexia	100	43
Abdominal pain	84	36
Diarrhea	36	15
Dysphagia	36	15
Epigastric pain	16	7
Increased intestinal movements	3	1
Others	5	2.4

TABLE VI

Frequency of toxic effects of Yperite on the cardiovascular system of 233 patients

Clinical manifestations	*Number of patients*	%
Palpitation	99	42
Left anterior chest pain	97	41.6

TABLE VII

Chest X-ray findings in 52 patients with sulfur mustard poisoning

Findings	*Number of patients*	%
Increased pulmonary vascular patterns	19	36.5
Cardiomegaly	1	1.9
Bronchopneumonia	1	1.9
Chronic bronchitis	1	1.9
ARDS	1	1.9

Other respiratory diseases were asthmatic bronchitis (2 %), pneumonia (1.5 %) and adult respiratory distress syndrome (ARDS) which occurred in one patient. None of these patients used face mask at the time of exposure.

The most common complaints referred to the eyes were itching and burning sensation (84 %). Other symptoms such as photophobia, excessive tearing, blurred vision, pain, decreased visual acuity and feeling of foreign body were recorded in 77, 75, 70, 68 and 52 % of the patients, respectively (table II). Objective conjunctivitis, blepharospasm, keratitis and sub-conjunctival haemorrhage were found in 48, 10, 3 and 0.9 % of the patients, respectively.

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Dermal erythema was found in 48 % of all patients. A majority of them (35 % of the total) had either single or multiple blisters. The mean burn area was almost 15 % of the body surface.

The most common complaints of neuropsychiatric system were headache, insomnia, general body pains and fear of the future as shown in (table IV). Anxiety, restlessness, agitation, confusion, depression and convulsions were found in 27, 13, 12, 8, 7 and 2.5 % of the patients, respectively.

The gastrointestinal symptoms such as nausea, vomiting, anorexia, abdominal pain, diarrhea and epigastric pain were recorded in 47, 43, 36, 15 and 7 % of the patients, respectively, but increased intestinal movement, as judged by auscultation, was only recorded in 3 patients (table V).

Toxicological analyses of the urine and the blister fluids of the severe intoxicated soldiers revealed sulfur mustard. Plasma cholinesterase activities were all within the normal range of the method applied (normal 2.3-7.4 Ku/L).

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TABLE VIII

Urine analyses abnormalities in 151 patients with Yperite poisoning

Abnormal findings	Number of patients	%
Hematuria	17	11.2
Glycosuria	7	4.6
Urobilinogen	4	2.6

TABLE IX

Haematological findings in patients with sulfur mustard poisoning

Tests	Normal	Number	No > Max	No < Min	% > Max	% < Min
WBC	4 - 10 × 10^9/L	225	28	3	12	1.5
RBC	4.5 - 6.3 × 10^{12}/L	190	9	3	4	1.5
Hgb	14 - 18 g/dL	195	1	12	0.5	6
Hct	39 - 52 ratio	212	6	4	2.8	1.8
MCV	77 - 91 fL	178	9	10	5	5.6
MCH	26 - 32 pg	178	6	19	3	10.6
MCHC	32 - 36 g/dL	178	15	13	8.4	7.3
Poly	40 - 65	225	61	11	27	4.8
Lymph	20 - 40	225	60	34	26.6	15
Mono	4 - 8	225	29	55	12.8	24
Eos	1 - 3	225	85	0	37.7	0
Baso	0 - 1	225	9	0	4	0
Platelet	1.5 - 0.4 × 10^{12}/L	193	1	5	0.5	2.5
Retic	0.2 - 2	140	50	0	35.7	0
ESR	0 - 10 mm/h	108	9	0	8	0

Transient hematuria, glycosuria and urobilinogen were found in 11 %, 4.6 % and 2.6 %, respectively (table VIII).

Eosinophila (38 %), reticulocytosis (36 %), neutrophilia (27 %), lymphocytosis (27 %), ESR elevation (8 %) were the common primary findings. Lymphopenia (15 %), neutropenia (5 %) and leukopenia (1.4 %) were found later in severe poisoned patients (table IX).

DISCUSSION.

The symptoms recorded in this study were more common than the signs. This could be due to psychiatric disorders and exaggeration of the severity of intoxication by the patients. Nevertheless, physical examination were not carefully performed in all patients due to a lot of admission in a short period of time and lack of medical staff and facilities. Clinical diagnosis of disorders such as ARDS and anxiety were made according to the Harrison's principles of internal medicine. Chest X-ray revealed less abnormalities than the clinical findings. This has already been reported (11) and we have also no explanation for this discrepancy.

Toxic effects on the skin depends on the use of protective suit at the time exposure. The soldiers were using protective clothing had only dermal lesions on the neck and rarely on the feet, armpit and genital area. Hot, humid weather and even temperate weather conditions strikingly increased the rapidity and degree to which mustard attacks the warm, moist skin of the perineum, genitalia, axillas, antecubital fossae, neck and the facial regions (1). Most of the patients had used protective suit but only a few of them had completely applied face mask. This could explain the high frequency for respiratory and general toxic effects rather than dermatic features. Therefore mask is more important than the protective suit, although it is more difficult and inconvenient for the soldiers to use face mask continuously, particularly in the hot weather.

For the patients who had symptoms and signs of parasympathic stimulation plasma cholinesterase activity was performed but there was no reduction whatsoever. However, these clinical features may be attributed to the sulfur mustard poisoning or some other toxic agents. Some patients described different smells such as garlic, vegetable, chocolate and different colours (grey, brown,

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white and black) which suggests that more than one chemical agents were used. Although the smell (garlic) and colours (brown) described for the sulfur mustard (1) is known to the professionals it may change by adding the essence and paints to confuse the enemy. Toxic effects of mustard gas have been studied in animals (13-14) and in human beings (1, 10-12). Toxicokinetic of sulfur mustard has rarely been reported in animals (12) and none in man. Apart from direct irritation effect on the eyes, skin and respiratory tract, deaths due to bone marrow depression, septic shock and ARDS may occur. In fact three of our patients died on the second week after exposure because of the septic shock, ARDS and pulmonary emboli due to deep vein thrombosis.

Laboratory findings particularly the haematological results is of great interest. Although haematological abnormalities have already been reported but the frequency of each abnormalities were not mentioned (1, 10, 11). The initial leukocytosis and polynucleosis might be due to bone marrow stimulation, but primary lymphopenia in severe cases suggests that the direct toxic effects of sulfur mustard haematopoietic system. Since sulfur mustard is powerful alkylating agent it may act rapidly and persistantly upon the deoxyribonucleic acid-replicating mechanisms of the individual cell during certain phases of mitosis (1). In severe intoxicated soldiers the white blood cells (WBC) reduced dramatically over the first few days after exposure and in one patient there was absence of WBC on the 7th day of hospitalization 2 hours before his death. Bone marrow aspiration and needle biopsy on this patient showed hyoplasia and focal necrosis with the absence of neutrophils. These findings are in agreement with previous reports (1, 11, 15, 16). In fact there have been biochemical abnormalities in few patients, but they were not specific and common enough to be discussed here. Further study is required to evaluate the sulfur mustard biochemical changes in sulfur mustard poisoning.

Acknowledgement. — We would like to thank all the medical and nursing staff of the poisons treatment unit of Emam Reza Medical Centre, particularly Dr. F. Sharifi and Dr. M. Vatankhah for their kind assistance.

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Medical use of mustard gas derivatives
A review

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SUMMARY.

Many of the alkylating agents used for the treatment of cancer are related to mustard gas, first used for chemical warfare in World War I. At that time it was noted that limited exposure to that agent caused depletion of the cells of the bone marrow, somewhat similar to the effects of ionising radiation. Following the report of this observation by Krumbhaar in 1919, a group at Yale University tried a nitrogen mustard, trichloro-ethylamine for the treatment of a transplanted malignant lymphoma in mice after they had observed the dissolution of lymphoid tissue in laboratory animals given this agent.

The first clinical trial on cancer patients took place in the early 1940s by Goodman and Gilman who treated patients affected by various forms of leukemia. These studies were published in 1963 by Gilman and not only showed the outstanding usefulness of the mustards, but also demonstrated their chief disadvantages : severe toxicities to the patients and the development of resistance by the malignant cells. A major application of nitrogen mustard today is the treatment of Hodgkins disease where it is combined with other cytostatics as vincristine, procarbazine and corticosteroids. The acute toxicities (bone marrow depression, vomiting) and mainly the late toxicities (induction of secondary malignant tumors) have intensified the search for less toxic products for the treatment of Hodgkins disease. Other mustard derivatives such as cyclophosphamide, iphosphamide and melphalan are still frequently used in cancer medicine today. Although their acute toxicities are less pronounced than those of nitrogen mustard, their use is also complicated by the induction of secondary malignancies.

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Report of three fatal cases of war gas poisoning

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SUMMARY.

In spite of the strict international prohibition on the use of chemical and biological weapons, Iraqi army made several chemical attacks against Iranian fighters. Thousands of the fighters became intoxicated on the 13th February 1986 in southern part of the Arvand river. Almost 300 of them were referred to the medical centres of Ferdowsi University in Mashhad. Of these, 3 were martyred despite intensive care, antidotal and supportive treatments and in 2 cases charcoal hemoperfusion were also performed.

The martyrs were 27, 18 and 17 years old males who were admitted to the medical centres almost 24 hours after the exposure. The eldest (case 1) had very severe and extensive second degree burns (> 80 % of the body surface), keratoconjunctivitis, acute tracheobronchitis, bronchopneumonia with marked lymphopenia (< 3 %) and primary leukocytosis which reduced gradually to total absence of WBC on the seventh day of hospitalization, 2 hours before his death. The second had lesser extent of burns (almost 35 % of the body surface), but had more severe bronchopneumonia and marked leukopenia (450/cmm) before his death, on the eigth day. The youngest (number 3) who had used protective clothings but no face mask or boots, showed burns with blisters on his face, neck and feet. He had vomitted once on his feet, on the way to hospital, which induced severe burns. He had similar toxic effects on his eyes, lungs and bone marrow. This patient died because of a severe adult respiratory distress syndrome (ARDS) on the 11th day after exposure.

Sulfur mustard was detected in the urine and blister fluid and vitreous fluid of the eyes in the 3 patients which were analysed

toxicologically (Method of Heyndrickx, De Puydt and Cordonnier, 1984) in the Toxicology laboratory of the poisons Treatment Unit. Plasma choline esterases were normal in all patients.

Autopsy needle biopsies of the martyrs revealed severe bronchopneumonia in all and ARDS in the 3rd case. Bone Marrow aspiration and needle biopsies showed hypoplasia and focal necrosis with absence of neutrophils in case 1 and marked neutropenia in others. Centrizonal congestion and enlarged mitochondria in the cytoplasm were seen in the livers of all the martyrs. Mild glomerularfibrosis, congestion and focal interstitial nephritis were found in cases 2 and 3. Focal wavy fibres and mild congestion of the heart were also seen in these two martyrs. Numerous colonies of microorganisms with filamentous/hyphal opportunistic infection and probably fusarium species were found in the lungs in two cases.

Further toxicological and pathological studies should be conducted on the plasma, urine and post-mortem materials of these martyrs to find out more precisely the toxic effects.

INTRODUCTION.

Poison war gas were first used by the Germans in 1915, and their use was prohibited by the Geneva Protocol of 1925 (1). Despite the ban, these agents have been used in many conflicts throughout the world. In August 1983, Iraq used the chemical agents for the first time in the Piranshahr zone in western Iran and has repeated its use on many occasions since then. The use of chemical war gases by Iraq was confirmed by the specialists sent by the Secretary General of the United Nations (2, 3, 4).

Thousands of Iranian fighters were intoxicated on the 13th of February 1986 in the southern part of the Arvand river. Almost 300 of these were transferred to Mashhah. Of these 3 were martyred. The present report deals with the limited autopsy findings using needle biopsies of various organs.

METHOD.

Needle biopsies of the heart, lung, kidney, liver and spleen were performed on three patients within ½ hour to 13 hours after death.

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All the biopsies were immediately fixed in 10 % buffered formalin except for the bone marrow biopsies which were fixed in Bouin's solution. After routine processing and preparation of wax blocks glass slides were prepared which were stained with Hematoxylin and Eosin, Periodic Acid Schiff, Gomori's methanamine silver stain and masson's trichrome.

The vitreous chamber fluid was also aspirated and analysed using the method of A. Heyndrickx (5).

CLINICAL FINDINGS.

Case 1. This 27 years old male (S.H.) was exposed to poisonous fumes, grey in colour on the 13th of February 1986 in the southern part of the Arvand river, and was transferred to Ghaem Medical centre, Mashhad, within 24 hours after exposure. He had extensive burns covering over 80 % of the total body surface. Supportive treatment was administered and was transferred to Emam Reza Medical Center, Poisons Unit where charcoal hemoperfusion was performed. His white count on admission was 16,800/cmm with 98 % neutrophils and the platelet count of 100,000/cmm. Over the next few days these progressively fell and just prior to death no white cells could be detected. He died of septic shock on the 8th day after exposure. Needle biopsies were performed 7h after death.

Case 2. This 18 year old male (N.S.) was exposed to grey coloured fumes on the 13th February 1986 in the southern Arvand river sector and was admitted to Emam Reza Medical Centre within 26 hours after exposure. He had extensive burns covering 35 % of the total body surface. He received N-Acetyl Cystein treatment and charcoal hemoperfusion. His maximum leukocyte count was 27,200/cmm which over the week dropped to zero. He developed deep vein thrombosis and died of pulmonary emboli on the 9th day since exposure. Needle biopsies were performed 13 hours after death.

Case 3. This 17 years old male (H.Y.) was exposed to grey coloured chemical war gases on the 13th of February 1986 in the Arvand River area along with the others. He was transferred to Emam Reza Medical Centre within 26 hours of exposure. He had 15 % of the total body surface burned, involving face neck and feet. He was wearing protective clothes but no mask or boots.

He had vomited on the feet on the way to hospital. He received N-Acetyl cystein treatment. His maximum white count was 18,900 with 98 % neutrophils. These progressively fell to 1,100/cmm, 16 hours before death. He died on the 11th day of ex-

TABLE I

Clinical findings in three males with severe sulfur mustard poisoning

Findings	*1*	*2*	*3*
Age (years)	27	18	17
Extent of burns (%)	80	35	15
Keratoconjunctivitis	++++	+++	+++
Tracheobronchitis	+++	++++	+++
Bronchopneumonia	+++	++++	+++
Maximum leukocytosis	16,800	27,200	18,900
Leukopenia	0	450	1200
Death after exposure	8 days	9 days	11 days
Cause of death	Septic shock	Pulmonary emboli	ARDS

posure with severe adult respiratory Distress syndrome. Needle biopsies were performed within ½ hour of death. Clinical findings are summarized in table I.

RESULTS.

Blister fluids and urine obtained during hospitalization and vitreous chamber fluid obtained at the time of autopsy were analysed by the method of Heyndrickx (5) at the Poisons Unit of Emam Reza Medical centre, revealed Sulfur Mustard.

Lungs.

Multiple needle biopsies showed extensive necrotising bronchopneumonia with prominent hyaline membranes in case 3, which also showed clusters of Gram positive cocci. In some alveoli there were filamentous/hyphael structures which stained faintly with PAS stain. These closely resemble fusarium species as illustrated by Cohen *et al.* (6) (fig. 1 and 2).

In case 2 there was extensive necrotising bronchopneumonia with necrosis of the cartilage. Filamentous/hyphael structures were also identified.

In case 1, only bronchopneumonia was noted.

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Liver.

The liver biopsies showed centrizonal congestion and in cases 1 and 3 prominent intracytoplasmic « inclusions », which were numerous, presumably representing giant mitochondria. However, electron microscopic studies are needed to confirm their etiology (fig. 3).

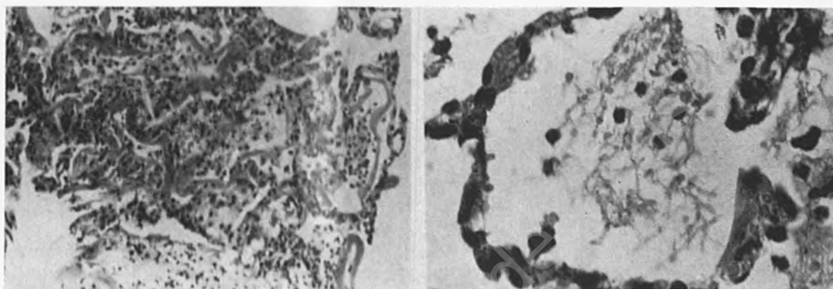

FIG. 1. FIG. 2.

FIG. 1. — Adult respiratory distress syndrome with prominent hyaline membranes (case 3).

FIG. 2. — Alveoli with filamentous/hyphael structures (case 3).

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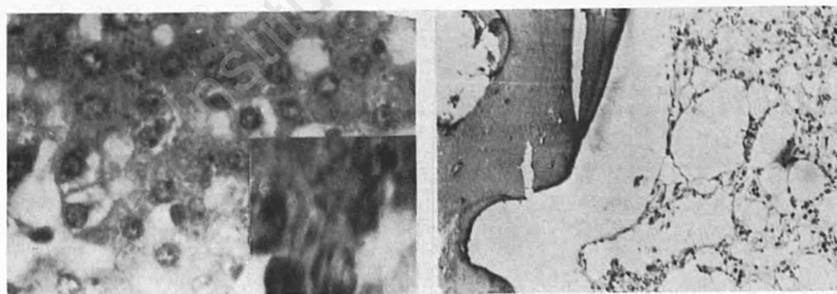

FIG. 3. FIG. 4.

FIG. 3. — Liver with intracytoplasmic inclusions, ? giant mitochondria. Inset with higher magnification under oil (case 3).

FIG. 4. — Hypoplastic marrow (case 2).

Spleen.

The spleen in case 3 showed congestion and decreased numbers of lymphoid follicles. Only a minute specimen was obtained in case 1 and none was obtained in case 2.

Kidneys.

The needle biopsies showed between 27 and 45 glomeruli in each of the patients and showed mild congestion and in case 3 focal necrosis of the tubules. In case 2 periglomerular fibrosis was detected in one glomerulus.

Skin.

Skin biopsy in case 1 showed extensive denudation of the epidermis with foci of regeneration. The upper dermis showed homogeneous eosinophilia and fragmentation with no inflammatory reaction. These findings are the same as previously observed by D'halluin *et al.* (7).

Bone marrow.

Needle biopsies obtained from the right and left iliac crests show slightly hypocellular marrow with maturation of both the myeloid and erythroid series but with marked left shift, in case 3.

TABLE II

Summary of microscopic findings in patients with sulfur mustard poisoning

Organ	*I*	*II*	*III*
Lungs	Bronchopneumonia	Necrotising hemorrhagic bronchopneumonia, necrosis cartilage, ? Filament/hyphae, ? Fusarium	ARDS AND necrotising bronchopneumonia, ? Filament or hyphae, ? Fusarium
Liver	Centrizonal congestion, ? Giant mitochondria	Centrizonal congestion	Congestion ? Giant mitochondria
Spleen	Minute fragment	—	Congestion, decrease in lymphoid follicles
Kidney	Mild congestion	Congestion, periglomerular fibrosis	Congestion focal necrosis tubules
Skin	Second burns, regeneration no inflammatory cells	—	—
Bone marrow	Marked hypoplasia Neutrophils absent Iron stain 2+	Hypoplasia, few neutrophils reduced markedly Iron stain 2+	Normocellular, left shift neutrophils reduced Iron stain 2+

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In some fields no mature polymorphonuclear leukocytes were identified and in other fields they constituted only 3 to 5 % of the cells. Megakaryocytes appeared adequate. Iron stain showed normal stores (fig. 4).

In cases 1 and 2 there was marked degree of hypocellularity with Fat to Marrow ratio of between 4:1 to 7:1 and only rare megakaryocytes were seen. Focal areas of necrosis was also noted. Pathological findings are summarized in table II.

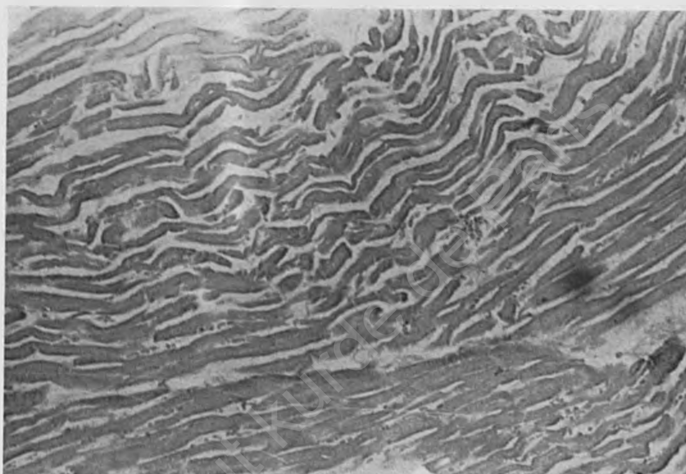

FIG. 5. — Heart with wavy fibers, suggestive of ischaemia. Note absence of inflammatory cells (case 1).

DISCUSSIONS.

Although chemical war gases have been used since 1915, very little has been published as regards its effects.

Three Iranian soldiers who had succumbed to the effects of poison wargases were studied by needle biopsies performed at the time of death. All had varying degrees of respiratory and dermal complications. There was necrotising bronchopneumonia and adult respiratory distress syndrome with areas of gram positive cocci. In two of the cases (no. 2 and 3) in addition filamentous/hyphael structures were identified. Although these may represent fibrin threads, they more closely resemble fusarium species morphologically (6). This raises the possibility that in addition to sulfur mustard there may have been contamination with mycotoxins. The other possibility is that the patients acquired these agents owing to their lowered resistance. However, it should

be noted that no trichothecene compounds were identified, which in any case, would not be ordinarily detectable at this late stage.

In all the three cases there was hepatic congestion mainly centrizonal in cases 1 and 2 which may be explained by the failing ischaemic myocardium as suggested by the presence of focal areas with wavy fibers (fig. 5). In cases 1 and 3 there were prominent intracytoplasmic inclusions which were quite numerous in some cells especially in case 3. These presumably represent giant mitochondria perhaps induced by the sulfur mustard. However, electron microscopic studies are necessary for confirmation of this finding.

The bone marrow showed severe hypocellularity involving megakaryocyte, myeloid and erythroid series. This has been previously described by D'halluin *et al.* (7). In case 3 although the marrow was relatively normocellular, there was a marked left shift with only a few neutrophils identified suggesting that the marrow was presumably progressing towards hypoplasia.

There is need to further study the effects of chemical war gases in animals to further define their effects and find appropriate therapy.

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Evolution of serum and erythrocyte magnesiumlevels in patients, attacked by the chemical warfare agent mustard gas (Yperite)

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ABSTRACT.

It is known that a decrease of serum and erythrocyte magnesiumlevels can occur immediately after a thermic aggression (burns) or a surgical operation.

This hypomagnesemia is generally moderate and short, normal values are registrated within 2 to 3 days.

In this report the evolution of the magnesiumlevels in patients, attacked by the chemical warfare agent sulfur mustard gas (Yperite), is discussed.

When the patients arrived at the University Hospitals of Ghent, 5 days after the attack, the serummagnesiumlevels were already normalized, but it was very remarkable that the erythrocyte magnesiumlevels returned to normal values only 8 days after the vesicant attack.

INTRODUCTION.

Although the effect of surgery, myocard infarct and thermic aggression on the concentration of magnesium is documented (6), no study of the immediate effect of chemical warfare agents, such as Yperite, on magnesiumlevels in serum and erythrocyte has been reported.

In the present investigation observations were made on burned soldiers attacked with Yperite during the Iran-Iraq conflict of 1986.

PATIENTS AND METHODS.

The blood samples were taken from 9 men, aged between 16 and 30 years old, with various degrees of chemical burning.

Blood was taken on admission of the best available vein, if possible without venous congestion, every day between 8 and 9 hours in the morning.

Serum and erythrocyte magnesiumlevels were estimated, using atomic absorption-spectrophotometry (AAS). AAS is the most preferred technique for the determination of magnesium in bio-

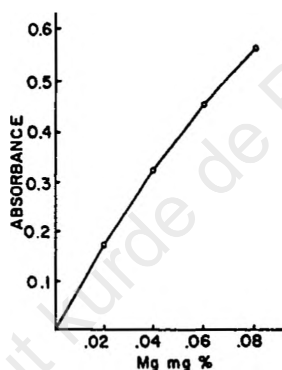

FIG. 1. — Typical absorbance-concentration curve obtained with aqueous magnesium solutions in 10-cm air-acetylene flame (2).

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logical fluids, since it is fast, accurate and reasonable simple, with sensitivities unexcelled with other elements (1).

Figure 1 represents a study done by Zettner and Seligson, note the slight deviation from linearity (2).

Magnesium is released from proteins by reacting with acids (Trichloroacetic acid/HCl).

The sample is diluted with 0.1 % Lantanium-oxyde in aqua bidestillata. The sample is analysed with an 372 Perkin-Elmer atomic absorption spectrophotometer, using an air-acetylene flame. The wavelength was the 285.2 nm of a mag/ca hollow cathode lamp.

Results are calculated with the aid of a standard curve (detection limit : 0.001 μg/ml).

Using AAS, we must always be aware of the chemical and clinical *(in vivo)* interferences, causing false elevated or decreased values (3).

TABLE I

Interferences (3)

	Causes of false positive or elevated values	*Causes of false negative or decreased values*
Chemical interferences	Hemoglobin	
	Trichloroacetic acid	
	Aspirin (prolonged therapy)	
Clinical interferences	Lithium carbonate	Ethylalcohol
	Magnesiumcompounds	Furosemide
	Medroxyprogesterone	Aldosterone
	Progesterone	Ammoniumchloride
	Triamtereen	Amphotericin B
	Vitamin D (chronic renal failure)	Calcium gluconate
		Calcium salts
		Ethacrynic acid (Edecrin)
		Insulin (large doses in diabetic coma)
		Mercurial diuretics
		Neomycin
		Oral contraceptives
		Thiazide diuretics
		Citrates (blood transfusion)

RESULTS.

A. Evolution of the serum magnesium.

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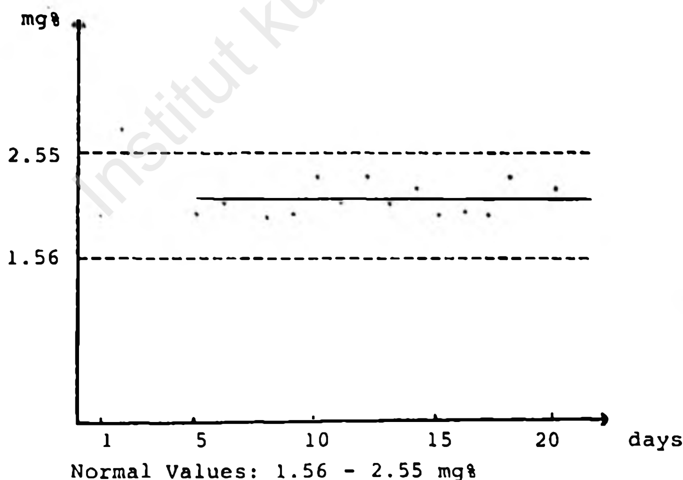

FIG. 2. — Evolution of the mean serum.magnesiumlevels of 9 burned soldiers.

The curve represents the mean values of the daily magnesium-levels of the nine men.

We notice that the serummagnesiumlevels on the day the men arrived in Ghent, meaning the fifth day after the vesicant attack

(mixture of mustard gas, Tabun and cyanide), were already normalized.

B. **Evolution of the erythrocyte magnesium.**

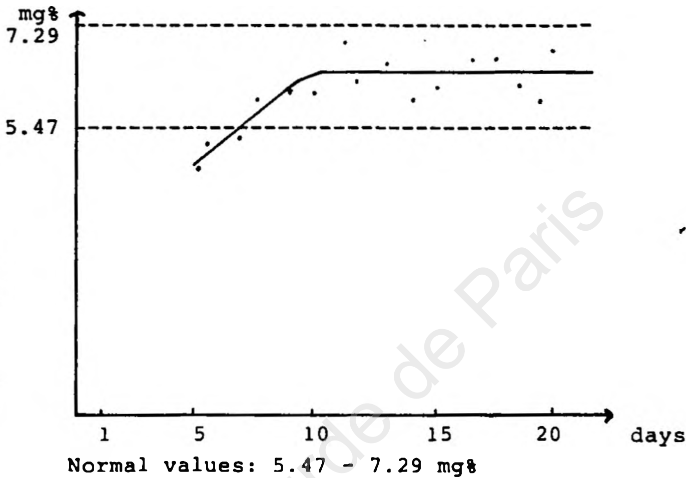

FIG. 3. — Evolution of the mean erythrocyte magnesiumlevels of 9 burned soldiers.

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The curve shows a clear hypomagnesemia in the erythrocytes on the fifth day. Normal values are measured after the third or the fourth day of hospitalization, which means eight to nine days after contact with the chemical warfare agents : sulfur mustard gas (Yperite), Tabun and cyanide (mixture of the 3 gases).

DISCUSSION.

The magnesium content of a 70 kg adult is about 20 gram (820 mmol Mg^{2+}). More than 50 % of this is found in the bone, associated with calcium and phosphorus. Most of the remainder is in the soft tissues. Magnesium ions act as cofactors in those enzyme systems, which also necessitates the presence of Adenosine-Triphosphate, where it forms a Mg^{2+}-ATP^{4-}-complex. Magnesium ions serve as activators in transfer and hydrolysis of phosphate groups, such as hexokinase, alkaline phosphatase (1).

Magnesium ions are essential, increased serum magnesium-levels (hypermagnesemia) leads to an increase in the atrioventricular conduction time of the electrodiagram. A magnesium deficiency (hypomagnesemia) causes changes in nerve conduction, muscular contraction and thermic regulation.

The development of a magnesium deficiency depends on one or more of a number of factors : deficient intake, failure of absorption or excessive loss (table II) (4).

TABLE II

Disorders associated with hypomagnesemia (4)

I. Disorders associated with inadequate and/or impaired absorption of Mg
- — Malabsorption syndrome
- — Kwashiorkor
- — Diet low in proteins and calories
- — Tetany

II. Disorders associated with increased Mg requirement and inadequate replacement of prolonged or severe loss of body fluids
- — Chronic glomerulonephritis
- — Surgical trauma
- — Myocardial infarction
- — Thermic aggression

III. Disorders associated with impaired renal conservation of Mg
- — Hypercalcemia
- — Hyperparathyroidism
- — Hyperaldosteronism
- — Diabetic acidosis
- — Excessive lactation

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Dietary deficiency is rare since normal diet contains an adequate amount of magnesium. Treatment with magnesium sulfate in cases of tetany, results in a rise in the serum magnesium-levels and a concomitant disappearance of tetanu and convulsions (1). An excessive loss has been reported after surgical trauma (open heart surgery, neurological surgery), after myocardial infarction and after thermic aggression. The hypomagnesemia is generally moderate and short, return to normal values is usually registrated from the third day (5). In our investigation, the serum-magnesium concentration is already normalized 5 days after the attack, but the erythrocytelevels remained abnormal during ± 8 days. The knowledge of this longer hypomagnesemia is important according to the role of magnesium in the muscular activity (magnesium deficiency tetany).

The hypothesis according to which there can exist a correlation between hypomagnesemia and a number of neuropsychiatric

manifestations, met simultaneously in the same patients, cannot be excluded.

In previous investigations (6) they have already mentioned the occurrence of mental symptoms (such as depressions, hallucinations and muscle cramps) in burned patients, due to electrolyte changes. We may suggest that in some cases, those symptoms may be due to or aggraved by magnesium deficiency, because a relief of the symptoms following magnesium therapy is noticed.

Our Iranian men promoted a higher nervosity and excitability, disappearing with normalization of the magnesiumlevels.

The link (7) is based on the close connection between magnesium concentration and the phosphorylation processes, which implies an effect on the energy generating mechanism of the mitochondria. An effect on nerve and muscle membrane potentials is well established. With a lowered concentration of magnesium (or calcium) the stimulation threshold of the motor nerve is lowered, and is conversely raised by an increase.

In muscle, because of actions on a number of enzyme systems (which are interrelated), the effects of magnesium are the opposite of those of calcium ; low concentration of magnesium enhance, but low concentration of calcium inhibit contractions.

Thus, decreased concentration of magnesium enhance irritability through increased nerve conduction, increased transmission of the impulse at the myoneural junction and increased contractility.

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First report of delayed toxic effects of yperite poisoning in Iranian fighters

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SUMMARY.

Sulfur mustard(yperite) is distributed to several organs after rapid absorption through skin or following inhalation. Literature data on the delayed lesions of yperite poisoning is very scarce and the relative contribution of each organ disorders has not been reported in detail. Thus, it was planned to record the frequency of late toxic effects on the Iranian fighters exposed to the chemical warfare agent.

Two hundred and thirty-six consecutive male fighters aged 16-38 years (X=21) who had a history of acute yperite poisoning more than 2 months before the study, were investigated. A questionnaire and a special examination chart was designed. Routine laboratory tests such as CBC and urine analyses were performed for all of them. Other investigations e.g. Chest X-ray, pulmonary function tests, ECG and EEG were done whenever clinically indicated. The symptomatic fighters were re-examined and treated with the consultation of other specialists, for at least once a month up to May 1986 (a period of 2 years).

The most common complications were found on the respiratory tract (78 %), CNS (45 %), skin (41 %) and eyes (36 %). Although asthma-like conditions was seen in 29 %, chronic bronchitis was the commonest chest complication (38 %). Other respiratory disorders were rhinitis and or rhinopharingitis (12 %), tracheobronchitis (11 %), laryngitis (7 %), recurrent pneumonia (5 %) and bronchiectasis (2 %). Neuropsychiatric abnormalities were recorded as neurosis (31 %), mental depression (26 %), personality disorders (21 %), peripheral neuritis (10 %), epilepsy (6 %) and psychosis (3 %). Skin hyperpigmentation (34 %) was

more common than hypopigmentation (16 %). Dermatic scar and scrotal oedema were also noted in some patients (8 % and 7 %, respectively). Chronic conjunctivitis was the main delayed toxic effects on the eyes (32 %). Nevertheless, some patients had other eye problems such as keratoconjunctivitis (3 %) and blindness (2 patients). Allergic reactions e.g. pruritus, urticaria and eczema were seen in 23, 8 and 2 % of them, respectively. All of these patients had eosinophilia. Loss of libido was recorded in 32 patients (14 %) and 3 of them had oligospermia (< 20 M/ml). Upper G.I. dysfunction were noted in 22 % of all patients, but high incidence of achilia and hypoacidity, hepatomegaly and permanent liver injury which were reported in the literature, were not prominent in this study, Transient leukopenia and renal dysfunction were only seen in 3 and 2 cases, respectively.

Radiomimetic and mutagenic effects have not been found yet. The study is going on and probably more delayed toxic effects may be detected in the future.

INTRODUCTION.

Sulfur mustard (SM) or mustard gas (yperite) is distributed to several organs after rapid absorption through skin or following inhalation. It may also induce general intoxication by ingestion of contaminated water or foods (1-4).

The term delayed toxic effect is defined to mean a lesion-caused by acute or subacute poisoning of the chemical warfare (CW) agent. The effect may be residual injury or by the unexpected onset of related symptoms after a protracted period of months or years, and being essentially irreversible. It must be emphasized that a lesion of this kind is not the same as one caused by chronic poisoning. A delayed lesion results, however, from a single dose or brief exposure — the onset of symptoms, months or years later, requiring no further intake of the substance (1, 4).

For decades SM was regarded as the « King of chemical warfare agents ». Despite the availability of highly toxic organophosphorous CW agent, this compound can not be omitted from any present-day appraisal of CW agents. Apart from soldiers, many thousands of persons engaged in the production, storage or stockpiling of CW agents are today risking their health (4).

Literature data on the delayed lesions of yperitepoisoning is very scarce and the relative contribution of each organ disorder

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has not been reported in details (1). Therefore, it was planned to record the late toxic effects of SM on the Iranian soldiers referred to the poisons treatment unit of Emam Reza Medical Centre in Mashhad.

PATIENTS AND METHODS.

In order to study the delayed toxic effects of SM on Iranian soldiers exposed to this CW agent, a questionnaire and a special examination sheet was designed. All symptomatic patients after 2 months post-exposure to SM were investigated. Routine laboratory tests such as blood cell counting, haemoglobin, haematocrit, erythrocyte sedimentation rate, routine urine analyses were performed for all patients, at least once every three months. Other investigations such as chest X-ray, electrocardiogram (ECG), electroencephalogram (EEG), arterial blood gases, pulmonary, liver and renal function tests were done whenever clinically indicated. The patients were re-examined and treated with the consultation of other medical specialists, for at least once a month up to two years.

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RESULTS.

Between June 1984 and June 1986, 236 male patients age 16-38 year (X=21) who had a history of SM poisoning were studied. Of these, 12 were hospitalized for periods of one to 6 weeks and the remainders were treated as outpatients.

The most common complications were found on the respiratory tract (78 %), central nervous system (45 %), skin (41 %) and eyes (36 %). Allergic reactions, gastrointestinal dysfunction and loss of libido were recorded in 33, 23 and 14 % of the patients, respectively (table I).

Although asthma-like conditions was the most troublesome and occurred in 29 % of the patients, chronic bronchitis was the most common complication (38 %). Other respiratory disorders were rhinitis/rhinopharingitis (12 %), tracheobronchitis (11 %), laryngitis (7 %), recurrent pneumonia (5 %) and bronchiectasis (2 %) as shown in table II.

Neuropsychiatric abnormalities (table III) were recorded as neurosis (31 %) mental depression (26 %), personality disorders

TABLE I

Common complications of sulfur mustard poisoning in 236 patients

Organs body	*Number of patients*	%
Respiratory tract	184	78
CNS	106	45
Skin	97	41
Eyes	85	36
Allergic reactions	78	33
GI dysfunction	55	23
Loss of libido	32	14

TABLE II

Late toxic effects of sulfur mustard on the respiratory tract in 236 patients

Findings	*Number of patients*	%
Chronic bronchitis	89	38
Asthmatic-like syndrome	68	29
Rhinopharingitis	28	12
Tracheobronchitis	26	11
Laringitis	16	7
Recurrent pneumonia	12	5
Bronchiectasis	5	2
Overall	184	78

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TABLE III

Neuro-psychiatric disorders in 236 patients with sulfur mustard poisoning

Disorders	*Number of patients*	%
Neurosis	73	31
Mental depression	61	26
Personality disorders	50	21
Peripheral neuritis	24	10
Epilepsy	14	6
Psychosis	7	3
Overall	106	45

TABLE IV

Skin changes due to sulfur mustard poisoning in 236 patients

Manifestations	*Number of patients*	%
Hyperpigmentation	80	34
Hypopigmentation	38	16
Dermal scar	19	8
Scrotal oedema	16	7
Overall	97	41

(21 %), peripheral neuritis (10 %), epilepsy (6 %) and psychosis (3 %).

Skin hyperpigmentation (34 %) was more common than hypopigmentation (16 %) as shown in table IV. Dermatic scar and scrotal oedema were recorded in 8 and 7 % of the patients, respectively.

Chronic bronchitis was the main delayed toxic effect on the eyes (32 %). Nevertheless, some patients had other eye problems such as keratoconjunctivitis (3 %) and blindness (2 patients) as shown in table V.

Allergic reactions including pruritus, urticaria and eczema were seen in 23, 8 and 2 % of them, respectively (table VI).

TABLE V

Late toxic effects of sulfur mustard on the eyes of 236 patients

Manifestations	*Number of patients*	%
Chronic conjunctivitis	75	32
Keratoconjunctivitis	7	3
Blindness	2	1
Overall	85	36

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TABLE VI

Allergic reactions in 236 patients with sulfur mustard poisoning

Manifestations	*Number of patients*	%
Pruritus	54	23
Urticaria	19	8
Eczema	5	2
Overall	78	33

Loss of libido was recorded in 32 patients (14 %) and 3 of them had oligospermia. Two patients were complaining of complete impotence. Upper gastro-intestinal (GI) dysfunction were noted in 52 (22 %) patients. In 12 patients, upper GI series were performed, of these, only 3 showed duodenal ulcer. Spasmodic colitis were also diagnosed in 14 (6 %) patients. Transient leukopenea and renal dysfunction were only seen in 3 and 2 cases, respectively. Two patients had cardiomegaly with abnormal ECG. The other laboratory investigations were not prominent in this study.

DISCUSSION.

Mustard gas produced acute toxic effects only at supralethal dosages, in which the central nervous excitation leads to convulsions and rapid death. Under field conditions even without protection, the development of symptoms does not usually occur until several hours after exposure (1, 3, 4). However, delayed toxic effects as already defined, include the lesions found months or years later post-exposure.

Respiratory tract disorder was the most common toxic effects in this study. Primary injuries of the respiratory tract, from asthma-like conditions to very severe emphysematous bronchitis, recurrent pneumonia and bronchiectasis have already been reported 1, 3, 4). However, chronic bronchitis, was the most common chest complication in the patients studied in my unit. Asthma-like syndrome induced the most therapeutic problem for the consultant chest physician and myself. Despite the high plasma theophilin concentrations ($>$ 10 mg/l) in some patients, persistant dyspnea was still remained. It was very interesting to see, that in one patient who was given a high dose of prednisolone by a chest physician, his dyspnea became worse. Some of the patients who had organic disorders presented neuropsychiatric symptoms, but most of the psychiatric disorders were seen in the soldiers with no organic effects. The patients who had severe psychiatric problems such as psychosis, referred to the psychiatric hospital of Mashhad University. But the recovery was not fully successful. Although grand mal convulsions were seen in 5 patients in the ward, their EEG were normal. In fact the epileptic patients were all EEG negative.

The patients who had hyper- or hypopigmentation were rarely complaining of their skin, but some patients with apparently normal skin, complained of itching and burning sensation of the skin. Dermal biopsy was performed in some patients, but no significant abnormality was found. However, microscopical examination of the skin lesions showed loosening of the epidermis with formation of a subepidermal gap, as previously reported (5-8). Molecular basis for mustard-induced vesication involves SM alkylation of purines in DNA which are processed to form apurinic sites. Apurinic endonucleases act at these sites to produce backbone breaks in DNA which cause activation of the chromosomal enzyme poly(ADP-ribose)polymerase (6, 7).

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Eye lesions and problem of blindness resulting from SM are matters which have received greater publicity than those relating to lesions and disability of other kinds (9-12). However, chronic conjunctivitis was a common complication of SM poisoning, where as the blindness was a rare outcome of the intoxication in this study.

Allergic reaction such as eczema, asthmatic like conditions with eosinophilia were common complications in our patients and need further immunological investigation. There has been no report up to date on the immunotoxicology of SM in the medical literature. Some patients who were apparently asymptomatic returned to the war zone and became intoxicated again very soon after a very mild exposure to SM. It seems that there should be a hypersensitivity reaction to this CW agent.

Loss of libido and even complete impotence were common complaints of the patients exposed heavily to SM. This has already been reported from the studies carried out on the victims of first and second world war (1).

GI dysfunction were noted in 22 % of all patients, but high incidence of achylia, perigastric overgrowth of the liver and cases of early gastric cancer which were reported (1, 13) have not been diagnosed so far in my patients.

Radiomimetic and mutagenic effects which were mentioned in the literature (1, 13, 14), have not been found yet. The study is going on and probably more delayed toxic effects may be detected in the future.

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Mycotoxins, «Yellow rain» and food contamination

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SUMMARY.

Mycotoxins may contaminate naturally (both directly and through the alimentary chain) food-stuffs and feeds in which they can develop; they are more typically synthesized on grain and on other low moisture vegetal products, but may also be found in low concentration in milk and milk products, in certain animals tissues and occasionally in fungal fermentation.

The most toxic for human health are: — Aflatoxins — Ochratoxins — Rubratoxins — Trichothecenes — Zearalenone — and those produced by Penicillum Islandicum.

They may enter the animal or human body by ingestion, inhalation or direct skin contact.

Their dosage and their relationship as ethiological toxic agents are often difficult, to check on account of to the great complexity of analysis methods and the frequent association with other ethiological factors. A further complication is the rather variable way in which the fungi produce their toxic metabolites in foods. The general problem of these poisons is very complicated by the use of mycotoxins in biological warfare, the well know « yellow rain ».

The A.A. stress the necessity of screening mycotoxins in food, in fact they may enter the food chain. This so polluted food may be exported in countries where they do not occur naturally.

Mycotoxins are produced by mould or fungi metabolism. Human food and feed may become contaminated with mycotoxins either by direct mould growth on food or feed or indirectly through the

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alimentary chain. They are more typically synthesized on grain and on other low moisture vegetal products, but may also be found in low concentration in milk and milk products, in certain animals tissues and occasionally in fungal fermentation. The mycotoxins responsible of human mycotoxicosis are referred in table I.

TABLE I

Types of human mycotoxicosis

Mycotoxin	*Casual fungus*	*Mycotoxicosis*
Neurotoxicosis		
Ergot alkaloids	Claviceps purpurea	Gangrenous and convulsive ergotism
Tremorgens	Penicillum spp.	Ilesha shakes ?
Citreo-viridin	Penicillum citreo-viride	Cardiac beri-beri ?
Hepatotoxicosis		
Aflatoxins	Aspergillus flavus and Aspergillus parasiticus	Aflatoxicosis
Islanditoxin (cyclochorotine)	Penicillum islandicum	Hepatitis (yellow rice disease)
Gastro-intestinal/ Haematotoxicosis		
T-2-Trichothecene	Fusarium sporotrichioides Fusarium tricinctum	Alimentary - toxic - aleukia (ATA)
Diacetoxyscirpenol	Fusarium equiseti	Drunken bread-syndrome
Nivalenol	Fusarium nivale	
Tenuazonic	Phoma sorghina	Onyalai
Dermatoxicosis		
Verrucarin A	Myrothecium verrucaria	Dermatitis
Roridins	Myrothecium roridum	Dermatitis
Pulmonotoxicosis		
Satratoxin	Stachybotrys atra	Inhalation toxicity ?
Genitotoxicosis		
Zearalenone	Fusarium graminearum	Estrogenic effects
Skeletrotoxicosis		
Fusarium G	Fusarium sp.	Kashin-Beck's disease
Nephrotoxicosis		
Ochratoxin A	Penicillum viridicatum	Balkan nephropathy ?
Rubratoxins	Penicillum rubrum	?

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The most toxic for human health are : — Aflatoxins — Ochratoxins — Rubratoxins — Trichothecenes — Zearalenone — and those produced by Penicillum Islandicum.

They may enter the animal or human body by ingestion, inhalation or direct skin contact.

Their dosage and their relationship as ethiological toxic agents are often difficult, to check on account of the great complexity

of analysis methods and the frequent association with other etiological factors.

A further complication is the rather variable way in which the fungi produce their toxic metabolites in food.

The problem of these lethal natural occurring mycotoxins has been made more difficult and severe on account of the fact that some have been claimed to be used especially trichothecenes forming the so called « yellow rain » in biological warfare together with the faeces of Asian bees.

We now examine separately these classes of mycotoxins characterized by production of single spot fluorescing blue under UV light when the toxic extracts are analyzed through paper chromatography, they are the following : aflatoxins, ochratoxins, rubratoxins, trichothecene.

Aflatoxins : produced by the *Aspergillus flavus* they are producing carcinogen causing multiple liver tumors with lungs metastasies.

Besides they cause aflatoxicosis. The vinylether bond in the terminal hydrofuorane group is responsible of this carcinogenecity.These toxins, consists of four major components as shown by thin layer chromatography.

The two faster moving spots were indicated as B_1 and B_2 because of their blue fluorescence under UV light, while the two slower spots, which had a green fluorescence were named G_1 and G_2.

Later on, other toxic factors were discovered in the milk of cows which had been fed aflatoxin-containing diets these two new aflatoxins were called M_1 and M_2.

Ochratoxins : it is the first major group of fungal toxins to be characterized after the aflatoxins. These are produced by *Penicillum* and *Aspergillus* species. They cause Balkan nephropathy.

Rubratoxins produced by another category of *Penicillum* species such as *Penicillum rubrum* cause also nephropathy.

Zearalenone, a common mycotoxin produced by *Fusarium* species, has marked oestrogenic effects in human beings and animals.

Trichothecenes produced by *Fusarium sporotrichioides* and *tricinctum* are one group of chemically related compounds associated with human and animal severe and lethal intoxications as alimentary toxic aleukia. The initial manifestations of this disease in men are loss of appetite and lack of growth ; later a staggering gait develops followed by collapse and death.

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They are claimed to have been employed in the biological warfare causing the « yellow rain » and therefore are of primary interest in this congress.

The mycotoxins assumed by animals from feed may storage and accumulate in the meat and in the secretory glands arriving to men through the alimentary chain as meat or animal alimentary products (f.e. milk).

On account of the fact that trichothecenes may have been used in the biological warfare, coming down, together with Asian bee faeces in the so called yellow rain, is evident that vegetal and animal food in the countries, where those mycotoxins are used, may be contaminated. Therefore is very important not to import vegetable, meat, and animal products as milk and cheese from these countries or at least to analyze these foodstuffs for trichothecenes and other mycotoxins presence.

In fact in order to be consumed as food they must have zero concentration of trichothecenes and other lethal or toxic mycotoxins.

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Applicability of capillary gas chromatography to the identification of chemical warfare agents and simulants by means of retention indices

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ABSTRACT.

Fused silica capillary columns have been evaluated for the screening of chemical warfare agents and simulants. The combination of these columns allows for the simultaneous screening of organophosphorous compounds, vesicants, irritants and simulants. The results show the high reproducibility offered by the capillary system, which permits the reduction of identification problems and analysis time.

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INTRODUCTION.

Capillary gas chromatography has proved to be a powerful tool in the area of underivatized compounds analysis (1-3), especially in the field of routine toxicological screening of environmental or biological materials. Due to the use of chemical warfare agents in recent conflicts (4), the need is existing for detection and identification procedures. Data of retention indices are available for several stationary phases in packed (5, 12) and fused silica capillary (1-3) columns. However, this retention index system is limited to compounds of some groups. There is only little information for our compounds of interest to the defence community (6, 7). This paper presents the retention indices for 15 chemical warfare agents and simulants on a CP ^{tm} Sil 5, CP ^{tm} Sil 8 and OV 1701 fused silica capillary columns, likely to be used for screening purposes. The authors chose to work with temperature-programmed conditions, as it has been shown that retention indices obtained in isothermal runs coincide closely with those determined under temperature-programmed conditions (8, 9).

EXPERIMENTAL.

Standards.

The n-C_6-n-C_{19} (Kit n. 21C) and the n-C_{19}-n-C_{32} alkanes were purchased from Alltech (Eke, Belgium) and were diluted in hexane p.a. (0.2 mg/mL).

Some standards were received from the Belgian Army, some were commercially available.

Instrumental analysis.

A Varian 6000 gas chromatograph equipped with a flame ionization detector and a Varian 4270 integrator were used for all analyses. Three fused silica capillary columns (25 m × 0.32 mm ID), coated with 0.2-μ films (Chrompack, Antwerp, Belgium) were used for the retention index determinations. The columns used were : *a)* CP ^{tm} Sil 5 ;b) CP ^{tm} Sil 8 and *c)* OV 1701, all injections were done directly (10) at 50°C. According to d'Agostino and Provost, 1985 (6), following the injection, the column temperature was held at 50°C for 2 min., and then temperature programmed at 10°C/min. to 300°C. The final temperature was maintained for 5 min. Purified helium is used as the carrier gas (10).

Calculation of retention indices.

A computerprogram originating from the authors for calculation of the retention indices either by n-alkanes or by other reference substances such as drugs in a test mixture and a library search program for the identification of the indices were developed. Written in MF BASIC for the Epson QX 10 personal computer with 256 K memory, two-disk drives and a dot matrix graphics printer, this software is user orientated and simple to operate (11), were applied.

RESULTS AND DISCUSSION.

An excellent reproducibility (SD $\leqslant$ 1) of the system, although not calculated in this report, was attributed to the direct injection technique (10). There was an increase in retention index value for each compound with increasing column polarity. The compari-

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TABLE I

GC Retention indices for some chemical warfare agents and simulants on fused silica capillary columns (abbreviations : RI = mean retention index ; $(RI)_L$ = mean retention index from literature (6, 7) ; $\Delta (RI) = RI - (RI)_L$

Compound	CP ^{tm} Sil 5			CP ^{tm} Sil 8			OV 1701		
	RI	$(RI)_L$	Δ RI	RI	$(RI)_L$	Δ RI	RI	$(RI)_L$	Δ RI
— Organophosphorous compounds									
Sarin	793	792	1	826	824	2	968	966	2
Soman	1012	1008	4	1049	1045	4	1190	1187	3
	1019	1013	6	1056	1049	7	1195	1193	2
Tabun	1085	1078	7	1139	1132	7	1142	1140	2
	1235	1210	25	1267	1257	10	1429	1421	8
Triethylphosphate	1099	1091	8	1372	1354	18	1574	1562	12
Tributylphosphate	1630	1616	14	1679	1659	20	1850	1831	19
— Vesicants and related compounds									
1,4-dithiane	1024	1018	6	1065	1060	5	1162	1157	5
Bis (2-chloroethyl) sulfide	1136	1124	12	1184	1173	11	1334	1326	8
Thiodiglycol	1142	1131	11	1195	1182	13	1467	1458	9
— Irritants, simulants									
Dimethylsulfoxide	788	786	2	845	843	2	1069	1067	2
Dimethylsulfone	853	851	2	926	922	4	1199	1195	4
Methylsalicylate	1177	1163	14	1201	1196	5	1310	1303	7

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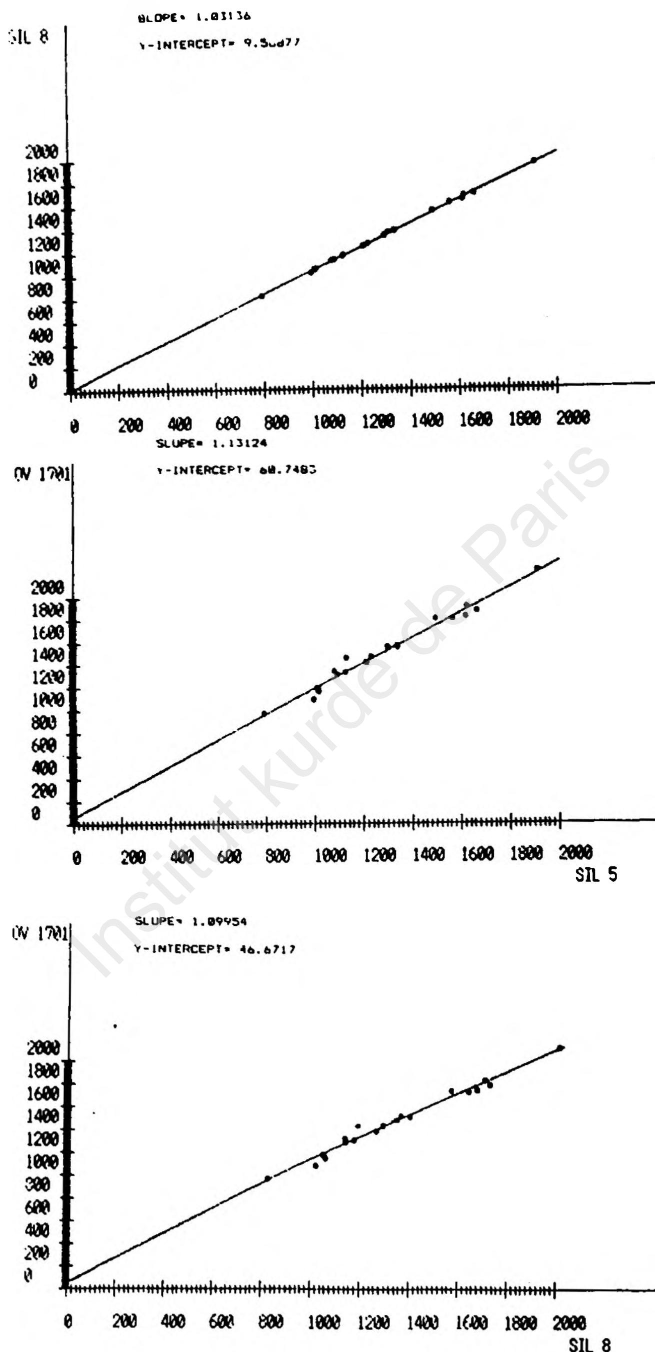

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FIG. 1. — Correlation of the retention indices of the chemical warfare agents on a CP ^{tm} Sil 5 and CP ^{tm} Sil 8, CP ^{tm} Sil 5 and OV 1701 and CP ^{tm} Sil 8 and OV 1701 fused silica capillary column.

son between our retention indices and those in literature, show a mean of 8 units for ΔRI, which is accepted for inter-laboratory measurements (12). The correlations observed for the retention indices of the compounds studied on the narrow bore fused silica capillary columns are shown graphically in figure 1, and the correlation coefficients for the pairs of columns examined are presented in table II. A combination of the CP ^{tm} Sil 5 and OV 1701 will give us the most information.

TABLE II

Correlation coefficients between GC columns

Column	*Column*	
	CP ^{tm} Sil 8	*OV 1701*
CP ^{tm} Sil 5	0.9996	0.9888
CP ^{tm} Sil 8	—	0.99[illegible]6

CONCLUSION.

This paper has compared the identification possibilities of three fused silica capillary columns by the retention indices for the screening of chemical warfare agents and simulants. It is hoped that these results will enable the toxicologists involved with chromatography to identify these compounds more easily.

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Chemical Warfare,
Treatment – Decontamination

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The clinics and therapy of victims of war gases

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Two years after our first experience with victims of war gases (F. Colardyn *et al.*, 1986 ; F. Colardyn and J. De Bersaques, 1984) twelve Iranian soldiers were transferred from Teheran to the University Hospital of Ghent.

Five days before their transfer, they suffered an air attack with, after several explosions, a black rain which persisted for some hours. A few hours after this event they complained of irritation of the eyes, the upper airways, dyspnea and itching of the skin, followed by the appearance of bullae and different skin lesions as has been described previously (H. de Keyser *et al.*, 1986). After transfer behind the frontline they were treated with IV fluid, antibiotics, bronchodilators and local skin therapy.

CLINICAL STATUS ON ADMISSION.

On admission these rather young patients (16-30 years) were deshydrated.

They showed extensive skin lesions with an involvement of 15 to 80 % of the body surface area. These lesions were most pronounced in the skinfolds and in the genital area and consisted of erythema, pigment alteration, epidermolysis with fat necrosis and bullae.

Besides these lesions one patient had two shot wounds.

All showed signs of irritation of the eyes with conjunctivitis and palpebral oedema ; corneal erosions were seen in three.

Fever of more than 38.5°C was present in eight.

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Broncho pulmonary system.

All complained of a sore throat and painful coughing with production of mucopurulent sputa. Sputum cultures yielded only a normal oropharyngeal flora. Diffuse bilateral rales were heard in all.

Signs of bronchopneumonia on chest X-ray were seen in five.

Four patients had arterial hypoxemia, which leads in two of them to endotracheal intubation with ventilation. One patient developed an Adult Respiratory Distress Syndrome.

After intubation bronchoscopy showed diffuse inflammation and necrotising tracheitis and bronchitis. After a few days sloughing of the mucosa was apparent.

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Gastro-intestinal system.

Most of them had anorexia, partially due to the painful irritation of the mouth mucosa, partially due to our Western food.

Six of them showed also a slight increase of AST/ALT. One vomited an Ascaris.

The haematopoietic system.

Eight showed, shortly after admission, a lymphopenia, five a severe leucopenia, one a thrombocytopenia.

The only patient who died had a total bone marrow suppression. In the other patients there was a normalization of the hematological parameters after some days.

Renal function.

Most of the renal parameters were within normal limits ; two showed a pathological sodium excretion, as can be seen in, for example, aminoglycoside tubulopathy.

Neurological system.

There were no pathological findings. Psychiatric disturbances were difficult to evaluate, due to the language barrier.

TOXICOLOGY.

— Cholinesterase activity in plasma, red blood cells and total blood : normal.
— Cyanide levels : normal.
— Mustard gas in urine, skindebris and bronchial sloughing material : absent.
— Methemoglobinemia : normal
(State Laboratory of Toxicology — Prof. Dr. A. Heyndrickx, Pharmacist).
— Thiodiglycol in the urine was strongly positive in the one patient who died
(TNO laboratory in Rijswijk (Holland) — Eng. Wils).

TREATMENT.

A *local therapy* was instituted by the Dermatologist and consisted at the start of chloramine dressings on the exsudative lesions. The other lesions were treated with silver sulphadiazine (Flammazine®) cream application.

The pain was controlled with tilidine-naloxone drops (Valtran®) and the itching was suppressed by antihistaminics.

The eyes were treated with an AB ointment.

Besides the local therapy a *general supportive therapy* was instituted with supplementation of albumine, vitamins and eventually calories.

As far as possible IV catheters were avoided, as it is known that placing IV catheters through damaged skin enhances the risk of catheter sepsis.

The broncho-pulmonary lesions were treated primarily by an intensive chest physiotherapy, avoiding antibiotics.

An exception was made for the neutropenic patients who were isolated and received a combination of selective gut decontamination, combined with IV antibiotics. As soon as the granulocytes rose again, IV antibiotics were stopped and the selective gut decontamination tapered off.

EVOLUTION.

One patient died within 72 hours after admission ; it was the patient who had a combination of ARDS, pancytopenia with an

empty bone marrow and multi organ failure ; he had also a high thiodiglycol level in the urine.

In the other patients the bronchial and skin lesions healed very slowly as did the eye lesions. The less pronounced skin lesions had the fastest healing ; but even after more than three weeks small bullae appeared provoked by slight mechanical trauma.

Besides the small bullae some showed hypertrophic scars, pigmentation alteration, hypotrophic skin areas and most still suffered from irritating cough and excessive sputum production at discharge.

Most of them also complained of photophobia and dryness of the eyes.

DISCUSSION.

As it was clear from the start that these patients suffered a mustard gas intoxication without an apparent associated toxic agent they were treated as indicated.

Final proof of mustard gas intoxication can only be derived from a combination of the clinical presentation, the fact that U.N. observers have detected unexploded bombs with mustard gas on the field and the indirect toxicological proof in one patient where we found high thiodiglycol levels in the urine. Thiodiglycol is one of the metabolites of mustard gas.

Mustard gas is mostly excreted by the urine (90 % within 72 hours), 6 % is eliminated in the faeces, 4 % is irreversible bound to the cells.

In the urine 50 % is excreted as thiodiglycol and 50 % is bound to glutathion or cysteine.

In contrast with our first experience we were more confident this time that only one toxic agent was involved and therefore therapy could be simplified.

In spite of the fact that these patients were more attainted than the first group and were sent earlier to us with more haematopoietic derangements, only one of the twelve patients died.

Septic problems were also less frequent than in our first group, where an extensive therapy was used with frequent intravascular manipulations.

Therefore we would suggest that in cases of a mono-intoxication with mustard gas a simple skin and eye therapy, combined with a general supportive therapy, and with the greatest emphasis on chest physiotherapy, can lead to excellent results.

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CONCLUSIONS.

We admitted twelve Iranian soldiers with a clinical picture of mustard gas intoxication. A formal toxicological proof could not be given, although the most intoxicated patient showed thiodiglycol in the urine. Lesions of the eye, the skin and bronchi were on the foreground. A general supportive therapy was instituted with in addition intensive chest physiotherapy and eventually an antibiotic program for neutropenic patients. Only one patient died.

At their discharge they still had residual lesions and it has to be foreseen that in the future they will suffer from some functional impairment.

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Dermatological aspects of intoxication by mustard gas

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This paper will present you the nature, the treatment and the evolution of the skin lesions as we could observe them on the 12 Iranian patients — victims of a chemical warfare attack — who were treated in our department, the department of Dermatology of the University Hospital of Ghent, in March and April 1986.

CHARACTERISTICS OF THE SKIN LESIONS.

Upon their arrival, about the sixth day after the event, all the victims showed a similar spectrum of skin lesions, but with a varying degree and extent :

— large erythematous patches sometimes covered extensive surfaces of the body. They felt warm and were painful ;
— in addition to those, there were areas of darker pigmentation with beginning desquamation ;
— the epidermolytic lesions were the most severe ones : to varying extents, all the patients showed loosening and shifting of the epidermis, which caused erosive and exudating areas. They suffered a lot of pain. At some places where the epidermis had disappeared, a white fatty necrotic material was visible in the wounds ;
— closed blisters appeared mostly on hands and feet, or on parts with limited epidermolysis. The blisters were filled with a yellowish fluid and could be very large.

Histological examination of these lesions shows loosening of the epidermis with formation of a subepidermal gap. The superficial cutis shows densification of connective tissue with a limited

amount of cellular elements present. This band will turn necrotic in a later stage. The cells of the basal layer lost their typical palissade structure and got a shrinking aspect. The nuclei are pycnotic.

These skin lesions are compatible with intoxication by Yperite. Yperite has a direct toxic effect on the skin : it disturbs the DNA and affects above all the rapidly deviding cells, that is for the skin the basal layer. The explanation for the difference in degree and extent of the lesions should be searched on the one hand, in the clothes, the position and the distance of the victim in regard to the event, on the other hand, in the concentration of the Yperite. In general, we can mention the genitals and skinfolds as the most affected areas. This is probably due to a higher degree of humidity (by perspiration) of the skin and prolonged contact with soaked clothing. That is also the reason why there are so frequently lesions at points of pressure of clothing ; e.g. the collar or the site of the belt.

SKIN TREATMENT.

The skin treatment was chosen to avoid infection by way of extensive erosive lesions, to stimulate good wound healing and to attenuate the subjective complaints of the patients. During the first days, we applied chloramine dressings on exudating areas because of their drying and purifying effect. On non exudating lesions, or in a later stage of the healing, we applied a desinfectant ointment: silversulfadiazine, known here as Flammazine ; the use of bandages allowed the patients to move progressively.

Great attention was given to rub the skin as little as possible. During the first days, and later if required, a painkiller was given before the local treatment. In a later stage, the predominating subjective complaint of the patients was the itching, on which antihistaminics seemed to have a favourable effect.

EVOLUTION.

The evolution of the skin lesions and the duration of healing depended on the type of lesion :

— the erythematous areas progressively took on a darker colour and after a stage of superficial scaling a quasi normal skin appeared. This process took about 4 to 10 days ;

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— the epidermolytic areas healed slowlier according as the erosions were deeper. The most superficial lesions showed re-epidermisation out of preserved hair follicles. A speckled pigmentation was very typical for this healed skin : the pigment is still present in the concentric area around the hair follicle, but was depleted on distance of it. The healing time for these lesions took about 14 to 20 days.

The deepest lesions, including the blisters, regenerated from the surrounding skin. This process, which shows a centripetal direction took longer depending on the size of the wound. In these cases, the healed skin also shows an irregular pigmentation pattern, hypotrophic areas on which mechanically induced blisters can develop and areas of hypertrophic cicatrization. This sometimes caused limitation of movements, eg. of the fingers.

CONCLUSION.

Intoxication by Yperite causes a polymorphic picture of dermatological lesions. The prognosis for these patients is rather reserved. There would be a higher incidence of skin cancer and it is evident that these patients should avoid any other cancerogenic factor on the skin, as there is, eg., sun exposition.

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Treatment of organophosphorus poisoning
Pharmaceutical aspects of antidotes

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INTRODUCTION.

The organophosphorus compounds of agricultural and medical interest are toxic esters of phosphoric and phosphonic acids. Insecticidal compounds such as parathion and malathion have been used extensively in environmental pest control, and selected compounds are utilized in veterinary and human medicine for elimination of ectoparasites (1). Restrictions on the use of organochlorine pesticides have increased demand for organophosphorus compounds. The wider distribution of these insecticides has led to an increased incidence of poisoning, both accidental and by deliberate ingestion of organophosphorus products (2). More toxic esters such as tabun, soman and sarin have potential military application as nerve agents. Although chemical warfare is banned under the Geneva Protocol of 1925, monitoring a global ban on development, production and stockpiling of chemical weapons is difficult. Contraventions of the Accord have occurred, the most recent being the use of tabun and mustard gas by Iraq in the Gulf War attacks of 1984 and early 1986 (3). The Soviet Union has considerable stocks of chemical weapons and the United States has recently approved production of a new binary weapon which will deliver nerve agents. This renewed interest in chemical warfare has led to recognition of the need for improved protection of personnel and development of effective chemotherapeutic treatment of nerve agent toxicity. Current research on organophosphorus antidotes is directed towards improving therapy for both agricultural and military products.

The primary toxic effects of the organophosphorus compounds result from accumulation of excess acetylcholine at receptor sites.

This is caused by inhibition of the enzyme acetylcholinesterase, which contains a serine moiety at its active site. When the hydroxyl group of this amino acid is phosphorylated by an organophosphorus compound, the product is much more stable than the normal acetylated derivative and regeneration of the active enzyme is retarded. The stability of the phosphorylated enzyme is dependent upon the nature of the alkyl group in the organophosphorus component of complex. If methyl or ethyl groups are present, reactivation of the enzyme may take several hours. When secondary or tertiary alkyl groups are present, significant spontaneous regeneration does not occur, and synthesis of new enzyme is required for esterase activity to return (4).

TREATMENT OF ORGANOPHOSPHORUS POISONING.

The toxic effects of specific organophosphorus compounds vary according to the rate and route of absorption, distribution characteristics, the rate of reaction with cholinesterase and the stability of the product. In all cases, treatment involves prevention of further exposure, blockage of the effects of excess acetylcholine and maintenance of adequate respiration. When possible, reversal of cholinesterase inhibition with a cholinesterase reactivator is beneficial, and administration of an anticonvulsant may enhance protection and recovery. Selection of an optimum regimen for therapy is complicated by the fact that therapeutic effectiveness is dependent upon the type of inhibitor, the extent of exposure and delays in initiation of therapy. Combinations of therapeutic agents with complementary activity are recommended for treatment of organophosphorus poisoning, since no single compound possesses the pharmacological properties required for satisfactory protection.

The standard anticholinergic drug used to treat acute organophosphorus poisoning is atropine, a potent blocker of the peripheral muscarinic effects of acetylcholine. It is administered by intramuscular injection. This drug has no activity against the nicotinic effects of acetylcholine, and is of limited value as a blocker of central muscarinic effects. Numerous analogues have been evaluated, but atropine sulphate remains the product of choice for symptomatic antidotal treatment of organophosphorus poisoning (5).

Inhibited cholinesterase can be dephosphorylated by nucleophilic attack on the phosphorus atom, resulting in reactivation. It

has been shown that oximes with an N-alkyl pyridinium structure are most effective (5). Selection of appropriate oximes is important as some of the phosphorylated oximes produced *in vivo* are themselves inhibitors of cholinesterase. Aging of inhibited acetylcholinesterase prevents reactivation (6). Dealkylation of the organophosphorus moiety of the inhibited enzyme occurs, preventing reaction with the oxime. The half-life of aging is approximately four minutes with soman (7) whereas it is several days with many pesticides. The rapid rate of aging with some organophosphorus compounds is a primary reason for the importance of prompt initiation of therapy with oximes. The reactivators may be administered orally or by injection, but the latter route provides therapeutic blood levels more rapidly and avoids the problems associated with swallowing tablets after administration of atropine.

Pyridinium oxime iodide (PAM), its methane sulphonate (P2S) and chloride (2-PAM C1) were the first oximes used therapeutically. Atropine sulphate with 2-PAM C1 is the standard treatment for organophosphorus poisoning in North America. Some European countries prefer P2S whereas others use obidoxime (Toxogonin), a bis-quaternary di-oxime, in conjunction with atropine. None of these oximes penetrates the blood-brain barrier readily, nor are they effective against « aged » inhibited enzyme. A new generation of asymmetric bis-quaternary mono-oximes including HI-6, HS-6 and HGG-12 has been synthesized (8). These compounds and many of their analogues have greater activity than the conventional oximes, particularly against soman. Often there is a higher incidence of toxic side effects, but this is not the case with HI-6 (9). Instability in aqueous solution is another cause of difficulty in developing a practical pharmaceutical product containing one of these oximes. However, there is considerable potential for future use of these second generation cholinesterase reactivators.

Benzodiazepines such as diazepam may be used to supplement atropine-oxime therapy. They are effective anticonvulsants which are lipophilic, readily crossing the blood-brain barrier. When used as supplements to conventional therapy, they reduced convulsions and increased survival in animals poisoned with organophosphorus compounds (10). An oral dose of up to 15 mg has been recommended for inclusion in the therapeutic regimen for treatment of acute organophosphorus poisoning in humans (11).

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Pre-treatment with a reversible cholinesterase inhibitor is a prophylactic approach which could reduce the problem of aging of inhibited enzyme. Pyridostigmine is a carbamate which binds to the hydroxyl group on the serine component of the acetylcholinesterase, protecting it from reaction with organophosphorus compounds. The carbamylated enzyme does not age and reactivation occurs readily. It has been suggested that 30-40 % inhibition would give adequate protection. An oral dose of 30 mg three times daily would achieve this level of inhibition in three weeks (11). Also, pyridostigmine has been suggested as a component of mixtures for injection in emergency situations, when longer-term pretreatment has not been used (12).

PHARMACEUTICAL ASPECTS OF PARENTERAL ANTIDOTE DEVELOPMENT.

Exposure to agricultural or military organophosphorus compounds may be sudden, and lead to acute poisoning. Rapid initiation of treatment enhances the probability of a successful outcome. Immediate therapeutic intervention is most conveniently provided using an autoinjection device for self-administration. Autoinjectors can deliver an intramuscular dose of the selected antidote without the need for removal of protective clothing. Injectors currently available may contain atropine sulphate alone, e.g. the Atropen®, or they may be filled with an atropine-oxime mixture. Oximes used in these injectors include 2PAM-C1, P2S and obidoxime. The aqueous parenteral solutions have a pH of approximately 2-3, which gives them a shelf-life exceeding two years at 25°C. The oxime injections are hyperosmolalic and this factor, combined with the low pH can cause a painful injection. High concentrations of 2PAM-C1, i.e. 400 mg per mL, cause localized tissue necrosis, but there is less problem at 250 mg per mL. Obidoxime has a lower osmolality at therapeutic concentrations and produces less problems at the site of injection.

The lack of effectiveness of conventional oximes as reactivators of soman-inhibited cholinesterase has led to the suggestion that alternatives should be used in injectors intended for military protection. The asymmetric bis-quaternary mono-oxime, HI-6 has been recommended for this purpose, despite the fact that it is ineffective against tabun (9).

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PARENTERAL HI-6 SOLUTIONS.

Pharmacological studies have shown HI-6 to be one of the most promising new oximes for treatment of nerve agent toxicity. It is well tolerated by humans and therapeutic blood levels are achieved in less than six minutes following intramuscular injection of 250 or 500 mg of drug (9). This route of administration gave 100 % bioavailability in animals (13). The problem with

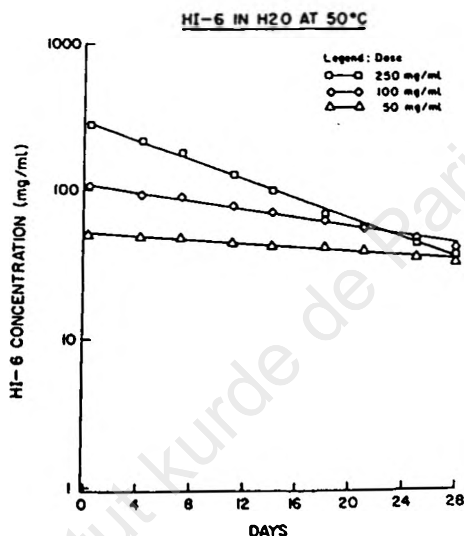

FIG. 1. — Stability of HI-6 : Effect of concentration. Results for half-life calculations are shown in table 1.

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development of this compound as a standard antidote for use in autoinjectors is the short half-life of HI-6 in aqueous solution at room temperature.

Accelerated stability studies with HI-6, (DRES # 32), were conducted using unbuffered aqueous solutions prepared with sterile water for injection. The solutions contained 250, 100, or 50 mg/ml of oxime and had an initial pH of 3.5. One ml aliquots were sealed into 2 ml glass ampoules. Solutions of each concentration were stored at 70, 60, 50, 37 and 25°C and representative ampoules were withdrawn at appropriate intervals for analysis of HI-6 content using a sensitive, specific HPLC procedure (14). Plots of log HI-6 concentration against time were linear at all temperatures with each concentration (fig. 1).

TABLE I

Half-life (days) of HI-6 oxime in aqueous solution at various temperatures

Concentration of HI-6 (mg/mL)	70° C	60° C	50° C	37° C	25° C	25° C*
250	1.54	3.97	9.41	36.80	99.45	136.69
100	3.65	8.95	21.69	86.63	313.64	313.13
50	6.16	17.06	48.16	208.20	823.22	909.47

* Calculated using the Arrhenius Equation.

The stability of HI-6 was shown to be concentration dependent, with more dilute solutions having greater stability. The similarity between the experimental results obtained for half-life at 25° and that obtained by calculation from accelerated stability studies confirm the validity of the latter procedure with this compound.

The pH of aqueous solutions of HI-6 drops on storage, and attempts to maintain the initial pH using physiologically acceptable buffer solutions in the formulation were not successful. The problem was greatest with the highest concentration of HI-6 which gave a terminal pH of approximately 2.0.

MULTI-COMPONENT FORMULATIONS.

There is considerable interest in formulating an injection which contains atropine and an oxime with other compounds which improve protection and recovery from poisoning by organophosphorus compounds. Incorporation of a benzodiazepine anticonvulsant has pharmacological advantages for therapy, but there are pharmaceutical problems associated with providing this product as a single injection. The oximes and atropine sulphate are readily soluble in water, but diazepam and other benzodiazepines which have been recommended are highly lipophilic. It is necessary to add a high proportion of a physiologically-acceptable organic solvent to the injection vehicle to prepare a « cocktail » containing atropine, oxime and benzodiazepine. A solvent containing 46 % propylene glycol, 11 % ethyl alcohol (95 %) and 43 % citrate buffer, (0.2 M, pH 3.5) was found to provide a physically acceptable preparation. Results from stability studies on representative mixtures showed acceptable stability for all compounds, except with HI-6. Pharmacological testing revealed that the absorption and distribution of atropine was delayed after IM

injection. This was observed through monitoring the cardiac effect of the drug. Radioimmunoassay techniques are necessary to quantitate the rate of release of the atropine from the injection site. Further studies are being performed to measure the bioavailability and pharmacokinetic characteristics of the atropine component of this and other injections. Atropine is the primary defense against the effects of organophosphorus compounds, and it is essential that the drug be distributed rapidly after injection.

Parenteral solutions prepared with the propylene glycol solvent have higher viscosity than simple aqueous solutions. This reduces the rate of ejection from an autoinjector, prolonging discomfort associated with injection. This effect is particularly apparent at lower temperatures.

The feasibility of incorporating pyridostigmine, a reversible carbamate cholinesterase inhibitor, with 2PAM-C1 in a single parenteral solution was examined using accelerated stability studies. Solutions containing pyridostigmine bromide, 5 mg, or 2PAM-C1, 350 mg, alone or in combination were dissolved in 1.75 ml solvent. Samples were prepared in Water for Injection, citrate buffer, 0.2 M, pH 3.5, and the propylene glycol solvent described above. All solutions were filtered through a bacteria-proof filter and sealed in 2 mL glass ampoules and batches were stored at 90, 80 and 70°C for 5 weeks. Representative samples were withdrawn at weekly intervals for analysis. The 2PAM-C1 was analysed by UV spectrophotometry (15) and pyridostigmine by gas-liquid chromatography using a neostigmine standard (16).

Plots of log 2PAM-C1 concentration against time were bi-exponential. Results were similar in all solvents and in the presence of pyridostigmine. At 90°C, the initial half-life of decomposition was 1.13 days, with the slower terminal rate giving a half-life of 129 days. With pyridostigmine alone, results were comparable in each solvent and the log concentration/time plots were linear.

At 90°C, the half-life of pyridostigmine in solution was 579 days, but in the presence of 2PAM-C1, no pyridostigmine remained after 1 week. A subsequent study, in which samples were withdrawn at intervals over ten days gave a calculated half-life of 0.91 days at 90°C for pyridostigmine in the presence of 2PAM-Cl. At 80°C the calculated half-life for pyridostigmine alone in solution was 1,880 days, whereas in the presence of 2PAM-Cl it was 3.33 days.

It can be concluded from these results that the stability of pyridostigmine is markedly reduced in the presence of 2PAM-C1, and formulation of an antidote mixture containing both of these ingredients may not be feasible. However, similar effects were not observed with HI-6 or obidoxime.

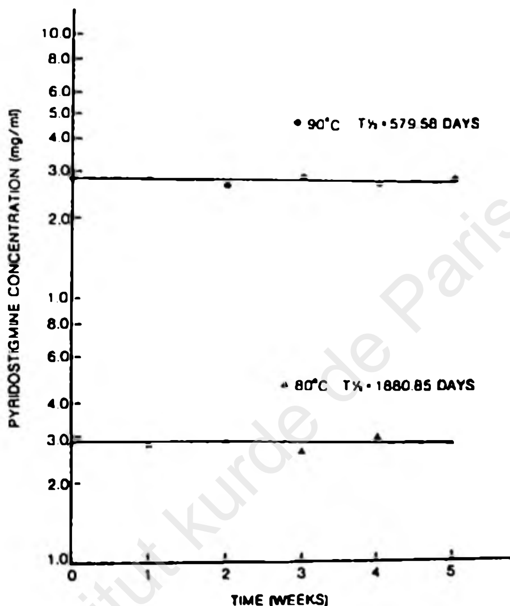

FIG. 2. — Log concentration/time plots for pyridostigmine solutions at 90° C and 80° C.

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NEW DELIVERY DEVICES.

The problems of incompatibility between components of a parenteral solution have led to the development of new devices which can contain separate solutions in a single injector. The Multipen® (17) is an example which can deliver up to three separate solutions sequentially through the same needle at a single firing. This multicompartimental injector enables physically and chemically incompatible solutions to be packaged in the same device, while eliminating the problems which would be associated with a « cocktail » of the same antidotes.

CONCLUSIONS.

1. Prompt administration of an injectable antidote containing a combination of drugs is required for optimum emergency treatment of organophosphorus poisoning.

2. Protection may be enhanced by pretreatment with a reversible inhibitor of cholinesterase such as pyridostigmine.

3. Formulation of hydrophillic and lipophillic components in the same parenteral solution may result in physical or chemical incompatibilities, or pharmacokinetic interactions.

4. Conventional oximes are most appropriate for incorporation into antidotes for organophosphorus poisoning at the present time. Stability of solutions is a problem with second generation compounds such as HI-6. However, these are active against soman, and are being actively considered for future use.

5. New autoinjector models may overcome incompatibility problems associated with some mixed formulations.

6. Further research and development are required to provide optimal products for emergency treatment of organophosphorus poisoning.

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A polymer coating to reduce or eliminate surface contamination by chemical and biological agents

by G.M. SCHOFIELD, S.L. BYERS* and R. LOCCI**

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I am here to tell you about some of the characterics and applications for a newly developed, unique polymer system called « *Clear-Shield* ». This new development in surface treatment technology has been found to reduce or eliminate surface contamination by biological and chemical agents.

Clear-Shield is a polymeric coating which protects against biological and chemical attack by forming a monomolecular layer on the surface. This layer or shield is transparent, chemically inert to most substances, and long-lasting.

Most recent work to date has been on glass and it is this to which I shall refer.

Research work in the United Kingdom by the Public Health Laboratory Service (PHLS) and BIOS, an independent laboratory, has shown at least a twenty-fold reduction in adsorption of bacteria and other contamination to glass surfaces treated with *Clear-Shield.*

Glass provides a habitat for microorganisms because of its own physical and chemical nature. For example :

Glass is not completely smooth.

Even new glass is uneven and pitted. Figure 1 shows a scanning electron micrograph of a new and clean glass slide.

After being placed into service glass becomes more uneven and pitted through environmental attack, abrasion, and a weathering

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process caused by moisture. Figure 2 shows a glass slide that has been artificially aged. Environmental attack and abrasion create more damage to the surface as shown in figures 3 and 4. The result is a microscopically pitted surface with « lattices » and « honeycombs », ideal hiding and breeding places for bacteria.

Glass holds static electricity.

The intensity of static electricity in glass can be increased by surface friction. As static charges on the surface increase the glass attracts and holds dirt.

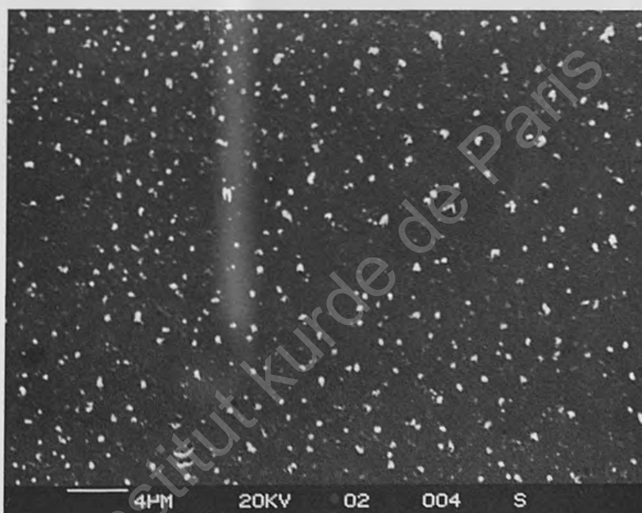

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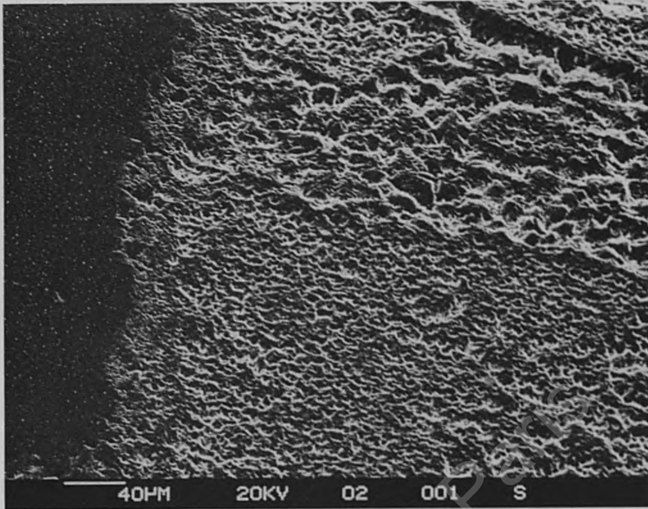
FIG. 1. — Scanning electron micrograph of a new, clean, microscope slide.

Glass is hydrophyllic.

There is a molecular layer of moisture on all glass caused by a chemical bond between silanol groups forming part of the glass surface and water molecules in the atmosphere. This layer of moisture traps and holds dirt and forms an ideal physico-chemical environment for microbial adhesion.

Many other substances adhere firmly to glass through chemical reactions.

Following are examples of substances that bond chemically to glass and cannot be removed by conventional cleaning methods :



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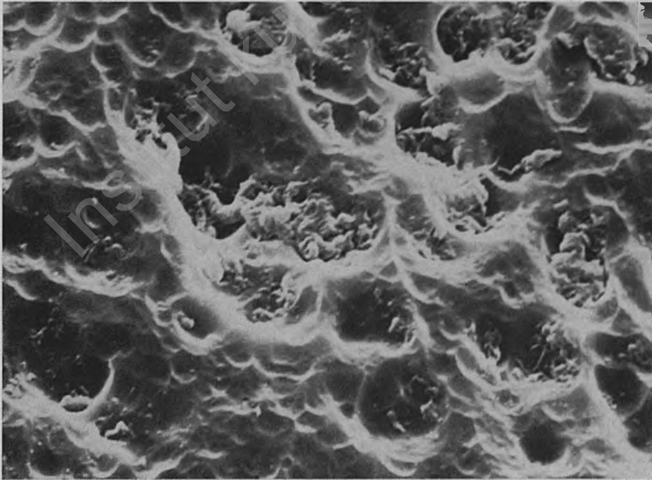

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FIG. 2. — Scanning electron micrographs of an artificially aged glass slide.

A

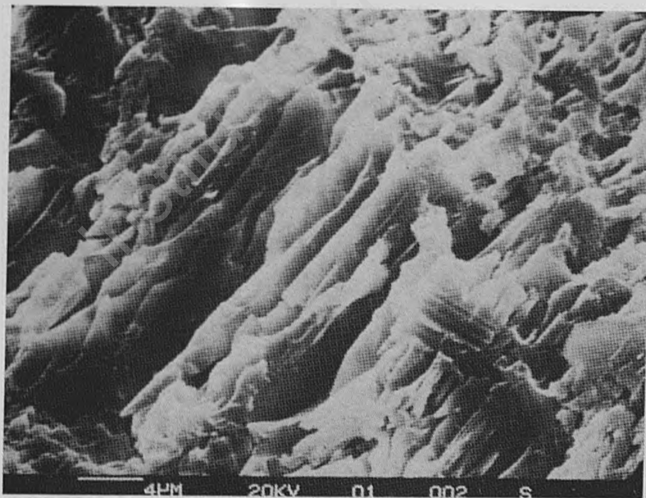

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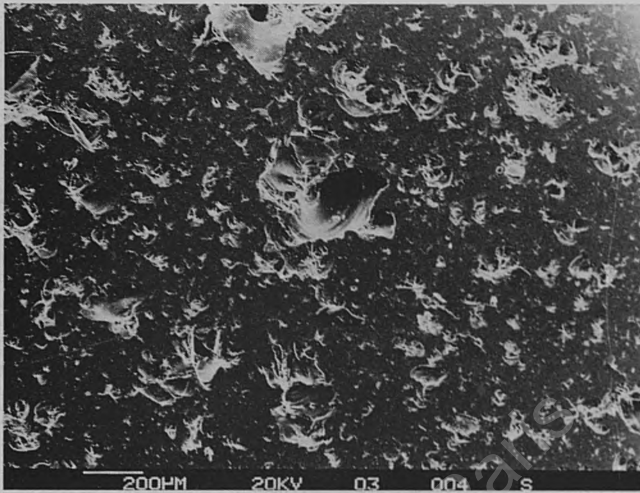
FIG. 3. — **Scanning electron micrographs of a hairline scratch.**



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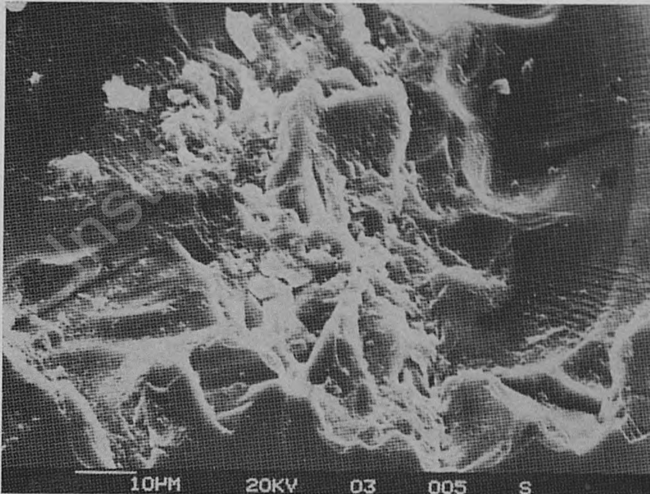

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FIG. 4. — Scanning electron micrographs of bead blasted glass slide.

TABLE I

Examples of substances that bond chemically to glass

Microbes

Carbonised food

Metal oxides, e.g.
- Lead
- Iron
- Aluminium

Limescale

Hydrocarbon pollution

Construction material, e.g. :
- Plaster
- Mortar
- Sealants
- Paints

Glass easily stains and discolours.

The substances in table I above leave deposits which stain and discolour glass, destroying its bright appearance, beauty and aesthetic qualities. The stains and deposits cannot be removed by conventional cleaning methods.

Clear-Shield has been shown to reduce or eliminate the habitats for microorganisms on glass by working as a « surface modifier ». It modifies surface properties without altering chemical analysis of the glass surface. It changes glass from hydrophyllic (water attracting) to hydrophobic (water repelling). It smooths out the surface, reduces the coefficient of friction and reduces electrostatic properties.

The coating is based on a proprietary polymer system, catalyst, and other surface active agents in a solvent carrier. Applied as a liquid to clean and dry glass, it reacts chemically with atoms on the surface to form a monomolecular layer that shows strong cross-linking between the polymer system and the surface. After curing, the polymer itself is chemically inert to most substances. This reduces or eliminates the adhesion of substances which normally bond chemically to glass.

Clear-Shield is unique because it is basically non-hazardous in its liquid form and chemically inert after curing. It self-cures at ambient temperature without any special conditions except that it be applied to a clean, dry surface. Application is by spray, cotton pad or dipping.

After curing, which is completed in a few minutes, the coating cannot be detected by the eye or an electron microscope.

Clear-Shield has been subjected to and passed standard test procedures for coatings. These procedures include :

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TABLE II

Summary of test procedures

Weatherometer (BS 3900), 2000 hours
Freeze-thaw, 20 cycles
Salt spray, 100 hours
Thermal shock, 20 cycles
Humidity cycle, 22 days
Autoclave (ISO 4802)
Coefficient of friction
Mixed alkali attack (ISO 695)
Haze/light transmission
Resistance to cleaning compounds and solvents
Paint adhesion
Migration
Removal of algae and limescale
Mechanical abrasion resistance

The resistance of *Clear-Shield* to adhesion by tenacious substances can be demonstrated by simple experiments using silver chloride solution or glycerol, two substances with quite different physical properties. Adherence of these materials to untreated glass is much stronger than to glass coated with *Clear-Shield.* Untreated glass dipped in these materials is covered by thick films or deposits whereas on the treated glass only occasional spots of contaminant remain.

Microbial film can be as harmful as any inanimate pollutant or toxic chemical. Microbial fouling in marine environments and oil pipelines are good examples. It is such attachment of microorganisms to surfaces that is of particular interest in microbiology.

In natural environments bacteria are usually found associated with an interface. This can be solid-gas, solid-liquid, gas-liquid, etc. Microorganisms associated with solid surfaces show the greatest metabolic activity and the most vigorous rate of growth and development. The physico-chemical environment of a solid-liquid interface confers many advantages for bacterial growth. Conversely, anything that discourages liquid formation also discourages microbial attack.

One bacterium frequently used in microbial adhesion work is *Staphylococcus aureus* (Ashkenazi, 1984 ; Jansson and Wadstrom, 1981 ; Locci, Peters and Pulverer, 1981), which is of medical importance because of the widespread threat imposed by it.

One particular experiment was carried out using treated and untreated microscope slides :

Test procedure.

Clean slides were placed in trays containing a suspension of approximately 3×10^9 cells ml^{-1} of *Staph aureus* and incubated on a rotary shaker at 100 rpm at 37°C.

After three hours the slides were removed, washed with phosphate buffed saline (PBS) and allowed to air dry. The slides were then placed into individual boiling tubes containing 40 ml of PBS, which were placed on a rotary shaker at 120 rpm at 37°C.

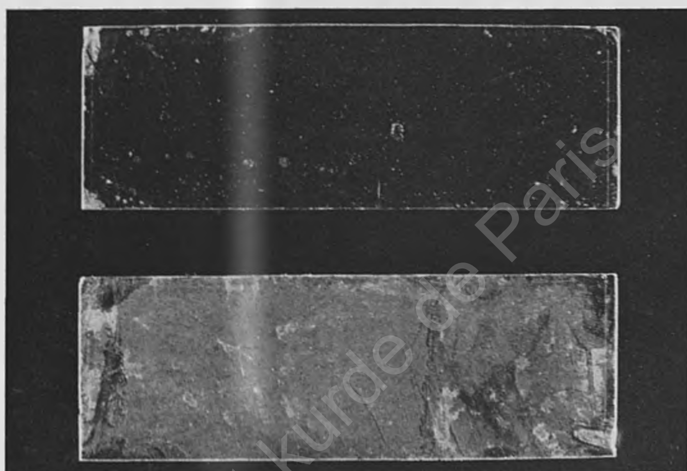

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FIG. 5. — Macroscopic view of treated (top) and non-treated (bottom) glass slides.

After one hour the slides were removed, allowed to air dry, and the bacteria counted in two ways :

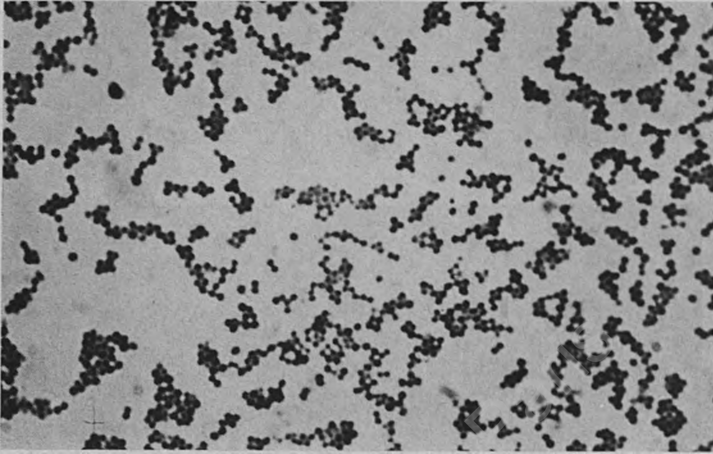
1. Actual counts using a 250 stereoscan electron microscope (Cambridge Scientific Industries Ltd., Cambridge U.K.) operated at 20 KV. Photographs were taken on 120 Kodak Tri-X film.

2. Viable wash-off counts.

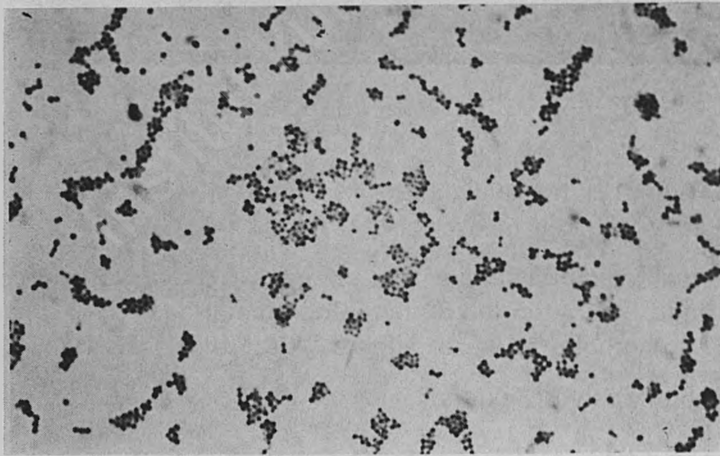
RESULTS.

There was a noticeable macroscopic difference in the appearance of non-treated and treated slides as shown in figure 5. This was entirely due to the number of cells adhering to the surface. Microscopically, counts were made of bacteria in 30 randomly selected fields of view on each slide.

NON-TREATED GLASS



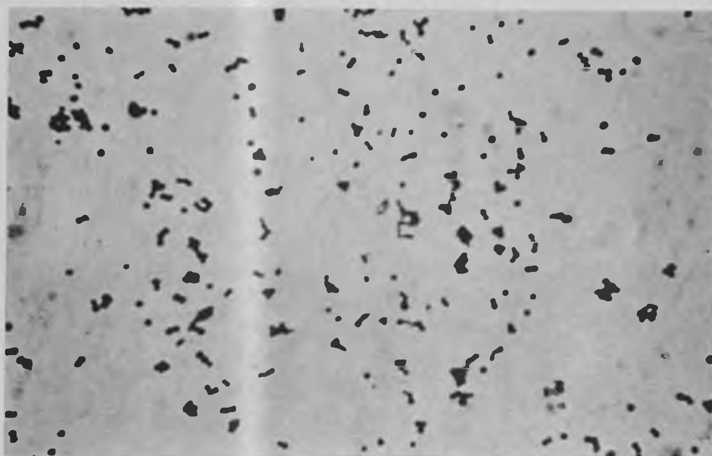
A. — Prior to washing.



B. — Post washing.

FIG. 6. — Micrograph of cells of *Staph aureus* adhering to glass slides.

TREATED GLASS



A. — Prior to washing.

B. — Post washing.

FIG. 7. — Micrograph of cells of *Staph Aureus* adhering to glass slides.

Results of the microscope counts and wash-off viable counts showed more than a 20-fold reduction in bacteria adhering to the treated slides.

Observations of typical fields of view are shown in figures 6 and 7. There are obviously fewer cells adhering to the treated slides.

In figures 8 and 9 the pattern of bacterial adhesion to the untreated slide is clearly demonstrated. However it was extremely difficult to find any cells on the treated slides. The only cells found are shown in figures 10 and 11.

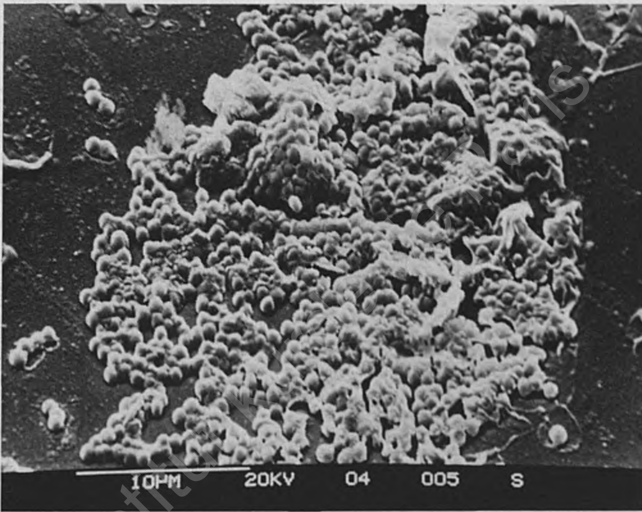


FIG. 8. — Scanning electron micrograph of cells of *Staph aureus* adhering to a clean non-treated glass slide.

It should be noted that only two groups of cells were found on the treated glass (fig. 10 and 11). These were along-side a salt crystal from the PBS and appear to have been protected by the crystal. All other areas that appear to be bacteria are either surface imperfections or salt crystals.

Further tests.

Similar experiments have been carried out with other bacteria including *Escheria coli, Bacillus pumilus spores, Candida albicans* and *Salmonella.*

As an example, slides treated with *Candida* were applied directly to the surface of poured Petri dishes of Saboraud's agar and removed and the plates then incubated at 30°C for 24 hours. The

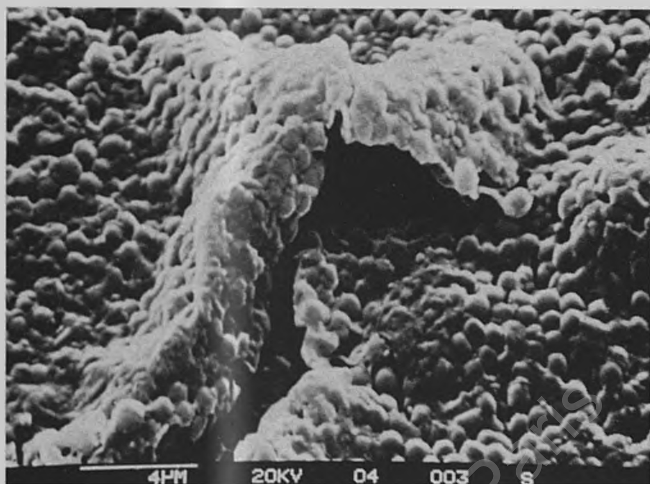


FIG. 9. — Scanning electron micrograph of cells of *Staph aureus* adhering to a clean non-treated glass slide.

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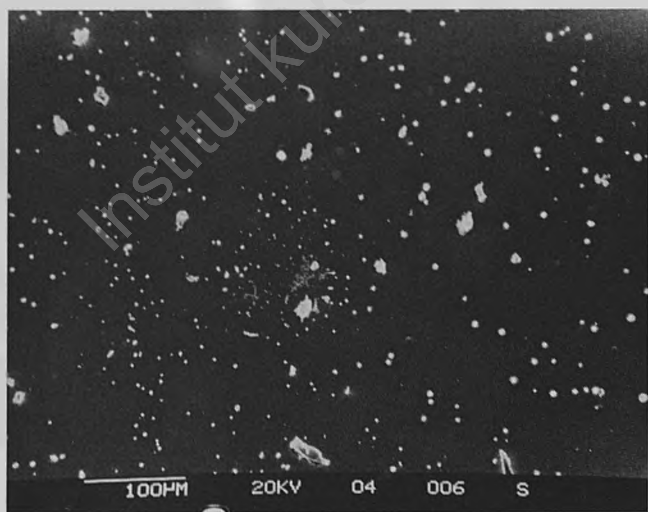

FIG. 10. — Scanning electron micrograph of cells of *Staph aureus* adhering to a glass slide treated with *Clear-Shield*.

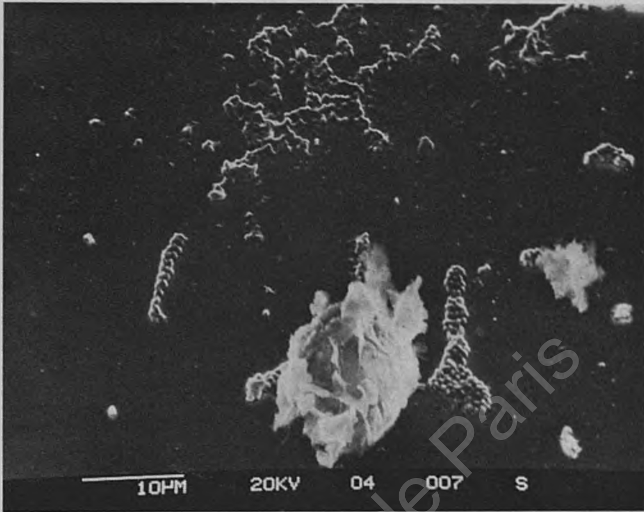

FIG. 11. — Scanning electron micrograph of cells of *Staph aureus* adhering to a glass slide treated with *Clear-Shield.*

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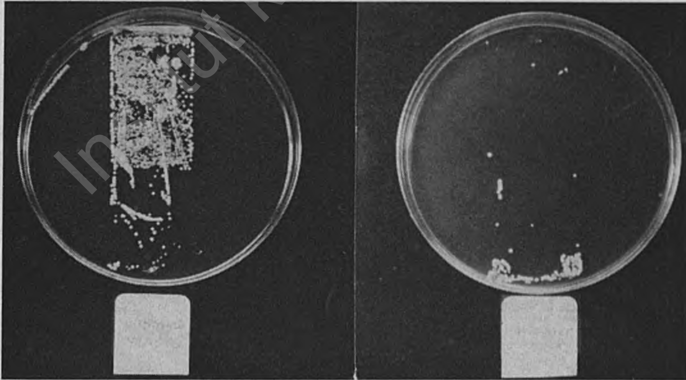

A. — Non-treated. B. — Treated.

FIG. 12. — *Candida albicans* contamination.

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number of colonies growing on each plate was counted under microscope.

Figure 12 shows a comparison of plates exposed to treated and untreated glass slides.

DISCUSSION.

New glass surfaces are not usually considered to be as prone to bacterial attachment as those which are aged, weathered or damaged by abrasion. However *Clear-Shield* has been shown to discourage adsorption of various contaminants to new, relatively smooth glass surfaces. The results on aged glass should be much more significant.

Tests carried out by two independent laboratories show at least a twenty-fold difference in adsorption of bacteria to new, non-treated surfaces when compared to treated surfaces. Numbers of bacteria on the grossly contaminated non-treated slides were probably an underestimate caused by problems of counting cells which were closely adhering and lying on top of each other. In addition, considering the amount of material adhering to the non-treated surfaces, the wash-off from coated slides was relatively much greater than from the non-treated surface.

Results clearly show that the *Clear-Shield* coating firstly impedes adherence of bacteria and secondly encourages desorption by washing.

It is therefore obvious that a coating substance able to confer such advantages would have wide applications within medical and hygiene fields. Such applications include glass surfaces where one or more of the following is important :

TABLE III

Potential applications - « clear-shield » for glass

Improvement in aesthetic quality and appearance
Reduction in microbial adhesion
Water repellency
Prevention of staining and discolouration
Protection against weathering and abrasion

In conclusion, the *Clear-Shield* coating has application to glass surfaces where protection against contamination by biologi-

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cal and chemical agents is desired and especially under wet conditions where the risk of microbial adhesion and growth is high.

*
* *

« *Clear-Shield* » is a registered trademark of Ritec (Clear-Shield) Ltd., 15, Royal London Estate, West Road, London N17 OXL, England.

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Treatment of intoxicated soldiers by war gases

by B. NEYRINCK*, A. WAUTERS** and A. HEYNDRICKX**

ABSTRACT.

In March 1985, three Iranian soldiers were brought over to « De Bijloke » Hospital in Ghent. They had been victims of a chemical war gas poisoning (Middle-East).

The video shows their clinical status on admission, the treatment, the evolution of their lesions, and their condition when they left the Hospital after one month of care.

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INTRODUCTION.

The video was filmed in March and April 1985. Three Iranian patients were brought over to the « Bijloke » Hospital in Ghent. They had been victims of a chemical war gas poisoning. They reached Belgium by airplane, 8 days after the exposure to the toxic gas (Iraq - Iran War).

One of the soldiers was wearing a protective suit during the attack. Unfortunately, he was not wearing any gasmask, so he was breathing the toxic gas and his lungs and eyes were affected.

The other soldiers were not wearing any protective suit or mask. Besides the pulmonary involvement and the eye irritation, they showed extensive skin lesions, which could be best compared with chemical burns. This became clear after toxicological investigation. Sulfur Mustard Gas (or Yperite) was detected in the patients' samples.

CLINICAL STATUS.

The first part of the video was filmed on the day of their admission to the hospital.

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** Department of Toxicology, State University of Ghent, Belgium.

Patient 1 and patient 2 had extensive skin lesions ; after the explosion of the bomb the skin had begun to itch early. At the same time erythema had developed. The skin colour had darkened and blisters, containing a yellowish fluid, had appeared.

When they reached Ghent (after 8 days) the erythema had darkened and became wine-coloured. They had alternating zones of pigmentation and depigmentation. The blisters broke open, leaving cutaneous detachment over wide areas.

All parts of the body were affected, but especially the armpit, the genitals (scrotum and penis) and the inner surface of the elbows and knees, probably because of the greater sensitivity of the skin (and) or the greater degree of sudation in those areas.

The patients all showed conjunctivitis and photophobia.

They complained from throatache ; they had a burning sensation in the mouth. Their respiration was wheezing and they had an irritative cough with production of sputum.

The most seriously affected soldier developed leukopenia, followed by secundary infections.

TREATMENT.

The second part of the video was filmed after a few days of treatment.

In the beginning towels soaked in Dakin Solution are put on the wounds. Dakin Solution is a 2 ‰ solution of Chloramine-T in water. This solution had already been used in Belgium during the First World War (1914-1918). Besides the disinfecting action, the Chloramine-T also reacts chemically with mustard gas molecules. This treatment with Dakin Solution is repeated every 2 hours.

After 4 to 5 days the wounds are treated with an ointment of silver-sulphadiazine 1 % (Flammazine®). The ointment is applied twice a day, and the wounds are covered with bandages. Before the bandages are applied, a narcotic analgesic, Tilidine (Valtran®) is given to relief the pain.

The eyes are rinsed with a 0.2 % solution of Mercuri-oxycyanide and an antibiotic ointment is applied (chlorotetracycline ointment — Aureomycine®).

If the patients complain from throatache, oral sprays are used (Hexomedine 0.2 % or sulphonamides).

Intravenous fluids for rehydratation are given, combined with vitamins, high-caloric nutrients and amino acids.

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Active charcoal is given orally (40 g, three times every 4 hours) in order to adsorb the toxins from the gastrointestinal tract.

High doses of vitamine C (3 × 1 g a day) and acetylcysteine (4 × 300 mg a day) are administered intravenously, as reducing agents.

Antibiotic therapy is instituted if necessary, after performing an antibiogram.

During the recovery and the healing of the wounds, the patients often complained about itching. In that case they received an antihistaminic drug, Fenistil®.

CONCLUSION.

The last part of the video was filmed after one month of care, just before the patients were released from the Hospital.

There are still pigmentation changes of the skin with diffuse scars.

The patients have symptoms of a chronic bronchitis, with an irritative cough. Their lungs are irreversibly damaged, like it was seen here in Belgium after the First World War.

The lesions of those patients show the importance of wearing protective clothing with gasmasks.

It also shows the importance of decontamination, and especially the speed of it.

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Clinical history and autopsy observations associated with the toxicological findings in an Iranian soldier exposed to yperite (mustard gas)

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SUMMARY.

We performed an autopsy on a 22 year old Iranian soldier, who was exposed to was gas 6 days before death.

The macroscopical observations can be summarized as follows : the skin shows extensive burns and defects of face, neck, thoracal, abdominal and genital area, legs and arms. The sclerae are subicteric.

The left upper arm and left leg show a major skin defect of resp. 2 × 1.5 cm ; 5 × 3 cm and 2.5 × 2 cm.

Other visceral lesions include necrotic tracheitis and bronchitis, generalized hyperemia of the gastrointestinal tract and hemorrhagic injection of the mucosa of bladder and pyelum of both kidneys.

A toxicological screening on chemical warfare agents performed on different samples was negative.

However, microscopical examination disclosed a necrotizing tracheitis and bronchitis, lung edema, extensive skin defects, atrophy of the lymphoid apparatus and of the bone marrow, hemophagocytosis in lymph nodes and spleen and acute tubular necrosis of the kidneys.

These findings correlate with an exposure to war gas of the mustard type.

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CLINICAL HISTORY.

A 22 year old Iranian soldier, who was victim of a war gas attack on 13 February 1986, was transferred by airplane to the University Hospitals in Ghent on 18 February 1986.

The clinical findings can be summarized as follows : severe skin burns, with exception of the feet, for almost 90 % of the body surface, penetrating wounds at the left arm and leg, and eye injury.

Bronchoscopic examination revealed a hemorrhagic mucosa, covered with necrotic membranes. Bone marrow biopsy showed a hypocellularity.

Laboratory investigations disclosed a severe agranulocytosis (white blood cells : 0 % ; thrombocytes : 29,000/mm^3). The clinical diagnosis of necrotising bronchitis, sepsis, shock and Respiratory Distress Syndrome was made. One day later, the patient died. An autopsy was performed.

MACROSCOPICAL FINDINGS.

The skin showed extensive burns and defects of the face, neck, thoracal, abdominal and genital area, legs and arms (fig. 1-3). The left upper arm and left leg showed major, penetrating defects of respectively 2 × 1.5 cm, 5 × 3 cm and 2.5 × 2 cm. The most prominent abnormality of the cervical region was a hemorrhagic injection of the larynx and vocal cords, which were covered with yellowish, necrotic membranes. Inspection of the thoracic cavity revealed a normal heart and vascular tree. The left pleural cavity contained 500 cm^3 hemorrhagic fluid, the right pleural cavity contained 300 cm^3 clear fluid. Trachea and stem bronchi were covered by an adherent necrotic membrane (fig. 4 and 5). The right lung weighed 830 g (mean normal weight = 450 g) ; the left lung weighed 908 g (mean normal weight : 375 g). Both were firm and contained yellow, adherent, mucosal membranes.

Inspection of the abdominal cavity showed the presence of 50 cm^3 of hemorrhagic fluid. The bowel was dilated and contained yellowish, necrotic material. The liver weighed 2,170 g. Its macroscopical appearance was normal. The spleen and kidneys were normal. The bladder showed hemorrhagic injection of the mucosa. The cerebrum, cerebellum, pons and medulla oblongata were normal.

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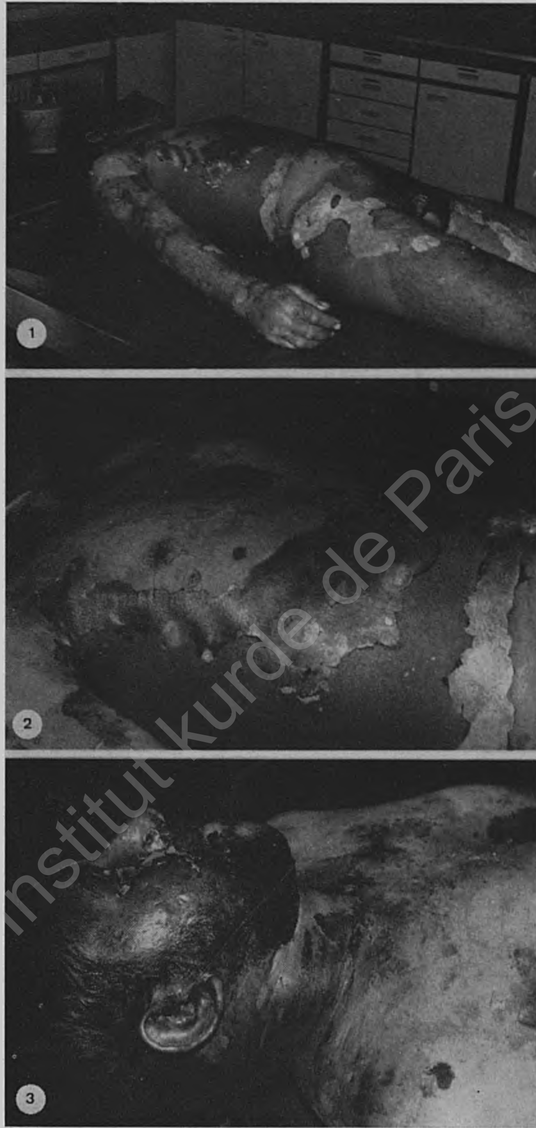
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FIG. 1. — Severe skin lesions with denudation of the dermis.

FIG. 2. — Extensive burns and defects of the thoracal and abdominal area.

FIG. 3. — Extensive burns and defects of the face and neck.

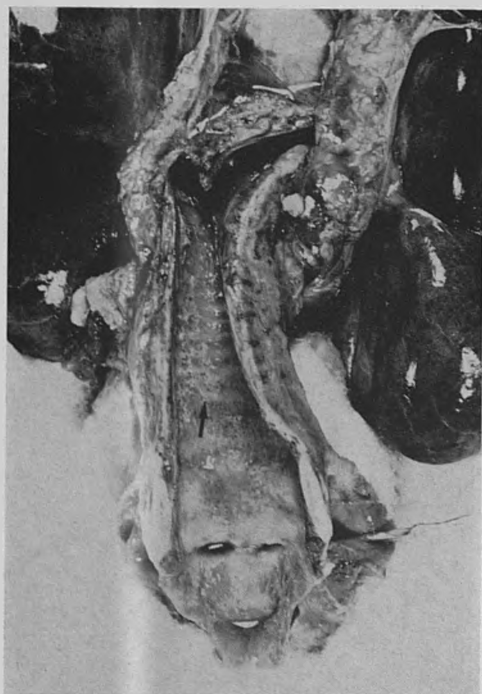

FIG. 4. — Adherent, necrotic mucosal membranes (↗) covering the trachea.

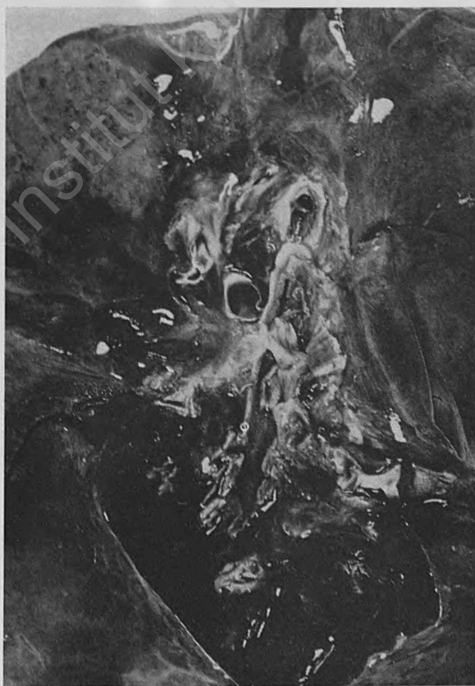

FIG. 5. — Adherent, necrotic mucosal membranes (↙) covering the stem bronchi.

MICROSCOPICAL FINDINGS.

The microscopical examination of the prelevements disclosed specific lesions.

The epidermis, consisting of a squamous epithelium, showed exfoliation of a large area (fig. 6). Arguments for an epidermal bleeding was the presence of iron pigment in the horn layer. The

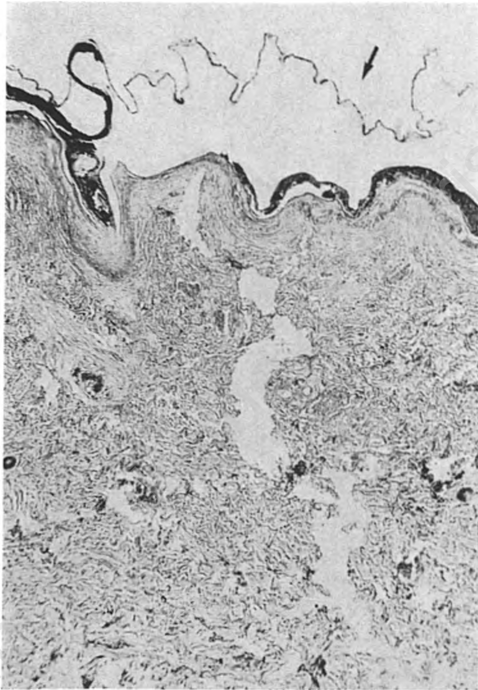

FIG. 6. — Exfoliation (↙) of the epidermis.
No inflammatory infiltrate in the underlying dermis.

underlaying dermis showed the presence of normal adnexes. An inflammatory infltrate was absent despite the defect of the epidermis. The hypodermis was normal. We concluded to burn lesion, without any inflammatory reaction.

The respiratory epithelium of the trachea had completely disappeared and was replaced by a necrotic membrane covering the lamina propria (fig. 7). An inflammatory infiltrate was absent. The deeper layers were normal. Identical microscopical observations were made in both stem bronchi. The lungs showed the same picture : the lobar bronchi and bronchioli were covered with an

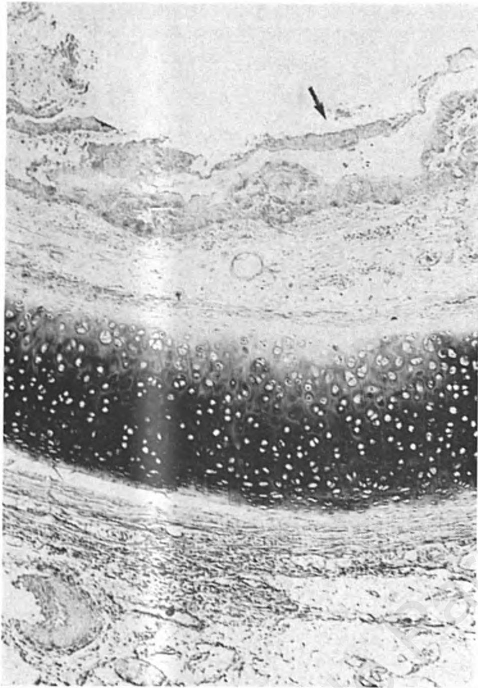

FIG. 7. — Adherent, necrotic mucosal membranes (↘) covering the trachea.

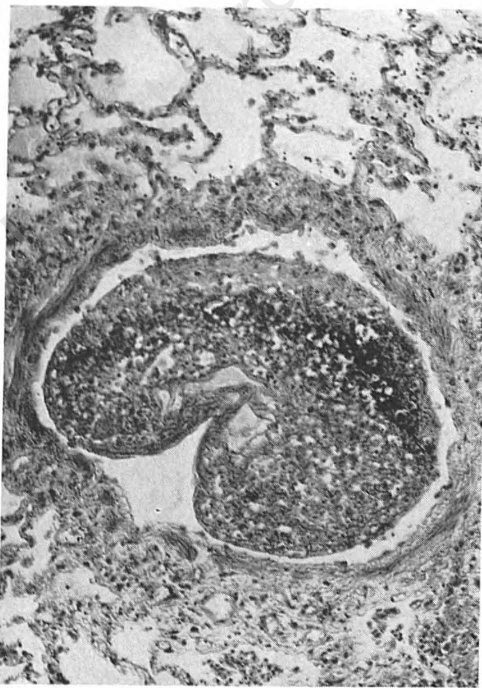

FIG. 8. — Adherent, necrotic membranes covering the lamina propria of stem bronchi, lobar bronchi and bronchioli.

adherent membrane (fig. 8). Only in a few areas, the respiratory epithelium was conserved. The small airways and alveoli were lined by an intact epithelium. Many alveolar sacs were filled with edematous fluid. A PAS-stain and Gram-stain confirmed the presence of bacteria and fungi in the necrotic membranes. A scanty inflammatory infiltrate was only seen in the right

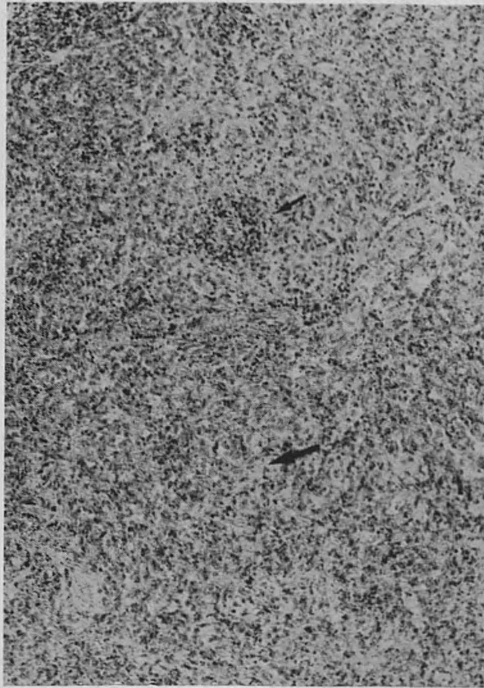

FIG. 9. — Spleen : reduction of the white pulp (↙) : hyperaemia (↙) of the red pulp.

upper lobe. The diagnosis of necrotising bronchitis and lung edema was made.

The most relevant finding of the gastrointestinal tractus can be summarized as a depletion of the lymphoid tissue and a discrete hyperaemia.

The spleen showed a reduction of the white pulp. Periarterial lymphatic sheeths were present. The red pulp showed hyperaemia and haemophagocytosis (fig. 9).

The bone marrow was hypocellular. The major portion consisted of mature fat cells. Lymphocytes, plasma cells and red blood cells were present. Megakaryoblasts, myeloblasts and normoblasts, on

the contrary, were absent, suggesting the diagnosis of atrophy of the bone marrow.

The paraaortic and parabronchial lymph nodes also showed a depletion. Haemophagocytosis was noted.

The adrenal glands revealed a lipid depletion of the cortex, indicating a severe stress situation.

Both kidneys showed normal glomeruli with hyperaemia of the capillaries. The large blood vessels were also hyperaemic. Some tubular lumina contained amorphous, eosinophilic hyaline and brown casts. Desquamated epithelial cells were mixed with the pigment. The interstitium was not enlarged, no inflammatory infiltrate could be detected. We concluded to an acute tubular necrosis with hyperaemia.The lobular structure of the liver was preserved. Centrolobular steatosis was present. The sinusoids were hyperaemic and the bile canaliculi contained some bile. The diagnosis of anoxic steatosis and discrete cholestasis was made.

TOXICOLOGICAL FINDINGS.

During the autopsy several samples were taken for toxicological analysis. However, it also needs to be reported that before the patient died, that means shortly after the patient was admitted to the intensive care unit, samples of blood, urine, skin and hair were taken. At the same time a metalic piece, probably a shell fragment, was removed from the patients leg and sent for analysis too. The samples taken before death were analysed according to the methods reviewed by Heyndrickx and Van den Heede, 1986 (1). The results of the different toxicological tests performed on those samples are summarized in table I.

The blood sample showed a normal methemoglobin level and a plasma cholinesterase activity reaching the lower normal range. The cyanide concentration in the blood as well as the plasma thiocyanate level were normal. The arsenic concentrations were also within the limits of the reference values, while traces of sulfur mustard or mycotoxins of the trichothecene family could not be found.

The results of the toxicological analyses performed on the samples taken post-mortem are listed in table II. Here again the cyanide levels were within the normal range while no traces of sulfur mustard, nerve gases or trichothecenes could be found.

TABLE I

Results of the toxicological tests performed on the samples taken before death

	Blood			Urine	Skin	Hair	Piece of metal
	Whole blood	*Plasma*	*Red cells*				
Methemoglobin	0.23						
Cholinesterase act.							
Morand-Laborit		41.9					
Nenner		1.41	14.91				
Cyanide	105						
Thiocyanate		21					
Arsenic	9.9			12.07			
Sulfur mustard				Negative	Negative	Negative	Negative
Trichothecenes	Negative			Negative			

Reference values :
Methemoglobin (whole blood) : 0-0.3 g %
Cholinesterase activity
Morand-Laborit (plasma) : 50 ± 10 %
Nenner (plasma) : 1.26-2.66 μmol.ml^{-1}min^{-1}
Nenner (red cells) : 9.85-16.41 μmol-ml^{-1}-min^{-1}

Cyanide (whole blood) : 0-100 μg/l
Thiocyanate (plasma)
non smokers : < 85 μM/l
smokers : > 85 μM/l
Arsenic (whole blood) : < 30 μg/l
(urine) : 0-100 μg/l

TABLE II

Results of the toxicological test performed on the samples taken post-mortem

Nature of the samples	*Cyanide μg/kg*	*Sulfur mustard*	*Nerve gases**	*Trichothecenes (Mycotoxins)*
Liver	13.3	—	Negative	Negative
Kidney	—	—	Negative	Negative
Stomach content	3.3	—	—	—
Skin	—	Negative	—	—
Hair	—	Negative	—	—

* Extraction, incubation of the extract with horse plasma (cholinesterases) and determination of the residual cholinesterase activity.

With the exception of nerve gases, where the detection was performed by a biological method described by Van Hecke *et al.*, 1955 (2), and Heyndrickx, 1956 and 1959 (3-4), the samples taken post-mortem were analysed according to the same reviewed procedures after slightly having modified some of the sample preparation methods.

CONCLUSIONS.

The results of the macroscopical and microscopical examination kept in with the diagnosis of an exposure to a war gas of the mustard type. The absence of cellular inflammatory infiltration was

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due to an immune suppression by the war gas. This hypothesis was corroborated by the fact that bone marrow and the lymphoid tissue of the nodes and the spleen were atrophic.

Although the symptomatology on admission in the hospital and the anatomo-pathological findings after death, led to the final conclusion that the victim had been exposed to a vesicant of the mustard type, no traces of sulfur mustard could be found. However, it needs to be taken into account that sulfur mustard is first converted to a sulfonium ion (fig. 10), which is thought to

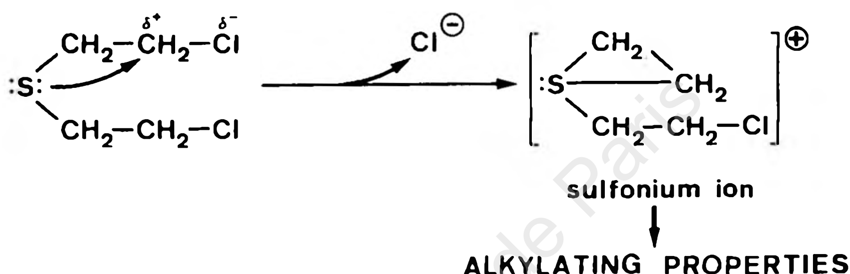

FIG. 10. — Conversion of sulfur mustard to a sulfonium ion with alkylating properties.

be responsible for its toxic action. A further biotransformation will lead to the formation of thiodiglycol, which will mainly be excreted under a conjugated form.

The sulfonium ion has alkylating properties and reacts with cellular macromolecules like : proteins, enzymes, DNA and RNA, containing sulhydro-, amino-, imino, hydroxy-, phosphate- or carboxylgroups.

The reaction with DNA can lead to a cross-linking of two complementary DNA-chains, which makes DNA unable to duplicate itself. Rapidly proliferating cells like the basal cells of the skin, the mucous membranes of the respiratory tract, the intestinal mucosa, the cells of the blood forming organs and the sex glands, are unable to repair the damage, what causes the cells to die.

Sulfur mustard is also inactivated by the use of chloramine-T (5), which is mostly used in the initial stage of the treatment, and not only acts as an antiseptic but also as a complexing agent for sulfur mustard leading to the formation of a non toxic reaction product (fig. 11).

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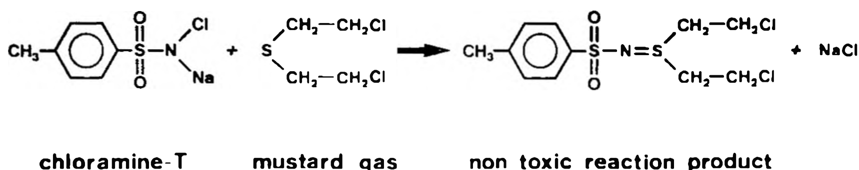

FIG. 11. — Reaction of sulfur mustard with chloramine-T.

Finally there was a time-lag between the exposure and the first sampling of 5 days, what implicates a partial or complete biodegradation and elimination.

As such the negative toxicological results certainly don't exclude the exposure to sulfur mustard.

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Report and conclusion of the biological samples of men, intoxicated by war gases, sent to the department of toxicology at the State University of Ghent, for toxicological investigation

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SUMMARY.

Treatment.

Skin :

— *decontamination with Dakin solution (2 ‰ Chloramine),*
— *Flammazine® ointment : silver sulfadiazine 1 %.*

Eyes :

— *rinsing with Ocal® solution,*
— *chlortetracycline (Aureomycine®) ointment.*

General :

— *rehydration with IV fluids (high caloric diet),*
— *active charcoal orally,*
— *antibiotics if cultures +,*
— *Vit. C / Acetylcysteine — reducing agents,*
— *blood transfusion,*
— *oral spray : hexomedine or sulfonamides,*
— *pain : Valtran® : Tilidine,*
— *itching : Fenistil® : antihistaminic.*

IF : *cholinesterase activity is lowered : Atropine sulfate (IV), Contrathion® (oxime) (IV).*

IF : *mycotoxins : hemoperfusion.*

Evolution.

After 30 to 37 days they left the hospital still (after one year) :

— *« chronic bronchitis », cough,*
— *lung,*
— *pigmentation changes of the skin with superficial scars.*

I. Different biological samples of men (blood, urine, skin, hair, gastric juice) were sent from the University Clinic of Vienna (Austria), the Humana Hospital Wellington in London (United Kingdom), the « Centre Hospitalier Universitaire Vaudois » in Lausanne (Switzerland) and the University Hospitals of Ghent (Belgium). The different reports were sent to those hospitals.

1. VIENNA.

First report of the toxicological analysis.

First series of samples (blood, urine and gastric juice) sent by the « Klinik für Anaesthesie u. Allgem. Intensivmedizin und der II. Chir. Univ. Klinik », Spitalgasse 23, A-1090 Vienna, Austria. The samples arrived in Ghent on February 18th at 9.30 p.m.

A. *Methemoglobin (blood).*
Reference value 0-0.3 g %.

Patient n. 7 : 0.17 g %.
Patient n. 8 : 0.18 g %.
Patient n. 9 : 0.16 g %.
Patient n. 10 : 0.21 g %.

B. *Sulfhemoglobin (Blood).*
Reference value : negative.

Patient n. 7 : negative.
Patient n. 8 : negative.
Patient n. 9 : negative.
Patient n. 10 : negative.

C. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Patient n. 7 : 43.2 %.
Patient n. 8 : 57.9 %.
Patient n. 9 : 54.5 %.
Patient n. 10 : 41.7 %.

D. *Cyanide (blood and gastric juice).*

Reference value (blood) : 0-10 µg %.

	Blood	*Gastric juice*
Patient n. 7	16.7 µg %	3 µg %
Patient n. 8	16.3 µg %	3 µg %
Patient n. 9	20.5 µg %	5 µg %
Patient n. 10	not enough sample	

E. *Thiocyanate (plasma and urine).*

Reference value (plasma) : non smokers : < 85 µM/L,
smokers : > 85 µM/L.

	Plasma	*Urine*
Patient n. 7	24 µM/L	21 µM/L
Patient n. 8	28 µM/L	24 µM/L
Patient n. 9	11 µM/L	46 µM/L
Patient n. 10	29 µM/L	30 µM/L

F. *Arsenic (blood and urine).*

Reference values : blood < 4 µg %,
urine 0-100 µg/24 u.

	Plasma	*Urine*
Patient n. 7	0.77 µg %	11.25 µg/L
Patient n. 8	0.59 µg %	28.70 µg/L
Patient n. 9	1.26 µg %	6.00 µg/L
Patient n. 10	1.03 µg %	4.75 µg/L

G. *Yperite, mustard gas (urine, gastric juice).*

Qualitative test.

Patient n. 7 : — urine : slightly +
— gastric juice : negative or below detection limit

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Patient n. 8 : — urine | negative or below
— gastric juice | detection limit
Patient n. 9 : — urine : slightly +
— gastric juice : negative or below detection limit
Patient n. 10 : — urine : slightly +
— gastric juice : negative or below detection limit

H. *Mycotoxins : trichothecenes (plasma, urine).*

	Plasma	*Urine*
Patient n. 7	negative	negative
Patient n. 8	negative	negative
Patient n. 9	negative	negative
Patient n. 10	negative	negative

Second report of the toxicological analysis.

Second series of samples (blood, urine and faeces) sent by the « Klinik für Anaesthesie u. Allgem. Intensivmedizin und der II. Chir. Univ. Klinik », Spitalgasse 23, A-1090 Vienna, Austria. The samples arrived in Ghent on February 19th at 9.30 p.m.

A. *Methemoglobin (blood).*
Reference value : 0-0.3 g %.

Patient n. 7 : 0.17 g %.
Patient n. 8 : 0.20 g %.
Patient n. 9 : 0.17 g %.
Patient n. 10 : 0.09 g %.

B. *Cholinesterase activity (plasma).*
Reference value : 50 %.

Patient n. 7 : 33.1 %.
Patient n. 8 : 49.6 %.
Patient n. 9 : 49.1 %.
Patient n. 10 : 33.7 %.

C. *Cyanide (blood).*
Reference value : 0-10 µg %.

Patient n. 10 : 6.2 µg %.

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D. *Yperite, mustard gas (urine) : qualitative test.*

Patient n. 7
Patient n. 9 } negative or below detection limit
Patient n. 10

Third report of the toxicological analysis.

Third series of samples (blood and urine) sent by the « Klinik für Anaesthesie u. Allgem.Intensivmedizin und der II. Chir. Univ. Klinik », Spitalgasse 23, A-1090 Vienna, Austria. The samples arrived in Ghent on February 25th at 9.30 p.m.

A. *Methemoglobin (blood).*

Reference value : 0-0.3 g %.

Patient n. 7 : 0.07 g %.
Patient n. 8 : 0.04 g %.
Patient n. 9 : 0.04 g %.
Patient n. 10 : 0.22 g %.

B. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Patient n. 7 : 25.7 %.
Patient n. 8 : 52.0 %.
Patient n. 9 : 48.0 %.
Patient n. 10 : 30.0 %.

C. *Cyanide (blood).*

Reference value : 0-10 μg %.

Patient n. 7 : 2.5 μg %.
Patient n. 8 : 3.6 μg %.
Patient n. 9 : 2.0 μg %.
Patient n. 10 : 6.4 μg %.

D. *Thiocyanate (plasma and urine).*

Reference value (plasma) : non smokers : < 85 μM/L,
smokers : > 85 μM/L.

	Plasma	*Urine*
Patient n. 7	10 μM/L	10 μM/L
Patient n. 8	7 μM/L	8 μM/L
Patient n. 9	5 μM/L	4 μM/L
Patient n. 10	5 μM/L	negative

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E. *Yperite, mustard gas (urine).*
Qualitative test.

Patient n. 7	negative or below detection limit
Patient n. 8	
Patient n. 9	
Patient n. 10	

2. GHENT.

First report of the toxicological analysis.

First series of samples sent by the University Hospitals of Ghent (samples received on February 18th at 10.30 and 12 a.m., and 5 and 5.30 p.m. respectively).

A. *Methemoglobin (blood).*
Reference value : 0-0.3 g %.

Ansari Azir (n. 1)	: 0.29 g %
Hassan Massantaba (n. 2)	: not determin. (clotted blood)
Nader Beyrami (n. 3)	: 0.23 g %
Mossadegh Rahmatolah (n. 4)	: not determin. (clotted blood)
Sahlabei Sadegh (n. 5)	: 0.34 g %
Parrkhi Mohammed Hassan (n. 6)	: 0.15 g %
Holan Hossein (n. 7)	: 0.13 g %
Talab Hossein Ashin (n. 8)	: 0.14 g %
Maslami Seyed Ramesan (n. 9)	: 0.17 g %

B. *Sulfhemoglobin (blood).*
Reference value : negative.

Ansari Azir (n. 1)	: negative
Hassan Massantaba (n. 2)	: negative
Nader Beyrami (n. 3)	: negative
Mossadegh Rahmatolah (n. 4)	: negative
Sahlabei Sadegh (n. 5)	: negative
Parrkhi Mohammed Hassan (n. 6)	: negative
Holan Hossein (n. 7)	: negative
Talab Hossein Ashin (n. 8)	: negative
Maslami Seyed Ramesan (n. 9)	: negative

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C. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Ansari Azir (n. 1)	: 51.7 %
Hassan Massantaba (n. 2)	: 49.7 %
Nader Beyrami (n. 3)	: 41.9 %
Mossadegh Rahmatolah (n. 4)	: 43.4 %
Sahlabei Sadegh (n. 5)	: 44.6 %
Parrkhi Mohammed Hassan (n. 6)	: 48.9 %
Holan Hossein (n. 7)	: 52.8 %
Talab Hossein Ashin (n. 8)	: 56.9 %
Maslami Seyed Ramesan (n. 9)	: 54.5 %

D. *Cyanide (blood).*

Reference value : 0-10 µg %.

Ansari Azir (n. 1)	: 14.0 µg %
Hassan Massantaba (n. 2)	: 16.5 µg %
Nader Beyrami (n. 3)	: 10.5 µg %
Mossadegh Rahmatolah (n. 4)	: 39.0 µg %
Sahlabei Sadegh (n. 5)	: 13.5 µg %
Parrkhi Mohammed Hassan (n. 6)	: 9.5 µg %
Holan Hossein (n. 7)	: 7.5 µg %
Talab Hossein Ashin (n. 8)	: 8.5 µg %
Maslami Seyed Ramesan (n. 9)	: 6.5 µg %

E. *Thiocyanate (plasma or serum and urine).*

Reference value : plasma : non smokers : < 85 µM/L,
smokers : > 85 µM/L.

	Plasma or serum	*Urine*
Ansari Azir (n. 1)	: 21 µM/L	21 µM/L
Hassan Massantaba (n. 2)	: 26 µM/L	21 µM/L
Nader Beyrami (n. 3)	: 21 µM/L	24 µM/L
Mossadegh Rahmatolah (n. 4)	: 7 µM/L	23 µM/L
Sahlabei Sadegh (n. 5)	: 6 µM/L	no sample
Parrkhi Mohammed Hassan (n. 6)	: 8 µM/L	100 µM/L
Holan Hossein (n. 7)	: 72 µM/L	36 µM/L
Talab Hossein Ashin (n. 8)	: 8 µM/L	42 µM/L
Maslami Seyed Ramesan (n. 9)	: 10 µM/L	no sample

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F. *Arsenic (blood and urine).*

Reference values : blood < 4 µg %,
urine 0-100 µg/24 u.

		Blood	Urine
Ansari Azir (n. 1)	:	1.88 µg %	7.2 µg/L
Hassan Massantaba (n. 2)	:	not enough sample	4.55 µg/L
Nader Beyrami (n. 3)	:	0.99 µg %	12.07 µg/L
Mossadegh Rahmatolah (n. 4)	:	0.94 µg %	8.20 µg/L
Sahlabei Sadegh (n. 5)	:	3.90 µg %	no sample
Parrkhi Mohammed Hassan (n. 6)	:	1.57 µg %	13.75 µg/L
Holan Hossein (n. 7)	:	3.51 µg %	15.45 µg/L
Talab Hossein Ashin (n. 8)	:	2.54 µg %	6.50 µg/L
Maslami Seyed Ramesan (n. 9)	:	3.70 µg %	no sample

G. *Magnesium (blood).*

Reference values : plasma 1.56-2.55 mg %,
red cells 5.47-7.29 mg %.

		Plasma	Red cells
Ansari Azir (n. 1)	:	2.80 mg %	3.77 mg %
Hassan Massantaba (n. 2)	:	2.30 mg %	4.98 mg %
Nader Beyrami (n. 3)	:	2.77 mg %	4.09 mg %
Mossadegh Rahmatolah (n. 4)	:	2.12 mg %	4.44 mg %
Sahlabei Sadegh (n. 5)	:	2.18 mg %	4.83 mg %
Parrkhi Mohammed Hassan (n. 6)	:	1.86 mg %	4.39 mg %
Holan Hossein (n. 7)	:	2.48 mg %	4.26 mg %
Talab Hossein Ashin (n. 8)	:	2.17 mg %	5.17 mg %
Maslami Seyed Ramesan (n. 9)	:	2.14 mg %	5.97 mg %

H. *Yperite, mustard gas (urine, skin, hair).*

Colour test, GC/ECD or GC/MS (Sim).

Ansari Azir (n. 1) :
— urine
— skin
— hair
} negative or below detection limit

Hassan Massantaba (n. 2) :
— urine : ± ?
— skin
— hair
} no samples received

Nader Beyrami (n. 3) :
— urine
— skin
— hair
} negative or below detection limit

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Mossadegh Rahmatolah (n. 4) : — urine, — skin, — hair: negative or below detection limit

Sahlabei Sadegh (n. 5) : no samples received.

Parrkhi Mohammed Hassan (n. 6) : — urine : slightly positive
— skin, — hair: negative or below detection limit

Holan Hossein (n. 7) : — urine, — skin, — hair: negative or below detection limit

Talab Hossein Ashin (n. 8) : — urine, — skin, — hair: negative or below detection limit

Maslami Seyed Ramesan (n. 9) : no samples received.

I. *Mycotoxins : trichothecenes (blood, urine).*

	Blood	*Urine*
Ansari Azir (n. 1)	: negative	negative
Hassan Massantaba (n. 2)	: negative	negative
Nader Beyrami (n. 3)	: negative	negative
Mossadegh Rahmatolah (n. 4)	: negative	negative
Sahlabei Sadegh (n. 5)	: negative	no sample
Parrkhi Mohammed Hassan (n. 6)	: negative	negative
Holan Hossein (n. 7)	: negative	negative
Talab Hossein Ashin (n. 8)	: negative	negative
Maslami Seyed Ramesan (n. 9)	: negative	no sample

Second report of the toxicological analysis.

Second series of samples sent by the University Hospitals of Ghent (samples received on February 19 th).

A. *Methemoglobin (blood).*
Reference value : 0-0.3 g %.

Mossadegh Rahmatolah (n. 4) : 0.12 g %
Sahlabei Sadegh (n. 5) : 0.20 g %

B. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Ansari Azir (n. 1)	: 47.5 %
Mossadegh Rahmatolah (n. 4)	: 41.7 %
Sahlabei Sadegh (n. 5)	: 50.7 %
Parrkhi Mohammed Hassan (n. 6)	: 51.3 %
Holan Hossein (n. 7)	: 45.7 %
Talab Hossein Ashin (n. 8)	: 59.5 %
Maslami Seyed Ramesan (n. 9)	: 49.0 %

C. *Cyanide (blood).*

Reference value : 0-10 μg %.

Ansari Azir (n. 1)	: 5.8 μg %
Mossadegh Rahmatolah (n. 4)	: 8.8 μg %
Sahlabei Sadegh (n. 5)	: 7.4 μg %

D. *Thiocyanate (urine).*

Sahlabei Sadegh (n. 5)	: 25 μM/L
Maslami Seyed Ramesan (n. 9)	: 42 μM/L

E. *Arsenic (urine).*

Reference value : 0-100 μg/24 u.

Sahlabei Sadegh (n. 5)	: 70.40 μg/L
Maslami Seyed Ramesan (n. 9)	: 33.60 μg/L

F. *Magnesium (blood).*

Reference values : plasma 1.56-2.55 mg %,
red cells 5.47-7.29 mg %.

	Plasma	*Red cells*
Ansari Azir (n. 1)	: 1.97 mg %	5.40 mg %
Mossadegh Rahmatolah (n. 4)	: 1.54 mg %	5.50 mg %
Sahlabei Sadegh (n. 5)	: 1.80 mg %	4.93 mg %
Parrkhi Mohammed Hassan (n. 6)	: 1.78 mg %	4.60 mg %
Holan Hossein (n. 7)	: 2.21 mg %	5.23 mg %
Talab Hossein Ashin (n. 8)	: 1.97 mg %	5.50 mg %
Maslami Seyed Ramesan (n. 9)	: 2.18 mg %	6.06 mg %

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G. *Yperite, mustard gas (urine, skin, hair).*

Colour test, GC/ECD or GC/MS (Sim).

Mossadegh Rahmatolah (n. 4) : — urine : negative or below detection limit

Sahlabei Sadegh (n. 5) : — urine, — skin, — hair : negative or below detection limit

Parrkhi Mohammed Hassan (n. 6) : — urine : negative or below detection limit

Maslami Seyed Ramesan (n. 9) : — urine : negative or below detection limit

Note : Analysis of a metalic piece found in the leg of Nader Beyrami (n. 3) : negative on Yperite (GC/ECD).

H. *Mycotoxins : trichothecenes (urine).*

Sahlabei Sadegh (n. 5) : negative
Maslami Seyed Ramesan (n. 9) : negative

Third report of the toxicological analysis.

Other series of samples sent by the University Hospitals of Ghent.

I. *Samples received on February 20th.*

A. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Ansari Azir (n. 1)	: 41.5 %
Hassan Massantaban (n. 2)	: 44.8 %
Mossadegh Rahmatolah (n. 4)	: not enough sample
Sahlabei Sadegh (n. 5)	: 40.2 %
Parrokhi Mohammed Hassan (n. 6)	: 48.0 %
Holan Hossein (n. 7)	: 43.4 %
Talab Hossein Ashin (n. 8)	: 61.6 %
Maslami Seyed Ramesan (n. 9)	: 50.9 %

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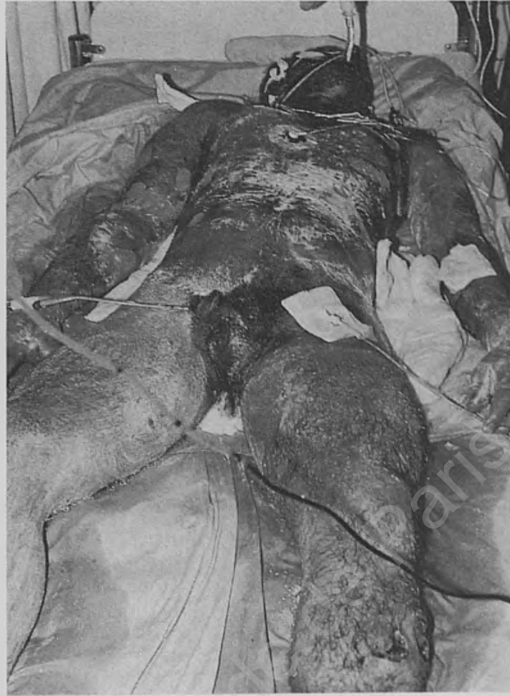

FIG. 1. — Nader Beyrami (patient 3).

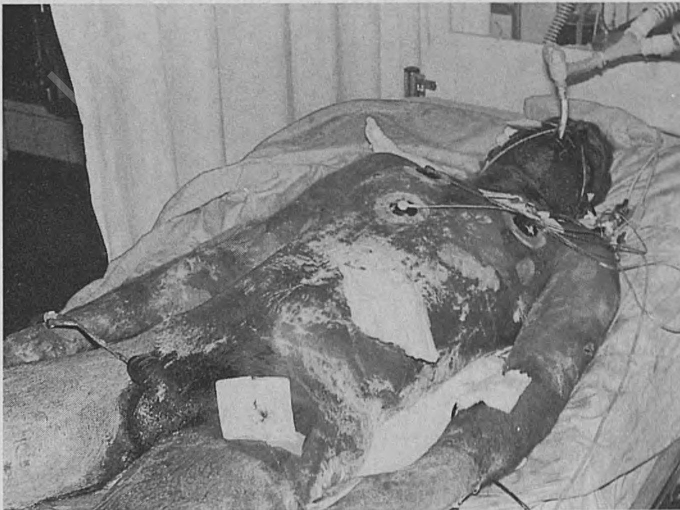

FIG. 2. — Nader Beyrami (patient 3).

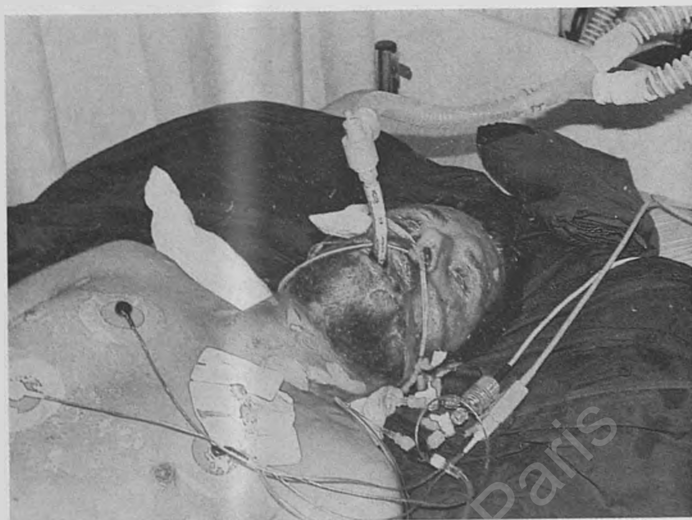

FIG. 3. — Nader Beyrami (patient 3).

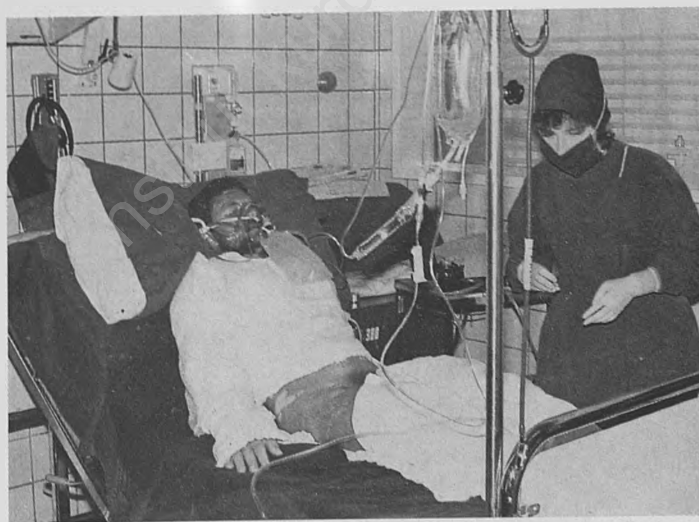

FIG. 4. — Nader Beyrami (patient 3).

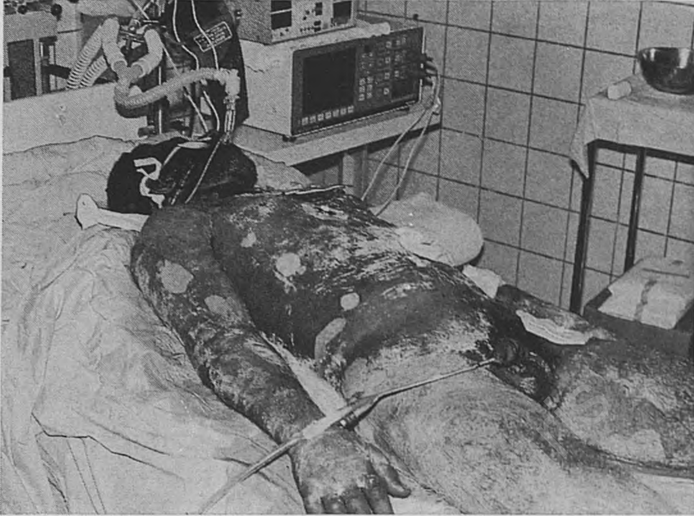

FIG. 5. — Nader Beyrami (patient 3).

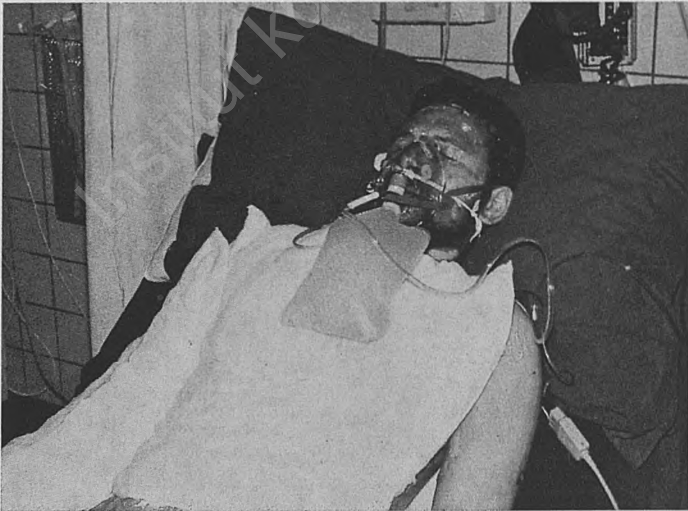

FIG. 6. — Nader Beyrami (patient 3).

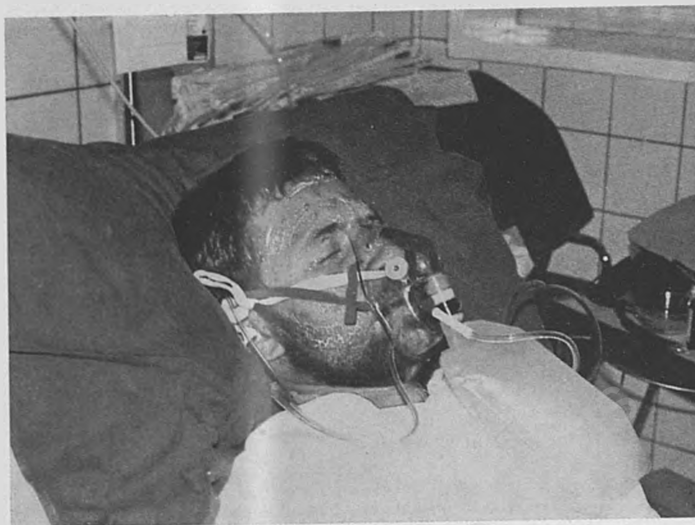

FIG. 7. — Narer Beyrami (patient 3).

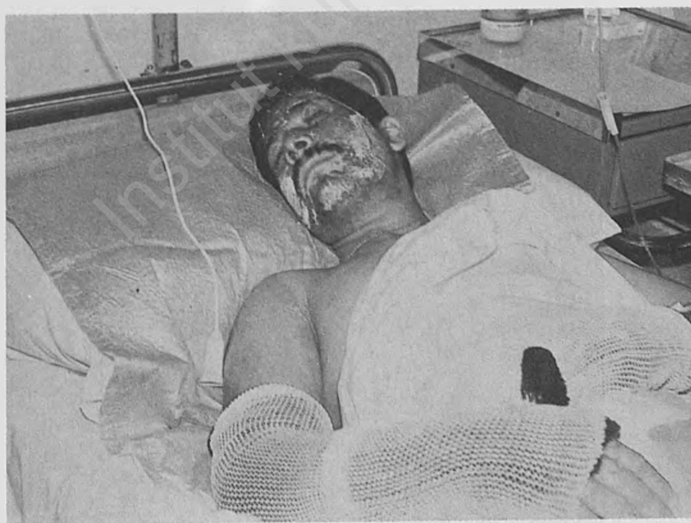

FIG. 8. — Mossadegh Rahmatolah (patient 4).

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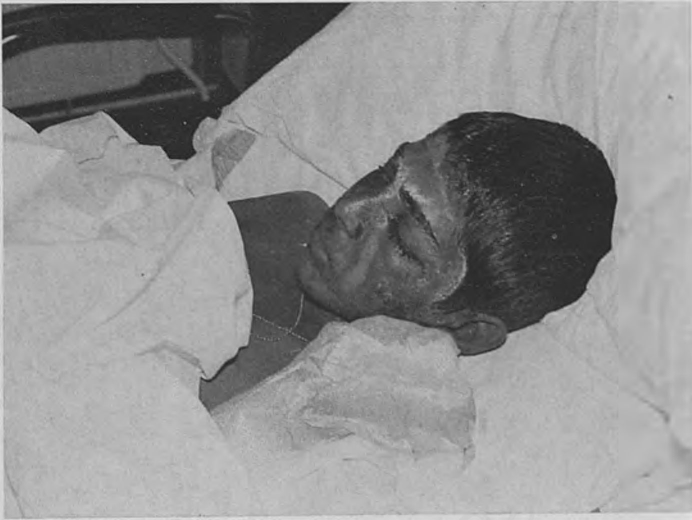

FIG. 9. — Sahlabei Sadegh (patient 5).

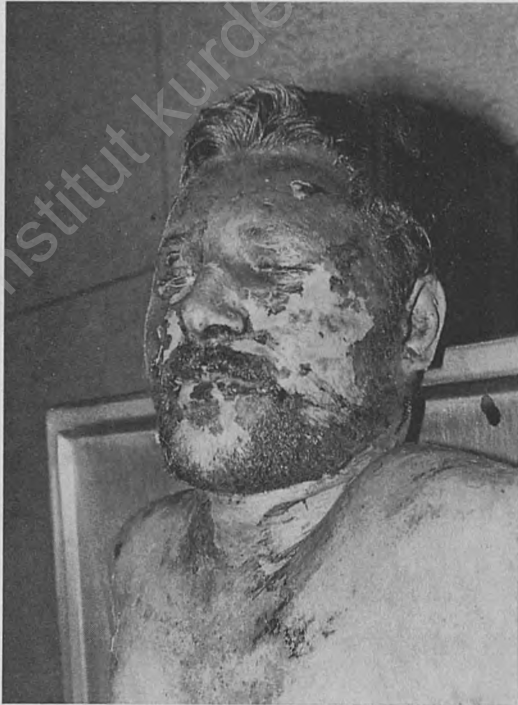

FIG. 10. — Nader Beyrami (patient 3) (autopsy).

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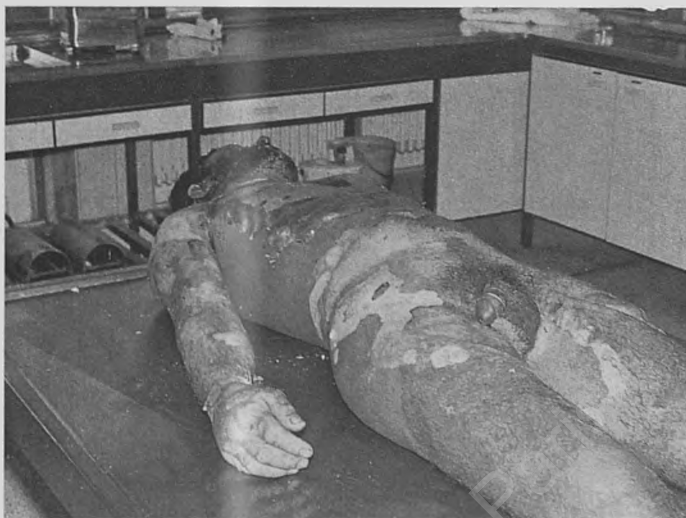

FIG. 11. — Nader Beyrami (patient 3) (autopsy).

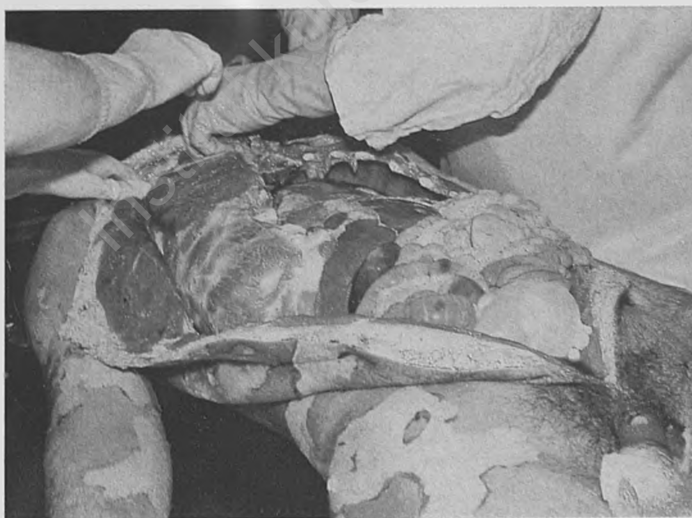

FIG. 12. — Nader Beyrami (patient 3) (autopsy).

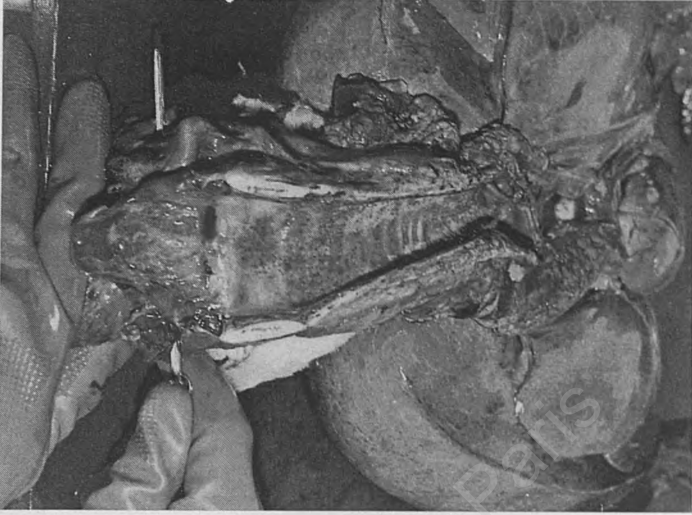

FIG. 13. — Nader Beyrami (patient 3) (autopsy).

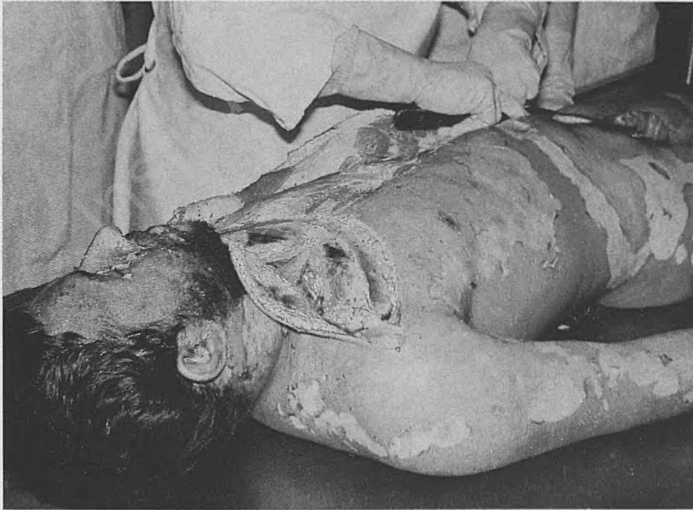

FIG. 14. — Nader Beyraml (patient 3) (autopsy).

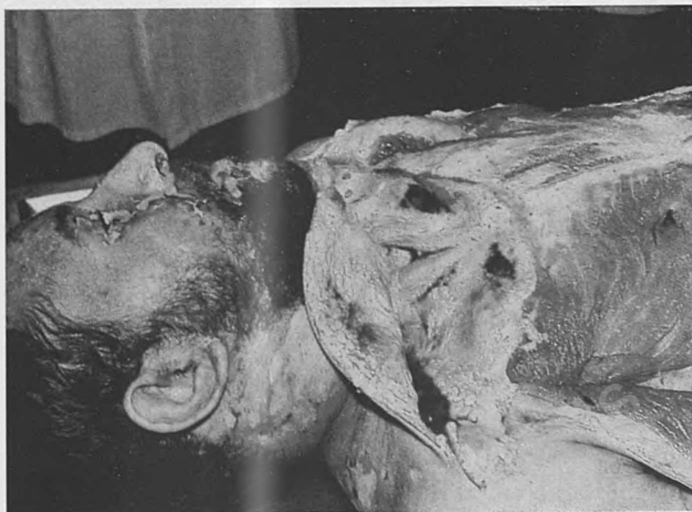

FIG. 15. — Nader Beyrami (patient 3) (autopsy).

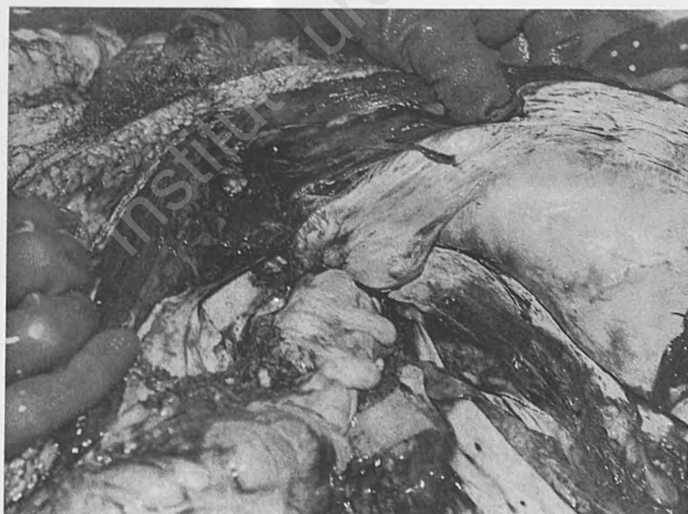

FIG. 16. — Nader Beyrami (patient 3) (autopsy).

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B. *Cyanide (blood).*

Reference value : 0-10 μg %.

Ansari Azir (n. 1)	: 10.9 μg %
Hassan Massantaban (n. 2)	: 5.8 μg %
Mossadegh Rahmatolah (n. 4)	: 12.7 μg %
Sahlabei Sadegh (n. 5)	: 9.2 μg %

C. *Magnesium (blood).*

Reference values : plasma 1.56-2.55 mg %,
red cells 5.47-7.29 mg %.

	Plasma	*Red cells*
Ansari Azir (n. 1)	: 2.07 mg %	5.28 mg %
Hassan Massantaban (n. 2)	: 2.05 mg %	5.63 mg %
Mossadegh Rahmatolah (n. 4)	: 2.05 mg %	6.56 mg %
Sahlabei Sadegh (n. 5)	: 1.94 mg %	4.86 mg %
Parrokhi Mohammed Hassan (n. 6)	: 1.90 mg %	4.70 mg %
Holan Hossein (n. 7)	: 2.30 mg %	5.13 mg %
Talab Hossein Ashin (n. 8)	: 2.09 mg %	6.14 mg %
Maslami Seyed Ramesan (n. 9)	: 2.10 mg %	6.24 mg %

D. *Yperite, mustard gas (hair).*

GC/ECD.

Maslami Seyed Ramesan (n. 9) : negative or below detection limit

II. *Samples received on February 21st.*

A. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Mossadegh Rahmatolah (n. 4) : 31.6 %.

B. *Cyanide (Blood).*

Reference value : 0-10 μg %.

Mossadegh Rahmatolah (n. 4) : 6 μg %.

C. *Magnesium (blood).*

Reference values : plasma 1.56-2.55 mg %,
red cells 5.47-7.29 mg %.

	Plasma	*Red cells*
Ansari Azir (n. 1)	: 2.08 mg %	5.57 mg %
Hassan Massantaban (n. 2)	: 2.01 mg %	5.88 mg %

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Mossadegh Rahmatolah (n. 4)	: not enough sample	
Sahlabei Sadegh (n. 5)	: 1.88 mg %	4.33 mg %
Parrokhi Mohammed Hassan (n. 6)	: 1.97 mg %	4.15 mg %
Holan Hossein (n. 7)	: 2.35 mg %	4.61 mg %
Talab Hossein Ashin (n. 8)	: 2.17 mg %	5.12 mg %
Maslami Seyed Ramesan (n. 9)	: 2.19 mg %	5.53 mg %

III. *Samples received on February 22nd.*

A. *Methemoglobin (blood).*

Reference value : 0-0.3 g %.

Mossadegh Rahmatolah (n. 4) : 0.15 g %.

B. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Ansari Azir (n. 1)	: 50.5 %
Hassan Massantaban (n. 2)	: 42.4 %
Mossadegh Rahmatolah (n. 4)	: 22.9 %
Sahlabei Sadegh (n. 5)	: 36.6 %
Parrokhi Mohammed Hassan (n. 6)	: 39.6 %
Holan Hossein (n. 7)	: 42.3 %
Talab Hossein Ashin (n. 8)	: 62.6 %
Maslami Seyed Ramesan (n. 9)	: no sample

C. *Magnesium (blood).*

Reference values : plasma 1.56-2.55 mg %,
red cells 5.47-7.29 mg %.

	Plasma	*Red cells*
Ansari Azir (n. 1)	: 2.09 mg %	6.19 mg %
Hassan Massantaban (n. 2)	: 1.94 mg %	6.91 mg %
Mossadegh Rahmatolah (n. 4)	: 2.06 mg %	5.97 mg %
Sahlabei Sadegh (n. 5)	: 2.08 mg %	5.15 mg %
Parrokhi Mohammed Hassan (n. 6)	: 2.03 mg %	5.18 mg %
Holan Hossein (n. 7)	: 2.31 mg %	5.94 mg %
Talab Hossein Ashin (n. 8)	: 1.90 mg %	5.53 mg %
Maslami Seyed Ramesan (n. 9)	: no sample	no sample

D. *Yperite, mustard gas (urine) : qualitative test.*

Mossadegh Rahmatolah (n. 4) : negative or below detection limit

IV. *Samples received on February 23rd.*

A. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Ansari Azir (n. 1)	: 37.7 %
Assan Massantaban (n. 2)	: 32.0 %
Mossadegh Rahmatolah (n. 4)	: 25.1 %
Sahlabei Sadegh (n. 5)	: no sample
Parrokhi Mohammed Hassan (n. 6)	: 40.2 %
Holan Hossein (n. 7)	: 38.0 %
Talab Hossein Ashin (n. 8)	: 57.0 %
Maslami Seyed Ramesan (n. 9)	: 38.8 %

B. *Magnesium (blood).*

Reference values : plasma 1.56-2.55 mg %,
red cells 5.47-7.29 mg %.

	Plasma	*Red cells*
Ansari Azir (n. 1)	: 2.42 mg %	5.72 mg %
Assan Massantaban (n. 2)	: 2.25 mg %	6.46 mg %
Mossadegh Rahmatolah (n. 4)	: 2.26 mg %	6.43 mg %
Sahlabei Sadegh (n. 5)	: no sample	no sample
Parrokhi Mohammed Hassan (n. 6)	: 2.02 mg %	5.36 mg %
Holan Hossein (n. 7)	: 2.57 mg %	6.00 mg %
Talab Hossein Ashin (n. 8)	: 2.00 mg %	6.31 mg %
Maslami Seyed Ramesan (n. 9)	: 2.18 mg %	6.18 mg %

Fourth report of the toxicological analysis.

Other series of samples sent by the University Hospitals of Ghent.

I. *Samples received on February 24th.*

A. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Ansari Azir (n. 1)	: 45.4 %
Assan Massantaban (n. 2)	: 44.2 %
Mossadegh Rahmatolah (n. 4)	: 25.1 %
Sahlabei Sadegh (n. 5)	: 27.4 %
Parrokhi Mohammed Hassan (n. 6)	: 44.6 %

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Holan Hossein (n. 7) : 36.6 %
Talab Hossein Ashin (n. 8) : 60.8 %
Maslami Seyed Ramesan (n. 9) : no sample received

B. *Magnesium (blood).*

Reference values : plasma 1.56-2.55 mg %
red cells 5.47-7.29 mg %

	Plasma	*Red cells*
Ansari Azir (n. 1)	: 2.32 mg %	5.67 mg %
Assan Massantaban (n. 2)	: 2.21 mg %	6.68 mg %
Mossadegh Rahmatolah (n. 4)	: 2.08 mg %	6.34 mg %
Sahlabei Sadegh (n. 5)	: 1.80 mg %	5.29 mg %
Parrokhi Mohammed Hassan (n. 6)	: 2.00 mg %	5.38 mg %
Holan Hossein (n. 7)	: 2.71 mg %	6.03 mg %
Talab Hossein Ashin (n. 8)	: 1.99 mg %	6.38 mg %
Maslami Seyed Ramesan (n. 9)	: no sample received	

C. *Yperite, mustard gas (urine, skin, hair, mucosa of the lung).*
Colour Test, GC/ECD.

Ansari Azir (n. 1) — hair : negative or below detection limit.

Mossadegh Rahmatolah (n. 4) — urine
— skin
— mucosa of the lung
} negative or below the detection limit

Sahlabei Sadegh (n. 5) : urine : negative or below detection limit

II. *Samples received on February 25th.*

A. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Mossadegh Rahmatolah (n. 4) : 38.3 %
Ansari Azir (n. 1) : 40.9 %
Assan Massantaban (n. 2) : no sample received
Sahlabei Sadegh (n. 5) : 35.6 %
Parrokhi Mohammed Hassan (n. 6) : 42.5 %
Holan Hossein (n. 7) : 37.5 %
Talab Hossein Ashin (n. 8) : no sample received
Maslami Seyed Ramesan (n. 9) : 39.7 %

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B. *Magnesium (blood).*

Reference values : plasma 1.56-2.55 mg %,
red cells 5.47-7.29 mg %.

	Plasma	Red cells
Ansari Azir (n. 1)	: 2.20 mg %	6.89 mg %
Assan Massantaban (n. 2)	: no sample received	
Mossadegh Rahmatolah (n. 4)	: 1.85 mg %	6.23 mg %
Sahlabei Sadegh (n. 5)	: 1.76 mg %	5.72 mg %
Parrokhi Mohammed Hassan (n. 6)	: 1.90 mg %	5.35 mg %
Holan Hossein (n. 7)	: 2.41 mg %	6.15 mg %
Talab Hossein Ashin (n. 8)	: no sample received	
Maslami Seyed Ramesan (n. 9)	: 2.14 mg %	7.00 mg %

III. *Samples received on February 26th.*

A. *Methemoglobin blood).*

Reference value : 0-0.3 g %.

Ansari Azir (n. 1)	: 0.22 g %
Assan Massantaban (n. 2)	: 0.10 g %
Mossadegh Rahmatolah (n. 4)	: 0.16 g %
Sahlabei Sadegh (n. 5)	: 0.18 g %
Parrokhi Mohammed Hassan (n. 6)	: 0.13 g %
Holan Hossein (n. 7)	: 0.14 g %
Talab Hossein Ashin (n. 8)	: 0.05 g %
Maslami Seyed Ramesan (n. 9)	: no sample received

B. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Ansari Azir (n. 1)	: 38.0 %
Assan Massantaban (n. 2)	: not enough sample
Mossadegh Rahmatolah (n. 4)	: 16.1 %
Sahlabei Sadegh (n. 5)	: 27.4 %
Parrokhi Mohammed Hassan (n. 6)	: 39.4 %
Holan Hossein (n. 7)	: 31.6 %
Talab Hossein Ashin (n. 8)	: 57.9 %
Maslami Seyed Ramesan (n. 9)	: no sample received

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C. *Magnesium (blood).*

Reference values : plasma 1.56-2.55 mg %,
red cells 5.47-7.29 mg %.

		Plasma	Red cells
Ansari Azir (n. 1)	:	2.24 mg %	6.21 mg %
Assan Massantaban (n. 2)	:	2.10 mg %	6.32 mg %
Mossadegh Rahmatolah (n. 4)	:	2.04 mg %	6.19 mg %
Sahlabei Sadegh (n. 5)	:	1.95 mg %	5.33 mg %
Parrokhi Mohammed Hassan (n. 6)	:	1.91 mg %	5.30 mg %
Holan Hossein (n. 7)	:	2.35 mg %	6.58 mg %
Talab Hossein Ashin (n. 8)	:	1.97 mg %	6.11 mg %
Maslami Seyed Ramesan (n. 9)	:	no sample received	

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3. LAUSANNE.

First report of the toxicological analysis.

First series of samples (blood and urine) sent by the « Centre Hospitalier Universitaire Vaudois » of Lausanne (Service d'Anesthésiologie, Prof. J. Freeman).

The samples arrived in Ghent on February 19th at 8 a.m.

A. *Methemoglobin (blood).*

Reference value : 0-0.3 g %.

Mohseni Ahmad-Reza : 0.09 g %
Ogaghlo Malec-Ashtar : 0.14 g %
Josefian Alireza : 0.05 g %

B. *Sulfhemoglobin (blood).*

Reference value : negative.

Mohseni Ahmad-Reza : negative
Ogaghlo Malec-Ashtar : negative
Josefian Alireza : negative

C. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Mohseni Ahmad-Reza : 40.4 %
Ogaghlo Malec-Ashtar : 48.4 %
Josefian Alireza : 59.2 %

D. *Cyanide blood).*

Reference value : 0-10 µg %.

Mohseni Ahmad-Reza	8.75 µg %
Ogaghlo Malec-Ashtar	10.30 µg %
Josefian Alireza	15.30 µg %

E. *Thiocyanate (plasma and urine).*

Reference values (plasma) : non smokers : < 85 µM/L,
smokers : > 85 µM/L.

	Plasma	*Urine*
Mohseni Ahmad-Reza	36 µM/L	99 µM/L
Ogaghlo Malec-Ashtar	32 µM/L	64 µM/L
Josefian Alireza	41 µM/L	93 µM/L

F. *Arsenic (blood and urine).*

Reference values : blood < 4 µg %,
urine 0-100 µg/24 u.

	Blood	*Urine*
Mohseni Ahmad-Reza	0.26 µg %	8.50 µg/L
Ogaghlo Malec-Ashtar	0.92 µg %	7.12 µg/L
Josefian Alireza	1.09 µg %	11.90 µg/L

G. *Yperite, mustard gas (urine), qualitative test.*

Mohseni Ahmad-Reza	negative or below detection limit
Ogaghlo Malec-Ashtar	negative or below detection limit
Josefian Alireza	slightly +

H. *Mycotoxins : trichothecenes (blood and urine).*

	Blood	*Urine*
Mohseni Ahmad-Reza	negative	negative
Ogaghlo Malec-Ashtar	negative	negative
Josefian Alireza	negative	not enough sample

4. LONDON.

First report of the toxicological analysis.

First series of samples (blood and urine) sent by the « Humana Hospital Wellington » of London (Department of Pathology).

The samples arrived in Ghent on February 21st at 0.30 p.m.

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1. *Description of the samples.*

Blood sample 1 : 6 tubes (lithium heparin) labeled : « Ebrahim Hendozadeh, Floor I, Room 119, n. 29092, Pre ».

Blood sample 2 : 4 tubes (lithium heparin) labeled : « Mohamdi Ali, c/o Mr. Ellis, Rm 118, n. 29091, Pre Perfusion ».

Blood sample 3 : 2 tubes (lithium heparin) labeled : « Mohamdi Ali, 19/2/86, Pre Perfusion ».

Blood sample 4 : 5 tubes (lithium heparin) labeled : « Mohamdi Ali, 19/2/86, Post Perfusion ».

Blood sample 5 : 7 tubes (lithium heparin) labeled : « Mohamdi Ali, 20/2/86, Pre Perfusion ».

Urine sample 1 : 1 tube labeled : « Hendozadeh, Pre ».

Urine sample 2 : 2 tubes labeled : « Hendozadeh, Post ».

Urine sample 3 : 2 tubes labeled : « Mohamdi, Pre ».

Urine sample 4 : 2 tubes labeled : « Mohamdi, Post ».

2. *Results.*

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BIBLIOTHÈQUE

A. *Methemoglobin (blood).*

Reference value : 0-0.3 g %.

Blood sample 1 (Hendozadeh) : 0.14 g %
Blood sample 2 (Mohamdi) : 0.29 g %
Blood sample 3 (Mohamdi) : 0.27 g %
Blood sample 4 (Mohamdi) : 0.08 g %
Blood sample 5 (Mohamdi) : 0.18 g %

B. *Sulfhemoglobin (blood).*

Reference value : negative.

Blood sample 1 (Hendozadeh) : negative
Blood sample 2 (Mohamdi) : negative
Blood sample 3 (Mohamdi) : negative
Blood sample 4 (Mohamdi) : negative
Blood sample 5 (Mohamdi) : negative

C. *Cholinesterase activity (plasma).*

Reference value : 50 %.

Blood sample 1 (Hendozadeh) : 44.7 %
Blood sample 2 (Mohamdi) : 42.7 %

Blood sample 3 (Mohamdi) : 42.2 %
Blood sample 4 (Mohamdi) : 40.8 %
Blood sample 5 (Mohamdi) : 37.9 %

D. *Cyanide (blood).*

Reference value : 0-10 μg %.

Blood sample 1 (Hendozadeh) : 8.3 μg %
Blood sample 2 (Mohamdi) : 17.0 μg %
Blood sample 3 (Mohamdi) : 11.0 μg %
Blood sample 4 (Mohamdi) : 8.0 μg %
Blood sample 5 (Mohamdi) : 14.0 μg %

E. *Thiocyanate (plasma and urine).*

Reference values (plasma) : non smokers : $<$ 85 μM/L.
smokers : $>$ 85 μM/L.

Blood sample 1 (Hendozadeh) : 43 μM/L
Blood sample 2 (Mohamdi) : 41 μM/L
Blood sample 3 (Mohamdi) : 33 μM/L
Blood sample 4 (Mohamdi) : 42 μM/L
Blood sample 5 (Mohamdi) : 38 μM/L
Urine sample 1 (Hendozadeh) : 44 μM/L
Urine sample 2 (Hendozadeh) : 45 μM/L
Urine sample 3 (Mohamdi) : 33 μM/L
Urine sample 4 (Mohamdi) : 21 μM/L

F. *Arsenic (blood and urine).*

Reference values : blood $<$ 4 μg %,
urine 0-100 μg/24 u.

Blood sample 1 (Hendozadeh) : 1.10 μg %
Blood sample 2 (Mohamdi) : 1.84 μg %
Urine sample 1 (Hendozadeh) : 118.6 μg/L
Urine sample 3 (Mohamdi) : 28.3 μg/L

G. *Yperite, mustard gas (urine), qualitative test.*

Urine sample 1 (Hendozadeh) :
Urine sample 2 (Hendozadeh) :
Urine sample 3 (Mohamdi) :
Urine sample 4 (Mohamdi) :
} negative or below detection limit

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H. *Mycotoxins : trichothecenes (blood and urine).*

Blood sample 1 (Hendozadeh) : negative
Blood sample 2 (Mohamdi) : negative
Urine sample 1 (Hendozadeh) : negative
Urine sample 3 (Mohamdi) : negative

II. Pictures of the patients at the University Hospitals of Ghent, taken during their treatment (fig. 1, 2, 3, 4, 5, 6, 7, 8 and 9).

III. Pictures of the patient (n. 3), who died in the University Hospitals of Ghent and which were taken during the autopsy (fig. 10, 11, 12, 13, 14, 15 and 16).

IV. CONCLUSION OF THE REPORT.

The results of blood, urine, skin, hair and gastric juice confirm that at least two wargases, in combination have been used : mustard gas (Yperite) and an organic phosphate which inhibits the human plasma cholinesterase (Tabun or analogues).

The possibility exists that a third component as cyanide or analogue has been used. The concentrations however are too difficult to evaluate, also due to the metabolisation and the lapse of time between the intoxication and the arrival at the different European university hospitals.

Some amounts found are very high and fatal doses.

There is no scientific doubt that those patients are lethal intoxicated by those chemical war agents.

The results of these investigations confirm the results which were published in the Proceedings of the First World Congress « New Compounds in Biological and Chemical Warfare : Toxicological Evaluation », concerning the mixture of gases that have been used and the ones which were detected in these cases.

The techniques and results as published (1) were checked by other international toxicological laboratories and confirm the techniques of extraction and the conclusions in biological samples of men and in environmental cases.

The bee faeces theory and yellow rain as said by Prof. Meselson and also food contaminants have no results and impact of our investigations.

These results were already published elsewhere.

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From the more than 400 samples that we analyzed from different patients of the world, no different conclusions had to be made.

These results show also that in the examined patients, the very high concentrations of methemoglobin in blood of men were related to « Yellow Rain » components. When substituting the Yellow Rain by other wargases, those high amounts of methemoglobin did not appear.

The lowered acetylcholinesterase activity of the human plasma was not related to the Mustard Gas (Yperite). By comparison of the toxicological results, the lowering was much too high, being possibly only related to Yperite.

Many control samples of humans, coming from the battle field and other places were analyzed for that purpose ; they confirm our conclusions.

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Chemical Warfare – Laboratory Findings
Analytical Methods

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Detection of mustard gas in urine by high resolution gas chromatography / mass spectrometry

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SUMMARY.

Mustard gas bis(2-chloroethyl)-sulfide (Yperite) could be detected by gc/ms in six of twelve urine samples after the work up procedure including steam distillation and liquid-liquid extraction. Its concentration ranged from 1 to 30 ppb. The identity of this compound was further proved by high resolution mass spectral data. In three of the six positive samples another mustard gas isomer — probably a dichloropropylmethylsulfide — could be found.

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After the first world congress had been held in Ghent in 1984 several methods have been proposed to detect mustard gas in biological material (1-5).

Gas chromatography/mass spectroscopy represents a reliable method which combines sensitive and specific detection and has been applied for its decisive analytical qualities.

After testing the urine samples for Arsene by atomic absorption spectrometry to screen for the presence of Lewisite, 20 mL of each sample were mixed with 20 mL concentrated hydrochloric acid, and solid sodium chloride was added in excess. The mixture was heated to boiling in a distillation apparatus and the volatile compounds were collected into 20 mL redistilled ether.

When 20-30 ml aqueous solution was obtained in the condenser — which had taken about 40 to 60 minutes — the distillation was stopped, the distillate saturated with sodium chloride, and the

ether layer was separated after extraction from the aqueous phase*.

The ether was dried with sodium sulphate and was very carefully removed at room temperature after filtration. The residue was dissolved in 1 ml methylenechloride, 100 mg silica gel (E. Merck, 0.2-0.5 mm) were added and slightly shaken for one hour. After removing the silica gel by centrifugation the solvent was again evaporated down to a volume of 50 microliters. Aliquots of this solution were analysed by gas chromatography -mass spectrometry.

The gc separation was performed on a 25 m long fused silica capillary, coated with methyl silicone and connected by a 0.1 mm fused silica capillary to a low resolution mass spectrometer VARIAN MAT 112.

The mass spectrometer was focused to mass 158, the accuracy of the mass determination tuned to within 10 ppm of the molecular ion of Yperite ; the resolution was set to 1500.

Six of the twelve analysed samples were positive and showed a concentration between 1 and 30 ppb bis (2-chloroethyl)-sulfide (Yperite).

The semi-quantitative determinations were performed by comparing the peak areas with the areas of standard solutions of appropriate concentrations.

Figure 1 shows a typical example of a positive urine, representing 10 ppb Yperite. Yperite was coinjected in another run in order to confirm, that the retention time of the sample component is identical with the retention time of Yperite. The detailed single ion monitoring trace — fig. 2 — gives no hint that the retention time is different.

Moreover another component (X) with the same nominal mass is observed with a retention time of 0.86 in relation to Yperite.

Three of the six Yperite-positive urine samples have shown this so far unknown compound.

The gas chromatographic-mass spectrometric analysis has been repeated with a high resolution machine Finnigan MAT 8430, coupled to a 25 m fused silica capillary. The resolution has been set to 10,000 and the fragments with the masses 157,972 and 159,969 have been detected in the urine extract.

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* In the course of the work up procedure two samples were significant for their distinct violet vapour during distillation. The separate analyses of all samples for iodine by X-ray fluorescence had proved that those two samples only had been positive, their concentration being 1.0 mg and 0.1 mg per milliliter (fig. 6).

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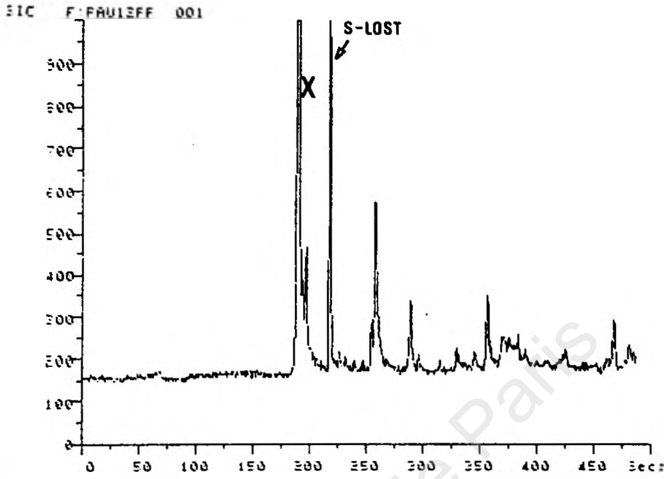

FIG. 1. — SID m/e 158 (low resolution) ; 25 m HP. Ultraphase (Methylsilicone) 100°/10°/180°C.

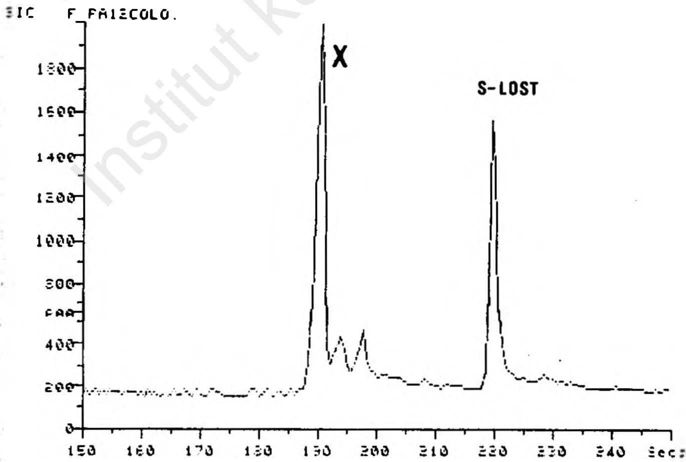

FIG. 2. — Detailed SID-trace after mixed injection with Yperite standard.

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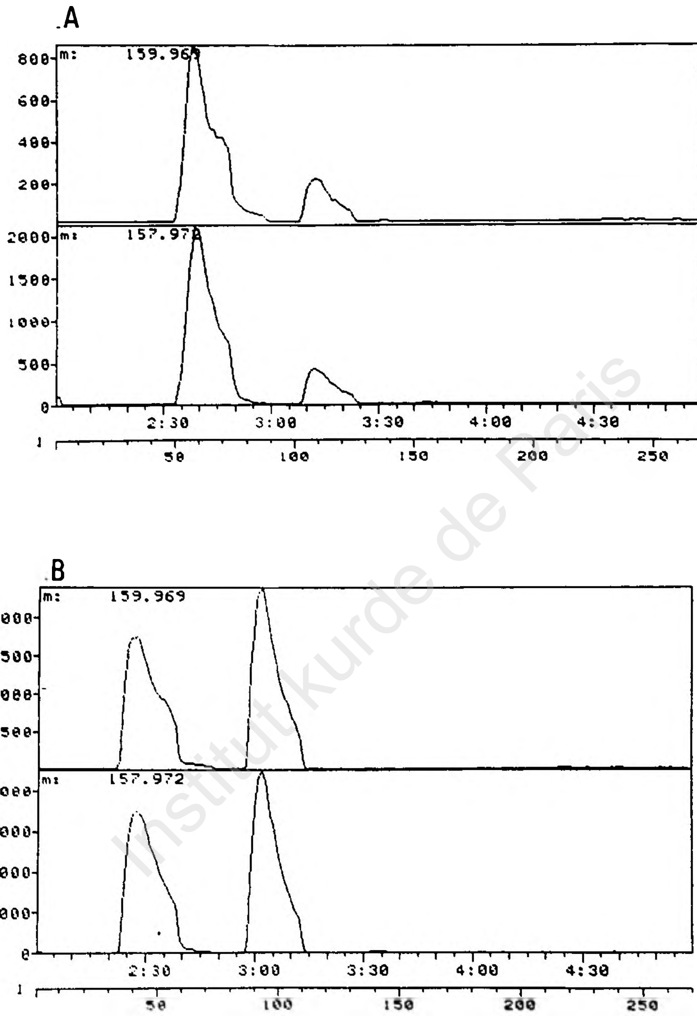

FIG. 3.

A. High resolution ion monitoring m/e 157.9724 and 159.9694, Finnigan MAT 8430, ratio 158/160 = 1.9 (1.8 standard), 25 m SE-54, of urine extract (fig. 1).

B. As in A, but mixed injection with Yperite standard.

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Yperite has got the same retention time as the second major component, a fact, which has been proved by mixed injection of sample and Yperite standard of similar concentration (fig. 3).

The component eluting with a retention time 0.86 in relation to Yperite, has exactly the same mass as Yperite. Furthermore the ratio of the masses 158/160 of both « compounds » has been measured with 1.9, while the ratio of these two fragments in the Yperite standard has been measured with 1.8.

The value of this quoted ratio is known to be typical for a two chlorine atoms containing compound.

Number of double bonded equivalent	*Error (ppm)*	*40-1000 amu mass*	*True mass*	*Min. % 0.00*					
				C	*H*	*N*	*O*	*S*	*Cl*
5.5	1	157.9724	157.9726	4	0	1	6	0	0
1.0	— 16		157.9699	1	2	0	9	0	0
9.5	— 15		157.9701	8	0	1	1	1	0
5.0	— 2		157.9721	3	2	4	0	2	0
4.5	7		157.9734	5	4	1	1	2	0
0.0	— 10		157.9707	2	6	0	4	2	0
1.0	4		157.9730	1	3	2	5	0	1
5.0	— 12		157.9705	5	3	2	0	1	1
0.0	10		157.9739	2	7	2	0	2	1
0.0	— 0		157.9724	4	8	0	0	1	2

FIG. 4. — Atomic combinations corresponding to mass 157.9724.

The exact mass determination at high resolution only offers three useful atomic combinations in respect to the elements C, H, N, O, S and Cl. Therefore the combinations $C_3H_2N_4S_2$ and $C_5H_3N_2SCl$ — as shown in figure 4 — are dropped in favour of $C_4H_8SCl_2$ because the ratio of the masses 158/160 demands two chlorine atoms.

Concerning the detection of Yperite in the low ppb range no further evidence for the identity of this compound has been collected.

The high resolution mass determination has been performed by Dr. U. Rapp, Finnigan MAT, and I am obliged to his kind assistance.

The experimental data by capillary gas chromatography, low and high resolution mass spectrometry support the statement that bis(2-chloroethyl)-sulfide is detectable in urine of patients affected by this compound.

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According to the high resolution mass spectral data the component eluting with the relative retention 0.86 to Yperite has the same molecular formula $C_4H_8SCl_2$, but a different structure.

E.R.J. Wils *et al.* (1985) have reported on two isomeric dichloropropylmethylsulfides in urines of mustard gas victims. These isomeres have been separated on a 50 m × 0.6 mm glass capillary, coated with SE-30. Their retention time was also shorter than that of Yperite. As these compounds have not been available for

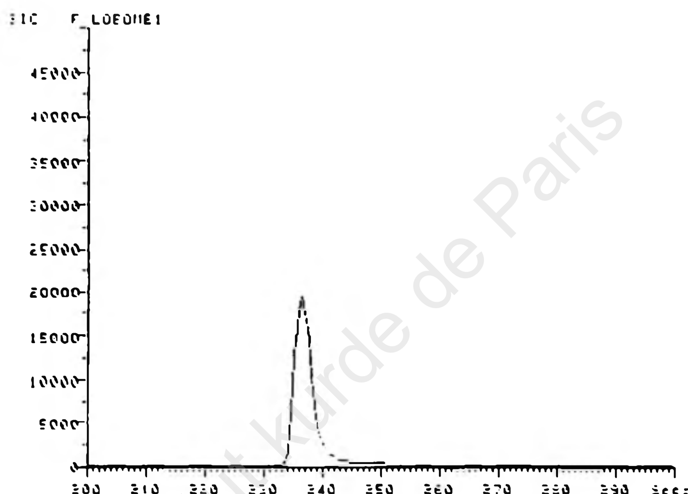

FIG. 5. — SID m/e 158 (low resolution), 25 m HP Ultraphase (Methylsilicone) detailed trace of the sample of the blind bomb.

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my investigations, a further proof in respect to chromatography could not be obtained, as far as the identity of this accompanying substance is concerned.

Due to the low concentration of these isomers in the matrix of urine extract, there is no chance of distinguishing them from Yperite, though the absence of the fragments of mass 63 and 65 in dichloropropylmethylsulfides might lead to the conclusion, that a distinction were possible.

The possibility that this isomer is an artefact from Yperite, can be excluded, because — following the work up procedure — it has neither been detected, when starting with Yperite, nor when using thiodiglycol. Moreover no such isomer could be found in the Yperite, obtained from a blind bomb in 1984 (fig. 5).

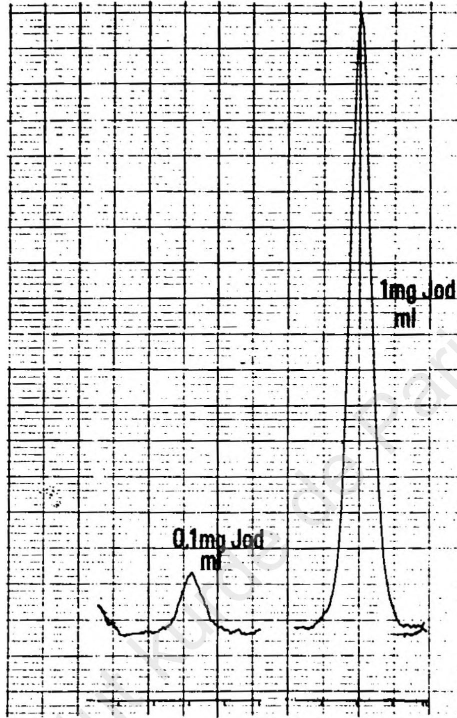

FIG. 6. — X-ray fluorescence determination of iodine.

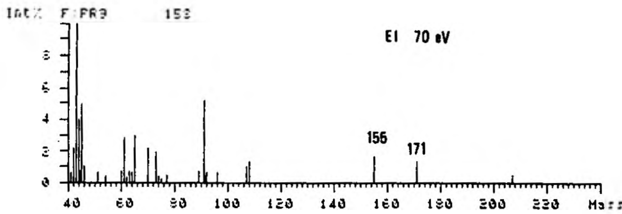

FIG. 7. — EI-70eV mass spectrum of p-toluenesulfonamide from urine extract.

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As far as the origin of this mustard gas isomer is concerned, it can only be excluded, that it is formed in the course of the work up procedure from Yperite or thiodiglycol. It may derive from an individual metabolism, from any contamination with products that form this isomer during the work up procedure, or from a mustard gas bomb with a different composition. So it is not possible to trace the source of these isomers by use of gc/ms only — and, due to the presence of chloride ions during work up —, it is not possible to distinguish between mustard gas or the hydroxylated metabolites.

Summing up the results of the gc/ms analyses of the twelve urine samples it can be reported, that six have been positive, concerning bis(2-chloro-ethyl)-sulfide in the range of 1-30 ppb and three of them have contained another isomer — probably a dichloropropylmethylsulfide.

After extracting them with chloroform and analysing the extracts by gc/ms, the presence of p-toluenesulfonamide could be detected by a complete low resolution mass spectrum (fig. 7).

These two patients had obviously been treated by chloramine-T (toluene-p-sulphon-chloramide) and afterwards by a iodine preparation, e.g. Povidone-iodine, in order to disinfect the skin-lesions.

These additional results are presented to point at the possible impairments when applying urine samples to field tests, based on a colour reaction.

It should further be taken into consideration, that the resorption of chloramin increases the methemoglobin concentration in blood.

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Yperite concentrations in the tissues of a victim of a vesicant exposure

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SUMMARY.

An Iranian soldier died at a toxicological intensive-care unit at Munich seven days after a vesicant exposure. At the forensic autopsy the typical symptoms of an Yperite intoxication were found. The vesicant was quantified in the tissues and in the body fluids by the following method : 1° Extraction by dichloromethane, 2° clean-up of the extracts by TLC on silicaplates, 3° extractive derivatization with gold-chloride, 4° quantitative determination by ET-AAS. The equal extracts, after heating, served for blanks. The following concentrations were found (mg Yperite/kg tissue wet wt.) : brain 10.7 ; cerebrospinal fluid 1.9 ; liver 2.4 ; kidney 5.6 ; spleen 1.5 ; lung 0.8 ; muscle 3.9 ; fat 15.1 ; skin 8.4 ; skin with subcutaneous fatty tissue 11.8 ; liquor from a skin blister : below detection limit ; blood 1.1 ; urine : below detection limit.

1. INTRODUCTION.

In 1985 an Iranian soldier (reported age : 24) died — ultimately of a pneumonia — at the toxicological intensive-care unit of the Technische Universität in Munich, seven days after a vesicant exposure. The findings of the autopsy (performed at the Institute of Forensic Medicine at Munich) confirmed the clinical diagnosis of an Yperite [Mustard Gas, Bis(2-Chloroethyl)-sulfide, S-Lost] intoxication and will be reported in detail elsewhere (1). In this paper the analytical efforts to quantify the vesicant in the tissues and in the body fluids of the deceased will be discussed.

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2. SAMPLES.

The tissues and the body fluids were taken at the autopsy and stored in polyethylene bags and — bottles at —20°C until they were analysed.

3. EXPERIMENTAL.

Heyndrickx *et al.* (2) have reported several methods (TLC, GC, GC-MS, AAS) for the analysis of Yperite in different matrices. In order to detect this agent in human tissues, we have optimized, combined and modified these methods by many investigations on spiked samples prior to the analyses of the real ones.

General remarks : At all analytical steps special care was taken that the temperature never exceeded 20°C. In any case the total analytical procedure from the defrosting of the tissue to the final AAS measurement was performed uninterrupted to avoid losses.

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3.1. Extraction.

3.1.1. *Equipment, Chemicals.*

Cutter : Malavasi s.n.c., Bologna, Italy.
Ultra Turrax : Fa. Janke and Kunkel, Staufen, F.R.G.
Rotavapor : Fa. Büchi, Eislingen, F.R.G.
Dichloromethane : analytical grade, E. Merck, Darmstadt, F.R.G.
n-Hexane : analytical grade, E. Merck, Darmstadt, F.R.G.

3.1.2. *Method..*

A sample of the tissue was homogenized (Cutter), and 1-7 g of the homogenate or of a body fluid (see table II) were mixed with an agitator (Ultra Turrax) thorougly with a 5-fold quantity of dichloromethane, subsequently shaked mechanically for 15 min. and than centrifuged. The dichloromethane layer was separated. This extraction procedure was repeated twice at the body fluids and three times at the other tissues, respectively.

The combined organic layers were evaporated to dryness at room temp. and reduced pressure on a Rotavapor.

The residue was weighed and resolved in 10 ml n-hexane. Half the sample or — if the total extract weighed more than 100 mg —

an aliquot of 50 mg was separated for TLC, while an other aliquot of the residue was heated to 150°C for 1 h to get an Yperite free blank.

3.2. TLC Clean-up Procedure.

3.2.1. *Equipment, Chemicals.*

Precoated TLC glass-plates 10 × 20 cm, SIL G-25 UV_{254}, Fa. Macherey and Nagel, Düren, F.R.G.
Solvent: Dichloromethane, analytical grade, E. Merck, Darmstadt, F.R.G.
Spray reagents : $PdCl_2$-solution (1 % in HCl 10 %),
$AuCl_3$-solution (1 % in water).
Yperite stock solution : 1 mg/ml in n-hexane.

3.2.2. *Method.*

Prior to the TLC separation the silica plates were cleaned by a blank run in the chamber with dichloromethane and dried. A maximum of 50 mg of the residue was solved in some 100 µl n-hexane, spotted in a band of 8 cm width on a TLC plate and developed over a distance of 15 cm. For visualization (of test spots only !) a $PdCl_2$-solution or a $AuCl_3$-solution (more sensitive) was sprayed on the plate. The R_f-value of the Yperite was approx. 0.90, distinctly higher than the front of the highest lipid fraction (triglycerides). The silica gel at a height of 12.5 to 15 cm was scraped off the plate.

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3.3. Formation of the Yperite-gold complex.

3.3.1. *Chemicals.*

Gold stock solution 1 mg Au/ml : Alfa Products, Danvers, MA, USA.
Toluene : analytical grade, E. Merck, Darmstadt, F.R.G.

3.3.2. *Method.*

The silica gel was transferred to teflon test tubes, 100 µl gold stock solution and 1.0 ml water were added and the tubes vortexed for 2 min. Than 4 ml of toluene were added and the mixture (water, toluene, silica gel) shaked another 2 min. mechanically. At least the toluene layer was separated by centrifugation.

3.4. **Quantitative Determination with ET-AAS** (Electro-Thermal Atomic Absorption Spectroscopy).

3.4.1. *Equipment, Chemicals.*

Perkin-Elmer AAS 3030 Zeeman, HGA 600, Printer-Plotter PR 100
Gold stock solution, see 3.3.1.

3.4.2. *Method.*

20 µl of the obtained organic layer (3.3.2.) — if necessary after dilution with toluene — were injected by hand in the AAS graphite tube. The AAS conditions are compiled in table I.

TABLE I

AAS conditions for the detection of the Yperite-gold complex

Wave length 242.8 nm ; slit 0.7 mm ; wall atomization ; purge gas : 300 ml argon/min.

Temperature programme :

Step	*Temperature*	*Ramp time (sec.)*	*Hold time (sec.)*
Drying	120° C	20	20
Charing	250° C	10	10
Atomizing*	2400° C	0	3
Cleaning	2650° C	1	4

* Gas stop.

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At any tissue the individual blank value (see 2.1.) was substracted. The calibration was performed by Yperite standard solutions, spotted on TLC plates and worked up simultaneously. The application of a standard addition method, as proposed by Heyndrickx *et al.* (2) seemed not to be necessary, because we know from several pilot tests with spiked samples, that dichloromethane extracts the Yperite nearly quantitatively from any human soft tissue and the applied AAS compensation method (Zeeman effect) avoids a depression of the gold signal by the cleaned-up organic matrices or other interferences.

4. **RESULTS.**

The results are summarized in table II.

TABLE II

Yperite concentrations found in the tissues

Tissue	Sample taken	Extract wt.	Yperite concentration
Brain	2.54 g	0.34 g	10.7 mg/kg
Cerebrospinal fluid	2.10 ml		1.9 mg/l
Liver	6.24 g	0.03 g	2.4 mg/kg
Kidney	5.14 g	0.16 g	5.6 mg/kg
Spleen	5.68 g	0.05 g	1.5 mg/kg
Lung	6.27 g	0.05 g	0.8 mg/kg
Muscle (thigh)	4.04 g	0.11 g	3.9 mg/kg
Fat (thigh)	2.79 g	1.37 g	15.1 mg/kg
Abdominal skin	0.78 g	0.21 g	8.4 mg/kg
Skin with subcutaneous fat	2.71 g	1.03 g	11.8 mg/kg
Liquor from a skin blister	4.00 ml		Below detection limit
Blood	20.00 ml		1.1 mg/l
Urine	20.00 ml		Below detection limit

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5. DISCUSSION.

Similar to other high lipophilic substances (e.g. pesticides, PCB's THC, etc.) Yperite seems to accumulate in the lipid compartments of the human body. The relative high Yperite concentrations, found in this case even seven days after the exposure, point out, that a metabolism of Yperite occurs rather slowly — at least after storage in the lipid depots. As described by Vycudilik (3), the urinary excretion of unmetabolized Yperite is extremely low ; but obviously this is not caused by a rapid disintegration of the agent, as supposed, but by its strong fixation to the lipid compartments of the body. The chemical stability of Yperite is reported very contradictory in the literature (3-6). Our investigations do not indicate any considerable chemical lability of this agent at least in the lipid tissues ante (see above) and post-mortem : in the presented case the tissues and the body fluids were stored after autopsy at —20°C (further conditions see 2.) for 12 months till they were analysed.

From our results it can be concluded, that the « tissue of choice » for the detection of Yperite in a corpse is the abdominal fat or an other tissue abounding in lipids. Among the body fluids blood seems to be more suitable for the detection of an Yperite exposure than urine. The last conclusion seems to be of special importance for the examination of living victims too.

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The toxicological analysis of chemical warfare agents in samples originating from Iranian soldiers

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SUMMARY.

In our Department lots of human biological samples have been tested for the presence of chemical warfare agents. All the samples originated from Iranian soldiers who were sent to Europe for treatment and stayed either in University hospitals in Ghent (Belgium) or in University hospitals of other Belgian or European cities.

Blood samples were tested on their cholinesterase activity in order to detect a possible exposure to nerve gases. Samples of blood, urine, faeces, sputum, skin, hair or yellowish fluid from blisters were further examined on the presence of sulfur mustard (Yperite), Arsenic (Arsine, Lewisite or other organic arsenicals), cyanide (hydrogen cyanide, cyanogen chloride) or mycotoxins of the trichothecene family, being toxic fungal metabolites which are considered since several years as constituents of a chemical weapon called « Yellow Rain ». In this paper details about the analytical toxicological methodology used are outlined. Some general considerations about the different toxicological findings collected during the last three years are also given.

INTRODUCTION.

In the spring of 1984 many Iranian soldiers became victims of chemical warfare agents, and some of them were transferred to European hospitals for treatment. In the early beginning no diag-

nosis could be made. It was said that the patients were burnt and one thought that it was due to Lewisite, an organic arsenical with vesicatory properties. However, it was found that the arsenic levels determined in blood and urine were within the limits of the so called normal or reference values. Later on it became clear that the « burns » were due to sulfur mustard, also called Yperite, an old chemical agent with long-term incapacitating properties in case of survival, and having been used for the first time in World War I near to the city of Ypres (Ieper, Belgium). Some of the patients showed cholinergic symptoms with a lowered plasma cholinesterase activity, what led to the supposition that nerve gases had been used at the same time.

Mycotoxins of the trichothecene family, which are toxic fungal metabolites found as contaminants in foods and feed, were also detected in some of the samples. As those trichothecenes were not detected in samples of soldiers who were fighting on the same battle field without having been attacked by chemical or biological agents, they were associated with the Southeast Asian chemical biological warfare phenomenon called « Yellow Rain ».

As they didn't stop using chemical weapons in the Gulf war, other victims were transferred to European hospitals again in 1985 and 1986, what means that in our department since 1984 lots of human biological samples have been tested for the presence of chemical warfare agents, because most European hospitals did call on Ghent for sample analysis.

As summarized in table I, the samples originated from Ghent, Brussels and Charleroi, what Belgium is concerned, while other samples came in from Austria, Switzerland, West-Germany, France, U.K. and Sweden.

In all cases blood and urine samples were analysed, and in most of the cases samples of faeces, sputum, skin (epidermis), yellowish fluid from blisters and hair were also sent.

What the toxicological screening is concerned, first of all the blood methemoglobin level as well as the cholinesterase activity were tested, the latter parameter being an indication for a possible exposure to nerve gases. Other interesting parameters were the arsenic contents of blood and urine, the blood cyanide concentration and the thiocyanate levels of blood and urine.

Finally all samples of urine, skin (epidermis), hair and fluid from blisters were tested for the presence of sulfur mustard,

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TABLE I

Origin of the samples tested for the presence of chemical and biological warfare agents

ORIGIN OF THE SAMPLES	NUMBER OF PATIENTS		
	1984	1985	1986
GHENT (BELGIUM)	5	3	12
BRUSSELS (BELGIUM)	-	3	2
CHARLEROI (BELGIUM)	-	-	4
VIENNA (AUSTRIA)	8	7	4
LINZ (AUSTRIA)	-	4	-
LAUSANNE (SWITZERLAND)	2	-	3
HAMBURG (W. GERMANY)	2	-	-
MUNICH (W. GERMANY)	3	-	-
RECKLINGHAUSEN (W. GERMANY)	10	-	5
PARIS (FRANCE)	-	-	1
LONDON (UNITED KINGDOM)	-	5	2
STOCKHOLM (SWEDEN)	-	-	1

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while mycotoxins of the trichothecene family were determined in blood, urine and faeces.

METHODS.

Methemoglobin.

The methemoglobin level was determined by means of the spectrophotometric method of Kiese, 1947 (1). As outlined in figure 1, the blood sample which contains an unknown amount of methemoglobin and an excess of hemoglobin is hemolysed and saturated with carbonmonoxyde.

As a result the hemoglobin is converted to carboxyhemoglobin, which can be measured at 577 nm, while the methemoglobin remains unchanged.

In a second stage the solution is treated with sodium dithionite in order to reduce the methemoglobin to hemoglobin, and the new generated hemoglobin is converted to carboxyhemoglobin too by reacting with the excess of CO.

The absorbance at 577 nm is measured again and the rise in absorbance is directly proportional to the methemoglobin level.

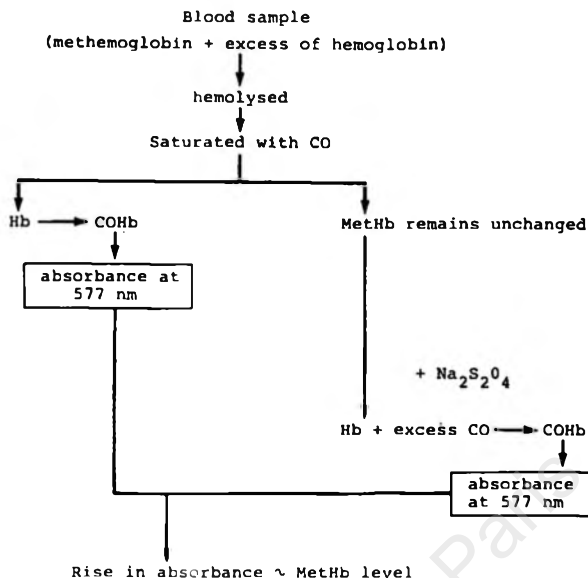

FIG. 1. — Principle of the method for the determination of methemoglobin.

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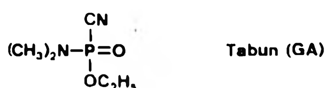
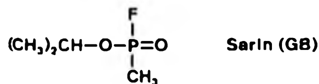
Blood cholinesterase activity.

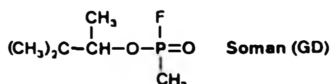
The blood cholinesterase activity can be indicative for a possible exposure to nerve gases. As shown in figure 2, there are nerve gases called G-agents like Tabun, Sarin and Soman, and V-agents like VX and similar derivatives.

What nerve gases and the Gulf war is concerned, physical evidence furnishing proof of the use of Tabun, was also collected by the UN team in 1984 (2).

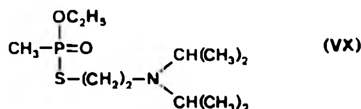
G-AGENTS







V-AGENTS



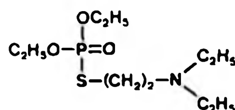


FIG. 2. — Chemical structures of some important nerve gases.

The blood cholinesterase activity was determined by two different procedures. The first one, according to Morand and Laborit, 1947 (3), is based on a manual titration with 0.01 N NaOH during one hour, after having added 0.5 ml of plasma to an acetylcholine solution as the substrate (Ref. value : 50 ± 10 %). The second method, described by Nenner, 1970 (4), is based on the same principle. However, the change to a potentiometric titration with 0.01 N NaOH during 5 minutes permits the determination of the enzyme activity in both the plasma and the whole blood. The red cell activity is then calculated by means of the hematocrit value (Ref. values : 1.26-2.66 $\mu M.ml^{-1}.min^{-1}$ for plasma ; 9.85-16.41 $\mu M.ml^{-1}.min^{-1}$ for red cells).

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Arsenic.

As illustrated in figure 3, an arsenic determination can be indicative for a possible exposure to either arsine, a systemic poi-

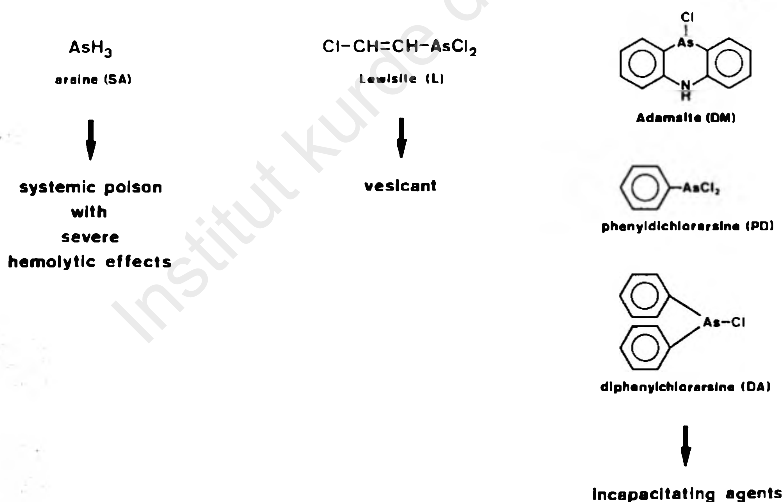

FIG. 3. — Examples of chemical warfare agents with an arsenical structure.

son with strong hemolytic effects, Lewisite, a vesicant, or other organic arsenicals with incapacitating properties.

A determination of arsenic was performed on blood and urine samples according to a procedure already described by Heyndrickx and Parisis in 1984 (5).

The method consists of a wet digestion of the sample with a mixture of nitric, perchloric or sulfuric acid, and a subsequent oxydation of trivalent arsenic (As^{3+}) to the pentavalent form (As^{5+}) by means of hydrogen peroxide. This is followed by a total arsenic determination based on an arsine generation, by adding sodium tetrahydroborate ($NaBH_4$) to a portion of the diluted digest, and a subsequent flushing of the volatile hydride into an electrically heated quartz cell, aligned in the light path of a Model 372 AA Spectrophotometer, where it is decomposed and the absorption of the arsenic measured.

Cyanide and thiocyanate.

The cyanide concentration of the blood can be indicative for an exposure to chemical warfare agents like hydrogen cyanide (AC), a suffocating agent, and cyanogen chloride (CK), a suffocating agent with irritating properties because of the chlorine atom.

Cyanide will be converted in the body to thiocyanate by the enzyme sulfur transferase and an endogenous source of thiosulfate, what implicates that the thiocyanate levels of blood and urine also need to be considered as usefull parameters.

The cyanide concentration in the blood was determined by a distillation of the sample after a previous acidification and a suction of the evolving cyanide in a sodium hydroxide solution, using a self-designed apparatus as illustrated in figure 4.

A part of the sodium hydroxide solution is further treated with primary sodium phosphate, chloramine-T and a pyridine-barbituric acid reagent, according to the reaction described in the Standard Methods for the Examination of Water and Wastewater, 1981 (6), with a final absorbancy reading of the developed blue color at 578 nm.

The thiocyanate levels were determined according to the method of Guerrero and Roig, 1973 (7). As outlined in figure 5, first an acid salted-out extraction is performed on a 0.2 ml sample amount.

This is followed by an absorbancy reading at 560 nm of the purple color formed after reaction with rhodamine B.

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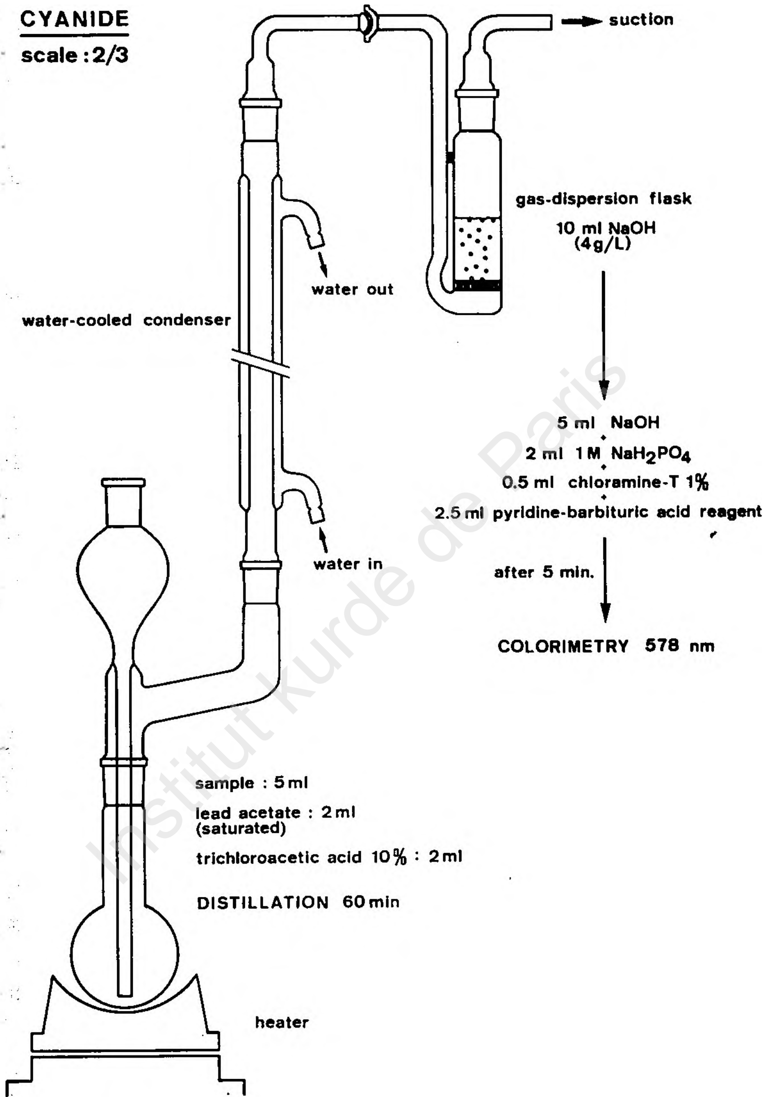

FIG. 4. — Self-designed apparatus for the isolation of cyanide from human blood or other biological samples.

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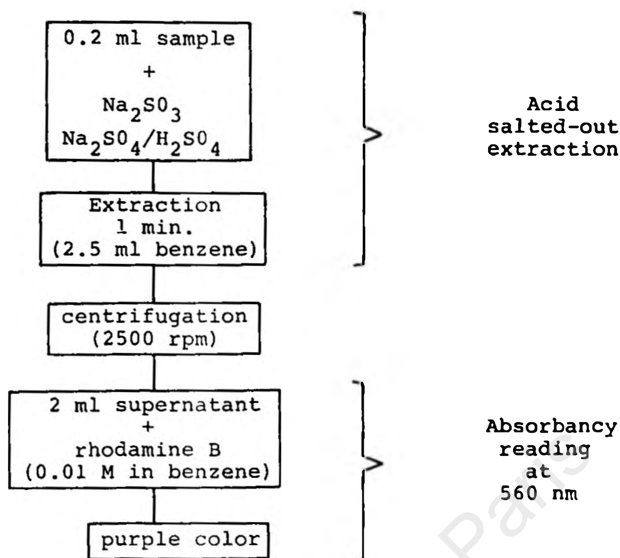

FIG. 5. — Method of Guerrero and Roig, 1973 (7), for the determination of thiocyanate in blood and urine.

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Sulfur mustard (Yperite).

When the first Iranian soldiers arrived in Ghent for treatment they all showed symptoms of an intoxication with vesicants of the mustard type. As illustrated in figure 6, vesicants of the mustard type consist of sulfur mustard and several nitrogen mustards. Sulfur mustard is also called Yperite because of its first

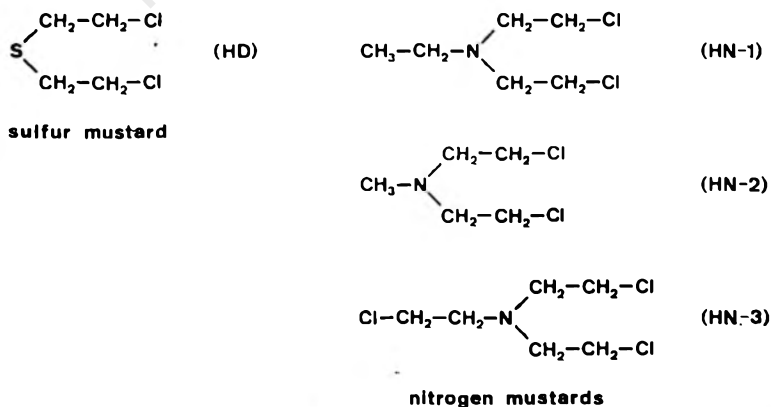

FIG. 6. — Vesicants of the mustard type including sulfur mustard and several nitrogen mustards.

use nearby the city of Ypres (Ieper) in Belgium during World War I.

Since the beginning of 1984, sulfur mustard has been used in the Gulf war as it was proven by analysing the liquid contents of unexploded bombs and impregnated soil samples collected by one of us on the Iranian battlefield.

As shown in figure 7, sulfur mustard is rapidly converted after exposure to a sulfonium ion with strong alkylating properties, giving rise to severe skin lesions and radiomimetic symptoms. By a rather complicated biotransformation process the sulfonium ion is further transformed to thiodiglycol, a final metabolite mainly excreted under a conjugated form. A partial oxydation to a sulfone and a sulfoxide can also take place.

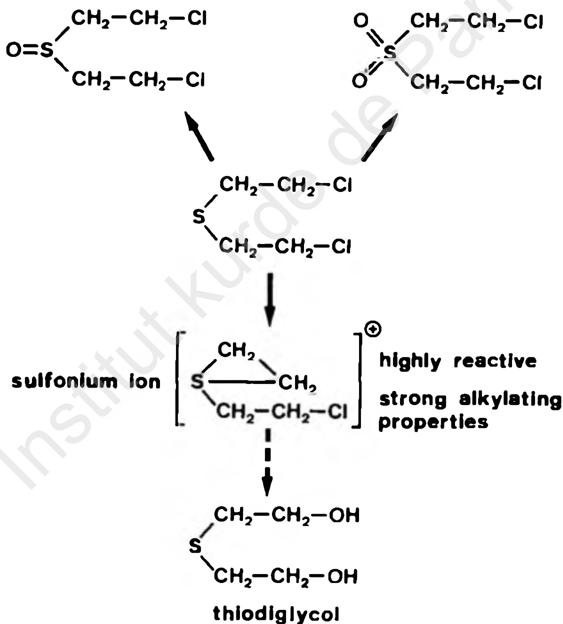

FIG. 7. — Metabolic fate of sulfur mustard.

As outlined in figure 8, the detection of sulfur mustard in human biological samples was done by performing at first a qualitative reaction, using the KANAG ADETOX FIELD TEST in a modified form applicable to biological samples, as it was already described by Heyndrickx *et al.*, 1984 (8).

It was also tried to perform a qualitative and quantitative estimation of sulfur mustard on another part of the sample by either

gas chromatography (GC), according to a procedure proposed by Heyndrickx *et al.*, 1984 (9), or by gas chromatography/mass spectrometry.

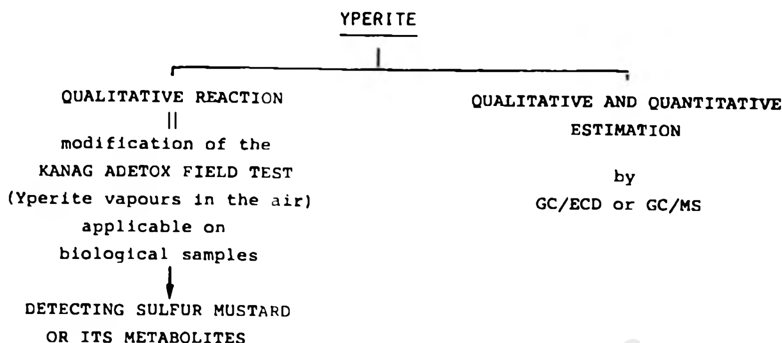

FIG. 8. — Proposed methodology for detecting sulfur mustard in human biological samples.

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The KANAG ADETOX FIELD TEST is manufactured in Switzerland (Louis-Schleiffer AG, CH-8714 Feldbach).

The test is designed for detecting chemical warfare agents like sulfur mustard in the air. It consists of a hand-pump with a stroke volume of 100 ml, detection lamellas containing a filter disk, and a series of reagents. When sulfur mustard is present in the air, the filter disk in the detection lamella will turn violet-reddish after having sucked the contaminated air through the disk and applying the appropriate reagents. As illustrated in figure 9, the system was used in a modified form by heating the sample in a bubbler absorber and first sucking the air through the sample by means of the hand-pump, so that the air eventually loaded with suspected sample components could pass the detection lamella.

As outlined in figure 10, the applied procedure for the qualitative and quantitative estimation of sulfur mustard consisted of a dichloromethane extraction of a sample previously saturated with NaCl. The extract was further purified on a SEP-PAK™ cartridge containing silica gel, and the final residue was reconstituted in either n-hexane or dichloromethane. The n-hexane fraction was examined by gas chromatography with electron capture detection using a Sil 5 fused silica capillary column. The dichloromethane fraction was analysed by GC/MS using a Hewlett Packard Model 5850 gas chromatograph equipped with a methylsilicone fused silica capillary column and directly connected to a

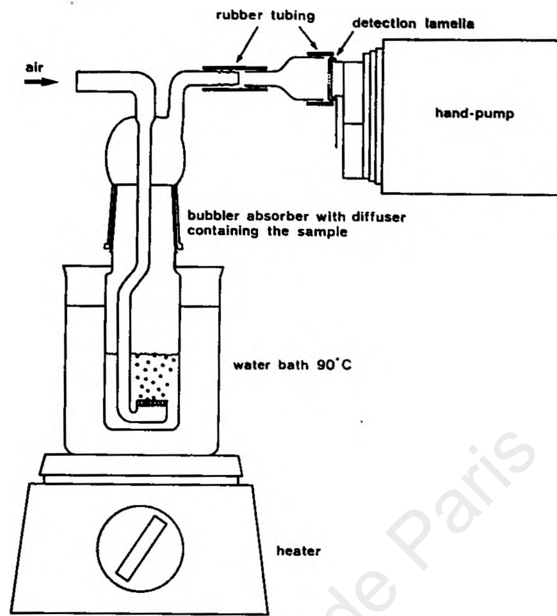

FIG. 9. — Detection of sulfur mustard or its metabolites using a modified KANAG ADETOX FIELD TEST.

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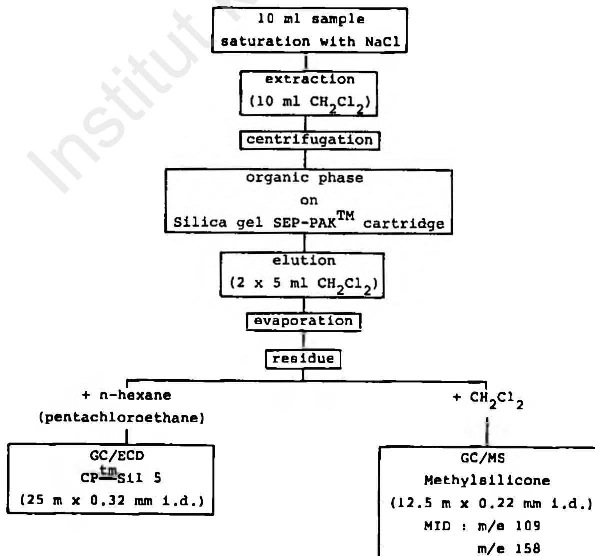

FIG. 10. — Applied procedure for the qualitative and quantitative estimation of sulfur mustard.

5970 Mass Selective Detector (MSD) operating in the MID mode by monitoring the molecular ion and the fragment ion with m/e 109.

Mycotoxins of the trichothecene family.

Trichothecenes are a group of fungal metabolites which are produced primarily by various species of Fusarium and to a less extend by some species of Cephalosporium, Myrothecium, Trichothecium, Trichoderma and Stachybotrys. All those fungi are plant pathogens which frequently invade agricultural products. Since several years, the mycotoxins of the trichothecene family are also considered as constituents of « Yellow Rain » a man-made weapon for the first time observed in Southeast Asia. As shown in figure 11, trichothecenes have a tetracyclic sesquiterpene structure with an olefinic bond at C9 and C10 and an epoxide group at C12 and C13.

Naturally occurring trichothecenes can be divided in 4 groups, depending on the substituents at five positions in the trichothecene nucleus. As illustrated in figure 12, group A toxins are either not oxidized or hydroxylated and esterified in the positions 3, 4, 7, 8 and 15. Trichothecenes of group B have a carbonyl group at position 8, while group C is only made up of Crotocin, a compound characterized by the presence of a second epoxide function at C7/C8. Finally toxins of group D are also indicated as macrocyclic trichothecenes due to the presence of a macrocyclic ring linking at C4/C15.

The toxicological screening for the presence of trichothecenes was limited to the toxins listed in table II. All those toxins belong to either group A or group B comprising compounds like T-2, HT-2, DAS, Nivalenol and Deoxynivalenol.

The isolation of trichothecenes from blood, faeces and urine was performed by extraction with a further clean-up on XAD-2, as outlined in figure 13.

As further outlined in figure 14, a part of the purified extract was first examined by gas chromatography with electron capture detection, after a previous derivatisation with heptafluorobutyryl imidazole, a procedure already described by Heyndrickx *et al.* in 1984 (10-12).

In case of a positive test, a further identity confirmation was done either on the same derivatized extract by GC/ECD after a standard addition, or on another part of the original extract by

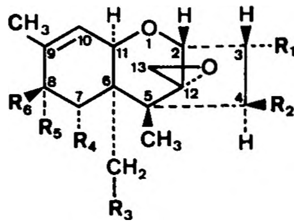

FIG. 11. — Basic structure of the trichothecene skeleton.

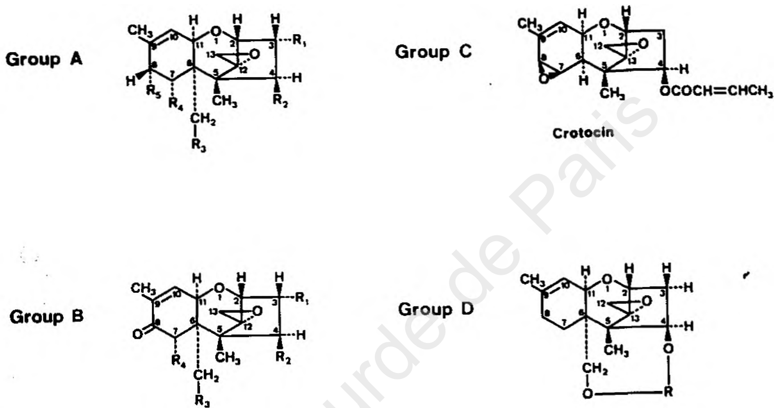

FIG. 12. — Classification of naturally occurring trichothecenes.

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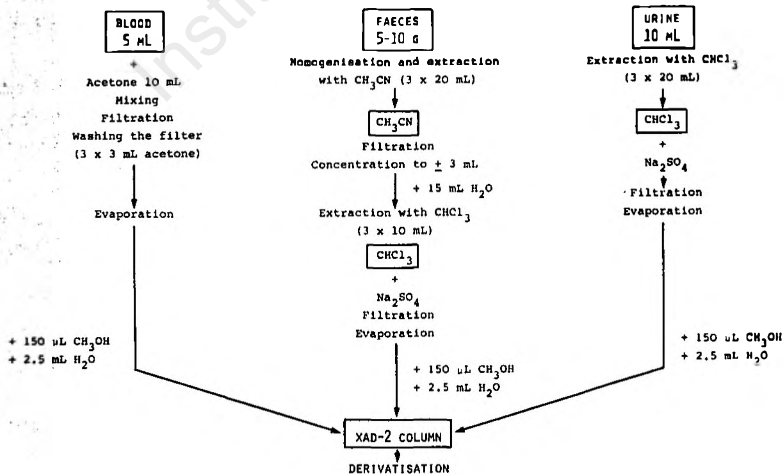
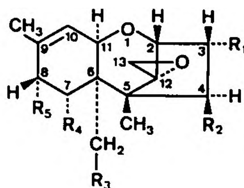


FIG. 13. — Procedures for extraction of human biological samples and further clean-up of the sample extracts.

TABLE II

A list of the trichothecenes detectable by the proposed analytical methodology

Group A



Name	R_1	R_2	R_3	R_4	R_5
T-2 Toxin	OH	OAc	OAc	H	$OCOCH_2CH(CH_3)_2$
Iso T-2 Toxin	OAc	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
HT-2 Toxin	OH	OH	OAc	H	$OCOCH_2CH(CH_3)_2$
T-2 Triol	OH	OH	OH	H	$OCOCH_2CH(CH_3)_2$
T-2 Tetraol	OH	OH	OH	H	OH
Neosolaniol	OH	OAc	OAc	H	OH
Verrucarol	H	OH	OH	H	H
Diacetoxyscirpenol	OH	OAc	OAc	H	H
15-Acetoxyscirpenol	OH	OH	OAc	H	H
Scirpentriol	OH	OH	OH	H	H

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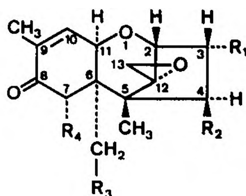
Group B



Name	R_1	R_2	R_3	R_4
Fusarenon-X	OH	OAc	OH	OH
Nivalenol	OH	OH	OH	OH
Deoxynivalenol	·OH	H	OH	OH
3-Acetyldeoxynivalenol	OAc	H	OH	OH

GC/MS. In the latter case the sample extract was silylated and dependent on the amounts of trichothecenes present, the instrument was operating in the SCAN or MID mode.

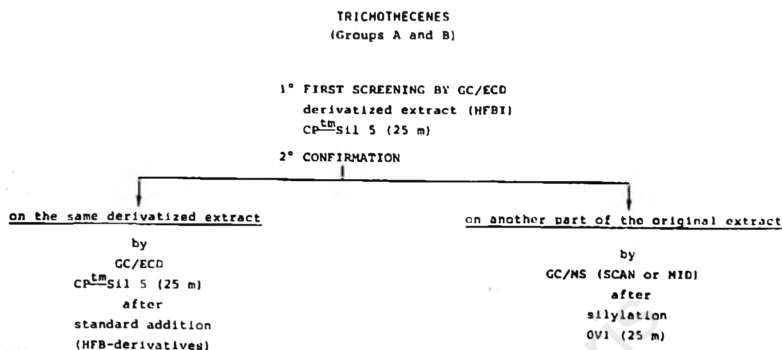


FIG. 14. — Proposed methodology for the detection of trichothecenes in human biological samples.

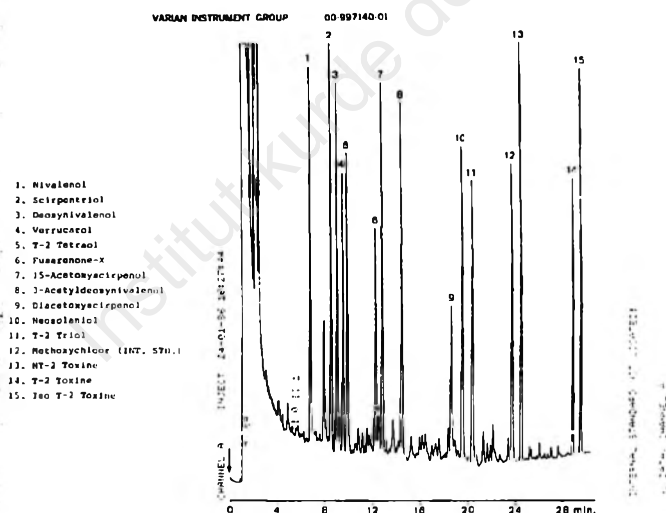


FIG. 15. — A separation of 14 trichothecenes (HFB-derivatives) on a CP^{tm}Sil 5 fused silica capillary column.

A gas chromatographic separation of the HFB-derivatives of 14 trichothecenes is shown in figure 15. Methoxychlor (peak n° 12) was chosen as the internal standard.

An example of an identity confirmation performed on the same extract is given in figure 16. Chromatogram A was obtained by injecting a derivatized extract (HFBI) of the urine, originating from

an Iranian soldier, who had been exposed to chemical warfare agents in 1985.

The chromatogram showed two suspected peaks, possibly corresponding with Verrucarol and T-2. Chromatogram B was obtained by injecting the same extract together with a small amount of a freshly derivatized standard mixture containing Verrucarol, 15-Acetoxyscirpenol, Methoxychlor and T-2. As a result a little peak shift was observed at both suspected peaks, while the peak-height of Methoxychlor was gently increased. By this

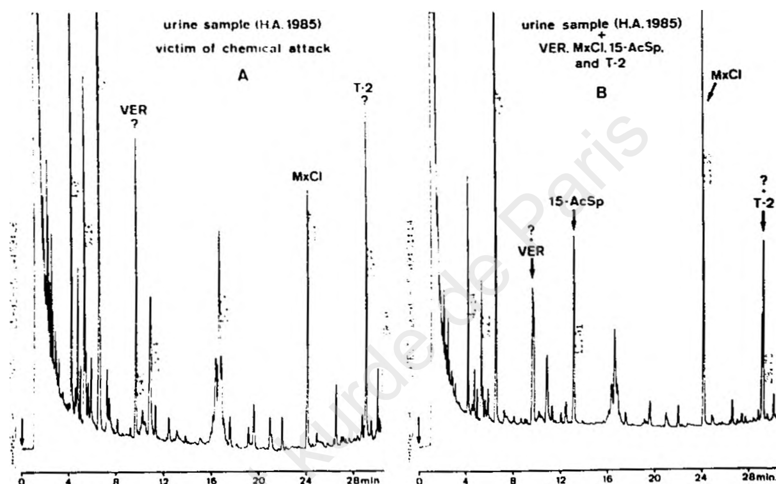


FIG. 16. — A : chromatogram of a derivatized urine extract with two suspected peaks ; B : chromatogram of the same extract after addition of Verrucarol, 15-Acetoxyscirpenol, Methoxychlor and T-2 (HFB-derivatives).

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experiment it was clearly demonstrated that the two suspected peaks were not due to Verrucarol and T-2.

The result of a gas chromatographic analysis performed on a corn extract previously treated with heptafluorobutyrylimidazole is shown in figure 17. The chromatogram of the derivatized corn extract showed a peak with the same retention time as the one of the derivatized Deoxynivalenol standard.

The remaining part of the original corn extract was silylated and analysed by GC/MS. The gas chromatograph was equipped with a 25 m capillary column (OV1) and the mass spectrometer (E.I., 70 eV) was operating in the scan mode. The reconstructed ion chromatogram (RIC), presented in figure 18, showed a small peak with the same retention time as the one of a silylated Deoxynivalenol standard analysed in the same conditions.

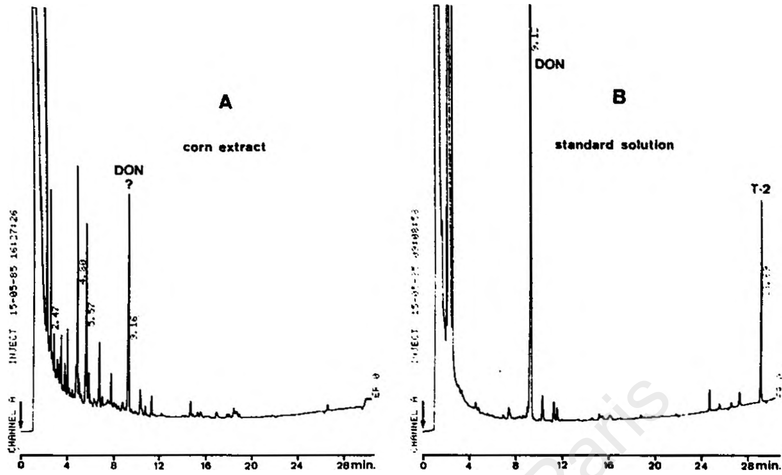

FIG. 17. — A : chromatogram of a derivatized corn extract (HFBI) ;
B : chromatogram of a standard mixture containing the HFB-derivatives of Deoxynivalenol and T-2.

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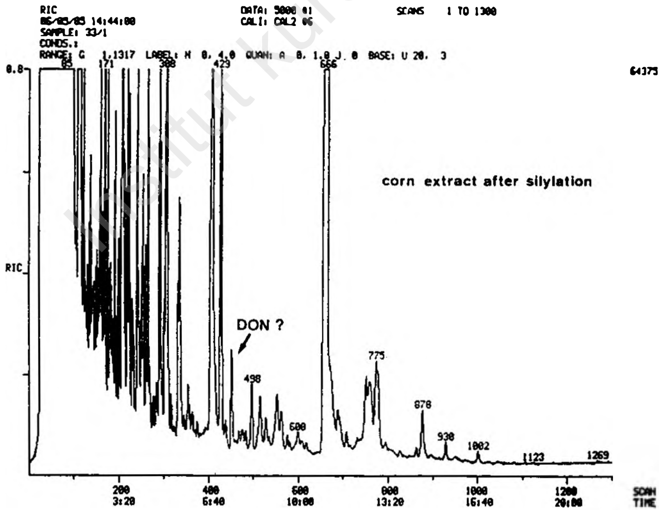

FIG. 18. — Reconstructed ion chromatogram (RIC) obtained after injection of the silylated corn extract.

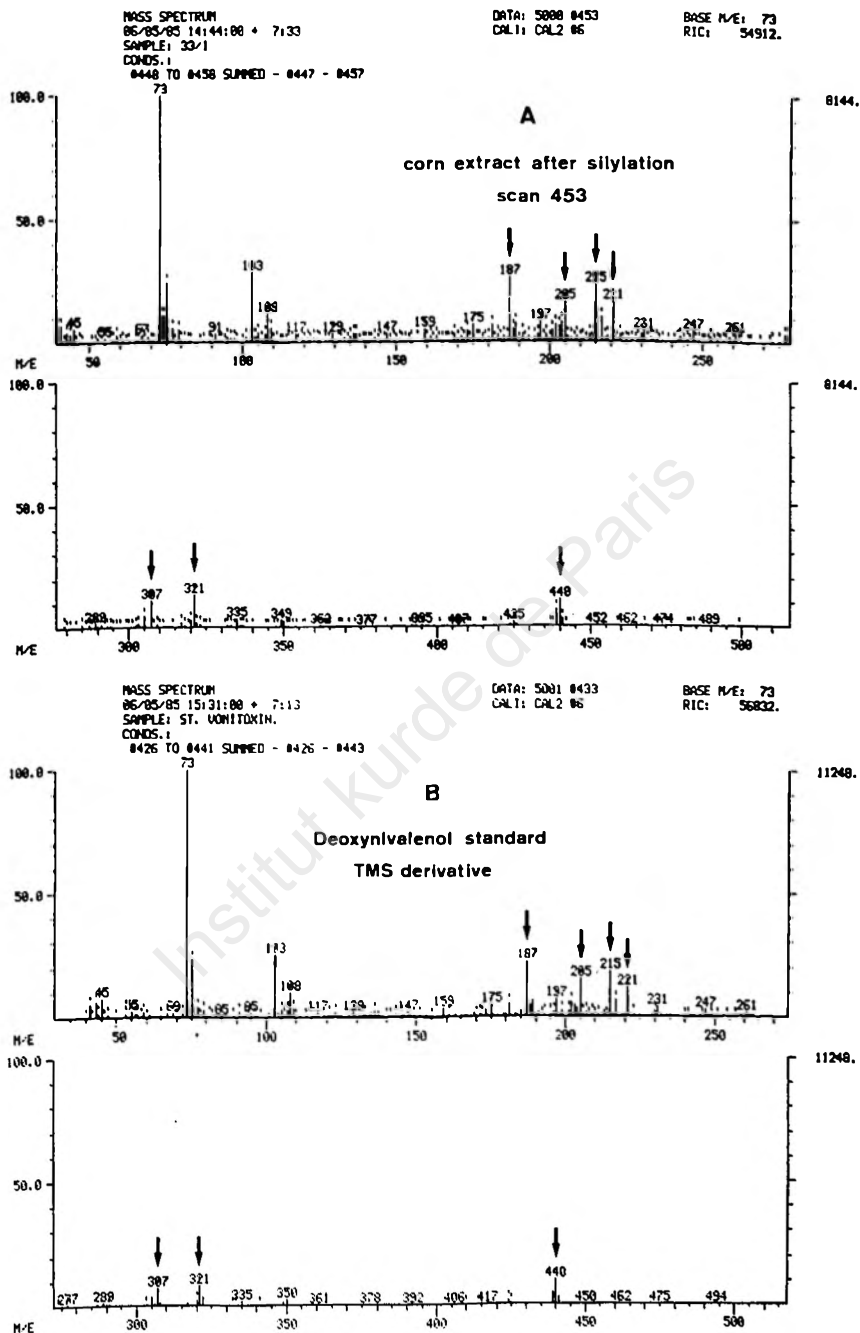

FIG. 19. — A : full mass spectrum (scan 453) of the unknown peak in the RIC of the silylated corn extract ;

B : full mass spectrum of a silylated Deoxynivalenol standard.

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As shown in figure 19, the mass spectrum (scan 453) of the unknown peak in the RIC of the silylated corn extract, was completely identical with the mass spectrum of a silylated Deoxynivalenol standard, confirming in that way the results of the former gas chromatographic analysis.

GENERAL CONCLUSIONS.

From the different toxicological findings collected during the last 3 years, the following conclusions are drawn :

1. None of the samples analysed in 1984, 1985 and 1986 showed enhanced arsenic values as compared to the reference values. That means that the use of inorganic or organic arsenicals is excluded.

2. Some of the samples analysed in 1985 and 1986 showed too high cyanide concentrations as compared to the reference values. The highest values found amounted 4 times the highest reference limit. This led to the conclusion that some of the soldiers had also been exposed to suffocating agents of the cyanide type.

3. In all the samples received in 1984, 1985 and 1986 it was impossible to detect sulfur mustard as such. There was only one exception where sulfur mustard as such was detected in a skin sample originating from London in 1985.
However, many urine samples showed even strong positive reactions with the KANAG ADETOX FIELD TEST, which was modified in order to enable its applicability on human biological samples. Blank urine samples taken from non exposed individuals, either or not under treatment with pharmaceuticals, didn't show any positive reaction with the modified system. This means that even to day, it still remains a question-mark whether or not these positive reactions were due to Yperite or to some of its metabolites.

4. Some patients showed lowered plasma cholinesterase activity values. In some cases this was in conformity with symptoms of nerve gas poisoning. In other cases where no symptoms of an organophosphate intoxication were observed, this was furthermore correlated with a liver pathology.

5. Another very important phenomenon was the fact that the blood samples analysed in 1984 showed very high methemoglobin

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levels of even up to 10 % (13). The blood samples analysed in 1985 and 1986 all showed normal methemoglobin levels. This could be brought in relation with another finding that mytotoxins of the trichothecene family were detected in several samples received in 1984 (14), while all the samples analysed in 1985 and 1986 didn't show any trace of those toxins.

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 either due to mycotoxins or to the other chemical agents, the more that according to eyewitness bombs and shells with different agents, giving rise to fumes or gas clouds, with a different taste and color, were used at the same time. This also means that the soldiers could become more or less intoxicated by different agents, according to their positions on the battlefield at the time the bombs or shells were dropped.

About six weeks after having performed the first analyses, the samples which were found to be positive for trichothecenes were extracted and analysed again by GC/MS, and no trichothecenes could be found.

When the gas chromatographic analysis with electron capture detection was repeated again on the same samples, also no peaks indicating the presence of trichothecenes were observed anymore, what led to a first conclusion that trichothecenes are easily broken down in biological samples, even when those samples are kept in the refrigerator. It also led to a second conclusion that the chromatographic peaks as observed during the first analysis were not due to interfering or co-extracted substances.

The instability of trichothecenes in biological samples was completely confirmed by some experiments with spiked samples, as it was found that after keeping the spiked samples for one week in the refrigerator, no traces of trichothecenes could be detected anymore, and these results were in conformity with some experiments of Mirocha, Minnesota, USA (15), who found that the addition of sodium azide to preserve the trichothecenes in blood samples, was ineffective.

The observation that trichothecenes are very instable in biological samples, and the controversial finding that those compounds were still detectable so many days after the exposure, point to the possibility of a storage depot for the toxins, what means that trichothecenes can remain in the human body for some time, before being eliminated.

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This theory is also supported by the animal experiments of American scientists who found that the elimination half life of trichothecenes depends upon the route of administration and that the skin appears to be functioning as a reservoir (14).

There were also remarks that the trichothecenes, if present in the samples of the exposed soldiers, were due to the intake of moldy food.

However, blood and urine samples, taken from individuals fighting or staying (like one of us) on the same battlefield, eating the same food, but not being attacked by chemical or biological agents, were analysed too by the same gas chromatographic procedure. No peaks with the same retention times of the trichothecenes were observed, what implicates that those findings also excluded any naturally occurring background of trichothecenes.

As a final conclusion it can be stated that in 1984 there were toxicological proofs for the use of sulfur mustard, nerve gases and trichothecenes, while in 1985 and 1986 there were toxicological evidences for the use of sulfur mustard, nerve gases, and also for an exposure to cyanide compounds.

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Les principes de la formation des personnels militaires à la défense contre les armes chimiques et biologiques

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1. GENERALITES - DEFINITIONS.

La défense des forces armées contre les effets des armes chimiques* est basée sur le principe fondamental suivant : un combattant (ou un élément de forces) dont la vie serait préservée mais qui ne serait plus susceptible de disposer des capacités physiques ou psychologiques nécessaires pour servir ses armes, pour donner, recevoir ou exécuter des ordres, pour juger des situations et réagir dans l'instant, deviendrait une proie facile pour l'adversaire. Celui-ci atteindrait ainsi aisément ses objectifs, qu'il ait anéanti ou non les personnels des unités attaquées. En conséquence, la défense chimique et biologique des forces militaires a pour finalité de permettre non seulement la survie, mais aussi la poursuite du combat malgré l'éventuel emploi de telles armes par l'adversaire.

Il s'agit, désormais, d'une doctrine généralement admise par les armées des nations occidentales. Elle suscite d'abord une remarque : l'objectif visé est très ambitieux. Elle pose ensuite un problème de fond : l'arme chimique est-elle une arme comme les autres ? Psychologiquement, non car si l'arme chimique était réellement une arme ordinaire, le raisonnement ci-dessus serait

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* Il existe une certaine tendance à confondre armes chimiques et armes biologiques et à utiliser indifféremment les expressions « défense contre les armes chimiques » et « défense contre les armes chimiques et biologiques ». En fait, les armes biologiques sont extrêmement diverses et la défense contre certaines d'entre elles échappe complètement aux actions locales. Il faudra donc comprendre ici : « la défense contre les armes chimiques et les formes des armes biologiques qui s'en rapprochent ».

inutile ; il est en effet banal, quelle que soit l'arme que l'on ait en face de soi, de viser à minimiser les pertes et à conserver le meilleur potentiel opérationnel possible. Puisqu'on éprouve le besoin de l'affirmer, plus encore puisque cette doctrine est considérée comme récente, c'est bien que le contexte est différent. Cette opinion est corroborée par plusieurs arguments spécifiques : le caractère effrayant attribué à l'arme chimique, son environnement psychologique et émotionnel, son emploi relativement limité au long de l'histoire, enfin le Protocole de 1925 qui en interdit, au plan des principes, l'utilisation sur le champ de bataille. L'arme chimique serait donc une arme tout à fait particulière.

Mais, dans la pratique, le raisonnement est tout autre. Il existe aujourd'hui par le monde des centaines de milliers de tonnes d'agents toxiques, une vingtaine de nations qui en ont la maîtrise, de sérieuses allégations d'emploi, la volonté délibérée prêtée à certains d'en faire un atout pour marquer un avantage décisif. On aboutit, qu'on le veuille ou non, à une certaine banalisation, à une certaine « conventionnalisation » de l'arme chimique.

Mieux vaut alors s'éloigner des définitions et des idées reçues et admettre que, si l'arme chimique n'est pas tout à fait une arme comme les autres, cela n'interdit pas d'ouvrir les yeux et d'adopter une position réaliste fondée sur l'hypothèse la plus contraignante : prendre toutes les dispositions nécessaires pour faire face à l'éventuel emploi de l'arme chimique sur le champ de bataille et éloigner ainsi le risque d'une surprise aux conséquences irréparables.

C'est pourquoi l'armée française, comme toutes les armées alliées, prononce désormais un effort d'envergure pour disposer, dans ce domaine comme pour toutes les autres composantes de la bataille, d'une capacité de résistance complète et cohérente, et pour tenter d'éliminer définitivement ce qui fut considéré longtemps comme un talon d'Achille.

Cette capacité de défense s'acquiert au prix, notamment, d'un équipement et d'une formation. La formation, objet du présent exposé, exige la conviction, met en jeu des moyens spécifiques et nécessite des structures d'enseignement. Nous allons examiner successivement ces trois composantes.

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2. LA PREPARATION INTELLECTUELLE : LA CONVICTION.

Deux obstacles sont à vaincre :

— l'incrédulité,

— les difficultés pratiques.

2.1. Aspect psychologique : l'incrédulité.

Les personnels susceptibles d'être confrontés au danger chimique ont une tendance naturelle au scepticisme. Celui-ci est fondé :

a) D'une part, sur le caractère impalpable du danger chimique ; il est très difficile de se préparer à faire face aux effets d'un risque insidieux, invisible, inodore, silencieux et qui prend ainsi un aspect quelque peu mythique ; il y a donc un effort constant à effectuer pour persuader les personnels de la réalité de cette menace et pour l'inciter à se sentir concerné.

b) D'autre part, sur certaines leçons apparentes de l'histoire ; nul n'ignore que si les armements chimiques ont connu un développement majeur lors de la première guerre mondiale, ils ont fait l'objet d'une abstention totale en 1939-1945. Cette constatation a longtemps stérilisé la réflexion dans la mesure où l'impression prévalait, même dans les sphères responsables, que l'arme chimique était définitivement dépassée. Il faut donc répéter sans relâche les raisons, aujourd'hui bien connues des spécialistes, pour lesquelles ce raisonnement est totalement caduc : motifs de l'abstention de 1939-1945, progrès énormes de la toxicité des agents et de la puissance des moyens de dispersion, existence et prolifération des stocks.

c) Enfin, sur une interprétation restrictive et erronée du protocole de 1925 ; beaucoup croient que ce texte élimine la guerre chimique comme la Convention de 1972 interdit la guerre biologique ; il faut donc expliquer que le protocole de Genève n'interdit pas la possession de l'arme chimique, que la plupart des nations se sont réservé le droit de l'employer en riposte et que, dans ces conditions, nous sommes loin d'être à l'abri : toute violation même mineure, toute provocation peut ainsi conduire à une explosion chimique généralisée.

2.2. Aspect pratique.

La défense contre l'arme chimique nécessite d'importants efforts dont les résultats sont abstraits et peu mesurables.

Sur le terrain, elle impose à l'homme le port prolongé de masques ou de tenues qui accroissent notablement son état de fatigue, surtout si les conditions météorologiques sont défavorables (chaleur, humidité...). Elle restreint son champ de vision, sa vitesse de déplacement, sa capacité à accomplir certains gestes simples ; elle gêne les actes physiologiques les plus élémentaires ; elle complique le service des matériels, elle impose l'utilisation de toute une gamme d'équipements supplémentaires. Si le combattant est enfermé dans un char, la protection ajoute aux contraintes inhérentes au combat proprement dit un effet de confinement qui peut être assez pénible.

Le chef a du mal à commander, ses hommes le voient et l'entendent mal, la fatigue réduit leur capacité d'attention, il ne les reconnaît que difficilement.

Dans les états-majors, les effets de deux phénomènes se combinent. D'abord, les contraintes inhérentes au travail de chacun, différentes mais de même nature que celles ressenties par le simple combattant ; vivre, voir, entendre, parler, lire, écrire, réfléchir, juger, décider, dans des conditions particulières. Ensuite, l'infléchissement du raisonnement tactique : le ralentissement, l'arrêt, le variantement d'une manœuvre, l'estimation des pertes, la remise en condition des unités, la prise de décisions spécifiques, toutes ces difficultés pour un danger que l'on ne voit pas et dont on imagine aussi mal la nature que les effets. Tout ceci crée une situation désagréable, parfois proche du malaise. Il faut donc beaucoup d'imagination ou beaucoup d'habitude pour se persuader qu'en procédant de la sorte, on ne perd pas son temps par rapport à l'étude et à la résolution des problèmes liés à des menaces plus concrètes et plus familières.

Cet effort doit être mené sans relâche : il s'agit d'une lutte qui n'est jamais complètement gagnée.

3. LES MOYENS D'INSTRUCTION ET D'ENTRAINEMENT.

Les nécessités de la formation pratique des personnels militaires sont multiples. Elles ont pour but d'accéder à la capacité d'opérer normalement en milieu chimique. Elles supposent :

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— l'instruction sur la mise en œuvre des matériels spécifiques,

— l'accoutumance à la contrainte, qui nécessite elle-même la satisfaction de deux besoins :

  — la familiarisation avec les moyens de protection,
  — la simulation d'une atmosphère réaliste.

3.1. La mise en œuvre des matériels spécifiques.

Comme vis-à-vis des autres menaces, la défense chimique suppose la mise en place dans les forces de matériels spécialement conçus pour satisfaire l'une ou l'autre des composantes de la défense. C'est le cas, par exemple, des détecteurs d'alerte, des appareils de décontamination, etc. Ceux-ci sont souvent plus complexes dans leur conception que dans leur utilisation mais ils doivent toujours être mis en œuvre par du personnel convenablement formé à cet effet et familiarisé avec leur maintien en condition.

Mais il ne s'agit pas d'un type d'action original à la guerre chimique. Nous ne le développerons donc pas davantage.

3.2. L'accoutumance aux contraintes.

3.2.1. *Les moyens de protection.*

Il est indispensable que l'homme soit entraîné en temps de paix à supporter les inconvénients causés par les équipements de protection (vêtement, masque, atmosphère confinée). On utilise souvent pour cela, pour des raisons de maintenance et d'économie, des matériels réservés à l'instruction tels que des vêtements d'instruction moins coûteux, disponibles en plus grand nombre et réutilisables. Dans d'autres cas, il est plus commode de mettre en service directement certains matériels en dotation, par exemple les masques, mais alors il est indispensable de respecter un certain nombre de précautions d'utilisation pour éviter de les détériorer et de surveiller très soigneusement leur entretien pour qu'ils soient en parfait état en cas de besoin.

3.2.2. *L'ambiance chimique.*

Ainsi que nous l'avons vu au paragraphe précédent, il est parfois difficile de convaincre les personnels à l'entraînement de

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l'intérêt ou de la réalité de la menace toxique. En outre, la protection est contraignante. Le personnel peut donc céder à la tentation de s'affranchir de la gêne qui lui est imposée, par exemple en prenant des libertés avec le port du masque ; on ne peut, en effet, mettre en permanence un instructeur ou un cadre derrière chaque soldat, à supposer que la tentation se limite à l'échelon des exécutants.

En outre, il est nécessaire de permettre à l'encadrement de contrôler l'instruction, de sanctionner les erreurs, de faire la chasse aux maladroits ou aux négligents et même, dans certains cas, de tester la qualité et l'adaptation des moyens de protection.

L'instruction de la défense chimique impose donc, pour atteindre son but, de matérialiser le risque avec un certain réalisme. Bien sûr, ce problème ne peut pas être résolu avec des toxiques qui sont, par définition, beaucoup trop dangereux.

On utilise alors toute une gamme de produits simulants qui nécessitent, en outre, des munitions et des moyens de lancement spécifiques. Ce sont le plus souvent des agents lacrymogènes qui « pénalisent » sans danger.

Les lacrymogènes, qui existent sous de nombreuses formes, d'une innocuité presque totale, sont dispersés par des procédés de simulation devenus classiques : épandages par avions rapides qui couvrent en quelques secondes de vastes surfaces avec une pseudo-contamination visible et intéressent donc des unités d'une certaine importance, épandages par avions lents ou par hélicoptères plutôt destinés à l'instruction de petites équipes, nuages créés par les échappements de divers moteurs, lancement de projectiles contenant des quantités limitées de produit liquide et explosant au-dessus d'une troupe... Ces procédés peuvent varier presque à l'infini ; certains font appel tout simplement à l'ingéniosité locale ; ils sont en général peu coûteux et très efficaces.

Ils peuvent en outre être étendus à des exercices de simple démonstration du fait chimique, sans emploi de lacrymogène ; par exemple, un épandage par avion rapide peut être non pénalisant : il illustre l'attaque, montre les dimensions et la nature de la contamination au sol et sur les matériels et permet de lancer des opérations de décontamination.

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4. L'ENSEIGNEMENT.

La conviction acquise, les moyens nécessaires mis en place, il faut transmettre les connaissances. Cet enseignement est adapté aux grades et aux fonctions mais il possède deux caractères essentiels :

— il concerne la totalité des personnels car nul, quel que soit sa tâche ou son rang, n'est à l'abri des effets de l'arme chimique,

— il se compose d'une instruction générale et d'une instruction spécialisée car si chacun est concerné, il faut des spécialistes pour conseiller les responsables et diriger les actions spécifiques.

4.1. L'universalité du problème.

Pour que la totalité du personnel soit concerné, il existe deux solutions. On peut soit constituer un corps chimique, chargé d'appuyer et de soutenir les unités sur le terrain et en temps de paix d'étudier et d'expérimenter les doctrines et de constituer un vivier pour l'instruction et l'information de tous. Cette solution comporte le risque d'une certaine déresponsabilisation de nombreux personnels vis-à-vis d'une menace qu'il est déjà trop tentant d'éloigner. C'est pourquoi on peut aussi faire, concrètement, de la défense NBC l'affaire de tous. Cette dernière solution, qui est notamment celle de l'armée française, introduit des responsables à tous les échelons dans tous les types de formations. Elle présente, en contrepartie, l'inconvénient de créer très peu de postes de spécialistes à temps complet.

En fait, les deux solutions présentent chacune ses avantages et ses inconvénients et le débat théorique reste toujours ouvert.

4.2. L'organisation de l'instruction.

Mais les deux procédés se rejoignent sur certains principes. Il est toujours nécessaire de disposer d'un centre d'instruction spécialisé. En France, il existe à cet effet une école de spécialisation organisant des stages très divers, assez nombreux et qui se trouve fréquentée par les personnels les plus variés, du simple caporal au colonel chef d'état-major de division. Si l'on

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désire que l'armée bénéficie d'une instruction complète et cohérente, une vingtaine de cours différents sont nécessaires. Mais bien entendu, la totalité de l'enseignement ne peut être dispensée dans une telle école, et l'instruction de type général doit être effectuée au sein même des unités.

A titre d'exemple, on décrit ici l'organisation de cette instruction dans l'armée française.

4.2.1. *Instruction générale (appelée également instruction toutes armes).*

Elle concerne tous les grades et tous les niveaux. En effet, chacun en fonction de ses responsabilités, doit acquérir des connaissances chimiques nécessaires à l'accomplissement normal de sa mission.

— Les militaires du rang reçoivent quelques heures d'instruction chimique lors de leur formation initiale et une instruction complémentaire tout au long de leur service. Cette instruction est évidemment dispensée au sein même du corps de troupe, de la même façon que les autres composantes de la formation militaire : sport, tir, armement...

— Les sous-officiers suivent une instruction à chacun de leurs niveaux de formation et notamment doivent acquérir les connaissances figurant au programme des certificats militaires permettant de franchir les grades et les échelons de solde.

— Les officiers sont instruits à trois niveaux :

— à l'école d'officiers, lors de leur formation initiale,

— à l'école d'application (école d'arme),

— lors du stage des capitaines qui intervient l'année de leur promotion à ce grade.

4.2.2. *Instruction des spécialistes.*

Celle-ci se déroule donc dans une école dont c'est la mission ; en France, c'est l'Ecole de défense NBC de l'armée de terre à Caen. L'enseignement de cette école concerne surtout, mais pas exclusivement, les cadres qui occupent des fonctions de responsabilités, soit dans la mise en œuvre de moyens spécifiques, soit en tant que conseiller du commandement. Les officiers suivent deux types principaux de stages qui durent deux ou trois semaines : officier de défense NBC du régiment, officier de dé-

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fense NBC d'état-major. Les sous-officiers peuvent suivre **3 types** de cours :

— sous-officier d'unité élémentaire ; ce stage de trois semaines correspond à la formation nécessaire aux sous-officiers de défense NBC de toutes les unités élémentaires de l'armée française ; ce n'est pas à proprement parler un stage de spécialité ;

— sous-officier chef de groupe de défense NBC de régiment ;

— sous-officier adjoint à l'officier de défense NBC de régiment.

Ces deux stages sont nettement spécialisés puisqu'ils sont couronnés par des certificats techniques de défense NBC ; aussi sont ils nettement plus longs (huit à six semaines) et supposent l'acquisition de connaissances approfondies dans les domaines de la menace, de la connaissance des divers phénomènes et des doctrines, procédures et équipements de défense.

Enfin, certains militaires du rang sont instruits à l'Ecole dans 4 branches spécialisées ; décontamination, reconnaissance, graphiquage, chef d'équipe.

5. CONCLUSION.

Ce court exposé visait à analyser les besoins en formation d'une armée moderne face à la menace de la guerre chimique, et à donner un aperçu des solutions auxquelles il est fait appel.

Ces besoins sont nombreux et complexes ; ils sont donc très coûteux en personnel et en matériel, mais leur satisfaction constitue une nécessité vitale pour éloigner les dangers d'une menace mortelle.

Il va de soi que les impératifs financiers, les délais et la disponibilité des personnes situent la défense civile sur un tout autre plan. Par ailleurs, nous l'avons vu, l'objectif n'est pas le même.

Mais si cet exposé, à travers l'intérêt que portent les armées occidentales à la menace chimique, a montré le poids et la nature des efforts de formation à consentir pour y parer et décrit certaines solutions transposables à la défense non militaire, il aura atteint son objectif.

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Chemical weapons : Chaos and misunderstanding

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INTRODUCTION.

In 1984 the Dutch government, recognizing the danger of unlimited and uncontrolled exportation of chemicals, announces its determination to start the control on the export of chemicals which can be used for the production of CW agents. And negociations were started with the European partners in order to standardize export limitations within the EC.

The reaction of the other nations has been far from enthousiastic however, and since April '84 a list of 11 chemicals has been effective for which an export license is needed for export outside the European Community.

However in the beginning of 1986 it was discovered that, in spite of these export limitations, Dutch companies have been involved in delivery of chemicals for the preparation of war gases to Iraq.

Therefore it seemed interesting to investigate the availability of chemicals for the production of CW agents and the way in which the export restrictions are enforced.

This investigation has been part of a 14-day project « Chemistry and Society » within the study program of first-year chemistry students at our University.

AVAILABILITY OF CHEMICALS FOR CW PRODUCTION.

In view of the limited time it was decided to restrict the research to the chemicals necessary for the production of the five wargases in table I.

These were chosen as being the most representative and because of the fact that these nerve- and blister gases are no gases but high-boiling liquids. This greatly facilitates synthesis, storage and handling. They are most effectively employed by vaporization, by either an explosion or spraying.

TABLE I

Compounds studied in this research

Name	*Formula*	*Mode of action*
Mustardgas	$S(CH_2CH_2Cl)_2$	Vesicant
Nitrogen mustardgas	$N(CH_2CH_2Cl)_3$	Vesicant
Lewisite	$\genfrac{}{}{0pt}{}{Cl}{H} > C = CH - AsCl_2$	Vesicant
Tabun	$EtO - P(=O)(CN) - N(CH_3)_2$	Anti-choline esterase
Sarin	$Pr_i O - P(=O)(F) - Me$	Anti-choline esterase

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The mustardgases are by far the easiest to manufacture (1). And also they are not as lethal as the nerve gases, easier to employ and moreover, they are cheaper.

The reaction mechanism and synthesis are readily found in chemistry books and even complete descriptions of the industrial processes are easily obtained (2).

The nerve gases are somewhat more difficult to produce, but still within the capacities of any chemist (3). They are however extremely poisonous (LD50 ~ .8 mg/person) and thus great care has to be taken, since they are readily absorbed through the skin. Lewisite takes an intermediate position both in synthetic problems as well as in lethality (4).

Thus in order to produce the CW agents studied, the chemicals in table II are crucial. However as can be seen from this table, the chemical industry is perfectly willing to supply these chemicals.

It should be realized of course, that these chemicals are not only used for the production of war gases, but that they are applied in a very large field : insecticides, plastics, photo-industry, electronics, etc.

TABLE II

Availability of key-chemicals, prices in US $/kg excl. VAT

Compound	*Janssen Chimica*	*Aldrich*
Thionylchloride	6.10	6.60
Thiodiglycol	77.—	51.10
Triethanolamine	6.70	9.60
Arsenic (III) oxide	28.—	89.80
Phosphorus oxychloride	11.40	14.30
Phosphorus trichloride	12.40	11.—
Sodiumcyanide	8.60	8.80
Sodiumfluoride	12.60	16.—
Dimethylamine	21.—	18.90

Upon calling a chemicals supplier as private persons, i.e. without stating a company or university name, we were able to obtain large quantities of both triethanolamine and thionylchloride. Not only were huge reductions on catalog prices given, there were no restrictions whatsoever regarding the delivery. Thus we could obtain 22.5 kg of triethanolamine at $ 4.43/kg (a 30 % reduction) and 60 kg of thionylchloride at $ 1.70/kg (a 70 % reduction).

This means that for only $ 240.— including VAT we could have produced 27 kg of nitrogen mustardgas. This quantity would be enough to kill 1,000,000 people (LD50 ~ 12 mg/person).

However, a more or less chemical background is still required for the synthesis of the nitrogen mustardgas.

But, for the non-chemists among us : do not worry ! Both Janssen and Aldrich also deliver the nitrogen mustardgas itself. They do not have much in stock, but are perfectly willing to synthesize 25 kg for interested parties. The price was not available but we could be sure to get considerable reductions compared to the catalog price. Also in this case there are no restrictions to delivery. This was confirmed by an official of the customs.

So, having established that anyone in the Netherlands can easily obtain large quantities of chemicals necessary to manufacture CW agents, lets turn our attention to the export.

EXPORT REGULATIONS CONCERNING CHEMICALS.

As mentioned in the introduction, since 1984 the Dutch government requires that an export license is needed for 11 key-chemicals, as is laid down in the « Export resolution Strategic Goods ». These 11 compounds are listed in table III.

Although this list seems extensive it is not. There are some chemicals suspiciously absent : mustardgas itself does not appear on the list nor any arsenic compounds crucial to the synthesis of lewisite or adamsite and neither do any of the following wargases, very successfully used in WW I : phosgene, chlorocyanide, chloropicrin and hydrogen cyanide.

TABLE III

List of key-chemicals for which an export licence is needed

A. Phosphorus oxychloride
B. Phosphorus trichloride
C. Chemicals containing a phosphor-methyl and/or ethyl bond
D. Esters of phosphoric acid
E. 3,3 dimethylbutanol-2 (pinacolylalcohol)
F. N,N-disubstituted-β-aminoethanols
G. N,N disubstituted-β-aminoethanethiols
H. N,N-disubstituted-β-aminoethanehalides
I. Phenyl-, alkyl-, or cycloalkyl substituted glycol acids
J. 3- or 4-hydroxypiperidine and its derivatives
K. Thiodiglycol

Apart from the shortcomings of the list their are 2 other major problems which make adequate control impossible :

1. There are no restrictions to the export of chemicals within the European Community. And since most countries hardly require any form of export license, chemicals for the manufacture of wargases can still be exported to anywhere in the world.

2. The second problem is the lack of knowledge about chemistry and esp. chemical names, within the departments responsible for giving and verifying export licenses.

Upon informing by phone whether or not it would be allowed to export thionylchloride and triethanolamine, the basic chemicals for the production of nitrogen mustardgas, officials from both the Ministry of Economic Affairs and the customs in Europoort (the port of Rotterdam) assured us that for these chemicals no export license is needed since they do not appear on the list of key-chemicals.

In fact however, triethanolamine belongs to the group of the N,N-disubstituted-β-aminoethanols and as such does appear on the list.

Due to a lack of knowledge however this is not recognized. Considering that most chemicals have more than one name one

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wonders how many tons of chemicals pass our borders each year unnoticed, for which in fact an export license would have been mandatory.

CONCLUSIONS.

Even though it is generally recognized that the Dutch government takes a leading position in controlling chemicals for the production of CW agents, lack of knowledge of chemicals within the various governmental Departments and the absence of agreement within the European Community regarding export regulations greatly reduce the effect of the measures taken so far. Therefore the present situation is such, that almost anyone can obtain large amounts of chemicals for CW production and convert these into wargases without any control.

The use of for instance nitrogen mustardgas (the easiest and cheapest to produce) by various terrorist organizations in Europe is at this moment highly feasable.

We therefore would like to make some recommendations.

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RECOMMENDATIONS.

— Fabrication and storage of all CW should be forbidden.
— The existing list of key-chemicals should be extended.
— Uniform export restrictions for chemicals on this list should apply within the European Community.
— Customs and government agencies dealing with export licenses of chemicals should have a chemical background.
— Governments should act as intermediate between buyers and suppliers of these key-chemicals in case of large orders.
— The suppliers of key-chemicals should keep an extensive registration of all buyers, even in case of small quantities. This registration should preferably be international and centralized.

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Combat casualty resuscitation in the contaminated environment

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SUMMARY.

The presentation through lecture and audio-visual aids will tackle the problem of the combat medic, military para-medic, Regimental Medical Officer and Regimental Medical Officer Assistant in treating combat casualties with ballistic injuries and associated trauma under contaminated conditions.

Jim Shortt will also demonstrate some of the equipment described in the lecture such as Military Anti-Shock Trousers (MAST), developed for the combat environment to assist circulation support.

Also, covered will be personnel and casualty protection, pharmaceutical prophylaxis and management of fractures and burns in combat when chemical and biological toxic agents are present.

This type of combat casualty care is called Emergency Victim Care (EVC) rather than Military First Aid.

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General Assembly

Conclusions

by A. HEYNDRICKX

Chairman

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1. The scientific conclusions of the First World Congress « New Compounds in Biological and Chemical Warfare : Toxicological Evaluation » that stated that chemical and biological warfare agents were used in the world namely : Yellow Rain (mycotoxins), Mustard Gas (Yperite), Tabun and Cyanide, are confirmed.
   Different independent internationally known scientists confirmed the techniques as used at the Department of Toxicology, State University of Ghent (Director : Prof. A. Heyndrickx), analysing environmental and human samples. Those techniques that were published completely in the Proceedings of the First World Congress could be repeated without any discussion.

2. The treatment of patients intoxicated by those chemical and microbiological warfare agents was discussed, compared to the treatment applied by different European university clinics, evaluated and improved. The civil population which has to pay the heaviest contribution was also discussed and methods set forward to protect them.

3. The Industrial Chemical Disasters as in Seveso (Italy) and Bhopal (India) were fully investigated, the cause of negligence described and the measures set forward, so that those massive intoxications of people can be avoided. The treatment also for intoxicated people was investigated.

4. Environmental disasters as in Cameroun, where toxic gases from natural origin are the cause of death, were chemically analytically and toxicologically discussed. Disaster plans of

action and the medical and toxicological teams needed are proposed.

5. The Tsjernobyl case was discussed ; also the environmental radiotoxicological evaluation of samples.

6. Terrorism. The analysis of explosives and the residual levels were discussed, the methods of detection to conclude to the kind of explosives that were used and the origin of them. A very important exchange between the different international specialists was achieved in order to combat these actions.

7. Drug problems. Control and distribution, the toxicity on man and the catastrophies of our society were discussed, and a plan of action given. The transplacental diffusion from the mother to the foetus and the difficult treatment of the newborn was discussed, evaluated and methods proposed for the intensive care unit.

8. The anti poison centers and the toxicology laboratories in the different parts of the World will exchange even more than in the past, on a direct contact basis between themselves, the results that are obtained and the methods used.

9. All participants agreed to cooperate fully and hope and are insisting, to have as soon as possible a Third World Congress.

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