The Problem of Chemical and Biological Warfare

Volume VI

Technical Aspects of Early Warning and Verification

SIPRI

Stockholm International Peace Research Institute

Almqvist & Wiksell International
Stockholm, Sweden

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New York

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Stockholm International Peace Research Institute

SIPRI is an independent institute for research into problems of peace and conflict, with particular attention to the problems of disarmament and arms regulation. It was established in 1966 to commemorate Sweden's 150 years of unbroken peace.

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SIPRI

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THE PROBLEM OF CHEMICAL AND BIOLOGICAL WARFARE

A study of the historical, technical, military, legal
and political aspects of CBW,
and possible disarmament measures

VOLUME VI

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Contents of the Study

Volume I. The Rise of CB Weapons

A description of the main lines of development in the technology underlying CBW and in the constraints affecting use of CB weapons. The period covered is approximately 1914–1945, although more recent developments in CW technology are also described. In addition, the volume includes an account of all instances known to SIPRI when CB weapons have been used in war, or when their use has been alleged; in this case the time-span is 1914–1970.

Volume II. CB Weapons Today

A description of the present state of CBW technology and of national CBW programmes and policies. It also includes a discussion of the attractions and liabilities of CB weapons, and of the consequences, intentional or unintentional, that might follow their use.

Volume III. CBW and the Laws of War

A description of the legal limitations on use of CB weapons. It comprises discussions of the field of application of the Geneva Protocol, particularly as regards non-lethal chemical weapons and anti-plant agents, of the existence, development and scope of the prohibition of CBW provided by the customary law of war, and of the application to CBW of general principles of the law of war. It also reviews the juristic works in this field.

Volume IV. CBW Disarmament Negotiations, 1920–1970

A review of the activities of the League of Nations and United Nations in extending and reinforcing the prohibitions concerning CB weapons, including a report of recent negotiations for international CB disarmament. The volume also contains an account of those instances when formal complaints of the use of CB weapons have been made to the two world organizations.

Volume V. The Prevention of CBW

A discussion of possible measures that might be adopted to prevent future CBW. The volume describes steps that might be taken to strengthen the legal prohibition of CBW, and the problems and possibilities, including those of verification, involved in the negotiation of CB disarmament.

Volume VI. Technical Aspects of Early Warning and Verification

A technical account of SIPRI research on methods of early warning and identification of biological warfare agents, together with a description of two experimental SIPRI projects on CB verification. The first project concerns the non-production of BW agents and involved visits to biological laboratories in several countries; the second concerns the nonproduction of organophosphorus CW agents and summarizes the results of a symposium.

PREFACE

The birth of this study of chemical and biological warfare can be traced back to 1964, when a group of microbiologists who were concerned about the problems of biological warfare started meeting under the auspices of Pugwash. After some meetings it became evident that there was need for more intense study than could be achieved through occasional gatherings of people who were busy with other work. In 1966–67 SIPRI, which was then starting up, decided to take on the task of making a major review of biological warfare. The study was soon extended to cover chemical warfare as well.

The aim of the study is to provide a comprehensive survey of all aspects of chemical and biological warfare and of the problems of outlawing it more effectively. It is hoped that the study will be of value to politicians, their advisers, disarmament negotiators, scientists and to laymen who are interested in the problem.

The authors of the report have come from a number of disciplines—microbiology, chemistry, economics, international law, medicine, physics and sociology and soldiery—and from many countries. It would be too much to claim that all the authors had come to share one precisely defined set of values in their approach to the problem. Some came to the problem because they were concerned that the advance of science in their field should not be twisted to military uses; others because they had taken a scholarly interest in the law or history of CBW; others because they had particular experience of military or technical aspects of it. What is true is that, after working together for a period of years, they have all come to share a sober concern about the potential dangers of CBW.

At an early stage it was necessary to face the question whether, if we assembled a lot of information on CBW and published all that we thought was relevant, we would risk contributing dangerously to the proliferation of these weapons. This proposition was rejected on the grounds that the service we could do by improving the level of public discussion was greater than any disservice we might do by transmitting dangerous knowledge. Secrecy in a field like this serves mostly to keep the public in ignorance. Governments find things out for themselves.

While the study has been in progress there has been much discussion of the subject. A group of experts appointed by the Secretary-General of the United Nations has produced a report on *Chemical and Bacteriological (Biological)*

Weapons and the Effects of their Possible Use. In the United States a rising tide of concern about CBW has given rise to Congressional hearings; a policy review, commissioned by the President, has led to the unilateral renunciation by the United States Government of biological weapons and to the decision to renounce first use of chemical weapons and to seek ratification of the Geneva Protocol. At the United Nations and at the Disarmament Conference in Geneva, CBW has received a lot of attention. A convention prohibiting the development, production and stockpiling of biological and toxin weapons has been concluded. Negotiations over a chemical disarmament treaty continue.

In response to an invitation from the UN Secretary-General, early drafts of parts of this study were circulated to his group of experts in February 1969. These drafts were also made available to the World Health Organization for the preparation of its own submission to the UN group of experts; this submission, together with the subsequent WHO publication based upon it, *Health Aspects of Chemical and Biological Weapons*, was prepared by a group of consultants that included Julian Perry Robinson from SIPRI.

The authors are conscious of the problem of avoiding biases. A disproportionate part of the information we have used comes from the United States. This is partly because the United States has been very active in the field of chemical and biological warfare in the post-war period. It is also because the United States is much more open with information than most other countries.

Since this is a team work and since, like most studies of this size, it grew and changed shape and changed hands in some degree as it went along, it is not easy to attribute responsibility for its preparation. The authorship of each part is indicated at the start of it, but these attributions do not convey the whole story. The team of people who produced the study met together often, shared material, exchanged ideas, reviewed each-others' drafts in greater or lesser degree, and so on. So it is a corporate product, and those who wrote the final drafts sometimes had the benefit of working papers, earlier drafts, ideas or material provided by others.

At first, Rolf Björnerstedt was briefly in charge of the study. After an interval, Carl-Göran Hedén took over. When he had to return to the Karolinska Institute—from which he has continued to give us his advice and help—Robert Neild assumed responsibility for the project. The other members of the team have been Anders Boserup, who from the earliest stages has found time to come frequently from Copenhagen to help on the project, Jozef Goldblat, Sven Hirdman, Milton Leitenberg, Åke Ljunggren, Theodor Nemec, Julian Perry Robinson and Hans von Schreeb.[1]

The work on rapid detection of the use of biological warfare agents (Volume

[1] SIPRI regrets that in Volume III of this study, Hans von Schreeb was mistakenly included in the list of team members responsible for that volume.

VI) was undertaken separately from the main study by Konstantin Sinyak, who came from the Soviet Union to work at the Karolinska Institute in Stockholm, and Åke Ljunggren, who went from Sweden to work at the Microbiological Institute in Prague. Both worked in close contact with Carl-Göran Hedén who contributed a study on automation. We are indebted to the two host institutes for the facilities and help they generously provided.

A great debt is also owed to many people outside the institute—too many to name—for the help they have given us. This includes those who attended the early Pugwash meetings on biological warfare, those who attended meetings at SIPRI on biological and chemical warfare, those who wrote working papers for us, those who gave their time to the biological inspection experiment and many people who have visited us or helped us with advice and material at different times. It includes people from many countries, East and West, and many disciplines. It includes people with many different kinds of expertise. The amount of help they gave us—and it was far greater than we had expected at the start—was clearly an expression of their concern about the problem. We are grateful to them all. The responsibility for what is said is, of course, ours.

August 1973

Frank Barnaby
Director

ATTRIBUTION

Carl-Göran Hedén edited Volume VI, adding microbiological and bioengineering considerations to the drafts provided by other authors. Åke Ljunggren wrote about indicators that might reveal BW development and discussed problems of detection and identification. Theodor Nemec described the SIPRI experiment on laboratory inspections and Johan Lundin summarized a symposium convened to consider inspection techniques for nerve gases. Two appendices, one on germ-free animals as producers of specific sera, and the other on gas chromatography/mass spectrometry in the detection of virus associated materials, were written by Å. Ljunggren and K. Sinyak, and co-writers, respectively.

CONTENTS

Introduction

This volume deals with some of the technical issues relevant to identification and early warning, which have served as background to the earlier volumes of the SIPRI six-volume study of chemical and biological warfare. It includes conference discussions and reports, some specialized background papers and two appendices (appendices 3 and 4) which describe laboratory studies initiated by SIPRI. Obviously the material must necessarily be rather heterogeneous, and it is unlikely that anyone will read the book from cover to cover. However, the specialists in the field, particularly those investigating various aspects of national or international control and inspection, should be able to find many items of interest if they consult the detailed table of contents.

The volume reflects a substantial amount of international collaboration; the biological inspection exercise, for instance, concerned 14 non-secret establishments in nine countries and, directly or indirectly, nearly 80 scientists. Other areas of study also required many discussions which are not recorded here. However, one discussion on the use of fluorescent antibodies (FA) has been included (appendix 2) in order to introduce the reader to the concern and the constructive and cooperative spirit which has permeated all our international contacts.

It might initially seem strange that the technical aspects of early warning against a CB attack, of inspection techniques suitable for investigating allegations and of verification of nonproduction are all presented in one volume. However, even if the political setting for each of these needs is quite different from that of the others, there are a large number of methodological overlaps. In fact, one might well think in terms of a continuous spectrum of approaches rather than in terms of distinct techniques. The need for speed in detection is, for instance, greatest in the case of warning against an attack, while, on the other hand, the need for precision in identification is relatively modest. At the other end of the spectrum are investigations of allegations, where speed is much less important than reliability of the analysis, which may require a very careful integration of background data with laboratory results. Finally, the techniques suitable for verification of nonproduction fall into an intermediary category, requiring both painstaking documentation and analysis of data, and quick spot-tests suitable for field

work. The latter might well involve both sampling techniques devised for CB defence and the use of certain early warning equipment for remote sensing of field testing.

The material presented here supplements the discussion of chemical and biological warfare (CBW) defences (see Volume II of this study)[1] and the considerations of the political setting for CBW inspections (see Volume V)[2]. In Volume V, for instance, we have analysed the need for verifying the observance of CB disarmament treaties.[3] In particular, appendix 2 of Volume V[4] is highly relevant to the discussions in this book.

In Volume V of this study we stress that the need for formal verification of CBW treaties is relatively slight for the nuclear-weapon powers and for the militarily weak countries, whereas the "middle" countries might see some attractions in such measures, at least as far as chemical weapons are concerned. With regard to biological weapons, on the other hand, it was felt that they had ceased to be an issue, mostly for political reasons. In addition, the technical problems appeared to be less difficult because the development and perhaps even the production of biological agents is more likely to be conducted largely within the scientific community than in a purely industrial environment. Consequently, information about where a BW programme might take place, who might be working on it and possibly which programmes were being undertaken at any given time is readily available from the open literature and from informal communication between scientists.

In chapter 1 of this volume we set out the evidence that lies behind this conclusion, and we indicate the significance of documentation not only for verification of nonproduction, but also for the analysis of allegations of biological warfare.

In chapter 2 we review the various problems of inspections concerned with biological weapons, first considering the various types and settings and then examining some of the technical features which might generate indicators. The possibilities for direct inspection at the stages of development, production, transport and storage of CB agents are also given considerable attention in the appendix on verification of CB disarmament in Volume V. With regard to the BW agents, we stressed that it is the combination of size and safety factors at the production stage which provides the best target for inspection. Since production capacity is, however, closely

[1] The Problem of Chemical and Biological Warfare. Volume II. CB Weapons Today (Stockholm: SIPRI, 1973).
[2] The Problem of Chemical and Biological Warfare. Volume V. The Prevention of CBW (Stockholm: SIPRI, 1971).
[3] *Ibid.*, pp. 113–20.
[4] *Ibid.*, pp. 137–89.

related to the shelf-life of the agents, we also give some attention to the problems involved in the storage of BW agents in the present volume. Chapter 2 concludes with a report on an experiment to develop techniques for the inspection of microbiological laboratories and appendix 1 to this volume contains the inspection documents used.

Identification of agents is certainly an important feature of inspections, but it is also relevant to early warning and public health needs. After discussing the latter, which is an area of great significance to any discussion of redeployment of military resources to civilian needs, we shall then provide a relatively comprehensive review of the subject in chapter 3.

Appendix 2 records the discussions at a meeting devoted to the potential of fluorescent antibody techniques, and appendices 3 and 4 are concerned with the possibility of using germ-free animals as producers of FA-sera and the potential of gas chromatography/mass spectrometry, respectively.

In the case of chemical weapons, the potential for direct civil application of the specialized early warning and verification techniques is not the same as that for biological weapons. It is true that one might build a strong case for the relevance of certain inspection and control approaches to environmental toxicology and to the global monitoring of pollutants [1], but the connections are more remote. Consequently, we concentrate our attention on techniques which are relevant to the disclosure of production geared to an important military product, namely, the nerve gases. With regard to the production of dual-purpose chemical agents such as phosgene, chlorine, tear gases and herbicides, the task of verification would be either to enforce a qualitative ban on prohibited filled weapons or to enforce a quantitative limitation on supplies of the agents, so as to ensure that supplies in excess of civilian requirements were not produced and diverted to military use. Technically delicate as this might be, it would, however, not constitute the same sort of challenge as does the control of facilities for making single-purpose CW agents. Of the single-purpose CW agents, we concentrate on nerve gases for several reasons. As pointed out in Volume V, the technology involved in, say, mustard-gas manufacture calls for sophisticated chemical engineering and skilled plant operation; a country that could provide these would probably also be capable of manufacturing nerve gas, and from a military point of view the nerve gases are far more attractive than mustard gas. Since there is also greater probability that a nerve-gas programme could be carried on clandestinely, we felt that the techniques for verification of nonproduction in the field of nerve gas are particularly significant. Inspection techniques for organophosphorus compounds were therefore selected as the subject for a SIPRI symposium, which is summarized in chapter 4 (see also the background papers, presented at the

symposium, in appendix 5). The speed and direction of the current CW negotiations indicate that this was a proper choice, but they also illustrate the problem of keeping the text up-to-date. This is true for many of the fields touched upon in this volume, for instance, the discussions of rapid methods and automation in microbiology which was the subject for a week-long symposium that involved over 300 participants from 30 countries (Stockholm, 3–9 June 1973). Unfortunately, the new facts reported on this occasion could not be included in the text.

Chapter 1. The significance of documentation in the verification of nonproduction and in the analysis of allegations in the field of biological warfare

I. *Problems of secrecy*

The conflict between science and secrecy seems to have been a problem at least since World War II, [2–9] and it seems to be a conflict of a rather basic nature. It might be possible to understand the problems involved by applying Shannon's "communication theory of secrecy systems". A chief concept in this theory is that of redundancy, which means that more symbols are transmitted than are actually needed to convey a piece of information. The reason for such redundancy is that it ensures that the message is intelligible even if part of the information is lost or distorted. A sort of redundancy occurs in scientific information, the most obvious being repetition of an experiment for the purpose of verifying an observation. Furthermore, the introduction and discussion of a scientific paper often repeat information from other sources, placing the new information in context. For proper reception, scientific information is obviously dependent upon redundant information.

A certain amount of insight into BW capability can be gained from analysing published information, since not everything can be kept secret. It is, of course, impossible to judge just how much information is kept secret without having access to all information but some appraisal can be made by considering where secrecy is difficult or expensive to maintain.

1. Scientific work is evaluated by the scientific community and often subjected to test by repeated examinations. [3–4] A government wanting to keep a project secret must thus shoulder the responsibility and expense of this testing, particularly if it forms the basis for important defence policies. This could mean that the average cost per project, considered to be around $50 000 in the United States, [10] would increase.

2. If scientific work is considered an investment, then free communication is essential for getting a maximum dividend from this investment. The loss incurred by secrecy is not easy to estimate, but it is well known that restrictions in the flow of information can mean that not all those who "need to know" receive the necessary data. In science it is, in fact, impossible for any one person

to know who "needs to know" since there is such a very large number of people in the scientific community, including people outside the field in question, who may react to a piece of information in a useful way. Restrictions in free communication therefore can cause delays in the development of, for example, a weapons or defence system.

3. Scientists are usually judged on the merits of their published work and are therefore anxious to publish. Prolonged secretive work in peacetime is known to cause psychological repercussions. [9]

4. Some disciplines, such as microbiology, are difficult to keep secret because of their multiple contacts with other disciplines. Applied microbiology is, for example, used as a tool of research in agriculture, biochemistry, genetics, pharmacology, and the brewing and fermentation industry.

5. Biological warfare represents a perverted sort of applied microbiology and public health, that is, most of the basic facts are known from open and civilian sources. For instance, the virulence of micro-organisms is studied both for its relevance to the field of natural infections and in order to produce living, attenuated vaccines. Such knowledge can obviously be used more or less directly to make a BW agent more virulent. Infectious resistance factors are another example found in nature. They have also been studied in the laboratory and have yielded much information of importance for genetics. Transferring such resistance to a BW agent will make the ensuing disease more difficult to treat.

6. Most of the agents that would be suitable for BW are known to give rise to accidental laboratory infections; indeed, this capability has been cited as a criterion for a suitable BW agent. [11]

II. *Presumptive limits of secrecy in BW*

Secrecy is thus expensive and not possible to apply to all fields important to biological warfare, since that would cripple several other disciplines. In this context, it is interesting to note how this problem has been approached in another field, where a phenomenon has both military application and civil use. For example, in the "airborne passive scanning infrared imaging system" it is essential that the "critical operating capability", or the amount of military knowledge that can be extracted by the technique, be kept secret. The civilian surveillance potential is, however, so great that complete secrecy would not be in the national interest. For this purpose a mathematical formula has been devised as an aid in deciding what is and is not to be kept secret. [12] The intention is to keep military potential secret in the hope that when a leak eventually occurs, a new system will be available.

This example of a desire to try to define limits of secrecy is not an isolated one. [2, 13] Applied to BW, the following would seem to be likely candidates for secrecy:

1. *BW agents selected for production.* The choice of BW agents must always involve exhaustive studies on how to make agents more efficient and how to protect the indigenous population. Thus it becomes important that information about infections in the production plant is kept secret since this would indicate the offensive capability. It is therefore hardly surprising that information about such infections has been conspicuous by its absence. [14]

2. *Methods of dissemination, including means to reduce the biological decay of the BW agent before reaching its biological target.*

3. *The results of field tests to determine actual efficiency and feasibility.* These field tests are of crucial importance to the weapon developer. A large testing area must be available if accidents are not to reveal BW activities. The Skull Valley [15] accident illustrates the need to have access to a more suitable testing ground, such as in the Pacific. [16]

4. *Study of tactical and strategic implications based upon the above points.*

5. *Those elements of defence which an aggressor can bypass but not those which deter him from BW attack.* The immunological characteristics of the population and the type of protection equipment available might belong to the first category, while the efficiency of civil defence, including any potent early warning system, belongs to the second.

III. *Accessible scientific information*

If, for economic or other reasons, secrecy is limited mainly to critical operating factors, then scientific literature throughout the world should contain some information about where BW or related subjects might be studied. While it is essential to point out that a survey of this literature would yield far from exact results, it should also be stressed that such exactitude may not always be necessary.

Until the late 1960s, such a study of the world's literature would have been a herculean task, since it would involve surveying more than 20 000 journals. Today many of these journals are covered by abstracting services, and a comprehensive world network (CODATA) is under development. However, just how large a proportion is covered is difficult to estimate in view of possible overlaps. The *Biological Abstracts* and *Chemical Abstracts* survey about 7 500 and 13 000 journals, respectively, and *MEDLARS* about 2 400 publications of medical importance. [17–18] *MEDLARS* covers roughly half of the approximately 800 journals [19–20] in which microbiological information can be found. This

indicates remarkably good coverage of medical microbiology. Furthermore, both *MEDLARS* and the *Biological* and *Chemical Abstracts* are available for search by computer. Thus a survey of scientific literature pertaining to BW is a practical proposition. Using the word *aerosol* as key, a search on the 1966–69 *MEDLARS* material was performed in April 1969 with the following results:

Combination with word 'aerosol'	*Numbers of articles*
Aerosol + micro-organisms	42
Aerosol + known potential BW agent	18
Aerosol + micro-organisms + biological decay	36
Aerosol + immunization:	
living vaccines	14
dead vaccines	19
Aerosol + sampling/detection	41
Aerosol + retention in lung of particles	25
Aerosol physics, optics, etc.	48
Total	**243**

In the table, each scientific paper is listed under only one heading and if more than one classification were possible, the one most applicable to the BW context was chosen. Apart from illustrating the above discussion, it shows that there is no shortage of material in the open literature.

The Biomedical Research Information Service [21] of the World Health Organization also provides easy access to published information. This service, which is also computer-based, lists the research projects currently being carried out, and where and by whom they are performed. Thus it becomes possible to compare and combine present and past activities. These systems naturally do not cover all information necessary for a BW survey, but much effort can be saved, some of which can then be directed at manual search.

IV. *Information from press, radio and television*

The press, radio and television are of great importance for supervision, both because these media can force governments to state their policies and also because they may reveal telling accidents. The press debate about Porton [22] and the US television film by the National Broadcasting Company (NBC) on CBW [16] illustrate this point. The usefulness of the press for this purpose is of course inversely proportional to the degree of governmental control. Nor should the value of the mass media in reporting epidemics be underestimated.

They can of course be both helpful and harmful. While it is easy to appreciate the speed and spread of communication, it is sometimes impossible to escape the impression that disproportions in reporting can cause unnecessary anxiety.

V. *Informal communication*

Informal communication between scientists is more difficult to evaluate. It can be argued that scientists in the medical profession, who have undergone some indoctrination to disregard national frontiers and who generally abhor BW, might communicate more information than others. If such a factor is of importance, then it will act less in countries where most microbiologists are Ph.D.s rather than M.D.s. The fact that inter-scientist informal communication will present the most vexing problems both for the security specialist [9] and for the information analyst is obvious from the following abstract from a report on the handling of toxicological information. [23] This, incidentally, is very similar in many respects to BW information, in that it is dependent on a multiplicity of sources.

Informal means of communication

According to the report by Spring and Honicker, there are at least 10 means of informal communication of drug-related information, as follows:

Unpublished but circulated reports.
Prepublication manuscripts.
Presentation of papers at large, open meetings.
Publication of proceedings of meetings.
Conferences, symposia and closed meetings.
Correspondence.
Round-robin letters.
Journal clubs.
Casual conversations.
Pharmacist-physician discussion.

These investigators reported that no clear patterns of use were apparent; much depends on opportunities presented for transfer of information. They concluded that, in general, investigators and teachers rely on informal means because information is available through informal channels long before formal publication. In their immediate fields of interest, investigators and teachers mainly used round-robin letters and invited-attendance symposia. In less immediately related fields they depended on meeting proceedings and actual attendance at general meetings. Practitioners, on the other hand, tend to rely

more on casual conversations, presentations at formal society meetings, and hospital staff meetings to remain abreast of their broad areas of interest. [23]

It may, however, be possible to tap such sources directly, for example, by asking specific questions at international conferences. In view of present knowledge of what is going on in other countries, it may not be necessary to ask a scientist about his own country. Such an interview, whether anonymous or signed, might yield a base line and if repeated might give some idea of possible capabilities.

VI. *World distribution of information*

It seems almost a truism to state that far less information comes from the East than from the West. In CBW-related literature, however, such disproportions seem to be less pronounced than is generally supposed in the West. Both the United States and the Soviet Union make great efforts to analyse each other's scientific literature. [24] Thus, the Aerospace Technology Division in the US Library of Congress translates articles from socialist countries on chemical, biological and environmental subjects of interest in the field of CBW. Likewise, it is known that the Soviet Union translates a large number of scientific journals. The question of how much information is obtainable from each country in this context seems to be of less importance. Both sides have ample scientific and technical knowledge of how to produce B weapons, but if or how they actually do produce such weapons is, of course, dependent on policy and organizational structure. However, if information is to be used outside the United States and the Soviet Union, as a kind of yardstick for measuring the efforts of these two countries, it would matter little from which source it originally came.

In this context it should be pointed out that there is a lack of information from the underdeveloped areas. Too few of their scientific journals are covered by the abstracting services and there is relatively little press, radio and television coverage. To some extent this may be counterbalanced by tapping the flow of information through the specialized agencies of the United Nations, but presumably this channel of information has to be widened. This should not be too difficult considering the fact that in the underdeveloped parts of the world the overall activity in higher education and science is small. This is emphasized in a 1971 UNESCO study. [25–26] The latter shows that 90 per cent of the world's science is undertaken by 14 nations and that 40 nations account for all but 1 per cent of the world's contribution.

VII. *Evaluation and analysis relevant to BW*

There are several subjects within, for example, the field of microbiology, which, because of their close relation to B-weapon research, can indicate actual B-weapon development, especially if combined with certain other subjects. The word "aerosol" by itself may not mean much, but to use "aerosol + micro-organism" in conjunction is slightly more revealing and the combinations "aerosol + BW agent", or "aerosol + biological half life", would be most revealing. The combination "BW agent + immunization" may be a point of interest, especially if ties with other projects are suspected. [27] The words "photoprotection" and "micro-encapsulation" are of obvious portent, as well as "virulence-enhancing factors" and "field trials on humans or primates". Those four factors belong to studies of BW feasibility, so the number of citations would be very low.

It is also possible to study the competence of individual authors and to define them in terms of their previous production records. One can also look for interesting combinations of talents: for example, microbiologist/toxicologist or for suggestive geographical concentrations at various institutions. It is also possible to follow authors and subjects to see if they suddenly stop publishing or if a subject suddenly disappears from the literature.

A study of the open scientific literature is possible today with existing computer programmes, although this is limited more by the ability and imagination of the analyst to formulate the question than by any other factor.

Since the efforts in the field of public health which are related to agents potentially useful in BW are reasonably well known in most areas, it is possible to relate the "natural" amount of research to new trends. [27] If, for example, Sweden were to study plague intensely, it would be rather remarkable, since plague has not existed in this area for a long time. If, on the other hand, Swedish scientists were not engaged in studies of tularemia it would also be strange, since this has been a recent problem in northern Sweden. Great caution must, however, be used in such interpretations. The Soviet Union, for instance, has small foci of plague in the southern part of the country and a great tradition in the study of the foci of disease, and, through its involvement in Viet-Nam, the United States came into close contact with plague.

The study of the geographical distribution of research is made easier by the fact that many BW agents are zoonoses. It is therefore possible to acquire information by mapping out the animal forms. FAO, OIE and WHO collect this information from veterinary reports and publish yearly compilations. [28] It then becomes possible, by adding this information to the data on human epidemiology, to arrive at some conclusions as to whether particular studies of potential BW agents are necessary or not. [29]

An analyst would have to exercise utmost caution in extrapolating chains of events from isolated observations since what seems logical to him may be less so in reality. There are, however, some known patterns in the development of B weapon systems. Experiments on animals, whether in the laboratory or in the field, are not enough to judge the potential of a B weapon system, so it is highly desirable to supplement them by studies on humans. [27] The supply of suitable humans for such purposes is, however, limited, and it is therefore reasonable to assume that such experiments are made late in the development and after field experiments on animals have revealed that it is possible to reach the target with a certain dose. Such knowledge of experiments on volunteers as was gained from the US television film by the National Broadcasting Company (NBC) [16] thus, for instance, makes earlier animal field tests probable.

VIII. *Possible future trends in the analysis of information relevant to public health and biological warfare*

Information analysis centres

The function of an information analysis centre is well summarized in the following quotation:

> An Information Analysis Center is a formally structured organizational unit specifically (but not necessarily exclusively) established for the purpose of acquiring, selecting, storing, retrieving, evaluating, analyzing and synthesizing a body of information in a clearly defined specialized field or pertaining to a specified mission with the intent of compiling, digesting, repackaging, or otherwise organizing and presenting pertinent information in a form most authoritative, timely, and useful to a society of peers and management. [30]

As such, evaluation and analysis are nothing new to science but have indeed been an integral part of scientific work. It is as necessary to be able to rely on scientific data as it is to be able to rely on maps for navigation. The study of any scientific paper will reveal this process of evaluation and analysis: the introduction is an analysis of available information, which is followed by the results of the author's experiments. In the discussion, these are finally analysed for their correctness and their position within the framework of existing knowledge. And finally, every scientific journal evaluates information before it publishes it. Scientific editorials, and even more markedly reviews, evaluate scientific work from special points of view. Reviews which are statements on present knowledge in a field become more essential the more information there is, but are also more difficult to write. In many fields, especially where the development is rapid, there is a need for an almost continuous review, and this is the back-

ground to the present trend of creating special information analysis centres which can be oriented around a discipline or a special subject. [31] In such centres, scientists cooperate with documentalists in order to evaluate and analyse the information for storage, retrieval and dissemination. In this context, available information-storage and retrieval systems are adequate [32]; the talent available to perform the analysis is more critical than computers.

Some attention must now be given to the various types of information analysis centres and to the way in which they handle the information. It might be added that in 1968 there were 113 federally supported analysis centres in the United States, [33] of the following main types:

Information Analysis Centre type I:
First, there is the individual or group that collects the world output of useful information in a particular field of science or technology (including the social sciences), organizes and stores it for retrieval, then condenses, analyses, synthesizes, or otherwise uses the information to create new knowledge. The new knowledge may be an analysis of sources of error, a correlation between properties, a decision on what to do next, or something else. This is a "discipline-oriented" centre.

Information Analysis Centre type II.
Second is the individual or group that collects the world output of useful information relevant to the solution of a set of problems encountered in achieving specific practical goals, organizes and stores the knowledge for retrieval, then analyses the information to attempt to solve specific problems of interest to the community it serves, or to determine what additional information may be needed to solve the problem. This is a "mission-oriented" centre.

Information Analysis Centre type III:
Finally, third is the individual or group that collects raw or partially processed observational results concerned with large-scale phenomena, organizes and stores the information, then analyses the results in order to obtain correlations, test theories or otherwise produce new knowledge. The phrase "large-scale phenomena" requires clarification. The phenomena referred to are those not containable within a single laboratory but instead associated with large-scale systems. For example the phenomena might be those encountered in the study of oceanography, in upper atmosphere physics, or in interplanetary space. Also included are the type of data gathered in measuring the transportation of people and goods, or those data about people that are obtained during a census. [31]

The work of such a centre is presented in table 1.1.

An information centre for BW analysis

The Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction, which was opened for signature on 10 April 1972 is a remarkable disarma-

Table 1.1. Information analysis centres

A. Input
   1. Search-procedure, tools, completeness
   2. Acquisition
   3. Copy format—hard copy, microfilm, fiche
   4. Data format—graphic, tabular, correlation, smothered
   5. Scope-optimum coverage by one group, undesirable fragmentation

B. Data selection
   1. Quality—reliability, accuracy
   2. Methods and procedures of quality assessment—characterization

C. Correlation/analysis/critique
   1. Applicability of statistical procedures–error
   2. Compatibility with other properties as well as internal consistency
   3. Data reduction
   4. Scope—optimum coverage of a number of properties, fragmentation
   5. Criteria of reliability

D. Storage/retrieval
   1. Data storage–raw data, processed data, analytic form, graphic form
   2. Bibliographic storage and retrieval
   3. Input and output formats

E. Dissemination
   1. Audience
   2. Information transmission
   3. Formats
   4. Networks

F. Use of ADP equipment
   1. Implications for record availability for future analysts
   2. Data transfer capability–software, hardware, reliability, compatability

G. Control of data generation
   1. Standards
      a. Data and materials
      b. Built-in control of paper writing

Source: Touloukian, Y. S. and Garvin, D. *Report of working group II. Data and information problems.* In Proceedings of the Forum of Federally Supported Information Analysis Centers. Committee on Scientific and technical information (COSATI), Federal Council for Science and Technology: Panel 6, Information Analysis and Data Centers. 7–8 November 1967, p. 36.

ment step, but we should realize that research aimed at production of known biological warfare agents or at the development of new agents and delivery systems is really not prohibited. This is particularly unfortunate against the background of the fact that no definitions are incorporated in the Convention which in fact leaves a disturbing twilight zone towards the chemical weapons. Also the maintenance of defensive preparations may generate suspicion, because production of certain quantities of biological agents will always be necessary in the development of protective masks and clothing, air and water filtration systems, decontamination equipment and detection and warning devices. This is one of many fields where the political risks could be reduced by international cooperation. The establishment of an information service might have such an effect, even if the main impact came from the removal of all secrecy surrounding microbiological research.

In order to guarantee compliance, the Convention entitles the parties to lodge complaints of breaches with the UN Security Council. However, it has been pointed out that, lacking international verification of nonproduction, it is difficult to see how it may be possible by legal means to collect evidence confirming the "validity" of a complaint as required. If the implication of the provision is that other extra-legal means may be employed to collect evidence on clandestine production of prohibited agents, it should be realized that the

parties are not in equal positions in this respect; many may not even possess such means. Here an information centre for BW analysis would open new lines of communication which would redress the obvious imbalance and function as a deterrent against evasions. If, in an information analysis centre of the type outlined in the previous section, political, military and economic expertise were added to the technical-scientific expertise, it might be possible to draw at least some tentative conclusions about BW developments. Such information can, of course, not be considered hard data but will probably take the form of questions that can be put to a country. The resulting answers or refusals to answer would add something to the existing information. It is self-evident that such an information analysis centre would be extremely useful in preparing the background for an inspection as well as in analysing the result of such an inspection.

The staff needed for such a centre becomes evident when one considers the types of information which would be analysed there. This has been described in full in a Pugwash article [35] and it suffices here to point out that information pertinent to BW can be found in such subjects as microbiology, infectious diseases, public health, zoology, botany, chemistry, bioengineering and meteorology. For evaluation and analysis it is desirable that the analyst be a practising scientist. [36] When this is added to the already suggested political, legal, military and economic expertise it becomes obvious that such a centre, with its access to computer facilities will be expensive to maintain. However, public health efforts would gain from such a facility, a sort of "International Public Health Information and BW-Defence Centre".

This combination might seem far-fetched, but a model has in fact existed (since 1951) in the form of the Epidemic Intelligence Service in the USA. [37–38] The balance between existing public health problems and present scientific capabilities will not in itself direct research efforts but may attract attention to places where knowledge is lacking. Such information would be valuable to those who decide which projects should be given priority [3], and politicians have indeed expressed a need for such information. [39]

A side-effect of supervision through a study of information would no doubt be that any nation that wants to develop biological weapons would have to be more stringent about secrecy. This may significantly increase the cost of development and would diminish the economic attractions of B weapons.

The creation of an information analysis centre for "public health and BW defence" would also help the individual scientist or scientific institution in the planning of research activities. Rapid access to published literature corresponding to the interest profile of the individual would be entirely within the scope of such a centre. An almost continuous review of many subjects would strengthen WHO's efforts to indicate areas to which research should be directed—a very important consideration in view of the coordination of international laboratory

efforts such as the newly established International Federation of Institutes for Advanced Study (IFIAS). Finally, such a centre would probably accelerate the standardization of scientific methods on a global scale.

Even if an information analysis centre could initially only perform some of these tasks, the individual scientist or scientific institution could ill afford to abstain from using it, since that would automatically give other laboratories a lead. If, in addition, the user were only charged a nominal fee for the services, the coverage provided by questionnaires asking for name, address and interest profile could be considerable. It follows that this information would be of great use in charting scientific activities, including those which might conceivably be related to chemical and biological warfare.

The case for combining the surveillance of communicable diseases with cooperative planning of biological warfare defence

Epidemiological surveillance is the study of a disease as a dynamic process involving the ecology of the infectious agents, the host, the reservoirs and the vectors, as well as the complex mechanisms concerned with the spread of infection and the extent to which this spread occurs. [40] On the one hand, it requires the systematic collection of morbidity and mortality reports, together with laboratory data, and on the other, it involves the analysis, interpretation and distribution of the information received.

Since an adequate surveillance programme permits early recognition of changes in the disease pattern and vector distribution, it is fundamental to any biological warfare defence system. It provides a solid base for the planning of such control measures as vaccination, and for an optimal utilization of the therapeutic resources. The speed required in biological warfare defence is such that it may necessitate such highly sophisticated approaches as the Command Automatic Data Processing System (CADPS) used by the United States Army. [41] This system provides an accounting system which reveals the medical situation at any given moment.

Surveillance programmes will vary according to local conditions, and they should naturally be aimed at covering the most vulnerable areas. Clearly, in the large cities in the developing countries, where the water supply and environmental health facilities cannot keep pace with the rapid population growth, problems are different from those caused by the controlled expansion of the megalopolis in the technically advanced countries. In both locations, political, social and economic factors affect the ecological conditions, though in different ways, and this emphasizes the importance of local surveys in order to evaluate the vulnerability of different types of society to BW attack.

Most national surveillance programmes lean heavily on the international

activities of the World Health Organization (WHO), which, in 1965, established an Epidemiological Surveillance Unit in its Division of Communicable Diseases. This organization has had long experience with quarantinable diseases (cholera, plague, relapsing fever, smallpox, typhus and yellow fever), in which control measures are normally based on a combination of the results of rapid laboratory diagnosis with the data from immunological and vector surveys—factors which are also significant in BW defence. Surveillance is also a feature of the work done by the WHO Influenza Reference Laboratories and is an integral part of the organization's campaigns against malaria and endemic treponematoses.

National surveillance programmes, like that of the Communicable Disease Center in Atlanta, Georgia, [42] contribute information on different diseases to the global picture (poliomyelitis, infectious hepatitis, influenza, salmonellosis shigellosis, diphtheria, etc.), but data from many underdeveloped areas of the world are incomplete. Surveillance of plague on the basis of a systematic study of the ecology of the disease has, for instance, been carried out in the Soviet Union, the United States and China, but in several other countries with natural foci, a long-term systematic study has not been carried out. [40] It is natural that such a lack may delay the detection of changes in the disease pattern indicative of a limited BW attack. In a technically advanced society, on the other hand, the normal surveillance of communicable diseases simplifies the detection of such an attack.

Obviously, a systematic study of the spread of infections in a population and a survey of certain diseases such as tularemia and Q-fever among animals—such as that carried out at the Institute of Epidemiology and Microbiology in Prague [43]—must provide a useful basis for BW defence planning. A national surveillance programme involving routine immunological testing and regular surveys of vector populations also provides a basis for retrospective investigations which may reveal important proofs to support a case of BW allegation, which is not an easy matter. [44–45] Particularly if supported by automatic devices (which would not necessarily have to be as rapid as those mentioned under the heading "early warning"), it might reduce the risk of an attack being launched. Cooperation between countries that aim at improving surveillance methodology might, together with improved WHO facilities, in fact, provide extremely important help in case of insidious attacks. [29] Multi-purpose immunological surveys are of particular significance in this connection because, if based on standardized techniques, they "permit much better comparisons of the epidemiological situation among populations living in different environmental conditions, than do existing morbidity and mortality statistics collected in areas with widely differing facilities for reporting and varied criteria of diagnosis and accuracy". [40] To be of real significance, such surveys should be broad and transnational and they would require advanced planning and data handling,

equipment for automated analysis and other facilities frequently unavailable to small nations. So far, however, the latter have not entrusted WHO with special powers and responsibilities in the field of surveys which could provide a defence basis against biological warfare—a strange fact if one considers that their own health services are far from adequate in this field. Those services might help to ward off epidemics which normally respond to quarantine measures, but they could hardly arrest the effects of an aerosol cloud or the spread of arthropod vectors from a massive release. The effects of such attacks might reach far beyond national boundaries and an effective limitation might require efforts of a magnitude that would exceed the capabilities of many nations. Such countries might be interested in buying an "insurance policy" which would guarantee immediate air delivery of substantial quantities of vaccines and antibiotics in case of attack, as well as providing personnel and advice. [29]

Several of the United Nations' specialized agencies have a certain competence in the field of BW defence. As mentioned above, WHO can be expected to be interested in the aspects of public health, and in addition, the Food and Agriculture Organization (FAO) should be concerned with plant and animal protection, and UNESCO in the associated basic microbiological research, documentation and science policy. From this point of view, and also considering the political dangers of impromptu appointments of international committees to study cases of BW allegations, the establishment of an independent International Microbiological Agency, similar to the International Atomic Energy Agency (IAEA), but of course smaller, has also been considered [46]. This would tie a control function in biological warfare to the peaceful application of microbiology, in the same way as the control of reactor fuels is tied to the peaceful uses of atomic energy within the IAEA. This proposal should receive renewed attention as a consequence of the recent acceptance of the biological disarmament treaty which envisages increased efforts in the application of microbiology to civilian needs. [47]

In addition to an effective system for nationwide reporting of infectious diseases, the planning of BW defence should involve the preparation of stand-by legislation for compulsory immunization, stockpiling of therapeutics, preparations for extra production of broad-spectrum antibiotics, microbiological research and, finally the spread of knowledge about unusual infections. [48] The administrative measures and the training of such personnel as police, customs officers, and so on, of course varies from country to country, but Sweden can be singled out as an illustration of the natural approach for a small country. [49–51] In Sweden a commission on medical catastrophes, under the Department of Health, coordinates all activities required by major fires and traffic accidents, explosions, accidental release of chemicals, natural catastrophes and so forth. This commission is also responsible for the necessary emergency meas-

ures to be taken against major epidemics as well as against poisoning via air, water and food. The production of detailed action plans, on the other hand, is the responsibility of special regional and hospital committees. These committees prepare detailed catalogues of measures to be taken in a number of conceivable situations. The regional committees consider such matters as sounding the alarm, the distribution of cases among available treatment facilities, internal communications, possible isolation of the stricken area, providing it with personnel and transport facilities, etc. The hospital committees, for their part, plan for the alerting of hospital personnel, for the distribution of responsibilities, for the use of medical supplies, for information to relatives, press and radio, for the relocation of patients under care before the emergency occurred, and, finally, for the registration, sorting and treatment of mass casualties.

International coordination of the type of planning just outlined would not only increase the overall level of preparedness against epidemics, but would also tend to make secret BW studies extremely difficult.

IX. *Conclusions*

In most countries microbiological research is public information, with the exception of a small fraction which is of direct military significance. The nebulous division between offensive and defensive efforts, the global need for extensive application in public health, the professional revulsion caused by perversions in medical research and, finally, a traditionally international outlook on the part of those concerned with fighting infectious diseases all tend to open the closed sector, particularly when it is growing. It then tends to throw off indicators which are easily analysed by modern techniques. The frequency with which such indicators are given out is a function of the size of the efforts and the sophistication in production methods but of course it is also profoundly influenced by the political and intellectual climate (increased sophistication tends to break the barrier between military microbiologists on the one hand, and university and other outside experts on the other). The existence of an international treaty makes it hard for a signatory to advocate a deviating political position on the national level, making voluntary inspection schemes a promising proposition. However, some general overview is also necessary, considering the rapidly changing panorama of molecular biology, microbial genetics and immunology as well as the advances in synthetic organic chemistry. Since new biospecific weapons might appear and synergistic combinations of old weapons might assume significance very rapidly, a continuous surveillance might well be advocated as a basis for regular treaty revisions. Verification in the field of biology will have to rely on the sum total of a very large number of

parts, each with a varying reliability due to the political, administrative, technological and scientific situation. Against this background, some sort of information analysis centre would be an essential part of an efficient verification machinery. It is important to note that a nucleus already exists within the framework of the UN specialized agencies. They focus on global needs with regard to public health, veterinary medicine and plant pathology, and might, in fact, become more efficient if a CBW verification mandate were added. This is a consideration which must enter any cost-benefit analysis related to inspection mechanisms relevant to biological warfare. With regard to collateral voluntary schemes, involving, for instance, an exchange of fellowship workers doubling as "inspectors", their regular research contribution would also have to be taken into account before the verification costs were calculated. [1]

Chapter 2. The problems of inspection concerned with BW agents

I. *The various settings for BW inspections*

Targets for inspection

Inspections may be directed against such different targets as production of agents or weapons, field testing of arms or, finally, their actual military use, and the impetus for the inspections may be either internal or external. The internal initiative is generated either as part of the routine of a regular verification programme or by indicators discovered through documentation or other means. The external initiative, on the other hand, would derive from organizations associated with an inspectorate and it would normally be based on an allegation.

Inspections can be either national or international and possibly both. The common emphasis is on international inspections because they have been regarded as a means of verifying and thus of reinforcing disarmament treaties. As pointed out in Volume V, however, this may not be a prerequisite for a treaty to fulfil its desired function. Because of the changing pattern of warfare, with increasing emphasis on guerilla and counterinsurgency operations, and the subversive activities in many countries, national inspection systems may also become important. These inspections do not face the same problems of access as an international effort but face the more serious problem of detecting small-scale efforts aimed at sabotage operations. The success of this type of inspection would be directly related to the degree of active participation on the part of individual microbiologists. If this were high, an inspection system operated by the national microbiological society and based on legislation aimed at licensing work on highly dangerous pathogens and on fixed rules for the handling and storage of material containing such micro-organisms, might provide a powerful brake on a dangerous development. However, it must be remembered that nature is always a source for new strains and that the opportunities to disguise the ultimate aims of research are almost limitless. In principle, the most effective insurance against professional aberrations would be a combination of sanctions and a criminal law that would reinforce the professional ethics which would certainly develop rapidly in an environment fostering the many peaceful applications of microbiology to human ecology. [47]

Personnel and equipment requirements

Inspection of potential production facilities requires expertise in microbiology, bioengineering and administrative procedures, and it must be regarded as a very demanding activity, because it is essential to have good knowledge both of scientific advances in many fields and of the state of the military art. In fact, the inspectors should be experts in imaginative means of evasion and in the design and use of detection devices of the "black box" type. They should know enough about cultivation techniques to be able to judge, for instance, the increase in capacity of a fermentor when it is fitted with a dialysis unit, or to evaluate novel techniques for growing diploid tissue cells as a virus substrate, such as their cultivation on the surface of suspended particles. However, they must also be experts in laboratory safety and biochemical separation techniques, such as electrofocusing and affinity chromatography, which might be used for the isolation of toxins.

In the inspection of field testing or actual use, the problems are political as well as technical, and the level of intrusiveness would vary according to the circumstances. On-site inspection would require no sophisticated equipment except personnel protection gear in cases where vaccination were not possible. However, a very high level of epidemiological and epizootological experience would be essential as well as facilities for taking and storing samples and for making clinical and pathological examinations. Equipment for microbiological identification work would be helpful but not essential, since the diagnosis would normally have to be verified by several independent specialized laboratories. Large-scale blood sampling followed by serological analysis would be essential, but in this case reliance would normally be on independent specialized laboratories.

These considerations indicate that the competence of the inspectorate for field testing would differ in emphasis from that required for the analysis of production facilities. However, any organization capable of studying allegations of use would also be competent to study allegations of testing, as long as the latter did not involve sophisticated remote sensing. Since the techniques which might be used in connection with the inspection of potential production facilities are amply exemplified in a later section, only the problems raised by inspections of allegations of use will be considered here.

Special problems in the study of allegations of use

Allegations of use can concern different levels of operation. First, there is the possibility of a full-scale attack over a large area which is exposed to an agent with limited case-to-case spread. Today the effects of such an attack can, of

course, only be discussed theoretically, but it seems likely that they will differ greatly from natural epidemic situations, even if the attack is superimposed on a pattern of increase in disease due to inadequate hygiene and population migration. The other extreme is when allegations arise from natural epidemic disease with uncharacteristic features co-occurring with political tension. In between these two cases are the possibilities of BW designed to mimic epidemic disease as well as abortive attacks due to miscalculation. Finally, there is the special case where a BW allegation is fabricated for political purposes, and is not based on observable changes in the disease panorama.

In the history of BW, several allegations have been made which have not been satisfactorily investigated within a reasonable time, or even at all. This is understandable, since the problems involved are extremely difficult. In order to verify an allegation, all natural causes must, for instance, be investigated and excluded, which is difficult since epidemic disease can be notoriously variable. Furthermore, many of the potential BW agents belong to the zoonoses which exist in foci, that is, geographically localized areas where fauna, flora and climate favour them. It is also known that a variety of human activities, including warfare, can alter the limits of such foci and that this might entail the spread of disease. [52–57] It may, at times, be difficult to differentiate a BW attack from a spread of disease caused by human passage through the focus or by ecological changes at its limits—as, for example, caused by altered agricultural procedures. An illustrative example of such human contact with a focus occurred in 1944 when 41 soldiers and sailors landed on the uninhabited South Bat island off New Guinea, where they came into contact with the rat-mite life-cycle of *Rickettsia tsutsugamuchi* which resulted in 26 of them contracting scrub typhus. [58]

From this discussion, it follows that an extensive epidemiological investigation of the area in question is a *desideratum* if not a *sine qua non*. Such a study should encompass not only human beings but also animals and insects. A search for the responsible agent and for its serological "fingerprint" must be carried out. Speed is necessary, since the agent may not survive for long outside a host. *P. tularensis*, for example, cannot survive on the soil surface for more than 24 hours under hot, summertime conditions. [59] Another reason for speed is that if blood samples are collected sufficiently early, it becomes possible to follow an increase in antibodies. Such an increase is usually evident 10 to 14 days after the début of the disease and is highly significant. However, in the absence of such early specimens, later ones could also be compared with samples taken before the attack. Here an important possibility is to make use of the sera kept in the WHO serum reference banks.

Conclusions

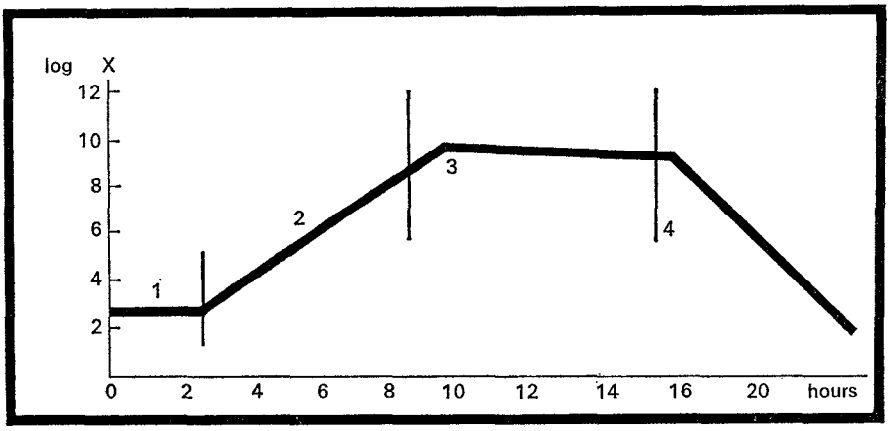
The chances for the success of any inspection effort are a function of the planning and competence which go into it. Inspection for production and for testing and use require different competence profiles, but they both need careful long-range preparation, including political and administrative measures aimed at assuring rapid access and freedom to take samples and to arrange for their analysis by the most competent experts. In the case of industries producing biologicals, the samples need not be living micro-organisms but material which has been inactivated in such a way that serological verification techniques might later be used. Virulence tests could be performed in the inspected laboratory under the supervision of the inspector.

With regard to inspection of allegations concerned with testing or use, the chances of success depend on detailed knowledge about the epidemiology of the area concerned before it was attacked. That knowledge is lacking in many regions of the world where it will thus be hard to arrive at a definite conclusion. Under any circumstances, inspection of an allegation of testing or use requires that data from many different fields be collected, processed and evaluated. Microbiological, military, epidemiological, demographic and meteorological experts must be available but even in this case, there is need for much preparatory work:

1. Alternative search strategies must be worked out.
2. Methodology both with regard to sampling and to laboratory procedures must be selected and tested.
3. Reference laboratories must be assigned and prepared.
4. Field personnel must be: selected for competence and such political acceptability as might affect access; trained in the appropriate methods including protection techniques; vaccinated against conceivable BW agents, where this is possible; and protected against other health hazards that may be encountered *en route*.

All of this is time-consuming and it is hard to say exactly how long the preparations would take. However, it would certainly not be less than three to six months, even if the highest political and economic priorities were given to the project. The *ad hoc* appointment of an inspection team composed of unprepared figureheads in science would indicate ignorance at the level of decision-making or political dispensation of an eyewash designed to hide unpleasant facts.

Chart 2.1. Growth phases in a bacterial culture



X=bact. per ml.

II. *Some technical aspects of the production and storage of BW agents*

Production of bacteriological BW agents

The technology of producing bacteria depends upon their rapid growth when placed in a suitable environment, as can be seen in chart 2.1, where the lag phase (1) represents an adjustment to a new environment, and the logarithmic phase (2) a period of rapid, logarithmic multiplication. The stationary phase (3) and that of decline (4) illustrate how micro-organisms die off as fast or faster than they are formed, a situation which occurs when the medium has been exhausted and/or inhibitory substances appear. If nutrients are supplied continuously and harmful substances are removed or neutralized as fast as they are formed, it becomes possible to keep the culture in its logarithmic phase. This is the case in a continuous culture [60] which, by remaining at the exponential part of the growth curve, yields almost incredible outputs. Table 2.1 indicates the efficiency of the continuous culture as compared with that of the more generally used batch culture. The fact that in table 2.1 a continuous culture yields almost ten times as much per volume of fermentor as a batch culture does, of course, not mean that the latter could not be used for large-scale production of BW agents. Much larger cultivation vessels would, however, be necessary, and those would be easier to detect both during manufacture and when installed. Continuous culture might also be preferred since it permits the quality of the product to be kept within fixed limits.

Skill and specialized equipment are necessary for the supervision of production both by continuous culture and batch processes. In the production of BW

Table 2.1. A comparison between continuous and batch cultures

Type of culture	Size of vessel (lit.)	Output[a] (bact/ml)	Time of cycle (hrs.)	Medium (lit./24 hrs.)	24-hr. yield[b] (number of cells)	Minimal loss during lyophilization (per cent)	24-hr. yield lyophilized bacteria (number of cells)	Days to reach 10^{17} cells[c]
Batch	1 000	10^{9}–5×10^{10}	50–100	250–500	2.5×10^{14}–2.5×10^{16}	*20*	2×10^{14}–2×10^{16}	500–5
Batch	100	10^{10-11}	5–10	250–500	2.4×10^{15}–5×10^{16}	*[20]*	2×10^{15}–4×10^{16}	50–2.5
Continuous	10	10^{10-11}	2–4	60–120	6×10^{14}–1.2×10^{16}	*40*	4×10^{14}–7×10^{15}	250–13

Notes:
[a] Fastidious micro-organisms. Many B agents are found in the lower range.
[b] Yields for batch cultures are too high, since they are calculated on the basis of zero time between batches.
[c] Time to 10^{17} chosen because it is considered to provide material for one attack.
Sources: Málek, I. and Fencl, Z. *Theoretical and methodological basis of continuous culture of microorganisms* (Prague, 1966); and Möller, G. (State Bacteriological Laboratory, Stockholm), personal communication.

agents, skill must of course be combined with experience in the handling of pathogens, and the question then arises where such a combination can be found. The obvious answer is that outside the defence laboratories only the vaccine producers qualify. Vaccine-producing laboratories thus form a possible pool of skilled personnel from which producers of BW agents could be recruited. Consequently their personnel rosters would be an important inspection target.

The production of vaccine entails the use of living, attenuated micro-organisms or virulent ones which are killed immediately after cultivation. Obviously the protective measures must thus be strict only during the first stage of the production and subsequently precautions are aimed more at protecting the vaccine than at the protection of the personnel handling it. Extensive safety precautions during the whole production cycle for a vaccine are hardly defensible economically and would hence be suspect.

Experience in continuous culture is less common among vaccine producers than might be expected. This becomes understandable only after studying how vaccines are produced. A batch of vaccine which is cultivated in a few days must then undergo a series of controls for potency and safety and this process can take as much as three months. After that, a decision can be made whether the batch can be sold and used or has to be discarded. Consequently it is safer not to put all the eggs in one basket but instead to work with several small batches at staggered intervals. For such work, batch cultures are adequate for everyone except for the large-scale producer. However, there may also be qualitative advantages in continuous culture stimulating a change-over from batch culture. Such gains must, however, be balanced against the cost and the time needed for a changeover. The presence in an inspected vaccine-producing plant of processes of a type that cannot be justified on economic or other grounds could no doubt be a useful indicator for detecting possible diversions to BW production. Table 2.2 illustrates the need for vaccines for the protection of a country's own population and lists the cultivation vessels necessary.

It is reasonable to assume that if a nation contemplated BW it would have to protect its own population, at least to a certain degree. In that case, alternative B in table 2.2 illustrates that a considerable increase in vaccine production would be necessary. This in itself might be indicative of a possible diversion to BW production, though of course, such a plan could be hidden by gradual stockpiling.

In this context, it should perhaps be pointed out that the form of vaccine production today is inherently irrational from an economic point of view, that is, small-scale production in many locations. Large-scale production of vaccines, centralized in a few places, would be cheaper and would also create better opportunities for standardization. [61–62] Furthermore, it would concentrate the research, which ought to result in improved vaccines sooner. No doubt the

Table 2.2. Estimation of the best cultivation vessel volume or cultivation method for vaccine produced for only newborns (case A) or for the whole population (case B)

Population $\times 10^6$	Case (see text)	Newborn $\times 10^6$	Est. need per year	Batchwise processes Max. no. of batches necessary (litres) 1	10	100	1000	Need for continuous processes
1		0.02	0.02–0.1 m^3	100	10	1	0.1	–
10	A	0.2	0.2–1 m^3	10^3	100	10	1	(±)
100		2.0	2–10 m^3	10^4	10^3	100	10	+(±)
1		1–5 m^3	1–5 m^3	5×10^3	500	50	5	+
10	B		10–50 m^3	5×10^4	5×10^3	500	50	++
100			100–500 m^3	5×10^5	5×10^4	5×10^3	500	+++

Sources: Möller, Å. L. *The present state and the future of human bacterial vaccine production.* International Symposium on Biotechnical Developments in Bacterial Vaccine Production. (Stockholm, 1965); *and* Immunobiological Symposium Series, Standard. Vol. 3. (Basle, New York, 1966), pp. 11–22.

present spread of small, uneconomical vaccine-producing plants is caused by the fact that every nation wants to be certain that it is not cut off from a vital supply in case of conflict. With the present possibilities for storing lyophilized vaccines for an almost indefinite period of time, this point is, however, of limited validity.

Production of virological BW agents

From a technical point of view, the production of virological BW agents rests upon the availability of living metazoan cells, since viruses cannot be grown without living tissue. The conditions for growth of such cells are very stringent and force the BW-agent producer to choose between one or the other of the following two approaches: (*a*) propagation of the virus in living embryos; or (*b*) growth of the virus in tissue cells cultivated in vitro either in suspension or on solid surfaces.

In the first case, the producer must have large numbers of embryos readily available and, whenever possible, he would choose fertilized eggs in view of their ease in handling. In fact, this approach is likely to attract attention from anyone intending to build a military capability on the basis of very limited resources. Many rickettsia, which could be potentially dangerous BW agents, can, for instance, be cultivated in this fashion. The indicators for an inspector to look for would be an abnormal interest in certain agents, coupled with competence in aerosol dissemination. The inspector would have to watch for large quantities of egg shell in the laboratory waste (high calcium content in the ashes), and for hatching machines as well as for tilting devices and environmental control equipment that might be used to convert existing space into egg

incubators. The size of chicken farms associated with the laboratory would also be estimated, and the normal product-mix would be studied from the point of view of possible changes in the production priorities. An abnormal level of safety in a laboratory making influenza vaccine would, for instance, be an indicator, as would a high level of, say, typhus antibody in the sera taken from the personnel in such a laboratory. This latter indicator is one of the most important categories of sample to which an inspector must have access. It goes without saying that the inspector and his staff must therefore be equipped to take blood samples and have access to an independent laboratory where a very comprehensive serological study can be performed.

When virus preparations are produced from tissue cells, cultivated in vitro, there is either a need for a seed stock of cells kept at very low temperature—normally in liquid nitrogen—or for special animal organs, such as monkey kidneys, that can provide the needed cells. In both cases there is also a need for very complex media, normally containing large amounts (5–10 per cent) of animal serum, often from calves. Those technical prerequisites easily provide indicators with regard to the size of the operations. Since fermentation equipment for bacteria can often be used for suspended cell culture, it would be examined with regard to exhaust gas treatment, to stirrer modifications and to safety features. However, most attention would probably be focused on the supply of medium ingredients and on their preparation for use, for instance by means of large-scale membrane filters. The type, size and safety features of harvesting equipment, such as centrifuges, would, of course, also be studied.

Storage

Storage of biological weapons is a much more complex issue than storage of conventional arms, since micro-organisms are living and therefore subject to decay and death unless their metabolism is slowed down or arrested. The metabolism of the micro-organisms can be inhibited in several ways, but only drying and lowering the temperature or a combination of the two procedures are of interest in the present context.

1. Drying in sterile soil or a similar environment has been used for the storage of spore-bearing bacteria. [63]
2. Various levels of low temperature are in use [63]:

(*a*) +4°C is commonly used for short-term storage, but a BW agent can hardly be expected to retain its efficacy for more than 1–2 weeks at this temperature.

(*b*) −20°C can be used, but care must be exercised since at this temperature eutectic mixtures with water are formed. It is known that some bacteria are

greatly weakened; *lactic streptococci*, for example, lose two orders of magnitude in 2 months. [64]

(*c*) −70°C or storage in the presence of solid carbon dioxide is definitely preferable to higher temperatures, but some loss of viability cannot be avoided.

(*d*) −170°C or storage in liquid nitrogen has been widely and successfully used for a range of micro-organisms. [65] It is, however, a complex and costly method requiring a considerable amount of space and, unless the micro-organisms are carefully handled, there is a risk of explosion when the material is removed from the containers. [63]

The last three forms of storage mentioned also require careful attention to the rates of freezing and thawing, so they are not commonly used if other procedures are successful.

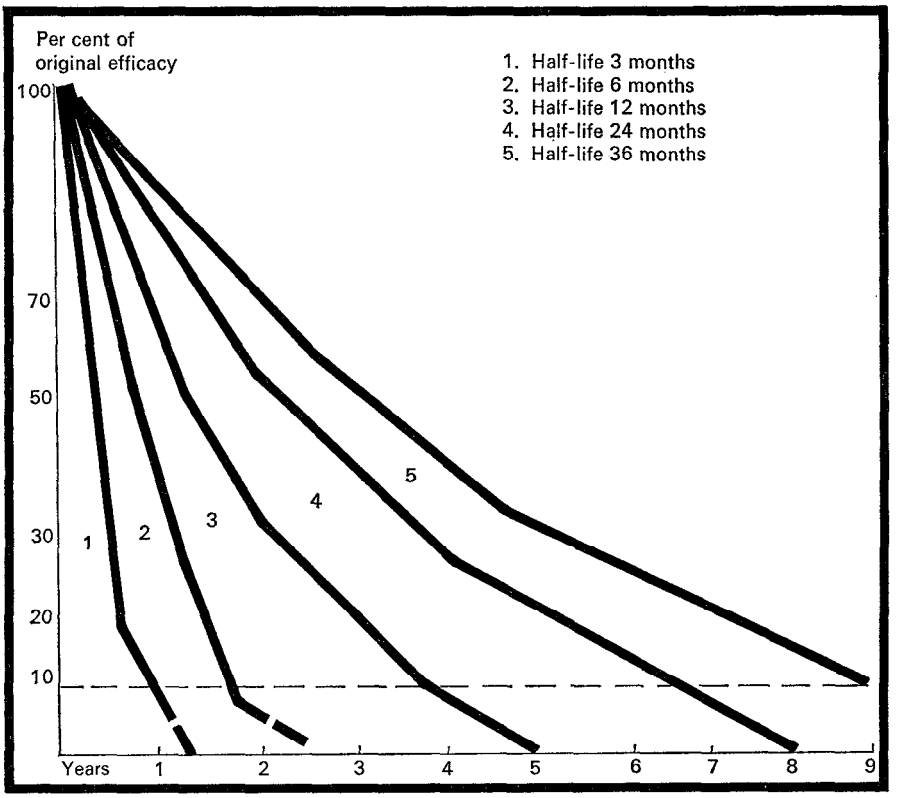
3. The combination of freezing and drying of the frozen cells in vacuum—lyophilization—has proved to be the most successful method for most micro-organisms, and one might doubt if biological weapons would be practicable without it. While the equipment and expertise required for satisfactory results should not be underestimated, the product can normally be expected to retain many, if not all, of its original characteristics for a considerable time. Three important qualifications must, however, be noted:

(*a*) Viability decreases with time, and satisfactory storage time is to a large extent dependent upon the purpose of the storage. For the purpose of keeping a type collection, [66] a loss of one or two orders of magnitude in 1–5 years, which is quite reasonable for fastidious micro-organisms [63], is a satisfactory result, but it can hardly be called adequate for a biological weapon. The loss of one order of magnitude means that the payload requirement is multiplied by ten. There are, however, a number of recent experiments using protective material which increase viability considerably. [67–68] Commonly used are sugars and proteins which, however, are comparatively easy to identify, and indicate preparation for a BW attack.

(*b*) Organisms may take time to recover and frequently they are not in optimum physiological conditions, requiring several subcultures before they regain their characteristics. This may be due to alterations in cell wall permeability which allow RNA and other substances to leak out. [69] Obviously such products would be of limited military value. [63]

(*c*) The question of virulence as opposed to viability may be one of the characteristics mentioned above, but is important enough to be considered separately. A consequence of a decrease in virulence is, of course, an increase in the infectious dose, which means increased payloads. Aging of micro-organisms in the

Chart 2.2. Decline in efficacy as a function of storage time



aerosol state is known to give this result [70] but little is so far known about the effect of lyophilization on virulence. However, it is interesting to note that in a Soviet experiment lasting 38 months, three out of four typical *Pasteurella pestis* strains lost varying amounts of virulence. [71]

These factors taken together indicate that decay in virulence and viability may constitute a problem and that the organisms are most probably dependent upon storage time. Thus, the shorter the time, the better is the chance that the product resembles the original with regard to these vital characteristics, and it seems reasonable to assume that considerations of this kind underlie the quote "the halflife of a few [biological agents] is something of the order of three to four years. Most biological agents have halflives of three to six months, but only if they are kept under refrigeration".[1] The graphs in chart 2.2 merely illustrate drops in efficacy during storage for some alternative half-lives. If one arbitrarily assumes that a decline of up to one order of magnitude is acceptable —and calculations do become more uncertain below this level—it is obvious

[1] See Volume V of this study, p. 157.

that most BW agents become unusable within comparatively few years. Longer periods of storage may be possible, but only at a very low temperature (−70°C) [72] which adds to the complexities.

It follows that considerable production has to be maintained merely to keep the stock of the stored products. The greater the decay, the greater production is necessary. Merely keeping 10^{17} bacteria in stock, for one attack against a small to moderately sized target would, if their half-life is 4 months, take as much medium as the diagnosis of some 50 000 clinical specimens. Obviously, such quantities can hardly be purchased on the open market without the risk of attracting attention.

It should finally be added that even when the agents have deteriorated beyond their military usefulness, they are still highly dangerous and must be destroyed. It also follows that if production were efficiently prohibited and followed up by inspection, one might soon be able to cease worrying about the threat of hidden stockpiles. No doubt there exists in the archives of the defence laboratories data on the storage life of BW agents which would be of great value to illustrate this point and indicate the extent of any stockpile threats.

Conclusions

There are obviously many instances where discrepancies between available resources and actual needs will provide tell-tale indicators of an intention to produce BW agents, but this concerns the use of more or less standardized equipment and normal approaches. If ever a completely unorthodox technique were found it would, however, also alert the inspector, who would be faced with interpreting its potential in terms of BW production. One example would be the "inverted" isolation technique, often used in the handling of toxic nuclear materials and occasionally in germ-free animal work. In this case, all operations are performed with the same equipment and techniques as those employed in any laboratory working with non-hazardous materials. The difference is that all equipment is kept in a hermetically sealed room, to which the operator or operators have access either wearing a plastic "halfsuit" or diver's equipment permitting entrance through a liquid seal filled with disinfectant. Modern fibreglass techniques and the sealing methods now used for plastic sheets could make it possible to close off a large suite of rooms hermetically and to handle dangerous pathogens with rather crude techniques. An inspectorate would consequently be extremely wary of any sizeable portion of a building to which access was not granted. Production techniques such as those outlined would, however, not guarantee against laboratory infections, and this would necessitate measures that could certainly be indicators of BW production. In summary, it can be stated that the production and storage of BW agents is a

complex activity which requires considerable investments in R&D as well as in skills and equipment. Accidents can either constitute a hazard to the environment or render the product harmless. The production of a biological weapon as a sideline to civilian activities is less likely to occur than manufacture restricted to a completely isolated facility. This latter would, however, require an exchange of information with the scientific community, and, as indicated in the preceding chapter, that exchange would be extremely difficult for the military security personnel to control.

III. *Inspection of nonproduction of biological warfare agents in declared research and production facilities: a field experiment*[2]

History and scope

The idea originated in the Pugwash BW Study Group, which was formed in 1964. This group was impressed by the Western European Union's system for inspecting arms production, including chemical weapons, and decided to investigate whether an inspection system might be feasible in respect to BW agents. The Pugwash experiment was based on the inspection of four laboratories in Stockholm, Copenhagen, Vienna and Prague.

The SIPRI project aimed to go further, inspecting more laboratories, using more inspectors and working out an inspection questionnaire and techniques as experience was gained.

The aim of the SIPRI project was to establish, by practical investigation, whether or not it was technically possible to discover if production of BW agents on a militarily significant scale could be carried out in non-secret microbiological research or industrial establishments.

The estimate of the quantity of agent that would be militarily relevant was 10 kilogrammes of microbial paste or spores, or hundreds of grammes of botulinum toxin. The basis for this estimate was that it would be a sufficient quantity to make an attack over an area of a few square kilometres with an expectation of reaching a high proportion of the occupants of the area by direct contact. This is an extremely low estimate—really too low—of what is a militarily relevant quantity.[3]

[2] The project on the inspection of nonproduction of biological warfare agents was carried out by SIPRI during 1968 and in the first half of 1969. It was conducted under the guidance of a board of scientific consultants: Professor C.-G. Hedén (chairman), Sweden; Academician I. Málek, Czechoslovakia; and Professor J. Rotblat, Secretary-General, Pugwash Conferences on Science and World Affairs. The secretary of the project was Mr Theodor Nemec, of the SIPRI staff.

[3] As noted in appendix 2 of Volume V (pp. 147–51) of this study, subsequent work suggests this fact.

Epidemics, epizootics or sabotage were not considered. The amount of pathogens or toxins required for this type of use is extremely small so in that case inspection is powerless. Furthermore, these means of attack are hazardous and unpredictable and consequently of limited military interest.

Twenty-two non-secret research and production establishments, ranging from a small university microbiological department to a large industrial company, in nine European countries were selected on the basis of advice from SIPRI consultants. The establishments were invited to cooperate in the inspection project and to nominate their representatives to serve on a SIPRI international inspection team. The United States and the Soviet Union were not included and the study was limited to Europe, where every effort was made to achieve a reasonable balance between laboratories in countries belonging to NATO and the Warsaw Pact as well as some non-aligned countries.

Of the 22 laboratories approached, 14 responded positively, although in a number of cases it was necessary, through personal contact and explanations from other scientists, to remove mistrust with regard to the motives involved. This obstacle was not restricted to the countries of any particular political system. One production establishment in a neutral country was forced to refuse receiving an inspection visit because this would have been contrary to the existing laws of that country. Another, a research laboratory in an East European country, declined, despite original acceptance, apparently due to a misunderstanding of the international character of SIPRI.[4]

It is understood that the 14 laboratories which participated did so with the knowledge of their relevant authorities.

The participant establishments were the following:

Research laboratories

1. The Medical Research Council Group for Bacteriological Bioengineering, Stockholm.
2. The Institute of Microbiology of the Czechoslovak Academy of Sciences, Prague.
3. The Institute of Virology of the Czechoslovak Academy of Sciences, Bratislava.
4. Bundesforschungsanstalt für Viruskrankheiten der Tiere, Tübingen, West Germany.
5. The Lister Institute of Preventive Medicine, London.
6. The State Institute of Hygiene, Warsaw.

[4] We probably helped ourselves a little in the selection. In all fairness, it must be said that we chose rather easy cases where we had personal contacts. It is hard to say how many institutes we would have had access to if we had picked institutes and industries on a completely random basis.

7. The University Institute of Microbiology, Copenhagen.
8. The Institute of Hygiene, Graz, Austria.

Production establishments

9. The Institute of Immunology, Zagreb, Yugoslavia.
10. The Lister Institute of Preventive Medicine, Elstree Laboratories, Elstree, England.
11. Wellcome Research Laboratories, Beckenham, Kent, England.
12. Institut Mérieux, Lyon, France.
13. LEO Pharmaceutical Products, Copenhagen.
14. Aktiebolaget ASTRA, Södertälje, Sweden.

Twenty-five scientists were directly involved in the inspection visits.[5]

The inspection visits

After having consented to an inspection visit, the laboratories were sent working documents on the inspection techniques and copies of the version of the questionnaire existing at the time (the original questionnaire was thoroughly revised and expanded during the visits—the one presented in the sample of documents in appendix 1 is the tenth version). Because of problems connected with visas and the organization of an inspecting team, the date of the inspection visit and the names of the inspectors were necessarily known to the laboratories several weeks—in the case of some laboratories, several months—in advance. As it was our aim to have scientists from both the East and the West on the inspecting team, the visa problems were considerable. In some cases visas required many interventions and were issued only hours before the departure of a given inspector. Similar difficulties were experienced both in Eastern and Western countries.

The pattern of the inspection visits differed from case to case and with the progress of the experiment.

The members of the visiting team gave a pledge to the visited establishment not to disclose observations which might endanger its scientific or commercial interests nor publicise the results of the visit. The establishments were also

[5] Prof. C.-G. Hedén, Prof. S. Gard and Dr Bengt Oom, Sweden; Acad. I. Málek, Dr J. Ricica and Acad. D. Blaskovič, Czechoslovakia; Prof. J. Rotblat, Prof. Sir Ashley Miles, Dr P. Lindop and Mr W. A. Fitzgerald, United Kingdom; Dr Triau and Dr Branguier, France; Prof. O. Maaløe, Dr J Leerhøy, Dr C. Treschow and Dr E. Frederiksen, Denmark; Dr J. Ungar, Switzerland; Prof. W. Kurylowitcz and Prof. J. Jeljaszewicz, Poland; Prof. D. Ikič and Dr T. Mannhalter, Yugoslavia; Prof. D. M. Mussgay and Prof. Dr G. Wittman, Federal Republic of Germany; Prof. Dr H. Möse and Doz. Dr V. Dostal, Austria.
In addition, 68 scientists from 13 countries (including the United States and the Soviet Union) took part in discussions about inspection techniques, either during the inspection visits or on other occasions.

promised that the filled-in questionnaires would be handled confidentially and used only as a background to the study.

The normal duration of the visit of a SIPRI inspecting team was 1 or 2 days; the maximum in one case was 4 days. The usual number of inspectors was two plus the secretary; the maximum was five on one occasion. As a rule the head of the inspecting team had experience from a previous visit in another (usually his own) laboratory.

The visit usually started with a meeting with the director of the establishment and the heads of departments, during which the questionnaire was filled in. A tour of the establishment followed with discussion with the workers present. After the conclusion of the inspection, another discussion took place during which opinions were exchanged about the improvement of the suggested techniques.

The cost of the project borne by SIPRI was approximately $11 000. The loss of time and money to the participating laboratories was considerable. In the case of a very large production establishment in a Western country, the cost of the preparations for the inspection and the visit itself approached SIPRI's expenditure for the whole exercise. It should be pointed out, however, that this estimate referred to a single, initial visit and that drastic cuts in the cost would accrue in the course of repeated visits.

The inspection documents

Appendix 1 represents the final version of the SIPRI inspection documents incorporating all modifications suggested during the inspection visits. The main item of interest is, of course, the questionnaire. The information it requests about the organization of the laboratory to be inspected, location of its buildings and mobile units, about the types and quantities of microbial agents produced, the numbers and competence of its personnel, laboratory and production equipment, restrictions on the movement of visitors, and immunization of personnel should give the inspectors a good picture about the nature of current and potential activities of the laboratory in question.

The questionnaire is to be filled in by the management well in advance of an inspection and publicly displayed so that the personnel of the establishment can become acquainted with the information contained therein. The personnel should be encouraged by the management to communicate freely with the members of an inspecting team. It is expected that eventual discrepancies between the answers given in the questionnaire and the actual state of affairs may thus be brought to the knowledge of the inspectors.

In most production processes there are alternatives. These alternatives cannot be put down in a routine check document. The questionnaire is first of all an

auxiliary tool. It is not the items in the questionnaire with which the inspectors are mainly concerned; their primary concern lies with those spots of information which, on the basis of overall experience, the inspectors consider to be critical. Some may appear in the questionnaire but certainly not all.

The main function of the questionnaire and the other documents, as far as our approach was concerned, was thus to force the potential evader into a limited number of postures and to make his life more difficult.

Ideas which emerged during the inspection exercise

The inspection visit would always have to be a minor part of a large-scale and continuous operation. The SIPRI experiment was severely limited in that SIPRI had neither the personnel nor the financial requirements for a proper pre-inspection documentation survey which obviously would be an essential phase of an officially established inspection operation.

The inspectors must have at their disposal all conceivable information about the laboratory to be inspected and must make a proper analysis of the information before the visit.

The efficiency of inspection will increase as the inspection system operates. After the tenth or fifteenth inspection visit to a laboratory, the inspectors will also have come to know the personnel, will have established personal contacts with them and gained their confidence. Only then can an on-the-spot inspection become fully efficient. This means that the inspection has to be a continuous effort. Inspection visits take place at intervals, during which the inspectors must gain supplementary information so that they can look for essential indicators during the visits. In order to recruit competent inspectors, one must give them some scientific challenge, possibly in the form of responsibility for the technical aspects of inspection. In the course of his work, a good inspector must necessarily become an expert in evasion techniques; that is, he must learn to know how his current inspection technique can be evaded and continuously study the possibility of countering evasions of different sorts by instrumentation. This aspect must be stressed.

It is necessary to develop a "black box" concept. In the interval between the inspections there should be tamper-proof devices which are hooked onto the apparatus used in the production process and which an inspector can monitor. They need not be in constant operation but can be shunted in at random, decided by the inspectors. Such devices can considerably improve the efficiency of a verification system. In the view of SIPRI inspectors, much emphasis should be put on microcalorimetry because of the fact that the heat generated by any microbiological process is rather typical. During different phases of microbial cultivation when different sugars are being fermented, heat is generated and one can obtain a sort of fingerprint of the process if one has a properly

constructed device. This should also record sterilization phases and other process variables. For instance, if the fermentor is used to make tetanus toxoid one should, after some experience, eventually distinguish a fingerprint which reflects this particular process run with a particular substrate, and if this same fermentor were suddenly used for producing botulinum toxin, it would probably show up in the fingerprint of this process.

There are of course numerous other possibilities, for example, programmed devices for sample removal. One can envisage automatic sampling devices of different types. However, such devices do not yet exist, nor can automatic microcalorimeters be picked off the shelf. They would have to be especially designed for the specific function outlined. One can envisage that there would have to be built-in power supplies which would automatically cut in during a power failure. One would have to design tamper detectors, etc. In industry there are examples of similar devices and techniques for checking certain products, such as techniques for sealing pipes and valves in alcohol manufacture. A lot of thinking has gone into this area and some existing devices could be more or less directly adapted to the verification process. The "black-box" concept obviously needs a major study which SIPRI could not undertake.

As such, inspections should be random processes, which leads to the important consideration of how frequent the visits should be. This problem was discussed during the SIPRI inspection visits and one of the ideas was to divide the laboratories into three groups. The first are those laboratories which would in principle not be inspected at all and would be covered only by documentation; that is, there would be a requirement to send in protocols, reports, reprints, and so on. This group would encompass the normal microbiological laboratory not studying pathogens but doing relevant work in molecular biology, genetics, bacterial physiology, etc., or being staffed with scientists qualified in the handling of pathogens. These laboratories would be outside the regular inspection pattern.

Another group would be laboratories of crucial significance which would need on-site permanent inspectors at all times. These could, for instance, be fellowship workers from other countries. However, the largest group of laboratories would be those exposed to random inspections. They could be divided into five categories:

1. Large-scale establishments which could convert facilities to a significant BW capability in less than 6 months without the need for supplementary personnel. Only specially authorized personnel have access to the premises.

2. Large-scale establishments which could convert facilities to a significant BW capability in less than 6 months, but without adequate personnel. Only specially authorized personnel have access to the premises.

3. Same as 1. but no special clearance needed for visitors.

4. Same as 2. but no special clearance needed for visitors.

5. Normal microbiological laboratory with good facilities for work with pathogens.

In a fully developed inspection system, a subdivision on regional activities might be desirable in order to reduce travel costs (for instance, North and Central America, South America, Africa, Western Europe, Eastern Europe and the USSR, the Near East, the Far East, Australia and New Zealand). Each such region might appoint a panel of 12 experts from which inspectors could be drawn. All activities should, however, be coordinated from a central office under the responsibility of an inspector general. This office would file all reports and assign the inspected laboratories an inspection rating.

The frequency of visits should be proportional to the level of risk, that is, the inspection rating. However, the choice should be random.[6]

To be able to run an inspection on an entirely random basis, the inspectors must have immediate access to the laboratory that is to be inspected. The inspectors must be currently provided with visas and this, of course, presents a major political problem which lies outside the scope of the SIPRI project. There are, of course, other things which could make unannounced access impossible, for example, the risk of infection. Inspectors would have to be informed about what agents they should be vaccinated for, so that a visit could take place without prewarning, or they would have to be provided with protective clothing and masks.

Everyone who participated in the SIPRI inspection visits was impressed by the fact that the inspection teams were accepted so readily not only in the university microbiological laboratories but also in the public health institutes and in industrial plants. Once they were accepted they never encountered any problems such as refusal to inspect facilities they thought relevant, and some of the firms

[6] *Possible arrangement:* Reports from all laboratories are submitted by 1 January each year and processed for computer analysis. On 15 March the deputy inspector generals (chairmen of the regional panels mentioned earlier) should meet to decide if earlier laboratory ratings should be changed. When the ratings have been fixed, a die is then thrown once for each laboratory. A-laboratories would then be inspected whenever the die shows the figures 1, 2, 3, 4 or 5 (an 83.3 per cent chance); B-laboratories when it showed 1, 2, 3, or 4 (a 66.7 per cent chance); C-laboratories when the die shows 1, 2 or 3 (a 50 per cent chance); D-laboratories when it showed 1 or 2 (a 33.3 per cent chance); and E-laboratories when it showed 1 (a 16.7 per cent chance). If a similar random selection is carried out by the central office on 15 September, the result would be that type A-laboratories would, on the average, be inspected every 29 weeks, B-laboratories once every 9 months, C-laboratories once a year, D-laboratories once every 18 months and finally the E-laboratories every 3 years.

certainly faced considerable problems in meeting their requirements. The inspectors emphasized that they were looking only for specific items and that they were not interested in the whole flow pattern which would probably have made the visit difficult from the point of view of interference with trade secrets. The inspectors indeed do not need that type of information. Experienced inspectors need only look at a few critical things, and once they have seen them they are satisfied and do not need to spend a lot of time looking at details. An inspection is not really a matter of mapping out the whole operation. In a sense it is like looking for a needle in a haystack. It is a hopeless task if one does not know how it is done, particularly for an outsider. However, the inspector has a metal detector. He knows what he is looking for from facts which are superficially not related. The expert need only see a few things in order to get a sufficiently clear picture of what can be done in a given laboratory. Of course, the inspectors have to be selected very carefully and the team can certainly not be picked on the basis of national representation alone. The team must consist of experts who spend a lot of time thinking about the problems of inspection and who come from fields where they are highly competent (bioengineering, microbiology and accounting).

In most processes there are alternatives, and the inspector must know a good deal about cultivation technology and microbiology in order to be able to evaluate alternatives. These alternatives cannot of course be put down in a routine check document.

Conclusions[7]

The efficiency of the SIPRI inspection technique could best have been tested by a positive experiment, that is, an attempt by one team to develop a BW capacity in one of the visited laboratories, unknown to the other team of inspectors. This idea had to be abandoned due to the costs involved which we estimated to be approximately $100 000. It would have been necessary to convert the laboratory where production was to be undertaken, with an expensive outlay on new equipment and safety measures, possibly even including the modification of buildings.

For an evaluation of the experiment we were forced to rely on the educated guesses of those associated with the project.

The following question was asked:

Suppose that a laboratory has been subjected to a series of five inspections by the same team, composed of a microbiologist (bacteriologist or virologist), a biochemical engineer and an administrator specialized in the study of production records; how

[7] See also Volume V, pp. 157–60.

effective (expressed in per cent of complete) do you think that a subsequent visit would be in disclosing an ongoing secret evasion providing a military BW capability (10 kilogrammes or more of highly virulent microbial paste, hundreds of grammes of botulinal toxin or an amount of rickettsiae or virus sufficient for an aerosol attack over many square kilometres)?

A fully developed documentation back-up permitting an analysis of production records as well as cross-checking of professional competence is assumed to be available to the inspectors. It is further assumed that the visit is unannounced following a frequency pattern geared to the chance for hitting on an actually ongoing evasion which is more than one in three.

This question was put to to 77 microbiologists and other scientists who were closely or peripherally involved in the project. Fifty-five replies were received, of which 51 provided a percentage figure in answer to this question. Those who had not directly participated in the inspection experiment gave answers approximately 20 per cent lower than those who had been directly involved. As noted further, various comments and qualifications were made. Regardless of these—they do not affect the picture much—it is not easy to interpret a percentage figure. Perhaps the important point to emphasize is that it means no more and no less than it says: that the average opinion of those involved in the experiment was that this kind of inspection had about a one in two chance of being successful. The respondents had the advantage of some acquaintance with the experiment and possibly the disadvantage of feeling committed to it.

The main point to note, however, is that the quantity of material being looked for—10 kilogrammes of microbial paste or spores—is extremely small compared with the amounts which we have now estimated are needed to constitute a military capability. It is less than the amount needed for one substantial aerosol attack, and it has been reckoned that an advanced country of medium size would need the capacity to produce, in a year or less, enough for 100 attacks if it were to possess a full military capability.

The comments made by those who answered the questionnaire mainly concerned the possibilities of evasion. These would, of course, be fairly high with respect to the small quantities to which the experiment was addressed. It was pointed out that some laboratories would present greater problems than others—for example, those where large quantities of potentially pathogenic material were handled normally, or those with a very small trusted staff, or those which provided know-how for a separate secret production unit. It was also pointed out that it would be virtually impossible to carry out unannounced visits. Apart from problems of visas or travel permits, a surprise visit could be refused on grounds of problems in the laboratory, such as risk of infection, escape of radioactivity or decontamination in progress. False records and double books might be kept. It was also suggested that efficiency would be increased if the inspection technique were not always known beforehand and

that inspection techniques should be further elaborated with regard to individual agents considered for biological warfare.

It is not possible to draw very extensive, firm conclusions from this evidence. This is partly because, as has been emphasized in Volume V, the possibilities to make inspection visits depend very much on the political background and atmosphere of cooperation or non-cooperation within which the operation is conducted. Our experiment was an exploration of the problem.

The experiment, together with our assessment of the required scale of military capability and the very special safety measures required to produce it, weighs against the view that verification is technically impossible regardless of the political circumstances. Plainly, more work on the subject is needed—and more publication of the work that has already been done. Meanwhile, the tentative conclusion from the SIPRI experiment and our analysis is that a substantial measure of on-site verification would be possible provided certain conditions were fulfilled: documentation, free access to all facilities and personnel, the possibility of visits at short notice or of permanent inspection by resident inspectors or by exchange scientists cooperating with them.

Chapter 3. Technical aspects on the detection and identification of microbial agents, particularly those that might be used in weapons

I. *Introduction*

As explained in the introduction to this volume there are so many methodological overlaps between the techniques used in early warning against BW attack, in investigating allegations and in verifying nonproduction that they can be handled together. However, early warning, detection, identification and verification of BW agents constitute a particularly complex issue for many reasons. First, there is the problem of tracing and identifying minute quantities of micro-organisms belonging to a wide variety of species in large volumes of air, water and food. All those vehicles contain varying amounts of harmless micro-organisms as well as organic material similar to that of micro-organisms, that is, protein, nucleic acid, lipids, ATP, etc. [73] Detection may also be deliberately made difficult by changing the biochemical characteristics used for identification, by encapsulation and by the use of more than one agent at a time. Obviously, no single method can be satisfactory for all possibilities. Secondly, the need for speed conflicts with the requirements of sensitivity and specificity, as indicated in table 3.1.

It is obvious that a combination of rapid, nonspecific and slower, more specific methods should be incorporated in each BW detection unit (sensor). The earlier a BW attack is discovered, the better are the chances of mitigating its effects. Ideally, detection should take place before exposure, but it must also be pointed out that it is often possible to take countermeasures during the incubation period if the agent can be identified in time. For some agents, for example, pneumonic plague and VEE, the incubation period can be as short as 24 hours.

Chart 3.1 illustrates the temporal relationships between detection and identification methods and countermeasures during an aerosol attack upwind of the target. In this way it becomes possible to distinguish, on the one hand, measures that prevent exposure—for example, the use of gas masks—and, on the other, those possible after exposure—for example, during the incubation period—such as medical and administrative measures. Early warning and identification

Table 3.1. Speed of detection versus sensitivity and specificity

Detection principle	Type of indication	Speed
LIDAR	Aerosol	Before arrival
Particle detection	Susp. particle size (1–5 μ)	Seconds
Key biochemical substance	Susp. micro-organisms	Minutes
Biological activity	Living micro-organisms	1/2 hour
Immunofluorescence	Presumptive diagnosis	1/2 hour
Rapid conventional method	Presumptive diagnosis	1/2–1 day
Conventional methods	Definite diagnosis	Days
Epidemiological methods	Definite diagnosis	Days, weeks

can be distinguished even if they merge into each other. If the aerosol attack is set up on the outskirts of the target itself, then the time available for pre-exposure measures dwindles to practically zero.

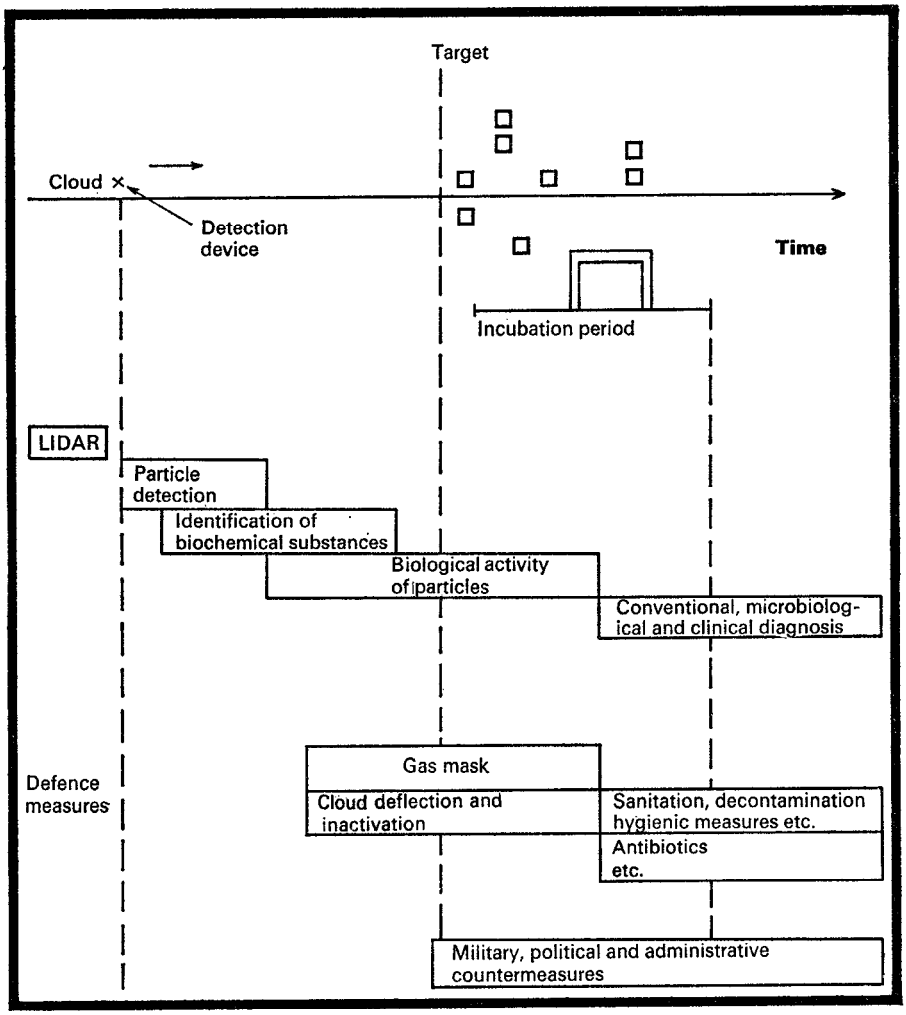
II. *Conventional diagnostic methods*

Clinical diagnosis in BW

Diagnosis of the actual disease will play an extremely important role, and the earlier such a diagnosis can be made, the earlier countermeasures can be taken. Since, from the point of view of the attacker, absence of immunity to the BW agent is desirable, if not absolutely necessary, it follows that suitable BW agents are such that are not common in the target area. Exotic agents might be used, and it is therefore not unlikely that those in the medical profession would have little experience with the diseases and would consequently encounter great difficulties in diagnosis and treatment. As this would add to the normal difficulties in the diagnosis of infectious diseases, a brief discussion of this aspect is perhaps appropriate.

Fever is a singularly unhelpful symptom since it is common in all infectious diseases. It also occurs in the course of other diseases but when large clusters of cases appear, fever caused by non-infectious disease can normally be excluded. The combination of fever plus a rash is believed easy to diagnose, at least by mothers with small children, but this is not always the case; the rash early in measles can, in fact, look just like scarlet fever or German measles, etc. As fever plus a rash, plus a marked cerebral involvement could signify both complicated measles or a purulent meningitis, the symptoms must be differentiated with the aid of further diagnostic procedures. In this context it is important that the physician then consciously or subconsciously uses geographical medicine to narrow down his choice. In most European countries it is not necessary to watch for epidemic or endemic typhus or, for that matter, dengue or relapsing fever. This example, taken from a textbook of infectious disease, indi-

Chart 3.1. Distribution of cases



cates the difficulties in diagnosing typhus, which is often considered in connection with biological warfare. Before the advent of the rash, which usually comes on the fifth day, other diagnostic probabilities would seem more likely.

It is obviously possible, or even probable, that the first cases of a disease which is new in an area will be incorrectly diagnosed both because the physcians are not familiar with it and because existing diagnostic techniques will not be employed since there is no call for them. A delay in diagnosis will mean a delay in treatment, and it is therefore reasonable to assume that the outcome of the disease will be more severe than in an area where it is familiar. Existing figures for mortality due to BW agents might therefore be on the low side, par-

ticularly if one considers such factors as nutritional status and hygiene, which can aggravate the situation.

Yet another aspect of infectious disease is relevant in this context. A study of the falling curve of death resulting from infectious disease could easily lead to the erroneous conclusion that these diseases no longer occur frequently. A reduction has undoubtedly occurred in the developed countries, but it must be emphasized that infectious diseases still cause a major loss of working hours. Infectious diseases are always present in a community, albeit in varying frequencies. A BW attack must therefore be differentiated from the normal infectious disease, which may show a higher incidence during hostilities due to lowered hygiene. Once a sudden increase in febrile diseases is observed, all cases of febrile disease must initially be regarded as suspect.

Finally, it should be emphasized that not only do several micro-organisms give similar syndromes, but the same micro-organism can give different clinical pictures. Diagnosis is therefore often the result of a careful collation of conflicting evidence derived by questioning the patient and persons in his environment, from physical examination and from laboratory examinations, that is, a process which has some similarity to that of military intelligence. Single, irrefutable signs of a specific disease exist but are, in fact, comparatively rare.

Microbiological diagnosis

The diagnostic laboratory serves several important functions in BW defence. It must aid in the diagnosis of clinical disease caused by a BW attack. Today, when warning systems are still in their infancy, this may be one of its most important roles. Also, it hardly seems likely in the foreseeable future that warning and identification devices will be capable of instant or very rapid identification for all possible micro-organisms and their conceivable modifications. It will probably prove necessary to send samples to a diagnostic laboratory.

Another task to be undertaken by the laboratory is that of generating accurate diagnosis of natural infectious disease. Without close surveillance of the infectious disease panorama, a natural epidemic might be mistaken for a BW attack.

A brief outline of conventional diagnostic methods is presented below, together with some currently used, or envisaged, rapid modifications. It can also be stated that many of the methods for automated detection have originated from conventional methods. The methods used for bacteria and fungi and those for virus and rickettsia are sufficiently different to warrant description under separate headings.

Identification of bacteria and fungi

Since bacteria and fungi are small and have few useful characteristics of shape and form, identification must be based on studies of them during growth and propagation under various circumstances in order to gain sufficient characteristics for a diagnosis. For growth and propagation, micro-organisms need energy which they can, for example, take from sugar that is then decomposed into acid and sometimes gas, both of which are easily detected. Since different species utilize different sugars, it follows that, by carrying out a series of such tests, one can gain an idea about the identity of the species under study.

Diagnostic methods based on growth and multiplication/reproduction are, however, time consuming. The shortest interval between cell divisions for bacteria is about 15 minutes, which means that one bacterium will take several hours to generate enough offspring to compose a mass that is visible to the unaided human eye. This material can then be treated and subjected to further tests, which also take time. Identification requires several tests and, even if many tests can run concurrently, the whole process is time consuming. At present, therefore, identification is rarely achieved before 24–48 hours have elapsed.

However, many approaches aimed at increasing the speed of identification are being studied. The one-dimensional, continuous strip culture [74] or its later variations [75–76] and the capillary tube methods are suitable for performing a number of analyses simultaneously and automatically. [77] If microbial growth and metabolic activities are made visible with suitable indicators or with the help of optical techniques, they can be detected much earlier than with the conventional methods. With one such method, 1 000 E. coli cells can be detected after 3 1/2 hours [78] compared with at least 8–12 hours with conventional culture methods. It is also possible to perform parallel tests on other media containing, for example, antibiotics, and in this way to gain information relating to the antibiotic sensitivity [78] of the organism in question. This is very important in BW defence.

For some bacterial species, shortcuts to identification are already available. The fluorescent antibody (FA) can be used for identification of bacteria immediately after isolation and is also used for the rapid detection of some bacteria in the original specimen (*cf.* below). In the latter case, cultivation is also usually performed in order to verify the diagnosis.

Phages, that is, viruses, that attack bacteria selectively can also be used, as in the rapid detection of anthrax which is possible within 7 hours with this method.

Identification of viruses and rickettsiae

Identification of viruses and *rickettsiae* is, on the whole, more difficult since they are only able to grow and reproduce inside the living cells from a host animal. Little is known about their metabolism and certainly not enough as

yet to aid in identification. (For a discussion of this point, see appendix 4). Identification usually depends upon immunological methods of which the fluorescent antibody technique (*vide infra*) shows promise owing to its speed. [79–81]

Identification of protozoa

Identification of protozoa rests almost entirely on the use of the microscope since few species can be cultivated with reliability. Due to this factor and the fact that protozoa are comparatively large and complicated organisms, their use in BW is probably limited to attacks via insect vectors. The recognition of these insect vectors is a problem for the entomologist.

Serological methods in BW identification

A factor common to bacteria, viruses, *rickettsiae* and to some extent protozoae and even some protein agents like ricin and other toxins is that, when introduced into a host, they are capable of stimulating the production of highly specific antibodies. Production of such antibodies, however, takes time and it is usually not until 1–2 weeks after the start of antigen exposure that a rise in their level can be established. Once formed, such antibodies remain in the body for many months and a single blood sample does not permit any other conclusion than that the antigen has been present at some earlier, unspecified time. A rise in the level of antibody, however, provides a strong indication of recent infection, whether symptoms appear or not.

Serological methods based upon antibody rise in humans or animals have, of course, no importance in BW defence except as one indicium that the agent in question has been present. An antibody can, however, be produced and extracted from a laboratory animal and it can then be used for identification of the organisms used to produce it. This is the basis of immunofluorescence, a serologic method whose importance in BW defence is so great that it warrants a fuller discussion in this volume (see pages 104–35). Among other uses of antibodies in detecting antigens, we could also mention the rapid diagnosis of smallpox by testing the contents of the pustules. Recently, this type of reaction has been speeded up by using electric current to force antigen and antibody together (immunoelectroosmophoresis).

III. *Rapid and/or automated BW detection*

Equipment suitable for BW detection should be capable of remote, unattended operation. Such equipment can be constructed according to any of three principles, *viz*, detection of physical particles, detection of key biochemical substan-

ces and detection of biological activity. Since the first of these only indicates the presence of an aerosol, the second only the presence of material possibly deriving from micro-organisms and the third that viable micro-organisms are present, it follows that the methods have to be combined in one way or another. The order in which they are given here is also appropriate since the methods follow roughly in order of their decreasing speed, increasing sensitivity and cost. The fluorescent antibody technique can be said to belong to the second group but it is described separately.

Sampling

Micro-organisms used in a BW attack are present in minute amounts dispersed in large quantities of air, water or food, and before they can be studied they must be concentrated and brought to the detection instrument. This task is done in so-called samplers, of which a large number of types are available and which operate according to several different principles. [82] The multitude of existing samplers appears to indicate that no one sampler is ideal for all purposes. [83] Obviously, a sampler that minimizes damage to living micro-organisms is necessary when detection devices based upon viability are used, whereas this need not be a primary consideration when searching for key biochemical substances by procedures in the course of which the micro-organisms are killed. The choice of sampler is thus intimately connected with the choice of detecting device/method since the overall efficiency of the equipment is dependent upon the careful combination of the two. [83] The efficiency of modern samplers is indicated by a device that can collect the particular contents of 10 m^3 of air into a small amount of liquid in 1 minute. [84] In discussing samplers, it should also be pointed out that different species differ in their ability to withstand the trauma of sampling. Spores have an excellent resistance, while a significant portion of plague and tularemia bacteria may not survive the sampling process. [85]

Detection of particles by physical means

Both in the United States and the Soviet Union, BW detection schemes appear to envisage physical particle detection as a useful early step for discovering aerosols. [73, 88] Such particle counters, however, respond to all particulate matter in the air, whether living or not and regardless of its origin. Also, there is unfortunately no fixed relation between these components except that the number of particles is always greater than that of the micro-organisms. [87] The need to make up a B-aerosol with a particle size in the range 1–5 μ in order to ensure effect becomes an aid in detection. With particle counters that can register size

as well as the number of particles, a sudden increase in the 1–5 μ range compared to other size ranges should be a highly useful indication of the presence of a B-aerosol.

Measuring and counting of particles can be done according to several principles. One of these is by magnification in which some form of scanner is coupled to a microscope. [88] The partichrome device described below is, in fact, a development of this method.

The volume-displacement method is a useful technique for measuring particles in a liquid. If a particle in an electrolyte passes a narrow gap between two electrodes, it will set up an increased resistance to the current passing between these electrodes and the change in resistance is proportional to the volume of the particle, which can then be easily measured. [89]

Particle measurement by light scattering will probably become an important method in BW detection since it is suited to continuous monitoring and is a comparatively inexpensive method. [90–92] The underlying principle is that while light passes directly through clean air, it is deflected or scattered by the presence of a particle and the amount that is scattered is proportional to the size of the particle. By utilizing a very narrow beam of light and catching the straight light in a trap, the scattered beams of light can be measured by photocells and thus recorded. [93–94]

Methods based upon detection of key biochemical components of micro-organisms

Micro-organisms contain substances which are more or less unique or, in the words of Ierusalimsky, "they are cut from the same material but they are put together differently". Detection of characteristic compounds will indicate the presence of micro-organisms, even if there is no indication as to whether they are alive or dead.

There are as yet few automatic devices for detecting key biochemical substances known in the open literature, but one device is the partichrome analyser developed in the United States and based on staining with the dye ethylviolet. This dye stains micro-organisms more efficiently than it does other aerosol particles such as dust. [95] The instrument derives its name from the scanning method where two photocells—one sensitive to blue light and the other to green—simultaneously register the light transmission of particles impacted on a sticky tape.

Information concerning other automatic devices is limited since they are either secret or only exist at a laboratory stage of development. Some appraisal of the possibilities of detection can, however, be gained from studying such principles of analysis, which, in view of their speed, specificity and sensitivity, can become useful.

Certain dyes, such as the thiocarbocyanins, can absorb light of a certain wave length. If certain biological compounds are added (protein, nucleic acids, etc.) they react with the dye and shift this absorption onto another wave length according to the character of the added substance. [96] The behaviour under ultra-violet light is characteristic of many biological substances, such as protein and nucleic acids. Small amounts of nucleic acid mixed with large quantities of protein can be observed as a result of a characteristic absorption in the ultra-violet spectrum. [97] Fluorescence, that is, the capacity to emit visible light under ultra-violet irradiation, is characteristic of some biological substances, for example botulinum toxin. Certain dyes capable of fluorescence also have a high affinity for certain biological substances. Acridine orange, for instance, becomes bound to nucleic acids, although more densely to the single stranded acid, usually RNA, than to the double stranded, usually DNA. RNA gives red and DNA blue fluorescence in ultra-violet light. This method is interesting in that the smallest amount of DNA that can be discovered corresponds to one-tenth of that found in an *E. coli* bacterium. [98] Field tests with this method showed, however, that at its present stage of development it is considerably influenced by non-specific fluorescence caused by some particles fluorescing spontaneously or in combination with the dye.

Special staining methods applied to bacterial extracts separated by gel diffusion, electrophoretically accelerated gel diffusion or isoelectric focusing in gels, might also yield taxonomic information. It has been possible to detect down to 0.03 micrograms protein per zone in an acrylamide or agarose gel and to reduce the time required for staining and decoloration down to a few hours. [99] A taxonomic application of discelectrophoresis to Leptospira has appeared [100] and the specific staining of certain enzymes, generating so-called "zymograms", should permit further refinements. [101]

The combination of electroosmophoresis with a serological identification procedure [102]—immunoelectroosmophoresis (IEOP)—has recently proved itself capable of detecting nanogram quantities of botulinum toxin in food. [103] Its potential for speed is illustrated by its use in detecting meningococcal antigens in liquor cerebrospinalis. The physician can in this case get the diagnosis 20 minutes after the sample is taken. [104–105]

Since the method is not only rapid, but also simple, cheap and applicable also to virus antigens [106], it is a technique of considerable relevance to BW defence. Its main problem has been the fact that it could only be used on acid or neutral antigens. However, this may be circumvented by modifications to the gel [107] and more recently by introducing carbamyl groups into the antigens. [99] The latter technique made it possible to obtain specific immunoprecipitates within 20 minutes from the following staphylococcal toxins: the α and β-haemolysins as well as enterotoxin B, all of which have isoelectric points in the

range 8.6–9.5, and from diphtheria, tetanus- and choleratoxin which have the isoelectric points 4, 5.1 and 6.6+7.8, respectively. However, not only was the method quicker than ordinary immunodiffusion, which might require as much as 3 days at room temperature, but it was also ten times more sensitive.

ATP, a common mediator in energy transport chains in nature, is a product of all living cells and can be detected by light emission when introduced into the enzymatic reaction system luciferine/luciferase from the firefly. Using currently available instruments, it is possible to detect amounts of ATP down to 10^7 micrograms which is equivalent to approximately 100–1 000 cells of the bacterium *E. coli.* [109–113] Theoretically, it is possible to detect single cells with this method. [114]

A pyrolizer—a device which heats the sample and converts the protein in it to NH_4+, measured in an ion detection chamber—is capable of detecting as little as 0.1 micrograms of nebulised albumin in the air. [73] The value of such a device has been questioned on the basis of the presence of non-microbiological protein in the air. This gives rise to noise in the system, but the device could probably be of help in detecting viral and rickettsial aerosols which contain albumin up to 99 per cent [95] if grown on egg and not subjected to purification by zonal centrifugation or other means.

It is possible to combine a pyrolizer with a gas chromatograph. The latter instrument will probably become very important in all kinds of rapid detection, whether for BW or public health purposes.[1] It offers great opportunities for discerning biochemical substances in an aerosol [115] but perhaps even more for the early detection of bacterial metabolites produced during growth of micro-organisms. [116] It separates substances during their passage, in an inert carrier gas, through a column in which the speed of passage is characteristic of the product. Substances which in themselves are not volatile can be studied after pyrolysis. An indication of its sensitivity in detecting metabolites of micro-organisms is illustrated by the estimate that it may only be necessary to study the excretory products of 1.5 bacterial cells for the purpose of detection. [117] Substances added to the aerosol in order to reduce the biological decay, such as carbohydrates, can probably also be detected by the gas chromatograph. [118]

Another instrument deserves special mention in this context: namely, the mass spectograph, which permits the analysis of compounds if split by a suitable method to units with a lower molecular weight than 1 000. Such splitting can be done by pyrolysis or hydrolysis, the latter probably proving to be the method of choice since the former will most probably give too complex and variable a picture. [73]

[1] See appendices 2 and 4 for data on the potential of gas chromatography and mass spectrometry.

Apart from the key biochemical components discussed above, there are others that might be studied with advantage. The presence of the amino acid d-alanine in an aerosol would be an indication of the presence of bacteria, since this amino acid is a fairly constant component of the bacterial cell wall regardless of species. Before such measurements can be made, the cell wall has to be broken down, and it is not known whether suitable methods for micro-determination have been fully developed. [119] Under any circumstances, the cell wall of bacteria will become a useful object for study, since different species of bacteria often have very characteristic wall structures. [120]

The composition of bacterial DNA also differs markedly between different species [121] and it is therefore possible that a study of this substance together with the cell wall might yield much information about different BW agents.

Common to all studies of key biochemical components is the finding that none of them is sufficient alone, since the air supports several of these components arising from natural sources such as algae, pollen, sawdust, and so on. [73] Normally, of course, small amounts of non-pathogenic micro-organisms also exist in the air. The presence of these components does not, however, indicate whether the micro-organisms are alive or not.

All the methods mentioned above require some manipulation of the original sample, which is usually destroyed during the analysis. This entails not only the drawback that some time is necessary for the analysis but also the requirement that part of the sample be set aside for other courses in the diagnostic strategy. A further drawback is that the instrumentation is fairly elaborate and therefore expensive to operate and to purchase.

Detection of biological activity

The principle of detection of biological activity is the same as that in the conventional laboratory; that is, micro-organisms are studied during their growth and reproduction which, as pointed out earlier, takes time. At least a few hours are necessary but the methods are valuable for indicating that the aerosol detected by particle counters and verified as containing microbiological components does indeed contain viable micro-organisms. The principles underlying such automated detection of biological activity have been described in the section on the conventional microbiological laboratory (see pages 64–66) and it is sufficient here to describe some of the actual pieces of equipment and their underlying principle.

The "Gulliver" device contains media in which the carbon part of the molecules is the radioactive C^{14}. [122–124] When this medium is metabolized by micro-organisms, radioactive gas is formed which can be detected by Geiger-Mueller counters. This method responds to as few as 10–100 micro-organisms

and, in practical tests, it detected life in a snowstorm on the top of White Mountain in California and in Death Valley within 50 minutes.[125]

The "Wolf trap" is a miniature growth chamber where changes in turbidity and pH are measured continuously since both these factors are indicators of growth and metabolic activity. [126] In the "Marbac", Eh in the medium is measured continuously. The "Minivator" and "Multivator" [127] contain multiple chambers where a given sample is simultaneously tested for its reaction to a variety of standard reagents, the principle being the use of media which, upon decomposition by microbial enzymes, form chromogenic or fluorescent substances. [73, 122] These devices, all of which have been developed for unmanned space research, can probably be used for BW detection, but little is known to date about their performance in this connection. However, they are obviously suitable for remote control. Apart from their lack of speed, it should be pointed out that in nature there are inert substances that can mimic micro-organisms; crystals can grow, glass can scatter light mimicking multiplication and some materials can absorb and desorb gases. [73] It should also be noted that the media can deteriorate spontaneously.

Fluorescent antibody techniques

Immunofluorescence (IFL) was first reported in the early 1940s and has since become an important diagnostic tool. [128-129] A bibliography in 1968 listed no less than 1 895 references relating to its use. [130] Such rapid progress was only made possible by several laboratories participating in its development, and as a consequence these laboratories have acquired great expertise in the application of the method. In recent years some applications have spread to the routine laboratories and this trend will no doubt continue. [131] In principle all micro-organisms can be diagnosed with immunofluorescence, although some are more difficult than others, and at present this requires access to the facilities of a specialized laboratory. [131] The general usefulness of the method both in identifying the micro-organism and in following its fate in the host, as well as its applications in studying several non-infectious diseases, will guarantee the future development of the method.

Principle

Micro-organisms (and many other substances) are capable of acting as antigens, that is, when introduced into an organism they cause the production of antibodies which counteract or neutralise the antigen. In principle, this defence mechanism is only directed towards the corresponding antigen; therefore, the antibody, if removed from the organism, can be used to identify the antigen. If the antibody is conjugated (linked) with a dye, it will still react with the antigen

and if this is a micro-organism the dye will make the cell visible. If the dye is capable of fluorescing—emitting visible light when illuminated with ultraviolet light (not visible to the human eye)—the micro-organisms, with their envelope of antibody and dye, will stand out against a dark background. This increases the sensitivity of the method and also makes it possible to find micro-organisms even if they are few in number and the species are mixed.

This method, which is called direct immunofluorescence, has the disadvantage that each antibody used must be stained with the fluorescent dye. In the indirect method, the specific unstained antibody is allowed to react with the micro-organism and the site of reaction is discovered by using an anti-antibody, that is, a substance prepared in an animal and capable of reacting with all antibodies from a different animal species regardless of their specificity. The fluorescent dye is linked to this anti-antibody and it is thus possible to work with only one fluorescent conjugate. This facilitates standardization.

In principle, the antigen-antibody reaction is specific, but one micro-organism gives rise to several antibodies, some of which may be similar to those initiated by other species. For example, this occurs with the plague bacterium, *Pasteurella pestis* and its relative, *Pasteurella pseudotuberculosis.* However, this can be remedied by absorption in which the antibody caused by *Pasteurella pestis* is allowed to react with *Pasteurella pseudotuberculosis.* When these bacteria are then removed from the mixture of antibodies, the resulting serum reacts only with *Pasteurella pestis*

Immunofluorescence and BW defence

Certain features are of special significance in this regard:

1. Most bacteria, fungi, rickettsia and some viruses conceivable as BW agents are within the scope of this method. [128, 130–131]

2. The method is highly sensitive and can identify living as well as dead micro-organisms, thus making it possible to detect organisms far from where they were released.

3. The method is rapid, that is, a result can be obtained within an hour, which in diseases like plague and anthrax might be of crucial importance for successful therapy. To gain the same information with normal culture methods might take 24–72 hours.

4. The cross reactions between different micro-organisms are not of entirely negative value since a reaction might be expected with several of the specially developed BW agents which an aggressor might use to confuse the defence. [132]

5. The method is very versatile. It can be used on various materials such as the output of an air sampler, various specimens from humans, animals and insects etc., and it is equally useful in identifying suspicious colonies on a solid medium.

6. Micro-encapsulation of BW agents in order to provide protection against drying and the effect of light might impede immunoflourescence unless steps are taken to remove this coating.

7. The equipment is fairly cheap, £250–450 [133], and the method can be used for a variety of public health measures. As a result, facilities for the use of this method in the detection of BW attack are to be found in most modern laboratories. This will soon apply even in the underdeveloped areas because the equipment is so much more efficient than the ordinary microscope in finding tubercle bacteria. As soon as more than 30–50 specimens per day are to be examined, it is actually cheaper to utilize this equipment, even in an underdeveloped country where one can calculate with low wages. [133]

The FA technique lends itself to automation. The FAST (Fluorescence Antibody Staining Technique), a device developed by the US Army, employs a high volume sampler, incubates the sample with fluorescent antibody, deposits the reacted mixture on a transparent tape, washes away unabsorbed antibody and dye, then scans the tape and records the number of fluorescent particles on the tape. It has been estimated that this device is capable of discovering bacteria at levels of less than one per litre of air. [73] It is, however, not known how many species of micro-organism this device is capable of discovering.

A very important recent development which opens up remarkable possibilities to speed up automatic FA-identification is the use of the dye laser to excite the fluorochrome. [134–135] Besides increasing the speed, this has the advantage that the wave length can be adjusted without the use of filters and that photo-destruction of the fluorescein can be reduced to a minimum.

Thus, there are many reasons for believing that immunofluorescence will become an important tool both in normal public health work and in the detection of BW. The effort needed to perfect the method should, however, not be underestimated. At the present time, such efforts are being made in the field of standardization of reagents which, in 1968, was the subject of a symposium [132] arranged by the International Association of Microbiological Societies (IAMS). The bacteria which give troublesome cross reactions, especially enterobacteria such as *Salmonella* and *Shigella* (see also appendix 3), are subject to intense study, as are several of the viral agents that can be used in BW.

For most micro-organisms, immunofluorescence in BW detection can be considered a "rapid presumptive test", [131] that is, verification by other diagnostic means is necessary in view of the critical political importance. The method can hardly be used as a basis for retaliation, but it can certainly indicate which defence methods should be utilized, for instance, antibiotic prophylaxis if an attack with *rickettsia* is suspected. The method is also most useful in the rapid unravelling of epidemic outbreaks and it provides an excellent rationale on which to base therapy in the acutely ill individual.

The potential of recent immunological labelling techniques

In the improvement of serological techniques, the availability of pure antibodies is always a great help. From this point of view the use of immunosorbent techniques is very important. One can, for instance, immobilize the antigen with glutardialdehyd [136] and elute the antibodies from the immunosorbent with 0.1 M glycine-HCl buffer at pH 2.8.

The availability of purified antibodies now paves the way for very sensitive serological techniques that may become extremely important in the detection and identification of small quantities of micro-organism. The possibility of using the competitive inhibition of the binding between radioactively labelled antigen and its homologous antibody with the aid of unlabelled antigen in unknown samples has, for instance, found wide application. [137] It opens up interesting possibilities for automation (see appendix 2, section III), but this is also true for some alternative labelling techniques which employ coupling methods also used in enzyme technology. One can, for instance, label proteins with bacterio-phage which is not inactivated until the protein is involved in a serological reaction [138], or one can use an enzyme as the label. [139–141]

Especially the technique of separating unreacted from reacted antigen by means of antibody fixed to a solid phase—a method also used in radioimmunosorbent technique (RIST) [142]—should be noted in the context of possible automation. In the enzyme-linked immunosorbent assay (ELISA), antibodies can be coupled both to BrCN-activated cellulose [143] or physically adsorbed to polystyrene tubes. In the latter case the reduced activity of an enzymatically-labelled antigen, caused by competition with the equivalent protein of the sample, could be determined directly in the tube by adding a suitable substrate system. It is easy to visualize how such a method could be adapted to a Technicon apparatus for continuous analysis, and since its sensitivity is equivalent to that of radio-immune assays and assays using antigen conjugated to bacteriophages (1–100 ng/ml protein can be determined), the approach has great scope for the study of bacterial antigens. It has, for instance, already been used for titrating antibodies to *Salmonella* O-antigens. [144]

Specificity, sensitivity and speed *versus* cost in BW detection

These four factors are, in one way or another, closely interrelated. Often a gain in one involves a loss in one or more of the others. Speed thus usually means loss in sensitivity, and increased cost (see table 3.2).

The actual cost of equipment and operation of a BW detection unit (sensor) is at present practically impossible to estimate in a meaningful way, due either to the cost of present equipment being regarded as classified, or to BW detec-

Table 3.2. Comparative values of detection techniques

Name	Type	Information	Amount or rate per organism	Amount or rate detectable	Number of organism required[a]	Time[b]
Wolf Trap	Growth	Viability in special growth media	Max. double each 15 min	10^4 bacteria ml/mv	10	2–3 hr
Gulliver	Metabolism	Specific substrate (glucose–C^{14})	3.3×10^{-3} cmp/hr	25 CPM	10^3	1–2 hr
Esterase	Enzyme	Living particle	10^7 molecules/min	10^{14} molecules	10^6	1–10 min
Phosphatase	Enzyme	Biological active	10^6 molecules/min	10^{14} molecules	10^7	1–10 min
DPN cyclic assay	Coenzyme	Living and dead particles	10^{-19} moles	10^{-14} moles	10^5	1–30 min
ATP-firefly	Coenzyme	Living and dead particles	10^{-9} μG	10^{-4} μG	10^5	1 min
Pyrolysis GC–MS	Organic analysis	Biological particle	10^{-15} moles	10^{-13} moles	10^2	5 min
BioSensor	Staining morphology	Bacteria	1 bacteria	20 bacteria	20	5 min
Immuno-adherence	Agglutination	Specific virus	10^3 aggregates	10^3 viruses	10^3	10 min

Notes:

[a] These values are the most optimistic values at present. One or two orders of magnitude higher are readily measured. These approaches are still under investigation and further improvements are possible in most cases either in methodology or instrumentation.

[b] Time can be reduced in many cases if a large number of organisms is measured.

Source: Mitz, M. A. The detection of bacteria and viruses in liquids. *Annals of the New York Academy of Sciences* 158: 651–64, 1969.

tion being still in its infancy. In fact, most detecting devices are still at the stage of being laboratory prototypes. [76] Furthermore, the cost and size of equipment to be used within the walls of a laboratory may bear no relation to the equipment designed to perform similar duties, unattended in the field. Since no single method can be considered adequate for solving all the problems of BW detection, a further complication in the calculation of cost is introduced: the need to use several methods simultaneously.

There is also reason to believe that the cost of a single BW detection unit (sensor) may not be the most important economic factor. In the detection of an aerosol attack, a single sensor would not be enough to establish the fact of a BW attack since it might, for example, react to micro-organisms from an animal passing close by or to a trickling filter for sewage. It would become necessary to compare indications from several sensors at different locations, taking into account both meteorological and terrain parameters, before it would be possible to give an alarm which is effective enough not to conflict with troop morale. Further, it would probably become necessary to follow the results from these sensors over a period of time. This obviously implies a command and control system where the information can be evaluated. It is reasonable to assume that such a system would account for a large part of the cost of BW detection. However, this cost would be smaller in countries which already have advanced civil or military defence communications, that is, in most developed countries. The underdeveloped countries, on the other hand, would have to pay correspondingly higher prices for the same amount of protection.

Trends in the development of automatic biodetection devices

Regardless of the future development in equipment for BW detection purposes, there is no doubt that efforts to detect micro-organisms will undergo continuous amelioration, regardless of whether they are present in aerosol form, in water or in food. Detection and sizing devices of particles are of great interest to manufacturers of aircraft instruments or electronic equipment, since it has been found that a high degree of cleanliness results in more reliable products. [146–147] Critical equipment is now assembled in rooms of a cleanliness standard comparable to or better than operating theatres, and continuous monitoring of particles is being performed. [147–148] In the field of air pollution, a continuous study of the nature of air pollutants will probably also yield better detection devices. In the field of medicine, the new therapeutic arsenal also means a higher degree of cleanliness since even minute amounts of micro-organisms can cause complications, for example, in a patient undergoing immunosuppressive treatment as preparation for organ transplantation.

There are thus several fields, apart from that of military activities in outer

space, which may promise better BW-detection devices. Present conditions can, however, be summarized in the sobering quotation: "although great strides have been made in automating biodetection and monitoring equipment, the cost of R & D in this field has tended to restrain development and limit application". [73] To this may be added that it seems probable that even in the distant future no device will be wholly effective, since it is already possible to devise BW agents that are extremely difficult to diagnose.

IV. *Aerosol detection by visual observation and by LIDAR*

Visual detection of aerosols is only possible during the time they are being sprayed since they become invisible to the human eye shortly after being disseminated. The behaviour of the aeroplanes spreading the agents may, however, be indicative. The agents may, for instance, be delivered with spray tanks across the wind and at an altitude low enough to arrive under the inversion layer close to the ground. Lookout precautions can, of course, be sharpened whenever meteorological or other reasons indicate an increased risk of attack. If an aerosol attack were suspected but there were no immediate casualties and no reaction from the gas alarms, there would be reason to suspect attack with B agents.

LIDAR, an optical analogue of radar, using pulses of light from a laser, can detect normally invisible particles in the air and can therefore be used to detect a CB attack at a distance. Its potential in this respect can be seen from an experiment in 1966 when an insecticide aerosol at a concentration of 2 400 ml per 10 000 m^2 was generated from a plane flying 30 metres above the treetops. This was detected at a range of 1 km. [149] A drawback in the use of LIDAR for BW detection is that the range is limited to some 30–40 km, and, therefore, more than one device would have to be used to protect a target. It should be emphasized that LIDAR is still in its early development and therefore no meaningful estimates of its price can be given, other than that it is high. At its present stage of development, the energy generated can damage the human eye even when it is reflected from an aeroplane to the ground. [149] Finally, it might be pointed out that, in view of its potentialities in the study of air pollution and a range of meteorological phenomena, it is reasonable to expect a rapid development of LIDAR.

V. *Detection of BW agents in food and water*

Detection of BW attack against food is complicated by the quite common presence of comparatively large numbers of harmless micro-organisms. The fluores-

cent antibody technique, which can discern small numbers of one kind of micro-organism mixed with large numbers of others, therefore comes in very useful in this field. With this method it is possible to make rapid presumptive tests for the presence of, for example, *salmonella, shigella,* plague and tularemia, and it would thus become possible to stop the delivery of food if a sample were found to contain suspicious micro-organisms. Using only a conventional cultivation procedure with its inherent delay of 24 to 48 hours, such actions would hardly be practical.

The detection of a BW attack against a water supply is not very different in principal from that of aerosol attack. The same methods for automated biodetection can be used, as well as the conventional diagnosis. The sampling technique, however, has a different emphasis in that the principal method is that of membrane filtration. In this method, which can also be used in aerosol detection, the water passes through a membrane of a certain pore size which retains the micro-organisms that can then be studied by one of the methods mentioned earlier.

Detection of botulinum toxin by its fluorescence in ultra-violet light has been mentioned before and another method unique to BW detection in water may be mentioned in brief even if its sensitivity is not very satisfactory. This method depends upon the lowering of the residual chlorine in the water when organic materials are added. [86] A considerable number of micro-organisms would have to be present before indication is given, and it is also an unfortunate fact that in many cities the chlorination of the water supply is variable and insufficient.

Finally it should be mentioned that modern separation techniques have opened up interesting opportunities with regard to the rapid identification of microbial toxins. It has, for instance, been shown that toxins can be identified within 20 minutes with the aid of immunoelectroosmophoresis involving the use of specific antisera. [99] Since the method is sensitive in the microgram/nanogram range and can be applied to significant biochemical problems like the determination of the isoelectric point characterizing practically important antigens like Au in hepatitis, it should rapidly reach widespread use. The great efforts which have gone into the development of a rapid, sensitive and simple method applicable to this antigen can serve as a pointer towards methods that should be considered also in the case of toxins. Radioimmunoassay, mentioned in the section on the potential of recent immunological labelling techniques [150] and passive haemagglutination [151], for instance, show great promise but ordinary gel-diffusion, electrophoretically accelerated gel-diffusion (IEOP) and complement fixation have also been used. However, the relative sensitivities vary considerably, in the case of the last three methods, for instance, in the relation 1:16:500. This can serve as just another illustration of the balancing act

always required between speed and sensitivity. [152] Miniaturization can then occasionally help, as in the detection of staphylococcal enterotoxin which can be done in the 0.1–1 microgram range with the aid of gel-diffusion in capillaries. [153–154]

Chapter 4. Possible techniques for inspection of production of organophosphorus compounds—Report of a SIPRI symposium

I. *Background*

The negotiations on chemical disarmament at the Conference of the Committee on Disarmament (CCD) in Geneva are at present mainly concerned with the type of verification which should be included in a treaty forbidding the production, development and stockpiling of chemical warfare agents and chemical weapons. Discussions have centred on international control, and on the technical means which are available for verification at different levels of access.

As part of its efforts to facilitate disarmament discussion by providing relevant technical data, SIPRI decided to undertake an experiment on the verification of nonproduction of organophosphorus chemical warfare agents. The project was intended to complement the BW inspection field-exercises conducted by SIPRI during 1968–69 (see pages 51–60 above). An exploratory symposium was held at SIPRI in June 1970 in order to clarify some of the intricate technical questions of verification.[1] For the most part, the symposium was devoted to a discussion of manufacturing techniques for the different nerve gases, the raw materials required, the types and quantities of by-product generated, the techniques for handling waste products, and points of similarity and difference between nerve-gas manufacture and the production of chemically-related civilian commodities by the organophosphorus industry. Matters remaining for further discussion included such problems as the delimitation of

[1] The symposium was held during 25–27 July 1970. The participants and papers presented were as follows:
Professor Jorma Miettinen (Finland): Chairman
Professor Y. Fukushima (Japan) "The amount of production and use of pesticide and herbicide in Japan, and some data on hazard study and environmental disruption"
Professor Paolo Malatesta (Italy) "An approach to the problem of inspections related to chemical war gases"
Dr Jan Moravec (Czechoslovakia) "Some aspects of the problem of convertibility of industrial plants"
Mr Theodor Nemec (SIPRI)
Mr Julian Perry Robinson (SIPRI) "The manufacture of nerve gases"
Mr Alan Pittaway (USA)
Dr Herbert Scoville, Jr (USA).

the nerve gases from those compounds which have only peaceful uses, and the technical methods which are actually available, or are capable of development, for use in a verification system. A further SIPRI symposium on possible techniques for inspection of production of organophosphorus compounds was held in September 1971.[2] Most of the participants submitted background papers (see appendix 5), and these formed the basis of the discussions.

The organophosphorus compounds were chosen because they are the class of compounds to which the most important CW agents—the nerve agents—belong, and also in order to facilitate the discussion of a feasible model of a verification system. The fact that the symposium was confined to these compounds was not intended to imply that CW agents other than the nerve gases are unimportant.

The aims of verification of an international agreement are to reassure the parties to a treaty that its provisions are not being violated and to deter potential violators. Most verification activities regarding chemical disarmament will encounter only normal industrial operations, and the deterrence concept implies that verification methods do not need to be 100 per cent efficient.

It was generally agreed that a national verification system would be indispensible. It would operate under conditions best suited to maximize effectiveness. And it could be devised in such a way as to take into account the administrative methods of the country concerned. Such a system would work best if it were under the control of the government, or possibly other civilian authorities. Different schemes can be envisaged for different countries.

The symposium also discussed how a possible international organization could process national data on the production and flow of elemental phosphorus. Diversions from established trends of the worldwide flow of phosphorus might be taken as indicating possible clandestine production of nerve agents. It was emphasized that the requirement that a verification scheme should also make adequate provisions for the signatories of a treaty to be able to judge data independently should be observed. But further studies must be undertaken before the feasibility of the method can be established. The experience of the Western European Union was, for example, also considered to be of interest. The work of devising a practicable system based on the processing of national data on elemental phosphorus production should be facilitated by the fact that there are only about 35 phosphorus-producing plants in the world today.

In general, the formulation of accurate technical methods of verifying the nonproduction of nerve agents—thereby solving the problem of those chemical

[2] The symposium was held in Stockholm on 21–24 September 1971, and was attended by 19 scientists from 13 countries. This chapter consists of a report prepared by the convener, Dr S. J. Lundin, and is supplemented by the background papers in appendix 5 which also includes a list of the participants.

agents which are of the greatest concern for reasons of national security—might well pave the way for the formulation of control systems for other CW agents.

The question of differentiating nerve agents from those organophosphorus compounds that have peaceful uses only was discussed in detail. It was concluded that it might be possible to obtain practicable differentiations. These might be based on the principal structural formulae of the compounds together with data on their physical, chemical and toxicological properties, and on their physiological effects.

The possibility that chemical plants, especially those producing organophosphorus insecticides, could be converted to the production of nerve agents or other CW agents cannot be excluded. However, both the size of the plant and economic considerations are probably limiting factors. So far as plant safety measures are concerned, if a plant were producing very toxic insecticides, the safety measures would possibly differ very little from a plant producing nerve agents.

There was general agreement that, should any type of on-site inspection eventually be agreed upon, adequate analytical methods that could be successfully applied already exist. However, further work is needed to perfect and apply the existing methods, bearing in mind the probable future developments of CW agents which are not known today.

Finally, it was proposed that a working group should urgently study the problem of which data on the production and flow of elemental phosphorus, and possibly some other phosphorus compounds, a national control organization might select and produce for further processing by a possible international verification organization.[3] Any scheme which is evolved should take into account possible application to other CW agents as well.

In the following, an extract of the discussions at the symposium will be presented.

II. *General comments*

In most arms-control discussions, the term "national control" is used to describe a situation in which a state, using only its own resources, verifies compliance with a treaty. It was suggested that this type of control should, instead, be termed "unilateral control". The term national control could then be reserved for use in a system in which both "national" and international organizations are used to verify an arms-control agreement.

[3] A group consisting of the following participants of the symposium is, in fact, being organized by SIPRI to continue work on this problem: Dr J. G. Kammüller, Netherlands; Dr S. J. Lundin (Convener), Sweden; Professor N. N. Melnikov, USSR; Dr J. Moravec, Czechoslovakia and Dr A. R. Pittaway, USA.

The need for both national and international verification organizations was emphasized. The possibility of maintaining groups of technical experts to assist the CCD and also to perform various types of on-site inspection was mentioned.

A complex aspect of the verification of production or nonproduction of organophosphorus compounds is related to the division of chemical agents into three groups: those which have no peaceful uses, those which are normally produced for industrial purposes, and those which have no peaceful uses themselves but which utilize processes or intermediates which do have such uses. The fact that nerve agents belong to the latter group complicates the problem of verification.

III. *National and international monitoring of production data*

International data processing of selected nationally-collected statistics may constitute a practicable, non-intrusive method of verifying the production of phosphorus compounds. The advantages were stressed of a standardized, continuous and routine flow of appropriate data from a country's production plants to an international organization *via* national organizations. The analysis of these data should be able to detect possible changes in the flow, due, for example, to the clandestine production of prohibited compounds. It should also be possible to trace the flow of a basic compound, such as elemental phosphorus, rather than final products. But a model must be constructed in order to evaluate the feasibility of these methods.

There are about 35 plants known to be manufacturing elemental phosphorus in the world. Even if secret plants to produce phosphorus were erected for military purposes, it should be possible to detect these, especially by satellite surveillance.

More information about the production data that would be available for national and international control organizations is required. But the measures and organizations which already exist in various countries to control the production of compounds such as ethyl alcohol, require statistical data similar to those which would be needed by national and international agencies to control the production of CW agents. Accordingly, a control system might be based on existing experience and practices.

Some information was given about the work of the Armaments Control Agency of the Western European Union (WEU). Methods involving different degrees of intrusiveness are used by WEU for inspecting plants—the inspection of primary materials and intermediates used in the process, the inspection of

factory records and accounts, and sample-taking for analysis. Special measures are taken to protect commercial secrets.

The advantage of a system based on international data-processing of statistical and economic information is that it diminishes the need for on-site inspection until definite suspicion arises as a result of the data-processing. In such cases, it would not be the task of the inspectorate to prove that the suspicion is true, but for the accused party to show that it is not true. This diminishes the need for 100 per cent efficiency in the verification methods.

IV. *Plant convertibility*

The question of whether chemical plants can be converted from peaceful to military uses was discussed. It is clear that the production of different types of organophosphorus compound requires different reaction sequences. The economics of production are also very important. Moreover, the production of organophosphorus CW agents would require extremely stringent safety measures, therefore it may take a long time to convert large insecticide plants from one type of production to another. (If, however, a war were imminent, economic factors and the need for optimal plant construction would probably be disregarded if the use of chemical weapons were intended.)

Medium-scale production units with capacities ranging from a few tons to some hundred tons per month are quite common in the chemical industry for batch-wise production of smaller amounts of organophosphorus and many other compounds—and these are often convertible to other types of production with relative ease. It should be noted that such units may not always function in the most economical way because of the need for a variable production capacity.

It may also be possible to produce nerve agents clandestinely by building plants so that distinguishing characteristics are concealed.

For economic and other reasons commercial pesticide industries tend to have only relatively small storage facilities—some hundreds of tons. Such capacities would not be relevant for constituting a significant nerve-agent capability. However, if large stockpiles of products were accumulated, this could be taken as a possible indication of the nature of the plant.

With regard to the problem of destroying stocks of nerve agents which have become obsolete, it was mentioned that adequate methods and equipment are now available for carrying out such destruction.

A serious problem, however, may not be convertibility, but specially built nerve-agent plants having only a stand-by capacity. Another problem will arise if so-called binary weapons are developed; these would require relatively

non-toxic intermediates which are stored in the ammunition and which are allowed to react to form the toxic nerve agent only when the ammunition is fired.

Plant conversion for other possible CW agents will, in some cases, be more feasible than for the organophosphorus compounds. Large-scale verification of chemical production plants would, however, not be practical. For other reasons also, it would be unrealistic to perform more than a few inspections.

In summary, plant conversion is a practical possibility. Such conversion may be very difficult or impossible to observe, at least from outside the plant. The factors mentioned indicate the importance of a non-intrusive verification method such as the monitoring of production data.

V. *Different properties of organophosphorus compounds*

The physical, chemical and toxicological properties of organophosphorus compounds have some characteristic features that may serve as a basis for agreement on the delimitation of the compounds, and thereby for the international regulation of their production. The types of toxicity to be considered are acute toxicity, long-time exposure toxicity, residual toxicity, etc. It was suggested that physiological properties should be added to these criteria in order to include other CW agents, such as the incapacitating agents. Economic factors were also included among possible criteria leading to a practical delimitation concept.

It was suggested that the use of organophosphorus insecticides should be limited on the basis of their toxicity and persistence in the environment. Accordingly, only agents of limited toxicity should be produced. Apparently, this proposal would encounter the following main difficulties. Different regions and types of agriculture require insecticides of very different characteristics. The exclusive use of insecticides of low toxicity may further the development of resistance to such products, and this may mean that insecticides of higher toxicity will have to be used in the future.

Novel techniques to combat insects also rely on new developments in the field of organophosphorus chemistry. Here is a special class of organophosphorus chemical insect-control agents that must not be overlooked in the development of suitable inspection methods based on chemical analysis. These substances act on insects by sterilizing the males rather than by killing the insects. They are, so far as is known, quite harmless to warm-blooded animals, and it is possible that the target organisms will not adapt themselves to the compounds. The compounds require starting materials different from the classic organophosphorus compounds for their synthesis, and different synthetic routes are used (for example, no esterification process is required).

VI. *Observation systems and laboratory methods for inspection*

The symposium considered several existing observation systems and laboratory methods suitable for developing into control methods. The extent to which there are procedures suitable for inspecting both production and nonproduction of organophosphorus compounds is not entirely clear, and clarification on this point would be valuable. However, since both concepts presuppose access to a plant or its immediate vicinity, it was considered useful to examine some of the analytical methods which are already in use to ensure that undue risks do not arise in or around organophosphorus-insecticide plants. Several more elaborate methods should also be examined, although they might require considerable research and development before they become elements of an inspection system.

With regard to identifying products and intermediates it is by no means simple to determine the structure of a completely unknown substance brought in for analysis. On the contrary, this can often be very cumbersome, even with the most sophisticated equipment—one of the limiting factors being the availability of an adequate data-processing system concerning compound properties. However, a normal inspection task would be to confirm a known production rather than to find out about a totally unknown substance.

One possible technical inspection method would be to check the activity of nerve and blood cholinesterase enzymes in the body. The activity of these enzymes is inhibited specifically by the organophosphorus compounds (and by carbamates), sometimes in very low concentrations. By using biochemical preparations of the enzyme, it is possible to utilize the same reaction to detect the occurrence of cholinesterase inhibitors in extremely low concentrations in the environment, for example, in water or air. Using this reaction and taking into account the different reaction rates of the enzyme to various inhibitors, it is possible to determine concentrations of the substances in a sample. This method may be suitable as an inspection technique—as applied, for example, in commercially available "detection sticks". Other enzymes, with different sensitivities to inhibition by various organophosphorus compounds, may also be used as a means of establishing inhibition spectra which could be used to differentiate substances.

So far as the observation of plant safety measures as an inspection method is concerned, there may not be any decisive difference between plants producing certain very toxic insecticides and possible nerve-agent plants, especially in the future. And industry's ability to manufacture safely very toxic compounds should not be underestimated.

Another suggestion was to investigate changes which may occur when a substance has escaped into the area around a plant: for example, changes may

occur in the microbial flora or in the biochemical behaviour of vegetation growing around production facilities. Organophosphorus compounds might also be detected in the material of the equipment in which they had been produced.

The analysis of liquid wastes may not be sufficiently clear-cut to guarantee this as an element of inspection. The difficulty here is that, increasingly, plants are giving their liquid wastes appropriate treatment.

The possibility of using mobile inspection teams was discussed. If each nation maintained its own inspection team, a serious waste of technical and scientific manpower would occur. International inspection teams would, therefore, be preferable. These could carry out inspections on request, but they would need guaranteed free access to perform their inspections. Control activity requires uniformity and the training of personnel; this is another reason why an international organization is needed. One cannot, for example, envisage that an *ad hoc* committee of scientists could be assembled to investigate an allegation and set out to solve all the problems connected with control in a short time.

It was generally considered important that national and international control organizations should be able to verify each others' results. An exchange of information, as well as of experts, between national and international verification agencies would, therefore, be of great value.

Another opinion, however, was that national control organizations alone would be better suited to perform the verification tasks. In any case, the existence of national organizations would imply that verification costs were shared proportionally.

One proposal was that an international centre should train national inspection personnel, teaching them common inspection methods and ways of evaluating the results of inspection. The trained personnel could then return to their own countries to work in national control organizations.

Appendix 1. SIPRI BW inspection documents

At the conclusion of an inspection, the following material should be available:

1. The documents to be submitted by the Director or Head of Department at the time of the inspection
   - Questionnaire with definition of terms . . . . . ☐
   - Personnel list, senior scientists . . . . . ☐
   - Personnel list, junior scientists . . . . . ☐
   - Personnel list, laboratory technicians . . . . . ☐
   - Personnel list, others . . . . . ☐
2. Documents to be filled in and then posted on the announcement board of the establishment within six days after receipt of notice about the inspection (together with example given in annexe A)
   - Questionnaire . . . . . ☐
   - Table of agents (annexe B) . . . . . ☐
   - BW Control Log (annexe C) . . . . . ☐
3. Private check list (annexe D) . . . . . ☐
4. Stock records and production documents . . . . . ☐
5. Purchase records for laboratory animals, "absolute" filters and ultra-violet lamps . ☐
6. Immunization records . . . . . ☐
7. Maps or model of facilities . . . . . ☐

Only available to inspectors:

8. Analysis of records of any previous inspection including a review or reprints, patents, reports, changes in organization as well as reasons for classification . . ☐
9. Material provided by sealed recorders and samplers previously mounted (the detailed nature of these devices is only known to inspectors, but they might, for example, be set up for recording cultures as far as microcalorimetric characteristics are concerned, or for registering the use of steam, fan motors, etc.) . . . . . ☐
10. Any report on the examination of strains of micro-organisms or cultures thereof held or used in the Institute . . . . . ☐
11. The Inspector General's specific instructions . . . . . ☐
12. Inspector's comments on current inspection . . . . . ☐

. . . . . .

13. Inspector's classification of establishment; and circumstances which led to classification at a first visit or change(s) in previous evaluation . . . . . .

. . . . . .

Date and place

Inspector's signature

I. *Inspection questionnaire to be filled in by the director of the establisment*

1. NAME AND ADDRESS of inspected establishment

2. CHARACTER of inspected establishment
   a. Government-controlled ☐
      Government-subsidized (wholly or in part) ☐
      Private ☐
   b. Nature of institution
      University Department ☐ Annual total expenditure . . . . . .
      Industrial Firm ☐ Annual total expenditure . . . . . .
      Research Institution ☐ Annual total expenditure . . . . . .
      Other (specify) . . . . Annual total expenditure . . . . . .
   c. Percentage of expenditure based on contracts with the Government and with other granting bodies . . . . . .
   d. Percentage of expenditure based on contracts with foreign institutions . . . . .
   e. Percentage of expenditure spent on production . . . . . .

3. ORGANIZATION
   Present organization, structure and activities.

4. SPACE AND FACILITIES AVAILABLE[a]
   a. Land area[a] . . . . . .
   b. Land area covered by buildings[a] . . . . . .
   c. Total floor space[a] . . . . . .
   d. Approximate percentage of space devoted to microbiological work . . . . . .
   e. Projected new buildings (bulk, taken over or rented)[a] . . . . . .
   f. Does the establishment have laboratory or field work facilities abroad which it controls? . . . . . yes ☐ no ☐
   g. Does the establishment own or have a controlling interest in any sea-going vessel? . . . . . yes ☐ no ☐
   h. Does the establishment own or regularly use any other grounds or facilities other than those listed (fields, buildings, mobile laboratories)? If so, designate A, B, C, . . yes ☐ no ☐

[a] In case of separate units these should be designated A, B, C, etc.

5. PRODUCTION of microbial agents as requested by inspectors

Agent	Quantities[a] over last calendar year		Quantity[a] stored at end of calendar year		Estimated total capacity geared to production of single agent[c]	Estimated production of single agent parallel to normal manufacture[c]
	Produced[b]	Distributed or sold	Live or toxic	Killed or inactivated		
Tissue culture			0	0		
Fertilized eggs			0	0		

[a] Kg dryweight bacteria and fungi; number of ED_{50} or million lethal doses (state animal or substrate used) of toxins or viruses, liters of tissue culture medium, number of eggs.

[b] Including discarded batches and quantities used for research.

[c] Estimated *maximum* capacity per month around-the-clock operation.

6. PERSONNEL

a. Have there been any laboratory infections during the last 12 months? If yes, name patient(s), state organism(s) involved and probable mode of infection.

. . . . . .

. . . . . .

b. Have there been any major changes in the activities of the laboratory personnel during the last 12 months?

. . . . . .

. . . . . .

7. PERSONNEL LISTS

Senior scientists

Senior scientists are personnel with formal training or special experience making them capable of working independently in the areas indicated. The area of current main activity is marked + + +, area in which there is a publication record during the last five years is marked + +. Area where work could be performed or directed is marked +. The names of persons experienced in large-scale handling of pathogens is underlined.

NAME	Number of years of employment in the Institution	% time outside employment	General Microbiology	Medical Microbiology	Veterinary Microbiology	Medical Virology	Veterinary Virology	Plant Pathology	Animal Husbandry	Plant Husbandry	Rickettsiology	Parasitology	Immunology	Biochemistry including Enzymology	Biophysics	Radiation Biology	Microbial Genetics	Histopathology	Entomology	Equipment design and construction	Epidemiology of infections	Quality control	Studies on aerogenic infections including aerosol technology

Consultants (permanently available)

NAME	Number of years of employment in the Institution	% time outside employment	General Microbiology	Medical Microbiology	Veterinary Microbiology	Medical Virology	Veterinary Virology	Plant Pathology	Animal Husbandry	Plant Husbandry	Rickettsiology	Parasitology	Immunology	Biochemistry including Enzymology	Biophysics	Radiation Biology	Microbial Genetics	Histopathology	Entomology	Equipment design and construction	Epidemiology of infections	Quality control	Studies on aerogenic infections including aerosol technology

Junior scientists

Junior scientists are personnel qualified to work in their main area of activity only under supervision or in collaboration. Current area of activity is marked + + +, area in which there is a publication record during the last five years is marked + +. Area where only minor supplementary training would be required for activity is marked +. The names of persons experienced in large-scale handling of pathogens is underlined.

NAME	Number of years of employment in the Institution	% time outside employment	General Microbiology	Medical Microbiology	Veterinary Microbiology	Medical Virology	Veterinary Virology	Plant Pathology	Animal Husbandry	Plant Husbandry	Rickettsiology	Parasitology	Immunology	Biochemistry including Enzymology	Biophysics	Radiation Biology	Microbial Genetics	Histopathology	Entomology	Equipment design and construction	Epidemiology of infections	Quality control	Studies on aerogenic infections including aerosol technology

Skilled laboratory technicians

Current area of activity is marked + + +, area in which there is previous experience is marked + +. Area where only minor supplementary training would be required for activity is marked +.

NUMBER OF INDIVIDUALS IN EACH CATEGORY	Number of years of employment in the Institution[a]	% time outside employment[b]	General Microbiology	Medical Microbiology	Veterinary Microbiology	Medical Virology	Veterinary Virology	Plant Pathology	Animal Husbandry	Plant Husbandry	Rickettsiology	Parasitology	Immunology	Biochemistry including Enzymology	Biophysics	Radiation Biology	Microbial Genetics	Histopathology	Entomology	Equipment design and construction	Epidemiology of infections	Quality control	Studies on aerogenic infections including aerosol technology

[a] Shortest and longest time within the category.
[b] Maximum and minimum within the category.

Other trained personnel

NUMBER OF INDIVIDUALS IN EACH CATEGORY		
Engineering—heavy	Workshop	
Engineering—fine		
Engineering—electrical		
Electronics		
Junior attendants	Animal Quarters	
Senior attendants		
Breeding of experimental animals		
Crop cultivation		
Ancillary personnel		
Other		

II. *Questionnaire*

1. NAME OF INSPECTED ESTABLISHMENT: . . . . . .

2. MANAGING DIRECTOR or Head of inspected department: . . . . . .

3. FIELDS OF COMPETENCE

A. Research

- General Microbiology . . . . . ☐
- Medical Microbiology . . . . . ☐
- Veterinary Microbiology . . . . . ☐
- Plant Pathology . . . . . ☐
- Entomology . . . . . ☐
- Bacteriology . . . . . ☐
- Virology . . . . . ☐
- Rickettsiology . . . . . ☐
- Parasitology . . . . . ☐
- Epidemiology . . . . . ☐
- Epizootiology . . . . . ☐
- Immunology . . . . . ☐
- Microbial Genetics . . . . . ☐
- Biochemistry . . . . . ☐
- Fractionation Techniques . . . . . ☐
- Biophysics . . . . . ☐
- Radiation Biology . . . . . ☐
- Studies on aerogenic infections, including aerosol technology . . . . . ☐
- Germ-free animal research . . . . . ☐
- Bioengineering and Biotechnology . . . . . ☐
- Quality control . . . . . ☐
- Sanitary engineering . . . . . ☐
- Other (specify) . . . . . ☐

. . . . . .

B. Development work

- Laboratory safety . . . . . ☐
- Design and testing of fermentors, germ-free animal units or sterile filling machines ☐
- Animal breeding . . . . . ☐
- Other (specify) . . . . . ☐

. . . . . .

C. Production

- Bacterial Vaccines . . . . . ☐
- Toxoids and toxins . . . . . ☐
- Viral vaccines and viruses . . . . . ☐
- Tissue cell cultures . . . . . ☐
- Diagnostics (microbiological) . . . . . ☐
- Media . . . . . ☐
- Sera . . . . . ☐
- Antisera . . . . . ☐
- Microbial cells . . . . . ☐
- Fermentation products . . . . . ☐
- Beverages . . . . . ☐
- Fermented foods . . . . . ☐
- Other (specify) . . . . . ☐

. . . . . .

4. ACTIVITIES

A. Research or recent publications on any of the agents specified by the Inspectors.

B. If studies were performed in the last 12 months on highly virulent or toxic materials, state number or total capacity of devices used:
   a. negative pressure hoods and laminar flow boxes with high efficiency filters or air incinerators (number of units) . . . .
   b. total number of glove ports . . . .
   c. aerosol-protected centrifuges and other harvesting devices (capacity converted to grams dryweight per day at maximum load) . . . .
   d. negative pressure rooms with UV lamps, exhaust air filtration and facilities for easy disinfection (m^2) . . . .
   e. aerosol-tight cages or rooms (maximum number in simultaneous operation on any occasion) . . . .

C. Manufactured products (including microbiological diagnostics) related to the agents listed in annexe B, or so closely related to them that a switch in production would be easily feasible. Indicate production and preparation processes: . . . .

D. Production for uses other than human and veterinary medicine: . . . .

E. Has the laboratory ever done any work on breeding of arthropods? . . . .

F. Do you obtain elsewhere bulk material for the production and processing of agents in annexe B? . . . .

G. Did any of your experiments during the last 12 months require total sterilization of effluent air or transport of living or dead animals in airtight containers? . . . .

H. Have any highly toxic or virulent biologicals been transported outside the premises in the last 12 months? . . . .

5. FACILITIES

A. Have you facilities which can be used for safe, large-scale handling of virulent organisms or toxic biologicals? . . . .
   a. Are there containers available (owned, hired or loaned) which could be used for safe transport by untrained personnel of highly virulent or toxic biologicals in batches exceeding 50 litres? . . . .

	Yes	No
b. Have you facilities for large-scale production of pathogens or toxins?	☐	☐
(1) in submerged batches larger than 100 litres (includes medium in case of dialysis culture)?	☐	☐
(2) by continuous cultivation >10 l (total laboratory capacity)?	☐	☐
(3) by surface cultivation >5 m^2 total area per week?	☐	☐
(4) submerged tissue cell cultivation >5 l per batch?	☐	☐
(5) surface tissue cell cultivation >2 m^2 per week?	☐	☐
(6) egg cultivation >500 eggs per week	☐	☐

B. Have you facilities for:	Yes	No
a. automatic handling of infected eggs?	☐	☐
b. aerosol equipments?	☐	☐
c. germ-free animal work?	☐	☐
d. animal post-mortems in protection hood?	☐	☐
e. effluent sewage decontamination?	☐	☐

C. What is your capacity for?
   a. autoclaving (total volume inside space m^3) . . . . . .
   b. autoclaving big items (inside volume in m^3 of biggest autoclave) . . . . . .

c. freeze-drying (total capacity expressed as kg of ice condensed per week at maximum utilization) . . . . . .
d. single batch freeze-drying (kg of ice condensed in largest unit under heavy load conditions) . . . . . .
e. spray-drying (total capacity expressed as kg of water removed per week at maximum utilization) . . . . . .

Temperature °C °C °C

f. cold-storage capacity (liters inside equipment space) . . . . . .
g. submerged cultivation of micro-organisms (total effective fermentor volume in liters) . . . . . .
h. cultivating large batches of micro-organisms (total effective volume in liters of largest fermentor) . . . . . .
i. submerged cultivation of virulent pathogens (total effective volume in liters of fermentors designed for exhaust gas treatment, operation at reduced pressure etc.) . . . . . .
j. cultivating large batches of virulent pathogens (total effective volume in liters of largest fermentor designed for reducing microbiological health hazards) . . . . . .
k. incubation in the +30–+40° C range (total volume in m^3) . . . . . .
l. egg-breeding (total capacity of egg-incubators expressed in number of eggs simultaneously accommodated) . . . . . .
m. animal breeding (specify the species and monthly output) . . . . . .
n. plant-breeding (area in m^2 and corresponding area under cultivation) . . . . . .

D. List all virulent human, animal and plant pathogens in your possession: . . .

E. Number of primates, excluding men, used in experiments during the last 12 months: . . .

F. Number of experiments or tests on human beings during the last 12 months: . . .

6. PERSONNEL

A. Does any part of your personnel pass security clearance before being hired? If yes, state reason: . . .

B. Is the use of identity cards required for entering any part of the inspected or nearby facilities? . . .

C. Is any part of your personnel required by contract or state regulations to regard specific research projects as classified for national security reasons? If yes, state whether the experiments involve human, animal or plant pathogens: . . .

D. Do you admit foreign visitors? . . .

E. Are their movements restricted? . . .

F. Are foreign scientists working in your establishment? . . .

G. Are their movements restricted? . . .

H. Are there any limitations on the publication of the results of work other than considerations of scientific quality or safeguarding of commercial interest? . . .

I. Is there a hospital or clinic associated with the establishment? . . .

J. Is your personnel required to report illness to a medically qualified person? If yes, name this person. . . .

K. Has any part of your personnel been advised to take prophylactically antibiotics or chemotherapeutics during the last 12 months? If so, how many? . . .

L. Is any part of your personnel required to make a complete change of clothes and to shower after performing particular tasks? If yes, describe the work involved. . . .

M. Is any part of your establishment subjected to a national or other inspection scheme aiming at reducing biological health hazards to the personnel? If yes, state the reason. . . .

N. Are there any restrictions on the movement within or outside the establishment of your personnel due to biological health hazards? If the answer is yes, give reason. . . .

O. What types of immunization have been performed and when? . . .

P. Do you keep immunization records Yes ☐ No ☐

Q. Is there a regular survey of any immune state performed on your staff or parts of it? If so, against what agents? . . .

. . . . . .

DATE OF POSTING

. . . . . .

Managing Director
or Head of
inspected Department
(name in block letters
under signature)

Annexe A

This explanatory note is to be attached to the document concerning inspection for nonproduction of biological weapons in microbiological research and production establishments.

This laboratory has undertaken not to engage in work specifically related to the development of biological weapons and is consequently subject to international control. A group of inspectors will pay a visit on . . . . . and require that the attached questionnaire is filled in and remains posted until their arrival. It has been completed to the best of the ability of those concerned but if errors have crept in, I expect to be informed so that they can be rectified.

Everybody should cooperate fully with the inspectors, who have pledged not to disclose any observations which might endanger the scientific or commercial interests of this establishment.

Date

Managing Director,
Head of inspected Department

Annexe B

Tentative table of agents

Bacteria and rickettsia

1. Bacillus anthracis
2. Shigella sp.
3. Brucella sp.
4. Vibrio cholera
5. Corynebact. diphteriae
6. Malleomyces mallei
7. B. pseudomallei (meliodosis)
8. Pasteurella pestis
9. Pasteurella tularensis
10. Salmonella sp.
11. Rickettsia sp.
12. Psittacosis

Viruses

13. Arboviruses
14. Smallpox and other poxviruses
15. Influenza and other respiratory viruses
16. Foot and mouth disease
17. African swine fever
18. Rinderpest
19. Rabies
20. Fowl plague
21. Blue tongue

Toxins, fungi and protozoa

22. Coccidioides immitis
23. Histoplasma capsulatum
24. Nocardia farcinica
25. Botulinal toxin
26. Staphylococcal enterotoxin

Annexe C

BW control log

This is to be kept by the laboratory prior to inspection. Any short-circuiting of sealed recorders necessitated by maintenance must be recorded giving the time involved and the name and address of maintenance crew head.

Date of use of pathogens listed in Annexe B	Cultivation unit for submerged bacterial batch > 100 l or continuous > 5 l holding volume	Cultivation unit for suspended tissue cells > 5 l	Aerosol experiment unit	Large-scale freeze- or spray drying	Larger simultaneous egg breeding than 100	Monkey(s)	Intended use	Person responsible for material

Annexe D

Inspector's private check list

This is a sample section only, since the questions will be used to cross-check questionnaires and also because it should contain technical information which ought not to be widely disseminated.

Referring to questionnaire I. 4: Outline on map or model:

a. Buildings added since its preparation.
b. Buildings projected.
c. All piping connections (water, sewage and steam) between buildings as well as all points where the services cross the border of the establishment.
d. Describe the position of all services for sewage treatment.
e. Location of electrical meters.
f. Location of gas meters.
g. . . . . .
h. . . . . .

Referring to questionnaire I. 5:

a. Does the Managing Director direct or participate in the governing board of any other microbiological establishment (research, production). If yes, name other assignment(s).
b. Is any part of your personnel paid directly by military funds or by foreign grants?
c. Are there any rules laid down for laboratory workers who enter the premises for any reason after closing hours? If the answer is yes, quote the rules.
d. . . . . .
e. . . . . .
f. . . . . .

Referring to questionnaire II. 3A:

a. Has any of you staff had previous experience in indirect (mechanical) handling of highly toxic chemicals?
b. Do you appoint safety officers?
c. . . . . .

Referring to questionnaire II. 4A and 4C:

a. Laboratory studies which might be of direct relevance to BW based on agents other than those listed in Annexe B.
b. Have you done work in *aerobiology*: aerosol generation and stability, animal exposure, micro-encapsulation involving particularly . . . . . , studies on virulence and stability enhancers such as . . . . . .
. . . . . .
c. Have you studied non-viable agents known to break down natural barriers to microbial infection, such as radiation, corticosteroids and antimitotic drugs?
d. Have you done work in *microbial physiology* or in cell research relevant to the viability of pathogens to storage (lyophilization), to induction of resistance to therapeutic agents or to agents which might neutralize natural or artificial protection barriers, agents such as, for instance . . . . . .
e. Do you study non-specific resistance?
f. Have you done work in *pathophysiology* such as studies on mixed infections or on virulence for primates?
g. . . . . .

Referring to annexe B:

1. a. Clostridia
   b. . . . . .
   c. . . . . .
   d. Marburg virus
   e. . . . . .
   f. . . . . .
   g. Mycotoxins
   h. . . . . .
   i. . . . . .
2. a. Do any of the pathogens mentioned in annexe B or in the previous paragraph normally cause disease in your country?
   b. Where do you export vaccines against the diseases caused by the agents referred to in the preceding two paragraphs?
   c. Do you carry out resistance testing with antibiotics or chemotherapeutics against any of those pathogens?
   d. . . . . .
   e. . . . . .
   f. . . . . .

Referring to questionnaire II. 5E:

a. How quickly could you convert your fermentors to make them leak-proof?
b. Name your source(s) of primates.
c. Have you any knowledge of infections brought in from tropical regions and spreading in your country?
d. . . . . .
e. . . . . .

Referring to questionnaire II. 6A:

a. What technique(s) is used for sewage-treatment?
b. What techniques do you use for leak-testing fermentors or aerosol-exposure units?
c. Have you facilities for making long welded seals in plastic materials?
d. In what connection do you use gaseous sterilants like peracetic acid or ethylene oxide?
e. . . . . .
f. . . . . .
g. . . . . .

Closing remark:
Knowing how the inspections are performed, how would you go about evading detection of activities which they are designed to find? Would you be prepared to accept an unexpected inspection? If yes, would you nominate your deputy responsible for receiving the inspection in case you were not available? Would you be prepared to allow the inspectors to sample any of your cultures? If no, state the reason.

Appendix 2. Fluorescent antibody techniques and some other rapid identification techniques suitable for BW defence—a round table discussion, May 1970

List of participants:

W. B. Cherry, National Communicable Disease Center, Atlanta, Georgia, USA

D. Danielsson, Department of Bacteriology, Örebro Hospital, Örebro, Sweden

Å. Espmark, National Bacteriology Laboratory, Stockholm, Sweden

C.-G. Hedén, Department of Bacteriological Bioengineering, Karolinska Institutet, Stockholm, Sweden

J. F. Hers, Department of Clinical Respiratory Virology, University Hospital, Leyden, The Netherlands

K. Å. Karlsson, Research Institute of National Defence, Sundbyberg, Sweden

Å. Ljunggren, National Bacteriology Laboratory, Stockholm, Sweden

R. C. Nairn, Department of Pathology, Monash Medical School, Prahran, Victoria, Australia (chairman)

T. Nemec, Stockholm International Peace Research Institute, Sveavägen 166, Stockholm, Sweden

R. Ryhage, Laboratory for Mass-Spectrometry, Karolinska Institutet, Stockholm, Sweden

M. Schaeffer, Bureau of Laboratories, New York City Health Dept., New York, N.Y., USA

K. Sinyak, Institute of Epidemiology and Microbiology, Kiev, USSR

I. *Research cooperation as a factor in BW disarmament*

Nairn: First on our programme will be a word from Carl-Göran Hedén on research cooperation as a factor in BW disarmament.

Hedén: I would like briefly to give some of the background of this meeting, which in a sense is a follow-up of a Pugwash-SIPRI conference on rapid detection methods for airborne bacteria and viruses, held in Stockholm in September 1966.

Our aims were threefold. First and foremost, today the possibilities of worldwide cooperation in carrying out research in areas related to CBW are great. We felt that if such cooperation can be realized, it could minimize the mistrust which now exists between scientists and between countries in regard to efforts made in the CBW field.

This would, however, be idealistic, wishful thinking if it were not for the fact that developments in the early 1970s have created a favourable situation for such cooperation: first, there is President Nixon's statement of 25 November 1969 followed in February by an additional statement on toxins. Toxins had not been included in the first statement—to some extent, I think, because the UN Expert Group had decided to differentiate between B and C by saying that biological agents were living agents, which, of course, transferred toxins over to the chemical side. I think that this was an unfortunate decision but the situation was rectified in President Nixon's February statement.

This initiative has opened up very interesting possibilities, because the large military establishments at Fort Detrick and Pine Bluff in the United States are being considered for a switch from military to civil administration and when that happens, they should also be open to cooperation of different sorts. I visited Washington, D.C. recently and had a long discussion with the President's scientific adviser Dr Du Bridge about this. I also talked with Dr Philip Handler of the National Academy of Sciences, Dr McElroy of the National Science Foundation and several others who are deeply involved in the matter. I think that it is quite safe to say that at the scientific level, there is a lot of interest in civil redeployment of the military facilities which could certainly be exceedingly important for a range of civil activities. Indeed, such activities can help both the underdeveloped countries and many industrialized societies.

Although the next sequence of steps is difficult to foresee, we have every reason to believe that several countries are now looking very thoroughly into the possibility of following suit on the basis of the US initiative. As

you know, there is a relatively strong pressure to civilianize the Porton laboratories in Britain, and similar moves are now being actively studied elsewhere, for instance, in my own country. Such considerations will also be in the focus of attention at the International Microbiological Congress (Mexico City, August 1970), which is organized by the International Association of Microbiological Societies (IAMS), and where several possibilities of the non-governmental organizations taking an active part in reducing the dangers of a further development, will be discussed.

Certainly the actions open to the non-governmental bodies are limited, and in Mexico we must of course consider only specific proposals which are realistic. Also there might be a general resolution, but there I am sceptical since we have had a large number of BW resolutions through the years which seem to have played a very minor role. So my aim would be to emphasize a forceful programme of action, bearing in mind that this might constitute a problem for IAMS' non-governmental member societies, since it may involve them in a conflict with their national loyalties.

The open meeting in Mexico City on 7 August will be followed by two closed meetings. The national societies have sent many delegates to the discussions and they have all received the same background material in the form of the UN study [155], the WHO study [156], and the provisional editions of the SIPRI study. The latter are relatively comprehensive documents, one part giving the history up to the present time and another reviewing the negotiations which have gone on within the UN structure. Finally, there is a part on verification and control. Three other books are being prepared and we are now considering adding an appendix on fluorescent antibody techniques. Thas is the background for this meeting.

Our second aim is concerned with the fact that the Pugwash Organization, which continues to be interested in rapid detection and identification of pathogenic micro-organisms, is planning a meeting in Geneva entitled Rapid Detection and Identification of Microbiological Agents, later on this year.[1] The present discussion might hopefully provide some input to that meeting.

Our third aim is related to the fact that, for about two years, SIPRI has been interested in doing exploratory CBW laboratory work on the basis of international participation, since this might perhaps be useful in establishing contacts and improving understanding. In the course of this planning, SIPRI gave one grant to Dr Ljunggren from Stockholm to work in Prague on fluorescent antibody techniques and another to Dr Sinyak from Moscow to work in Stockholm. His topic was not fluorescent antibody techniques but something which is related to early warning and identification, namely

[1] It was actually held in Geneva on 18–21 February 1971.

gas chromatography/mass spectrometry. We thought that they might both benefit from meeting a group such as that now convened in Stockholm.

Nairn: Thank you, Dr Hedén. Could you expand a little on the redeployment perspective?

Hedén: There are several hundred people at Fort Detrick for whom there is essentially no financial provision after 1 July. In order to keep the establishment running, an executive decision must be made. I find the probable delays in this worrying, because I happen to regard Fort Detrick and Pine Bluff as an international resource, and not only as a national resource of the United States. If no money is forthcoming, the whole organization will be mothballed which would represent a loss to the world at large and also a hazard if the policy of the United States with regard to CBW changes. However, judging from the recommendations that I understand now exist, a redeployment is likely. One problem, however, is that so many possible departmental interests must be considered.

The Department of Agriculture might want the insect-breeding facilities for biological control purposes such as the ferromones, sterilized males, insect pathogens etc. The Department of Health, Education and Welfare has a logical interest in vaccine and cancer research; the Department of the Interior might want the exposure chambers for pollution trials and, finally, NASA could use the toroids for simulating zero-gravity in studies on the ecological repercussions of space flights, etc. In fact, I am worried that this might essentially turn Fort Detrick into a jack-of-all-trades, which could mean a decline in the quality of the work done. Personally I would like to see a concentration and expansion, but this seems unrealistic at the present time when the financial situation is so difficult.

II. *Potentials and limitations in fluorescent antibody techniques*

Nairn: Now we should clarify the purpose of this meeting. It would seem that since the meeting has been placed at the end of this fluorescence symposium [129] some people have got together who know about labelled antibodies and the purposes for which they might be used. Perhaps on the basis of your presentation we might usefully restrict ourselves to indicating technological areas that could be further explored at future meetings.

The purely mechanical problems of sampling, particle concentration and recognition of the microbiological nature of any particle—for example, by nucleic acid detection—are not really my province and they do not seem to present any serious technical difficulty. My brief is to introduce the

problem of specific identification of any micro-organisms and the role of immunofluorescence in this. For reasons which I shall soon outline, I should like to enlarge the scope of this brief to include the role of radioisotope-labelled antibodies for such identification.

Let us first say a word about the employment of immunofluorescence for our purposes. Semi-automated detection is already being practised, *albeit* in a model system with a known micro-organism and corresponding specific antiserum. Dr Cherry will be able to recount the latest developments in this field in the United States. The National Communicable Disease Center in Atlanta, Georgia has collaborated with the US Aerojet Corporation in the development of mechanized immunofluorescence for routine serum antibody tests in the diagnosis of antitreponema activity in syphylitics. The staining procedures are mechanized and human intervention is only required at the stage of microscopical assessment of stained preparations. The cost of engineering a robot microscopist still seems likely to be greater than that of employing a human observer at this stage.

There is no doubt that the processing of specimens from collection to staining and transferal to the microscope stage is fairly simple. A concerted programme to develop pure, specific antibody conjugates for relevant bacteria is still required and, when successful, will do much to solve all the serious present-day problems. The solution for virus tracing is far less easy: these may need a cell culture stage in their identification, and mechanizing this for several different viruses would be a major achievement in itself.

Whatever microscopical preparations of micro-organisms are obtained for examination, anyone with long practical experience of immunofluorescence will know about the analytical care required for the microscopy to avoid fallacious positive or negative conclusions. False positives are probably no less serious for our present purpose than false negatives since they might distract from the true nature of the assailing organism, for example, if it were really a virus with a bacterial distractor.[2]

Moreover, distractors in the air sample to guarantee false positives could be readily included in the germ cloud. Non-microbial particles of high net positive charge and of the right size would give non-specific staining only distinguishable from specific micro-organisms by introducing full immunological controls into any automated testing system; that is, we shall always require non-immune conjugates for testing antigenic specificity of the target particles.

The actual recording device—the fluorescence microscope—may not yet

[2] Two agents, one of which is difficult to detect, are mixed together in order to distract attention.

permit a fully automated system, despite the recent commercial introduction of much of the equipment needed for this. Incident light microscopy with dichroic mirror filters are a great advance and, combined with sensitive microfluorimetry, should make mechanization of this stage possible. However, I wonder if technology has really advanced far enough to put this into practice. Any automatic focusing device would undoubtedly use much of the fluorescent emission, which is already only bright enough for fluorimetric detection.

This thought has suggested to me that we might side-step the engineering problem of microscope precision by adopting radio-labelled antibodies in preference to immunofluorescence. This would greatly increase the sensitivity of the system and would relieve us of the problems of precision engineering for high-power microscopy. There are two worthwhile approaches to the use of the radio-labelled antibodies for our purpose which I will come back to later: (*a*) measuring the coating of the organisms with radioactive antibody; and (*b*) measuring deletion from a radioactive antibody solution after contact with the specific micro-organisms.

Hedén: As I have hinted before, I would particularly like to place emphasis on areas of study which could become a focus for international cooperation. Is there any concrete project which one could advance in Mexico and suggest as an effort which would be vitally important to public health?

Nairn: Does anyone have any views on what else we, as a group, might usefully do for international cooperation?

Schaeffer: I think that possibly, in addition to having a precise technical area to cover, we could perhaps pursue some discussion as to what we, as individuals and cooperatively as representatives of professional organizations, can do in our own countries, to restrict the uses of technology to civil purposes. Rapid detection methods are now more important than ever, particularly since automation has become a focus of interest. A large American organization claims that it is now on the point of developing an automated procedure for the isolation and detection of viruses. This is a large undertaking, and I doubt if it is feasible at this time. My feeling is that, as microbiologists, most of us have tended to wrap ourselves in mothballs and conclude that, in our scientific contributions or public health activities, we are doing a great deal for humanity. But we have forgotten that we have a greater role to play, either by example or by becoming vocal. What role should we play, both philosophically and politically, as well as contributing scientifically?

Nairn: This is a very good idea, but again we ought to put this at the end of the agenda. Otherwise we are not going to make best use of the individual specialized knowledge in this particular field.

III. *Present status of labelled antibody techniques relevant to BW, against the background of earlier discussions*

Nairn: As Professor Hedén said, a group met in 1966 to discuss these very matters. That meeting dealt with detection methods for microbiological agents. Then there was a subsequent meeting, about which I know very little. It was held in Prague and, as Dr Ljunggren is working in Prague now, perhaps he could report on it. What was the date of that meeting?

Hedén: The Pugwash Marianske Lazne meeting was held in 1969 and soon afterwards, plans were made by the academies, the Czech Pugwash group and SIPRI for Dr Ljunggren to work with Dr J. Sterzl in Prague.

Ljunggren: The 1969 meeting comprised a report on the work SIPRI had done thus far, and it reviewed the SIPRI inspection experiment. It emphasized the importance of open work in the BW area and the need to open up new channels, one of these being this exchange of scientists. There was also a discussion of possibilities for future work, but that did not concern rapid detection very much so I will not go into it here.

Nairn: Perhaps I should quickly summarize what the 1966 meeting concluded. In 1966 the general recommendations and conclusions were that it was possible to organize an early warning method based upon two main things. One was on the detection of life, that is, nucleic acids, in any microbial clouds, and the emphasis was on using Acridine Orange and examining them by ultra-violet light with the techniques very well known to Professor Caspersson at the Karolinska Institute in Stockholm. This method could pick up very small quantities of nucleic acid and recognize that what we were dealing with was, or had been, living matter.

Secondly, it was considered possible to automate fluorescent antibody methods at a cost of about $250 000. Everyone was well aware of the antigenic variants of organisms, and a list of organisms which might be examined in this way was put forward at the meeting. I do not suppose it has been very much altered since then, and I am not sure that I have the actual list with me, but people like Dr Cherry are well aware of what they are.

This brings us to the beginning of the present meeting, where the next item on the agenda was a contribution from myself on the limits of the usefulness of the fluorescent antibody technique.

We have heard some quite amazing things at the immunofluorescence conference which, as a matter of fact, have altered my thinking in the last couple of weeks. I have been thinking about automation, of labelled antibody detection of organisms, and I would like to say a few words about how far I think we have now reached. Then I suggest that we ask Dr Cherry some special microbiological questions.

Although the value of immunofluorescence for the study of micro-organisms is great, we should not lose sight of the fact that laboratory diagnosis and experimental microbiology may present very different problems from those posed by biological warfare. We tended at the last meeting to treat biological warfare as an extension of the laboratory although this actually is not the case, because, presumably, in biological warfare we would often be dealing with large numbers of isolated micro-organisms of a single strain, as opposed to the clinical and experimental situation where you have to identify a particular micro-organism against a biological background. I think with these different problems the solutions may be different, and those emanating from the present conference may not be appropriate for biological warfare.

Immunofluorescence is at its best as a technique when you use it to indicate where your target material is located. It defines the exact biolocation of a micro-organism, but for some detection purposes, as distinct from localization, I wonder if immunofluorescence should not now be given priority over immunoradiation. I see this as a different problem, because radio-labelled antibodies possess the great value of more sensitive detection—where microscopical precision is not an issue at all. And the degree of sensitivity has been shown in several papers in the meeting to be very substantial. It so happens that virtually all our background knowledge of using labelled antibodies as a diagnostic tool comes from immunofluorescence. Immunofluorescence has provided the validation of anything that we can do with labelled antibodies—the background work will never be lost. In any case, it may still provide the ideal second line of identification.

In immunofluorescence, semi-automated detection is already being practised. It has been done in a model system with a known micro-organism's specific antiserum. In the United States there is an instrument now working quite well and selling for about $10 000 which will pick up *Treponema pallidum* antibody. It is essentially a batch laboratory process and may not be what we want, but it shows what can be developed. Another laboratory (in Brighton) has a continuous line of cultured cells growing on film which

obviously could be used for automatic virus detection, so it looks as though continuous flow processes are available.

It is interesting that the project reported from the National Communicable Disease Center (CDC) in Atlanta on the recognition of syphilis antibodies is one which is automatic right up to the stage of a microscopist. The people in Leyden feel that this can also be automated with a photofluorimeter. Using a 90 X water immersion objective, they passed a film through the light path. The difficulty of focusing as an engineering problem had, however, probably not really been solved.

Automatic self-focusing devices are simple for focusing a picture on a screen, where there is plenty of light and you can use part of the illumination to provide a different trajectory of light, so that when it goes out of focus it automatically returns. But the trouble with immunofluorescence is that there is not enough light to use split-beam methods. The engineering aspect may perhaps not be impossible, although it may be extraordinarily difficult. Obviously, since the power needed for micro-organisms will be very high, say 1000 X, even the thickness of the micro-organism itself will make a difference in focusing and independent human intervention will possibly be needed at this stage. This is why I thought that radio-labelled antibodies might be better, because focusing would not be a problem. I think ^{125}I is the best radio label at present in existence. The method would be to get as pure antibody as possible and I think elution methods are needed, possibly with antibody from germ-free animals. If we can get pure or relatively pure antibody and it can be labelled with ^{125}I, then some continuous flow process can immediately be introduced. We can have antibody flowing through a scintillation counter, a sodium iodide crystal with a hole in it, with continual counting and continuous sampling of particles so that when any antibody is substracted, the counts in the supernatant will fall and there will be an increase in the sediment.

One can imagine a solution of pure antibody globulin coupled with ^{125}I circulating through a scintillometer and giving regular counts until exposed to a concentrate of specific micro-organisms which would delete radiolabelled antibody. Given an instrument such as a continuous flow centrifuge to separate particles and liquids, the count in the antibody solution would diminish and that given by the particles would increase. I know that this will work as a manual batch process in the laboratory. With present technology it could undoubtedly be automated. Cross-checking particles against the supernatant should provide certain results and poses no problems for a modern multi-channel scintillometer. A control section with immunoglobulins not directed against the particular micro-organism would offer an ideal check on the specificity of the detection procedure.

The test organism that we have used as a public health measure in Australia is *Brucella ovis* but it could provide a very useful continuation programme from which data could be extracted. It may be of interest to Dr Danielsson that immunoradio-tracing unfortunately does not readily lend itself to any millipore techniques. The millipore filters take up the radioactive material nonspecifically and destroy everything, and I wonder if he would comment on that. He is already running the semi-automated detection system of water microbes by millipore filtration procedures and it seems to me that this project would lend itself more than any other to some real-life model testing system of surreptitious microbial contamination of water supplies.

IV. *Problems in sampling*

Schaeffer: As you pointed out earlier, Mr Chairman, the sample collection is primarily a matter of engineering. However, I feel that we do not have a really good collecting method. Aside from the mechanism itself, there is the problem of what happens to microbes in the process of collection. When I was in the US Public Health Service, we did some work with the people at Fort Detrick with regard to sampling devices, since we were concerned with all detection possibilities, including the detection of possible attacks of BW. I learned early that the procedure of collecting is very deleterious, both for bacteria and viruses. For instance, when looking for micro-organisms in air by passing the air through filters, cotton, or any simple filter, I was amazed to find that cotton itself is tremendously toxic, particularly to bacteria, but also to viruses. Even in the simple act of bubbling air containing virus particles through water, the bubbling procedure itself was very destructive. The surface tension is possibly a factor. It would seem as if this exploded the microbial cell. I found that when putting in a known amount I could only collect some micro-organisms.

The problem connected with sampling seems to be more than just engineering. I think we have an important biological phenomenon taking place that we do not quite understand.

Nairn: Dr Hers made this very clear in his contribution.

Hers: In principle, very little is known about the survival of different sorts of viruses and bacteria in the air under different conditions of humidity, rain, temperature, irradiation of the sun, and so on. Professor Winkler, in Utrecht, has made several studies on the survival of bacteria and viruses in air. He found a decrease in the micro-organisms which survived in the air.

Conditions such as humidity, temperature, etc. have a tremendous influence. Sampling is not the same as making good aerosols. I known that our sample collector devices, let us say, beyond 5 000 metres, are very unsatisfactory and we need better designs. In September last year I attended the Third International Congress on Aero-Biology in Sussex and saw some sampling devices. The conclusion from the conference, which I think will come out within a few weeks, was that we do not have any good samplers. [157]

Cherry: If you are thinking about survival in terms of viability, this is one question. Survival in terms of the organisms remaining intact but non-viable, so that one can detect them with isotope-labelling, retaining their surface specificity and so on, is another problem.

Nairn: This point, which Dr Cherry raised, is a very important one for the use of any kind of labelled antibody. It is quite important to use a method which can identify dead as well as living organisms.

Hers: I agree.

Cherry: If the particles, say bacteria, are actually autolyzed during the collecting process, this is bad. But if they are retained intact and possibly collected in some sort of a preservative medium, autolysis probably could be prevented. Then isotope or fluorescein-labelled antibody, or other techniques could be used for detection or identification, if this were actually a problem. There are reels of data from Detrick studies covering many years on the survival of all sorts of pathogens under a variety of conditions.

Hers: The influence of environment is of such an enormous variation that you do not know what to do.

Cherry: For some organisms.

Hers: Yes, for some organisms. I will come back to the question of sampling again. In the survey of the conference on aero-biology, the main topic was laminar flow studies, which became focused on kidney transplantations and similar operations. Infection in such patients is extremely widespread in spite of laminar flow. These data led to the conclusion that it was very difficult to sample even bacteria.

Hedén: One other thing is relevant in this connection and that is related to Meselson's suggestion at the Pugwash BW Study Group—that one might be able to detect biological agent testing by sampling at a point very far removed from the proving grounds. But in the study group we found that no one really knew what happened to the antigen structure, from the

fluorescent antibody point of view, when particles had been airborne at a relatively high altitude for a couple of days or even a week.

Cherry: From a theoretical standpoint, I want to ask if the organism under consideration is dangerous; has it retained its viability after travel in the cloud and is it hazardous to man or animal? It has probably retained its surface, unless it is extremely labile. The chances are good that its surface structure is pretty much intact even though it has been exposed to rather severe and hazardous conditions.

Hedén: Meselson's idea involved detection of organisms which had been living but which had died and drifted off, so it was essentially not a question of living cells.

Nairn: Of course, they do survive and maintain their antigenicity under the most remarkable conditions. In freeze-drying, for example, they are perfectly all right, and it was your group, Dr Cherry, that identified pasteurella in putrified carcasses of rats.

Cherry: The most striking example was a guinea pig carcass, which was purposely put on a shelf in the laboratory. And I am sure it must have been tightly closed because it stayed there for seven years. This rotten carcass was then exhumed from the bottle and smears were made and *Pasteurella pestis* stained beautifully from the rotten tissues.

Schaeffer: But that is a special situation, and now and then this will happen. However, micro-organisms at high altitudes are exposed to sunlight and other radiations, and most of us do not realize that light is much more destructive to many of the organisms we work with than some of the antiseptics or antibiotics we use.

Nairn: They may not be culturable, but they will probably retain their antigenic surface structure so that they will be detectable; and if they are not, the chances are that they are non-viable.

Schaeffer: Yes, but the fact is that we really do not have enough information.

V. *Antigen structure in relation to identification and antibody formation*

Hedén: The question of the destruction of microbial antigens is relevant not only to the detection of bacteria in a cloud, but also to the structure of a

micro-organism when it is used to immunize an animal to make antibodies. As Dr Ljunggren is studying this on germ-free animals, perhaps he might now tell us something about antigens from this point of view.[3]

Ljunggren: My programme in Prague is to compare sera from germ-free and conventional animals with special reference to the *Salmonella* group. Logically, sera from germ-free animals should lack natural antibodies caused by the natural flora, and therefore they ought to show less cross-reactivity.

Starting with conventionally reared rabbits and using direct immunofluorescence, I found that sera from animals immunized with heat-killed smooth *Salmonella typhimurium* LT2 were bound to several rough mutants and that the rougher the mutant, the more the fluorescence increased. Thus, mutants of chemotype Ra were hardly stained at all, whereas the Re chemotype was stained to almost the same titer[4] as the smooth parent strain. The serum which should only have contained antibodies against smooth determinants thus obviously contained antibodies against deeper lying structures in the cell wall. They became accessible to reaction when the lipopolysacharide (LPS) became less complex.

The presence of multiple antibodies in a serum is obviously unsatisfactory, and the possibility that these undesirable antibodies against deeper lying structures are a result of natural antibodies boosted anamnestically by the immunization is one background to the use of germ-free animals. For this purpose, germ-free piglets were used. Pigs have the advantage of having a six-layered placenta, and maternal immunoglobulins can therefore not pass over to the foetus and confuse the picture. However, the use of piglets had two disadvantages: first, that they grow too rapidly, reaching a size not compatible with normal incubators, and secondly, that intravenous injection can only be performed by the intracardial route. The experiments were therefore started using sera from pigs which had survived colonization with live smooth LT 2 in the hope that this process of immunization could be useful. Unfortunately, however, it proved that the Ra chemotype mutants, which, when using rabbit serum, were hardly stained at all, were stained by the piglet serum to practically the same titer as the smooth LT 2. This was presumably caused by rough strains occurring during the infection. The work is being continued by immunizing the piglets with intravenous injections of heat-killed bacteria and by trying to immunize the conventional rabbits with the LPS in the hope that this latter procedure will give rise to less antibody against the deeper lying structures.

[3] Presentation abbreviated, since a more complete description is given in appendix 3.
[4] A titer is a measure of immunological potency.

Nairn: Two major attempts have been made at making pure antibodies: one is germ-free animals and the other is trying to use specific antigens. This is obviously very sensible. Does anybody have any comments on the use of germ-free animals? Has anybody used them?

Cherry: It's amazing! Here is a new technique that seems promising but it becomes complicated by the fact that rough variants occur which would ordinarily cause no problem at all and which upset the works.

Schaeffer: Something like a relapsing fever type of phenomena! May I ask which is more important with regard to the antibody: that it be completely pure, or that it does not have any crosses which would be annoying? If the latter is true, would you not be better off using a specific pathogen-free animal that can cope better with your injection of polysaccharide than minor antibodies from unrelated organisms which might give you a little trouble?

Ljunggren: Yes, but the question for those *Salmonella* is: what should I keep them free of?

Schaeffer: Of *Salmonella.*

Ljunggren: Yes, but I must also keep them free of the *Citrobacter,* for example, because the *Citrobacter* serum with the same 0-antigen as this one cross-reacts practically totally.

Schaeffer: Quite. Is it possible for you to keep them free of related organisms, not of related antigens, but not entirely germ-free?

Cherry: He has an unusually complex problem if he is trying to develop an antibody for *Salmonella* screening at the genus level because regardless of how pure the antibody is, you are not going to be able to avoid cross-reactions with enteric organisms if the material is then used back against mixed flora that contain enteric organisms. This is because of the numerous opportunities that exist for true serologic relationships between other enteric bacteria and *Salmonella.*

Schaeffer: It is a very useful clinical problem.

Cherry: You would not think of the *Salmonella* as a BW agent, except the typhoid organism.

Nairn: But it would not matter if the serum cross-reacted with other sero-types. You could sort that out.

Cherry: Sero-types of *Salmonella?*

Nairn: Yes or other cross-reacting organisms. What I think matters is that your antigenic specificity is important so as not to create irrelevant globulins. Because if you have labelled antibody against large amounts of other things in your reagent, then you get too much background in any automatic detection system.

Danielsson: Do the sera of the germ-free animals also react with certain types of *staphylococci?*

Ljunggren: No, but this needs further checking so I am grateful for the reminder.

Cherry: We only looked for antibody or something that simulated antibody against *staphylococci* and we only looked in germ-free mice. We did find it, although we could not find it in mice three weeks old; but by the eighth week we found it in all of them. Although we never proved this, as we had to discontinue the work, I feel certain that the stimulus was sterile food which might have been contaminated at many steps during processing.

Hers: At the conference in Sussex, some speakers talked about the danger of the *staphylococci* invasion in connection with the suppression of transplantation reactions. There, *staphylococci* might well be transmitted by food.

Cherry: I should think that, by now, someone must have looked at the sera of some germ-free animals—mice—for a variety of antibodies.

Hers: Fodder can often be a serious problem. As you know, ferrets are highly susceptible to distemper. We import ferrets from North Africa and England and we keep certain breeds. Some years ago, I think in 1966, we had distemper in one of our stables and had to destroy more than 1 000 animals, which cost an enormous amount of money. Since that time we decided to sterilize all fodder, to prevent an infection hazard.

Nairn: In any case, you would want to give a diet which was minimally antigenic on all accounts, because antigenicity from its mere content of foreign animal protein is just as important.

Hedén: Certainly there have been completely antigen-free foods tested, but I do not know if they have been tested on a very extensive basis.

Nairn: Quite apart from the *staphylococci,* Dr Cherry, milk proteins might be just as distracting. I mean, if we want to increase the signal-to-noise ratio, it is important that there is *no* antigen present.

Schaeffer: I realize the problems of feeding in germ-free animals is a tremendous order. Would it be possible to do something like hydrolyzing proteins; you know, the preparation they use for intravenous feeding, with added vitamins and making sure that there are no whole proteins?

VI. *The contribution of the individual scientist to improved public health measures and international understanding*

Nairn: I would now like to introduce the topic of personal contributions by biological workers in their national environments.

Hers: We have done one experiment to simplify and to speed up diagnosis as much as we can. We are working with three different unrelated viruses. These are inoculated in different sorts of tissue culture, and we detect them on one mixture of antibody.

Nairn: What could you have done to make the Dutch Government aware of your success in that particular field and also to make them influence public opinion?

Schaeffer: I should say that there has been more activity on the part of scientists in the United States in recent years. They have been a bit more vocal. There have been resolutions passed by the US Public Health Association; at its last annual meeting in Boston, the American Society of Microbiologists (ASM) had a very exciting session on biological warfare. Riley Housewright was there and I think did a very good job. I think that it would be difficult for an organization which uses techniques in a destructive fashion to attract very many, if any, of our outstanding scientists. A few, perhaps, are always available for a salary. So, we appear to be moving in the right direction, but I don't think that we have gone far enough and I am asking what can we do in small ways as well as in big ways? How can I influence my colleagues?

Hers: During the past years we have had several seminars in the Netherlands about the same problem, initiated primarily by the universities, Leyden University being one of the first to hold a seminar on ABC weapons. All one can do is to discuss the matter with other scientists and this will give them a possibility to introduce the subject in their scientific programmes.

Cherry: I think it is important that we do what little we can, and talk to associates and our scientist friends. Also we are sometimes asked to speak to high school groups about science. I have not heard this discussed, but my

feeling is that the winding down of BW by the Nixon Administration is also a good starting point because the layman is terrified of BW. I do not think nuclear warfare is as frightening to the layman as BW, because BW is such an unknown factor.

Schaeffer: Frankly, I think that the one reason the Nixon Administration went ahead is that they must be convinced that they are not giving up a great deal and it is an easy thing to do. I have no fear of BW, not because I know so much about medicine and biology, but because I do not think that it is going to be, at any time, at least with our present knowledge, any decisive factor in warfare, but has only nuisance value and psychological effects.

I don't think Nixon was motivated by generosity, but it was a wise initiative to take. I am hoping that the Department of Health, Education and Welfare will cooperate. They have not accepted the proposal yet. We need places like Fort Detrick for Lassa fever, for instance, which has recently been imported from Africa into the United States. It is a very dangerous virus and is highly contagious.

These are areas in which we can be influential, I think. We can try to convince Mr Finch[5] to accept an area like this—totally, not just a part of it. This is one of the ways in which our meetings and national scientific meetings, etc. can be brought out into the open and make the public aware of the need. Then they can exert pressure. This relates to Dr Cherry's idea with regard to scientists who are very often called upon to speak to high school students, college students and so forth; young people could be given more information about this and about how useful these things are for daily preventive medicine, public health and rapid diagnosis, and so on. This is where the effort should be, instead of on the other side.

Hers: In the Netherlands there was an enormous emotional response from people who listened, but who do not understand the problems, and my task was to talk about infection and what we know and do not know. So many people with limited knowledge are talking about such things. They made statements, for instance, in which biological weapons were called "weapons of the poor". Now, our lab does a lot of microbiological work and it is very, very expensive. Obviously this slogan is wrong, and I think that one thing we should do, as scientists, is to say that one must understand that B weapons are very expensive.

[5] At the time of the Conference, Secretary of the Department of Health, Education and Welfare.

Nairn: This is an important change of thought and it is probably very sound to have this recorded, because biological weapons really were considered to be the nuclear weapon of the poor and it was thought that the major powers should put a good deal of effort into defence so that they could prevent dissemination among the poor nations, so to speak.

Hedén: We are now discussing an awfully complex problem, and I think that it is very dangerous to make off-the-cuff statements. One can certainly work out quite a few scenarios where the applications of BW in conflicts between developing countries might cause considerable problems. I think that one must weigh all the different types of scenario, and this is actually what we are trying to do in the SIPRI studies.

One thing that is very important with regard to all disarmament efforts is the timing. The Nixon statement came too early, as far as I am concerned. It failed to stress the redeployment angle, and the need to do something which could have a positive effect in a large part of the world, where there are great problems. Certainly, nobody needs BW, I agree; in fact, you need it like a hole in the head! But it is a tall order to switch the potential, so, ideally, there should have been a comprehensive preparatory study and then a political "show" at the proper time.

But then, of course, statesmen are in a difficult position, because the crisis frequency increases. J. Platt, for instance, has brought this out; the frequency of crisis is becoming so high that our present decision-making processes easily become strained to breaking point and then initiatives will, of course, not always be optimally timed.

Schaeffer: If he had asked for my advice, I would have suggested that he say that what we need is defence against biological warfare which Nature is constantly waging against us, and far more effectively than any nation can do. And what we will do then is to work on defences against just that: Nature's biological warfare around the world. He could have capitalized on this and gained some very good publicity. I think that if the Department of Health, Education and Welfare took over establishments for military microbiology and used them effectively, then we could still make great progress.

Hedén: However, we should learn a lesson from the situation and consider the specific research areas which we should be prepared to advocate—areas which would make use of the available facilities and expand international cooperation. We have, for instance, discussed germ-free animals as antibody producers. It is an interesting area because technically sophisticated isolation facilities are needed. But we should be aware of the fact that many

other important projects can lay claims on whatever BW facilities might become released for civilian needs. One can, for instance, make a very strong case for research on biological control, because this might require microencapsulation methods, a lot of the aerosol technology, climatological know-how, and so on. This is an example of an area which I think should be given very careful study from the point of view of international collaboration. In the developing parts of the world, there is a very rapid agricultural take-over by monocultures, so-called green revolutions, which require a high level of insecticides.

However, in the industrialized countries we are greatly concerned with environmental problems, including biocides and this concern is apt to spread to the developing countries who might want alternatives—and rightly so, because it seems unfair that we should let them operate under a chemical hazard that we would not accept in our part of the world. Diagnostic work related to husbandry and veterinary vaccines seems to represent another critically important area. Take, for instance, the impact of foot and mouth virus in India and Latin America, rinderpest in Africa, etc. As you line those things up you will find that it is very easy to advocate a major effort in the microbiological field. What then are the things which we, around this table, should like to see carried out on an international cooperative basis in such a context? I repeat this question because it is conceivable that in a few years' time we might find ourselves faced with a major fellowship exchange programme. Based on existing facilities, what are those fellowship workers going to do? What areas related to rapid diagnosis should we recommend? This is something which I should like us to discuss.

VII. *The scope for improvements in the rapid identification of micro-organisms*

Danielsson: I would like to dwell on a combined fluorescent antibody (FA) technique for a moment.[6]

Cherry: I had a point about isotope work. I am afraid I am not quite so optimistic as you, Dr Nairn, about the use of radio isotope-labelled antibodies for rapid detection. I know the Detrick people have considered this problem several times over the last 16 or 20 years or so, and as far as I know they have not done very much with it. Some years ago we made one little trial with tritiated *E. coli* serum. This is an exchange reaction and the

[6] Dr Danielsson's technical description of a membrane filter/minicolony FA technique for H. *influenzae* and *Pasteurella* is deleted since it is published in reference [158].

reason we used tritium was because it has a fairly long half-life and also because you can get a high specific activity. It is done in a dry form. The exchange occurs over a period, I think, of 2 or 3 weeks. We sent the material away for labelling and got it back still in dry form. We then hydrated and tested it and found that the loss in titer was tremendous. I do not know whether this was due to radioactivity that had been introduced or whether there were other factors.

Hedén: Was it exposed in vacuum?

Cherry: Yes.

Nairn: I do not think that method is very good. We tried to use it ourselves for electron microscopic autoradiography, and we gave it up because it seems as though radio chemical "cooking" of the antibody takes place. This does not happen with radio-iodine in which the maximum labelling is one iodine atom per globulin molecule. I was told by one of the workers at this conference in Stockholm that the antibody lasts for 6 months. We get no loss of titer whatever from the labelling. Radio-iodine labelling is less of a problem than with FITC.

Cherry: Why do you say that you want a maximum of one atom per molecule of protein? Do you mean that it is ample in BW detection situations when you have a small number of bacteria in your sampling apparatus and therefore only a few oportunities for antibody to react?

Nairn: We have a substantially lower degree of labelling with success in the case of *brucellosis.* We have got about a 1 per cent labelling and it is really quite efficient.

Cherry: Have you quantified the number of organisms you can detect this way?

Nairn: No, but I think it is quite large. This stems from the observation I made at the beginning that, in BW, one will expect to be dealing with a large number of organisms and it is not the same as the clinical situation.

Cherry: Is that a logical assumption? I ask the question because you do not know where the sampler will be in relation to the cloud, let us say. You may be on the very fringes of the thing. You do not know where the cloud has been liberated or how diffuse it has become by the time the samplers are in operation. You may be dealing with a low number of organisms.

Nairn: But the concentration techniques should provide you with sufficient organisms no matter where you are.

Cherry: The other point I wanted to make was that it seems to me that the specificity problem with isotope-labelled antibodies is going to be analogous to that with FITC labelled antibodies. Possibly, there might even be other problems, but I am sure that isotope-labelled antibodies may be taken up by extraneous material.

Nairn: These would give you problems of false positives, which, of course, do not really matter nearly so much as false negatives.

Cherry: With the fluorescent antibody technique you have the advantage, at least if you are reading it visually, of the morphology of the organism, if it is a bacterium. In viruses, you may not have morphology but you have this additional advantage of visualization which you might not have in automated procedures.

Nairn: It would be interesting to do both in the way Gerald Johnson demonstrated at the immunofluorescence meeting, that is, double-labelling with FITC and iodine so that you get the best of both.

Hers: Mr Chairman, what we need is sensitive methods and I think perhaps it would be sensible to use screening methods as a first step, and then more specific techniques. The second step might use automated isotope labelling, perhaps, and the third step fluorescent antibodies where you have the morphology too. What do you think about that?

Nairn: I think the reason why the isotope technique was given up at Detrick and at Porton was the absence of sufficiently pure antibody. The background from labelled globulins is so high that you cannot really do any work. What we are using with *brucella* is eluted antibody; putting it on to the organisms, washing them, and then taking it back off again and labelling that. We think that this will increase the specificity and sensitivity tremendously.

Hers: I would like to ask, how do you concentrate the micro-organisms?

Nairn: We have only done this as a laboratory exercise by the ordinary conventional methods. We tried millipore filters, which do not work because of the nonspecific binding of the radio-labelled globulin, so we just have it in a tube. I have no doubt that it is possible to make a kind of small-scale Sharples centrifuge which gives a continuous flow of supernatant and deposit. Dr Hedén, do you know if such an apparatus exists?

Hedén: Well, certainly the Anderson zonal centrifuge exists and I think that the Aerojet people in the United States have been working on a separator of this type. It is not commercially available as far as I know,

but it is intended for FA. However, what I think has not been done and what should be done is to use the Albertson phase separation technique where you make the micro-organisms move from one phase to the other. By choosing the proper phases you can get enormous concentrations of cells into a droplet. Beyond certain concentrations, aquous polymers like dextran and polyethyleneglycol do not mix, and by choosing the proper salt concentration you can concentrate the cells in the dextran phase, which can represent a very small volume. In fact, you can do this with a growth medium, so that you get a growth medium with two phases. Dr M. Puziss and I have, for instance, grown tetanus bacteria in this way: at the bottom, the dextran phase, leaving the big bulk of the polyethylenoglycol as a nutrient supply. [159] For cultivation purposes it is an interesting technique because you can stir up the bottom phase in the polyethylenoglycol getting emulsion droplets where the cells behave as in a dialysis culture machine with a vast dialysis membrane. You can get very high toxin concentrations that way. Here, then, is a simple technique which does not necessarily involve membranes or filters. I think one should explore this in the FA field.

Espmark: It should not be forgotten that this technique is also very suitable for purifying and concentrating viruses and also has the potential for distinguishing different viruses. The method is also very simple.

VIII. *Current efforts in the automation of FA techniques*

Nairn: Are there any other points, Dr Cherry?

Cherry: There are two things that I thought might be of some interest. One would be the latest automation that I am familiar with, for Group A Strep, which I think has a great deal of potential for us since detection is being stressed. This is a machine that has been built by Aerojet General. There is only one machine, a prototype, so far. Let me describe the machine first.

In the United States, FA tests for Group A *Streptococci* have become the most massively used FA technique. Some state labs are only doing a few tests but, on the other hand, some are doing as many as 350 000 throat swabs for Group A Strep per year. It is estimated that if we could automate this procedure so that it became cheaper and more available, then probably 150 000 000 throat cultures would be processed for Group A Strep annually. In 1963 Hochberg stained *streptococci* suspended in a test-tube with fluorescent antibody, eluted the antibody following staining and dissociated the antibody by changing the electrolyte concentration. He then measured the fluorescence of the dissociated antibody in a fluorometer. This was an

interesting technique, but it has not proved to be satisfactory because only a certain percentage of the antibody is eluted. Also, when it was applied to throat cultures rather than to pure cultures of Group A *Streptococci*, the unlabelled organisms present refracted light and gave an additional signal that confused the interpretation of the read-out. The method was also technically difficult because it involved two or three steps of centrifugation, and filtering was not successful as a substitute for centrifugation, because the antibody bound nonspecifically to the millipore membrane and gave false positive readings. The next step was taken in our laboratory by Dr Moody, who has been interested in this all along because he is the developer of the technique for identifying or grouping *streptococci* by this method. He worked on this from the standpoint of spotting the stained organisms. Again, they were stained in suspension with FA. They were then spotted on filter paper—he ran through several different types of paper before he found one that was satisfactory for this—and after excitation the spot was read for fluorescent emission in a fluorometer at 360 nanometers, Now, let us come back to the present and the machine that Aerojet has developed, again in cooperation with Moody and with the former Heart Disease Control Program of the US Public Health Service. Probably over $300 000 has been put into the development of this machine. They now use a mylar tape, which is about 2 inches wide. Initially, millipore tape was used but proved to be too expensive. Therefore, they stamped holes at about 2-inch intervals along the length of the mylar tape. Into each of these holes, which are above 5 millimeters or so in diameter, a 4 millimeter diameter millipore disc is cemented. The sample is then placed on this millipore disc.[7]

The original model that came to the National Communicable Disease Center for evaluation and went on to the Connecticut State Laboratories for field evaluation with the actual throat cultures did not have the processor which the latest model has. The latest model has just been evaluated at CDC with pure cultures of *streptococci* and has now gone on to Con-

[7] The clinical specimens, throat swabs, are incubated in broth overnight. After centrifugation and washing, cells from the culture are spotted by pipette onto the moving tape which comes off a roll. As this tape passes along, about every 2 inches there is a small millipore disc on which the sample is deposited. Beneath the disc there is a suction station, which immediately pulls the liquid through the millipore disc, leaving the organisms on the millipore membrane. The tape passes over rollers into an incubation chamber with high relative humidity and an immersion type heater. There is a lot of condensation, far too much, particularly with 50°C heat. A humid chamber is needed to keep the samples from drying out since the staining takes place during the movement of the organisms on the millipore disc through this area. At a certain point there is a wash station; in fact, several stations can be added very easily, facilitating an indirect fluorescent antibody test if required. In addition to a strip-chart recorder, a printer has also been used to give a digital readout representing the amount of fluorescence seen by the sensor.

necticut for further evaluation. In the original model you simply stained the organisms in suspension and pipetted them on to the tape which went under a sensing-head consisting of a modified fluorometer. This gave a strip chart tracing the fluoroscence of the organisms as they passed under the sensing-head. This worked very well, but Aerojet General was interested in automating the process further so the incubation could be carried out automatically rather than following the earlier procedure; culturing in test tubes and transferring to the tape.

You can insert a sample every 20 seconds, which means a passage of 180 specimens per hour or at least a thousand specimens per day. The estimate is based on the use of two technicians to keep the machine operating fully. At $4.00 per hour per technician, plus the cost of the reagents, which will be manufactured by Aerojet General, and the cost of the tape, it is estimated that the cost per test for Group A *Streptococci* specimens will be between 6 to 14 cents. The tape may be patented; you will have to buy the tape from them in order to use the machine. Also, they will probably lease the machine rather than sell it; they are going over the lease arrangements with the FTA slide processor. This price of 6 to 14 cents compares with $1 per test by manual grouping using the FA technique, or, probably, considerably more than $1 owing to inflation and so forth. So this has a potential for large capacity, low cost, and versatility and, I think, for adaption to other immunofluorescent tests.

This has been Max Moody's project and I am most enthusiastic about it as I think that it is adaptable to almost any kind of automated fluorescent antibody test. But now it is at a critical point because politics have become involved and, because of the cut-back in the aerospace programme, Aerojet General's biology group has been reduced to about half the number of professional people they have had in the past. They have also separated them from the parent company.

Danielsson: Do you think it is time to re-evaluate an indirect technique now that better defined antibodies are available?

Cherry: There is some saving in work, although labelling is so routine these days. But, you are right, it probably should be re-examined.

Nairn: Some organisms, such as *Shigella,* would require an extra step. That is one of the problems, it is not?

Danielsson: With such a machine, there would not be much trouble with an extra step.

IX. *Problems in the identification of viruses*

Espmark: Very little has been said about viruses, I think.

Nairn: Quite so. Perhaps we should move into this field now. Dr Hers will first talk about the problems of virus identification in air samples.

Hers: It seems to me that if a virus can be picked up from the air and cultivated in susceptible cells, the diagnosis can be made very early. In our immunofluorescence experiments, we have increased the speed of diagnosis: we made diagnosis in 16 hours, but I think we can speed this up to 4 hours. Although we have made several attempts at picking up viruses from the air, we are very doubtful that airborne viruses in general can be collected this way.

Espmark: Many of these tests for rapid detection of viruses, when applied to biological warfare defence instances, would certainly have to be performed under less than perfect conditions in many cases. I doubt whether diverse dots, without any sort of defined morphology in preparations, could be said to be due to virus. In my opinion we are dependent on the guidance of morphology within cells when we interpret the signs of presence or absence of viral antigens. Hence, it seems to me that the possibility of a specific diagnosis directly from air samples is rather doubtful at the present time.

Another point is that the viruses that would be used for biological warfare would certainly not be purified, but would contain a large amount of host material, that is the substrate, and the range of substrate would probably be rather restricted. It would be primate cell material or possibly chicken material. And I feel that the antigenic mass of the substrate would be far larger than the antigenic mass of the specific viral agent. Hence it would perhaps be feasible to demonstrate the substrate in the air sample and thus show that a BW attack has occurred. Although this would be much better than to know nothing, it would evidently not tell us anything about the nature of the agent.

Besides short-term culture of air samples for virus demonstration by, for example, immunofluorescence, it would perhaps be even more important to be prepared for a quick diagnosis on diseased people infected early by the attack. Important types of specimen for this purpose are samples from the respiratory tract, throat samples, sputum, etc. Urine is also easily accessible; in fact many viral agents appear in cells in the urine, for instance *herpes* viruses and many of the *myxo* and *entero* viruses. In the case of exanthe-

matous diseases, vesicular material or skin biopsies for sectioning are pertinent materials. It is very easy to get biopsy material with present punch instruments. A little more doubtful and a little more difficult to get is biopsy material from internal organs, for example, liver and kidney biopsies. These could, however, be obtained with needles, which would not confer any great risks.

After cell material from these different locations has been prepared as smears by centrifugation or otherwise, or by sectioning, it is stained for immunofluorescence. Due to the preliminary information we have as to symptomatology, we can hopefully choose the right battery of sera for this purpose. (A series of slides was then shown.)

Nairn: It is quite clear that you need morphological identification for viruses. This will not only be useful in early diagnosis of cases, but also in allegations of the uses of microbiological warfare.

Espmark: We should not ignore the possibility of making antibody tests for early diagnosis in cases where antigen is not so easily detectable.

Hers: If you are speaking of purified viral antigen, you cannot detect that as such, but you can detect it adsorbed onto or in cells, or in parts of cells. It is not necessary for the virus to live. An older publication from Canada describes the first diagnosis of a respiratory disease directly from throat washings. They did spectrofluorometry following an antigen test. We have used this in our vaccine production. Influenza virus was harvested from the amnion fluid of eggs and subsamples of the sediment from the Sharples centrifuge were diluted and FA-stained. For the final identification you need a living host, for example, a tissue culture. (A demonstration of slides followed.)

Nairn: Mixture with biological host material is not necessarily a serious distractant. It may be helpful in collection and in providing a larger mass of matter.

Hers: I should like to stress that impression smears, even from vesicles, are highly valuable. We can make a very thorough diagnosis that way. Although rabies is very rare in the Netherlands, it is a very important public health problem, because it is always imported from Germany. The indirect FA testing for rabies in the Netherlands is now performed on brain smears from diseased animals.

X. *The potential of gas chromatography/mass spectrometry for the detection of tissue culture substrates and marker molecules*

Hedén: Dr Espmark stressed the fact with regard to air detection that we should put more emphasis on detecting the substrate molecules, i.e., monkey tissue or whatever, from the limited range available to a B-weapon manufacturer. As far as early warning goes, this might be quite relevant. Perhaps Dr Sinyak, who has given a lot of thought to this and especially to the use of gas chromatography could make some comments. Gas chromatography might possibly also be adapted to antibody labelling techniques, so it might be interesting to consider how sensitive the method is. Would it be possible to label an antibody with a molecule which is very easy to pick up with gas chromatography? But let us first hear what Dr Sinyak has to say.

Sinyak: The present study was started to investigate the lipid compounds of tissue culture fluids. Initially the samples were treated with oxygen and then registered. This type of oxidation was convenient because of its simplicity, but it was difficult to control. In fact, 3 or 4 per cent of the samples gave negative results. Therefore, we developed an alternative, quite promising technique. (A description, summarized in appendix 4, followed.)

Nairn: How quickly could your method work, Dr Sinyak?

Sinyak: In order to study a sample very carefully, it requires half an hour. But this time can be shortened to 15–20 minutes.

Nairn: That is fantastic! Can a virus profile also be identified in the profile of the host cells?

Sinyak: No. We identify only the tissue culture fluid.

Nairn: Obviously here is a very quick, special way of picking up host tissues which could be important to us. Are there any comments?

Hedén: It would be very interesting to hear what Dr Ryhage thinks about the limits of the gas chromatography technique as far as miniaturization is concerned. Suppose you label an antibody with something which is easy to pick up. What is the limit?

Ryhage: It should be possible to use gas chromatography in the nanogram range, but by using the combined gas chromatograph/mass spectrometer (LKB 9000) you may select, let us say, the molecular ions of the three C_{20}-fatty acids with 1, 2 and 3 double bonds from a complex mixture by focusing on these three ions using the special technique of alternating ac-

celerating voltage. In this case the sensitivity could be increased a thousand times as compared to running a complete spectrum for each compound. This should allow you to work in the picogram range. However, the components studied are usually only a small fraction of the complete mixture. The injected amount would therefore be in the nanogram range.

Hedén: I should perhaps have emphasized that I was not thinking of analysing antibodies as such, but rather of labelling antibodies with something that you could easily pick up on a mass spectrometer, maybe heavy nitrogen or a special fatty acid. How does the sensitivity then compare with the isotope technique?

Nairn: There are far more problems than that. We would have to build up a completely new technology, because of the problems which we call non-specific staining.

Cherry: We have been interested in this approach to the rapid detection of disease, perhaps even before symptoms occur. It has been reported for virus diseases by Matruka and Alexander in their papers using an electron capture detector, but with rather poor graphs. We have very little to go on so far, because we have just started in the area of analysing body fluids. We have looked at a number of normal spinal fluids for baseline, and we recently got *Mycobacterium tuberculosis* and *Meningococcus meningitidis* spinal fluids, both of which had two or three very good peaks, which were not present in any of the normal spinal fluids and were different in those two diseases. But we do not know what the peaks represent. They might be altered host metabolites, or they may be cell constituents, or metabolic products of the cell itself or of the spinal fluid. There is, I think a tremendous potential here for rapid and early diagnosis of infectious disease.

Ryhage: We have not used the combined gas chromatograph/mass spectrometer to any great extent in virus work, but if we can identify characteristic differences in the mass spectra from parts of the gas chromatogram, it is possible to distinguish between different cell materials.

Sinyak: But there is certainly a problem in selecting the proper column because the same sample gives very different GC-spectra on different columns. One should know what to look for.

Cherry: I think the combination of GLC and mass spectrometry is the most potent analytical tool on the horizon for us in microbiology for detection and analysis of micro-organisms and detection of disease. Right now it is where FA was 20 years ago.

Nairn: Are there any vital points that you feel have not been brought out? Could we sum up the situation as we see it?

We believe that there are rapid detection methods for recognizing micro-organisms and, in the case of viruses, for recognizing their carriers; that gas liquid chromatography and mass spectrometry should be developed as fast as possible to provide what should eventually turn out to be a more direct and better method for identifying things than using antibody. However, in the meantime, as Dr Cherry said, we are still 20 years away and we are well aware of the technology that had to go into fluorescent antibody. We should cash in on the technology that we have acquired in fluorescent antibody and use it—it seems to be essential for virus diagnosis—where morphology is so terribly important. In the case of the recognition of microbes, it looks as though radioisotopes may provide an alternative method but fluorescent antibody is still going to be extraordinarily useful for the final clinching diagnosis.

XI. *The question of laboratory* versus *field studies*

Professor Hedén, a point in your letter referred to the problems involved in the field study of allegations. You were thinking of providing means of rapid access to an area where such a problem existed. I have given this matter a good deal of thought and have come to the conclusion that at the present level of technology, it would be of much greater value to organize centres of expertise all over the world, to which suspected material could be sent. [160]

A panel of laboratories practising first-class diagnostic immunofluorescence, perhaps under the auspices of the World Health Organization, strategically distributed to keep transport time for material to a minimum, should be set up.

The laboratories could from time to time indulge in practice exercises to maintain their state of ready capability. They would need to be supplied originally with reagents, but not instruments, from some central source, say here in Stockholm. In the first place, specific high titer antiserum conjugates and a supply of control antigenic preparations for checking techniques would be required and must, of course, always be on hand.

The only other thing necessary would be an instructed field organization for satisfactory transmission of suspect material, whether automatically or manually collected.

I do not believe that mobile field units could tackle the problem half as well. The resources of an established laboratory of personnel, equipment

and reagents are to be preferred and these could never be shipped *in toto* to a disaster area.

However, after having listened to Dr Hers at this meeting, it looks as though there are sometimes gains to be made by doing diagnostic work at the time of sampling.

Hedén: Perhaps we can also hear from Dr Karlsson in respect of a recent epidemic of tularemia in Sweden.

Karlsson: Yes, we had a serious epidemic outbreak of tularemia in the north of Sweden two years ago and there were a lot of dead sylvan animals which were sent in for examination. They were picked up in the fields of the epidemic area.

Cherry: I am sorry, what animals?

Karlsson: Hares. In all these cases we used the fluorescent antibody technique as well as histopathological and/or conventional bacteriological investigations. Recently, Borg showed that the histopathological examination is very reliable in tularemia of hares and we used his method and criteria. The bacteriological examination was made on liver and spleen suspended in physiological saline and the suspensions were also injected into guinea pigs. On the FA test we used FITC-labelled anti-tularemia conjugate from a man who had just recovered from tularemia. Such field material was, of course, decomposed to a rather high degree, and we first had to make an experiment to see how this affected the possibility of using the FA technique. We therefore infected rabbits with small doses of tularemia and after death the organs were stored at room temperature for a long time. Every 2 or 3 days we then examined them by using imprint smears (demonstrations of slides showing liver smears made up to 25 days *post mortem*.) The FA technique was also compared with cultivation. There was a good likeness between these two methods, and we had nine cases where the FA test was positive and the cultivation negative. We also had one group consisting of 33 cases where we had positive FA tests and autopsy, but negative inoculation results. Finally, we had material from hares and some other animals where we did not have time to make guinea pig inoculations, but where we compared the FT technique with autopsy. There was a very good agreement between these two diagnostic procedures.

Nairn: Could the material be sent to you in your laboratory, or does a skilled and experienced person have to go to the area?

Karlsson: This particular investigation was done in the laboratory, but of course, it is also possible to do such investigations in the field.

Hers: I have heard a rumour that the English are working with mobile units for examining rabbits. Do you know anything about that?

Karlsson: No, I have not heard any details about this. We use the FA microscope and since the imprint smears are very easy to handle, I think that it is quite possible.

Hedén: Experience from allegations in the past in Korea, for instance, illustrates the problem of getting samples. People get to the area so late. Also, such allegations are normally made under conditions of considerable political and military stress and transport is not so easy as it usually is.

Hers: The mobile unit that we were using in the Netherlands was of tremendous help. I think that we need this sort of public health equipment, although trains and other means of transport are very fast. However, real outbreaks also travel very quickly. Our laboratory acts as a reference laboratory for the World Health Organization (WHO) in the Netherlands, and our task is to make diagnosis as soon as possible and to get isolates to prepare vaccines. To do so, one must have the strain. Our mobile unit collects specimens from the diseased people directly. The unit is equipped with a tissue culture set as well as low temperature cabinets, and is completely self-supporting. It operates by radio telephone, so that we can speak to everyone without losing contact with the base laboratory. We sample from the airports and harbours if suspected cases of disease are coming from abroad. Special facilities are given by the Ministry of Transport; we can park everywhere in the Netherlands and can use all roads. This resulted in an enormous rise of isolations of the viruses we were studying and, thus, we accelerated diagnosis. Both outbreaks of influenza A-2 (Hong Kong) were very rapidly recorded in the Netherlands, earlier than for example, in the United Kingdom. We had several cases months before the outbreak was demonstrated in the normal diagnostical way. [161]

Nairn: Obviously, we need both sorts of facilities, centres in as many convenient parts of the world as we can and mobile units.

Cherry: In those centres we need the reagents for handling any situation that may come up and people who understand how to use them and interpret the results. This was a point that I made in a document I have just given to Professor Hedén, that there should be some centre for distributing the reagents.

Hedén: What about the WHO? That should be a good location, and Geneva has a good airport.

Nairn: We should have had David Rowe here.

Hers: Undoubtedly, this is WHO's project.

Cherry: In my experience, it does not matter how good the reagents you have may be, if the person who is going to use them and look at the smears has never seen *Pasteurella tularensis* in tissue before. He is not going to make a reliable diagnosis. Rather than having a mobile unit in the field or bringing everything into the laboratory, I would go myself or send someone who knew how to collect the specimen and who could make a smear directly or collect tissues from the autopsied animals. The samples should then be put in ice or frozen and ideally I would bring them back personally so that I know that they returned the way they should come back to the laboratory.

Hedén: Since it is getting late, I would now, on behalf of SIPRI, like to express our thanks to our Chairman, who has been successful in getting so much out of all the participants, and to all of you who have taken time off on a Saturday to join this round table discussion.

Appendix 3. Rapid detection of BW agents: a report by Dr Åke Ljunggren on his studies in Prague, 1969–1971

I. *Background*

This SIPRI study owes its outlines to the Pugwash movement and in particular to the committee for BW from which the idea of an East-West exchange of scientists working in the field of rapid detection and identification of BW agents gradually arose. The method chosen was immunofluorescence (IFL) in view of its applicability to both BW and public health.

Two reasons dictated the choice of micro-organisms. First, *Salmonella* presents several difficulties and secondly, its surface structure is now fairly well charted.

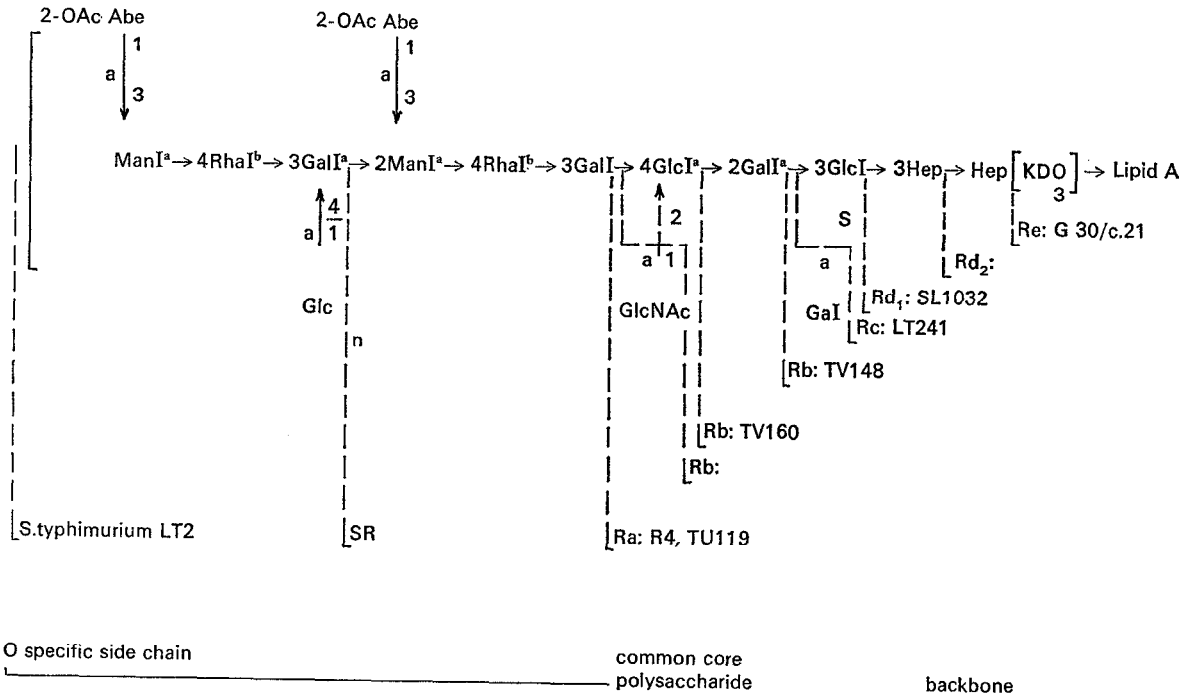
II. *The surface of Enterobacteriacae*

This section is an attempt to discuss some of the results of this investigation, especially where they concern the bacterial surface structure and its role in the interaction with the host. The study was carried out at Professor J. Sterzl's laboratory at the Microbiological Institute of the Czechoslovak Academy of Sciences in Prague during 1969–71.

The surface structure of micro-organisms is of central importance. If, for example, it is in any way defective, the host may be able to phagocytize and kill the invader. Conversely, if the surface possesses protective characteristics, the micro-organism may well be able to multiply in the host. Recent advances in our understanding of the chemical fine-structure of the surface in some of the members of the *Enterobacteriacae* family will no doubt expand our knowledge of the relation between the surface and pathogenicity. Further, we can assume an improvement in serological diagnosis in this family since the surface is the site of the reactions between antibodies and micro-organisms.

The cell envelope of gram-negative bacteria consists of several layers. Innermost is the plasma membrane and, outwards, there are several intermediate layers of which the most substantial consists of a peptidoglycan which gives the

Chart 3A.1. The chemical structure of the Salmonella typhimurium LT2 Lipopolysaccharide



cell wall its rigidity. The outer membrane, which is of most direct concern to the subject of this study, consists of lipopolysaccharide (LPS), phospholipid and lipoprotein. [162] This layer can be separated from the rest of the bacterium and subjected to further study. It is commonly termed endotoxin owing to its ability to induce toxic manifestations in the form of fever, leucopenia, etc., typical of many infections caused by gram-negative bacteria. The toxic activity of the various parts of the endotoxin is not yet fully elucidated. According to one view, the lipid alone is responsible [163] but some believe that the other structures contribute in an essential way. [164]

The protein part of the endotoxin has only recently been studied in some detail. It may be of very similar construction in different genera of *Enterobacteriacae*; the sharing of an antigenic determinant between *Escherichia* and *Serratia* has been demonstrated. There is also evidence that the protein participates in the endotoxic activity. [165]

Both protein and lipid parts of the endotoxin are outside the scope of this study, which principally concerns the polysaccharide (PS) part. The PS is not responsible for the endotoxin effect. It does, however, give rise to antibodies and is thus of great importance both in the protection of the individual and in serological diagnostic procedures. The PS consists of the core, which lies closest to the cell wall; extending from the peripheral end of this core is the the O-specific side chain. The core (see chart 3A.1) [166] comprises a number of sugars which, in all members of the *Salmonella* genus, are identical and bound to each other in the same manner. The construction of the core and the sugars present differs between different genera of gram-negative bacteria.

The O-specific side chain (see chart 3A.1) [166] consists of multiples of identical units, each constructed from a few sugars. Electron microscopy reveals that these side chains extend up to 1 500 Ångströms from the visible boundary of the cell. [167] Thus, the simile of a micro-organism covered by fairly dense "hair" would not be inapt. Obviously, such a covering will be highly important physically, for example, in reactions between antibodies and micro-organisms. It is the chemical structure of the repeating units, that is, which sugars are present and the steric configurations resulting from the bonds, which determines the antigenic structure. Thus, the 4-determinant is due chiefly to the abequose-mannose structure, the 5 is in the acetyl-abequose and the 12 in the glucose-galactose (see the O-specific side chain in chart 3A.1). The 9 determinant in, for example, *S-typhi* is due to the presence of tyvelose rather than abequose. More than 50 such serologically distinguishable antigenic structures or O-antigens are known. The O-antigens often exist in combinations, for example: 1, 4, 5, 12 in *S. typhimurium* and 9, 12 in *S-typhi*, and this further aids in distinguishing the various serological species. *Salmonella* species are thus defined by their antigenic structure, first with respect to their O-antigens and subsequently in

greater detail according to the H or flagellar antigens. The flagellar antigens lie beyond the scope of this study and are mentioned here only for the sake of completeness.

III. *S-R variations*

Strains of gram-negative bacteria having the O-specific side chains are often termed smooth or S-forms, after their smooth colony form. It has long been known that mutants can arise that have a more or less rough colony form. These forms, termed R-forms, also lack serological O-specificity, as well as practically all pathogenicity, and they also autoagglutinate. Endotoxins from R-forms are, however, as toxic to animals as are those from S-forms. [163] The lack of pathogenicity in the R-forms is explained by the absence of the O-specific side chain which, in the S-forms, shields the micro-organism from the protective forces of the host. The R-forms have only the core, or part of it. They are classified as chemotypes and, on the basis of their content of monosaccharides, are characterized by the length of the core or core residue. Thus, Ra has the whole core while Rb–Re denotes steps in the shortening of this core residue. The Ra is to some extent pathogenic while the lower chemotypes are progressively less so. Thus, the less complex their LPS, the less difficulty do the bacteria present to the host. These R-mutant strains are usually stable. They differ from the smooth parent strain only in one characteristic: the structure of the polysaccharide. It is therefore hardly surprising that such strains are of great interest in many fields of research. For the purpose of this study, the R-form is especially interesting since it becomes possible to use two mutants from the same strain, the only difference between them being the presence or absence of one sugar in the core. Unfortunately, the S–R variation is less distinct than might be assumed from the presentation above, since S-forms normally contain small amounts of R determinants. Conversely, many, if not most, R-strains have some S determinants on their surface. These mutants have been given the unfortunate term leaky, on the assumption that the mutation leading to the rough form is not complete, but that an altered protein, that is, enzyme which retains some of the characteristics of the parent form, is synthesized. As a consequence some core stubs are capped by O-specific side-chains. [168]

Serological reactions can be no better than the quality of the antisera used. Ideally, the antiserum should contain only the relevant antibodies, in high titer and in the right immunoglobulin fraction, for the reaction used. In the case of many micro-organisms, these requirements are not always easy to fulfil and those belonging to the *Enterobacteriacae* family present some definite problems, making it a challenging subject for study:

Table 3A.1. Reactions between an anti-LT2 conjugate and some rough mutant strains

Strain[a]	Chemotype	Dilution of rabbit anti-LT2 conjugate							
		1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
LT2	S	+++	+++	+++	++	+	+	−	−
S6	S	+++	+++	+++	++	+	+	−	−
R4	Ra	+	+	−	−	−	−	−	−
TV119	Ra	+	+	−	−	−	−	−	−
TV161	Rb	+	+	+	−	−	−	−	−
TV148	RB	+	+	+	−	−	−	−	−
R7	Rb	++	++	+	+	−	−	−	−
LT2M1	Rc	+++	+++	+++	++	+	+	−	−
SL1032	Rd_1	+++	++	+	+	+	−	−	−
G30/C21	Re	+++	+++	+++	++	+	+	−	−

[a] All strains are derived from the smooth parent *S. typhimurium* LT2. S6 was a rough mutant which reverted to smooth, while the other strains portray an increasing roughness.

1. The LPS is a comparatively poor antigen by itself. [164] Traditionally, the form of antigen used for immunization is whole bacteria killed by heat which inactivates the protein antigens but not the polysaccharide antigens. [169] The antibody level may fall during prolonged immunization.

2. The antibody response is more pronounced in the IgM than in the IgG fraction of the immunoglobulins. This is unfortunate in the case of direct IFL, where IgG is the desired fraction and where IgM is seldom desirable since it is known to be a contributor to nonspecific staining [170], probably due to the fact that IgM easily becomes overlabelled. When IgG and IgM were labelled under identical conditions, it was found that their F/P (ratio of fluorescein to protein) were 9 and 1, respectively, which is in line with the findings of White [171] and of Brooks. [170] If mixtures of the two immunoglobulins are used, the F/P could just as well represent the ratio between the immunoglobulins. The now advocated use of the fraction of a conjugate known to have a specified range of F/P and obtained by chromatographic separation on materials and with buffers, [172–173] does, in fact, virtually exclude the IgM.

3. The possible presence of so-called natural antibodies and their effects are discussed in the section on germ-free animals.

4. Antibodies against R-determinants have recently been demonstrated in not-insignificant titers in sera from animals immunized with heat-killed smooth bacteria. [174–176] This was easily verified using the IFL technique and is illustrated in table 3A.1. The staining in this experiment, however, is probably due to a spectrum of antibodies staining several different determinants. Part of the staining is probably due to anti-S-antibodies combining with S-determinants on so-called leaky mutants, since there is a lowering of titer after ab-

sorption with the S-antigen, whether in the form of whole live bacteria or the LPS. Another part of the staining is probably due to the R-determinants but the progressively more intense staining with increasing roughness might be due to deeper lying antigenic determinants becoming accessible as the core structure becomes more denuded. The results here are not fully comparable with those of Schlecht [174], for example, who obtained precipitating activity only with some mutant chemotypes. The difference may be due to the difference in technique used in the present study, both in prolonging the immunization and in the use of whole bacteria. Schlecht used LPS as the precipitating antigen which, of course, excludes reactions with other components of the cell wall.

It should be pointed out that, for ordinary diagnostic procedures, the presence of such anti-R-antibodies is probably of little importance. In diagnosis, the only point of interest is the S-determinants on the O-specific side chain, and if the bacteria have R-determinants in the form of uncapped cores they will be covered by the "hair" of neighbouring side chains and anti-R-antibodies will not be capable of reacting. For diagnostic purposes, diluted antisera are usually used, which means that the R-activity, which is much lower than the S-activity, is diluted beyond the effective threshold. The situation becomes more difficult when different anti-O sera are pooled and thus diluted, while the anti-R-antibodies exist in all of them and are not diluted. There is also the possibility that these antibodies against the R-determinants may cross-react with similar determinants in related micro-organisms. The experiments discussed later indicate that such antibodies can react with R-forms of *E. coli.* Since such R-forms exist in nature, they could give rise to false positives, a highly undesirable possibility in BW-detection, which could occur unless the sera were carefully absorbed.

In summary, it is obvious that antisera against *Salmonella* contain antibodies against deeper lying common structures in the cell wall, as well as the desired antibodies against the specific structures of the surface layer. This is *per se* an unfavourable situation and is probably due to the form in which the antigen is used. There are three main approaches to eliminating unwanted antibodies in diagnostic sera:

1. Absorption of the undesired antibodies with their antigens is commonly carried out. It is, however, somewhat laborious and a certain loss of the specific antibodies can scarcely be avoided.

2. Purified antigens can be used.

3. The use of germ-free animals has been advocated.

IV. *Germ-free animals*

Conventionally reared laboratory animals are normally colonized soon after birth with a normal microbial flora, both externally and in their gastrointestinal tract. This normal flora can give rise to antibodies without obvious symptoms of disease. Such antibodies, which have occurred without known stimulation, have been termed natural. This type of antibody, can obviously mask the findings in studies of infectious diseases, especially if the organism under surveillance is closely related to a species occurring in the normal flora. The use of germ-free animals might contribute towards eliminating difficulties. The term germ-free animals is used exclusively for laboratory animals that have been delivered aseptically by caesarean section and then kept in sterile surroundings in special incubators, fed sterilized foods, etc. It stands to reason that such procedures are costly both in terms of skilled manpower and materials.

Germ-free animals which are stimulated with one antigen can therefore be expected to develop only antibodies against this antigen in their serum and not antibodies against the normal flora. Theoretically, therefore, such sera should be better for distinguishing between the pathogenic species of the *Enterobacteriacae* and those belonging to the normal flora. This theory was the original basis of the SIPRI experiment.

The animal used in the investigation was the piglet, which has the advantage of having a six-layered placenta that effectively prevents the transfer of maternal immunoglobulins to the foetus which might obscure the results of a study of antibody response. Among the disadvantages resulting from the use of piglets is the fact that they quickly grow to a size which is not compatible with incubators of normal dimensions and that intravenous injections can only be administered via heart-puncture, a procedure that is not without risk. The piglets were immunized with heat-killed LT2 in increasing doses (0.5–3 ml). In the irst experiment there were 10^9 bacteria per ml which, in view of the lack of clinical and immunological response, was increased to 10^{10} per ml in the second experiment. With this dose the piglets showed definite reactions after each injection. The level of specific anti-LT2 IgG, measured by immunodiffusion before and after absorption with live LT2, reached a level not higher than 300 gamma/ml, even during prolonged immunization with amounts of antigen producing clinical symptoms. During this stimulus the total IgG rose to about 2 000 gamma/ml. The reason for this discrepancy is not known, but it seems probable that some form of nonspecific stimulation caused the high total IgG. For comparison, conventionally reared piglets were immunized with the same antigen. This resulted in the same low level of specific IgG. This unresponsiveness to stimulation with heat-killed *Salmonella* for the purpose of

initiating the formation of IgG has also been observed by others. [177] Sera from the germ-free piglets showed the same tendency to react with R-determinants as did sera from conventionally reared rabbits. From this result, it may be assumed that the ability to form S- and R-reactive antibodies during immunization is not dependent on the presence or absence of natural antibodies, but instead is due to the presence of R-determinants in the antigen. It might be added that it is questionable whether the natural antibodies are of importance in IFL since conjugated normal rabbit serum could not be shown to stain either the S- or the R-strains.

In conclusion, it might be said that the use of germ-free piglets resulted in low levels of the desired immunoglobulin and that the same broad reactivity as that found in rabbits was observed.

V. *Study of specificity of IFL*

The R-mutant can also be used for studies of the specificity of antigen-antibody reactions. The antigenic structure of the O-antigen is now well characterized and it is possible to use mutant strains which exhibit only minor differences in the chemical structure. Thus, the strains TV 148 and TV 160 both belong to chemotype Rb and are derived from the *Salmonella typhimurium* LT 2 strain. They differ only in that TV 160 has one more molecule of galactose at the end of its core. [168] The ability of a serological method to detect this comparatively small difference between two antigens can be considered a measure of the specificity of the method.

Conventionally reared rabbits were immunized with the two strains mentioned until immunodiffusion showed distinct precipitation lines. Conjugates containing IgG and with F/P about 1 were used to stain a variety of S- and R-strains (see table 3A.2). Obviously these conjugates contain antibodies capable of reacting with a variety of antigenic sites but no other definite conclusion was possible. Absorptions with the *E. coli* S-16 R-strain were performed on the assumption that some staining was due to the deeper-lying wall structure. Absorption with the smooth LT2 strain was done to eliminate reactions between S-antibodies caused by S-determinants in the immunizing strain and S-determinants in the bacteria to be stained. These two absorptions resulted in some lowering of titer but marked cross-reactivity was observed among the Ra and Rb chemotypes. Then, the two sera were cross-absorbed: firstly, the anti-TV 148 serum with TV 160, and *vice versa*. After further absorption with the Ra strain TV 119, the sera reacted with the immunizing strain and in insignificant titer with an Rc strain. Final absorption with the immunizing strain extinguished all staining. Immunodiffusion showed that the precipitation line

Table 3A.2. Serial absorption of anti-TV 160 IgG conjugate

		Dilution of anti-TV 160 IgG						
Strain	Chemotype	1/2	1/4	1/8	1/16	1/32	1/64	1/128
Before absorption								
LT 2	S	+	±	±	− − −	−	−	−
R 4	Ra	+ + +	+ +	+	−	−	−	−
TV 119	Ra	+ + +	+ +	+ +	+	+	−	−
TV 160	Rb	+ + +	+ + +	+ + +	+ + +	+ +	+	+
TV 148	Rb	+ + +	+ + +	+ +	+	−	−	−
LT2M1	Rc	+ + +	+	+	+	−	−	−
E. coli S-16	R	+ + +	+ +	+	+	−	−	−
E. coli 086	S	−	−	−	−	−	−	−
After absorption with E. coli S16								
LT 2		+	±	−				
R 4		+ + +	+ +	+				
TV 119		+ + +	+ +	+	+	−		
TV 160		+ + +	+ + +	+ + +	+ + +	+	+	−
TV 148		+ + +	+ + +	+ +	+	−		
LT2M1		+ + +	+ + +	+	−			
		±	−					
After absorption with LT2								
LT2		−						
R 4		+ + +	+ +	+				
TV 119		+ + +	+ +	+	−			
TV 160		+ + +	+ + +	+ + +	+ +	+		
TV 148		+ + +	+ +	+	−	−		
LT2M1		+ +	+	−				
After absorption with TV 148								
R 4		+ +	+	−				
TV 119		+ +	+ +	+	−			
TV 160		+ + +	+ + +	+ +	+			
TV 148		+	±	−				
LT2M1		+ +	+	−				
After absorption with TV 119								
R 4		±						
TV 119		±						
TV 160		+ + +	+ + +	+ +	+			
LT2M1		+ +	±	−				

disappeared during this absorption. Lastly, absorption was carried out with the homologous LPS, which also extinguished staining. It is thus clear that the antibody reacted with the LPS part of the micro-organism in the specific reaction. It is also obvious that the method used is sufficiently specific to discern between two polysaccharides which differ only in the presence or absence of one sugar.

The problem of specificity therefore does not lie in the IFL method *per se* but in the preparation of sufficiently specific antisera. The use of heat-killed

micro-organisms, whether smooth or rough, results in antibodies against both S- and R-determinants as well as antibodies against deeper lying structures. Such ballast antibodies might possibly react with similar antigenic structures in other species of *Enterobacteriacae* or even with other genera whose antigenic structure depends upon the polysaccharide structure. The next step in the investigation will therefore be a series of studies of synthetically produced disaccharides and oligosaccharides coupled to a carrier, in order to investigate whether this might offer a possible alternative for the production of antisera without the ballast antibodies. Such a study has other practical connotations since, if such antibodies can be produced in laboratory animals, it might also be possible to induce them in humans. The importance lies in the prospect of being able to produce a vaccine which does not contain the LPS and is therefore devoid of endotoxic activity.

VI. *Study of sensitivity*

The amount of labelled antibody needed to give positive staining is a measure of sensitivity. This information is useful in many fields, not least of which is that of logistics since the more antibody is needed, the more has to be produced, and the organization of production may be greatly influenced, especially if large-scale continuous testing is envisaged.

Positive staining can be defined in many ways: the one selected in this study is that + + + represents typical somatic staining where the cell wall is stained so that the cell appears as a rectangular shape surrounding a dark centre, and + + and + represent lower staining intensities where the whole bacterium shows the same staining intensity. For practical purposes, the difference between + + + and + + is fairly easy to confirm: either one observes the profile surrounding a dark centre or one does not, which is less subjective than optical grading of fluorescence.

In this experiment, an anti-LT 2 IgG labelled with fluorescein to an F/P of 0.8–1.2 was rendered specific against factor 04, and 05 and 04 respectively, through absorption with relevant strains. A serial dilution of this conjugate was used to stain fixed amounts of LT 2 and the last dilution giving + + + staining was recorded. Simultaneously the conjugate was absorbed with excess LT 2 and the amount of IgG before and after absorption was studied by immunodiffusion. The difference, the specific anti-factor IgG, was found to be 6.10^{-5} g/ml. This serum gave + + + staining of 10^8 bacteria in dilution 1/16 which corresponds to $2.5 \cdot 10^6$ molecules of IgG per bacterium. Both the factor-sera gave similar results and it is interesting to note that other experiments

using the indirect technique needed amounts of specific IgG which are about one order of magnitude less in the intermediate layer.

The method used is undoubtedly somewhat crude but it does indicate that the amounts of reagents needed are not inconsiderable.

VII. *Relevance of findings to CBW*

1. The IFL method itself is highly specific. It can be used to distinguish two polysaccharides which differ only in the presence of one galactose molecule.

2. "False positives" are anathematic to CBW detection. The possibility of ballast antibodies reacting with irrelevant bacteria has been discussed earlier.

3. The amounts of reagents needed for positive staining are not inconsiderable. Together with the low yield of IgG during immunization, this dictates the use of sera in comparatively low dilution.

4. Continuous, automated BW detection by IFL will need large amounts of reagents. If the yield of specific IgG is low and further reduced by absorptions, the supply of serum may well become a problem. A detection device using one drop of serum diluted 1/5 per minute will, under these circumstances, use amounts of serum every day which one rabbit takes 10 days to produce. Other animals cannot be used since the diagnostic scheme for *Salmonella* is dependent on rabbit antisera. Methods which will increase the yield of the specific antibodies are therefore of great importance.

Appendix 4. Some studies on the possibility of using gas chromatography and gas chromatography/mass spectrometry of fatty acids in the rapid detection of virus-associated materials[1]

I. *Introduction*

The isolation and identification of pathogens or the demonstration of the antibodies they elicit are the main links in the specific diagnosis of infectious diseases. They are supplemented by various achievements in microbiology and virology, such as immunofluorescence, but in principle they retain their traditional orientation.

It was early established that an infectious agent which affects an organ or a group of cells in the body, disturbs the normal metabolism, resulting in substantial biochemical changes. Some of these changes appear clinically (jaundice in infectious hepatitis yellow fever) or can be demonstrated by specific tests often aimed at some enzyme (aldolase, transaminase, etc.). However, biochemical changes have limited value as specific diagnostic indicators, partly because our knowledge about the biological changes which occur at the level of the cell or organ is inadequate. Rapid routine methods which permit a detailed study of the components of the proteins, carbohydrates and lipids have only become available in the last decade or so. In this context, thin-layer chromatography, gas chromatography and mass spectrometry form a particularly significant triplet.

The development of gas chromatography improved the simplicity, speed and accuracy of the analysis and also resulted in a better separation of many of the component systems of biological origin providing new opportunities for applications in microbiology and virology. Reports have also been published on the successful use of gas chromatographs with high resolution and sensitivity for the determination of lipid components in tissue cultures, [178] lipid and carbohydrate components in Neisseria [179] and Clostridia. [180] Henis, Gould and Alexander [181] advocated their use for biochemical studies in microbi-

[1] This paper by K. Sinyak, C.-G. Hedén, R. Ryhage, C.-G. Fri and L. Sinyak describes the studies performed when K. Sinyak worked at Professor Hedén's department, the Department of Applied Microbiology, Karolinska Institute, Stockholm on a grant from SIPRI.

ology and for the detection and identification of micro-organisms. In a recent paper [182], Mitruka, Carmichael and Alexander reported on the application of gas chromatography for the detection of activity of certain viruses in inoculated cell cultures and in animals.

For several years SIPRI has been engaged in a study of disarmament in the field of chemical and biological warfare. It was then decided to engage in some laboratory work, focusing on the problems of rapid detection and early warning. Two lines of approach were chosen: a model study on the specificity of fluorescent antibodies produced in germ-free animals, on the one hand, and an evaluation of the potential of gas chromatography/mass spectrometry for the rapid detection of materials which might be associated with virus aerosols, on the other. This paper concerns the second project.

II. *Material and methods*

Cell cultures

In the experiments, HeLa, Vero (a continuous line of African green monkey kidney cells), SIRC (a continuous line of rabbit eye corneal cells), BYK-21 (baby hamster kidney cells) and LuQ (diploid lung cells) were used. The cell cultures were grown in 0.2 and 1.0 litre Roux culture flasks. The monolayers were washed with Hank's salt solution and overlaid with 0.25 per cent trypsin solution, and within 10–15 minutes the detached cells were pipetted and centrifuged at 1500 rpm for 10 minutes and then resuspended in Eagle's medium. The SIRC and Vero cells were released from the glass by a solution consisting of 40 per cent by volume of 0.02 per cent versene, 60 per cent by volume of 1 per cent trypsin and 10 per cent by volume of PBS A (trypsin buffer). The HeLa, BHK and diploid cells were released by standard techniques (0.25 per cent trypsin). For passages of cells, culture flasks (0.2 l) were seeded with 2 million Vero cells and after 2 days in the thermostat a good monolayer could be observed in all flasks.

Nutrient media and chemicals

Cell lines were cultivated in Eagle's medium. One per cent of l-arginine (1.74 G/l) to the Eagle's growth medium for SIRC cells was added. Before use, 10 per cent of inactivated calf serum was added to the growth medium. The maintenance medium which was usually used for infected cells and their control cells contained 2 per cent of inactivated serum. Inactivation was carried out in a water bath at 56°C for 30 minutes.

As will be mentioned below, the maintenance medium yields a complex lipid

Chart 4A.1. Chromatogram of extract from the Hank's salt solution

spectrum (see chart 4A.10), but Hank's salt solution yields only two small peaks (chart 4A.1), which may be derived from the organic solvents used for extraction. Those different background peaks must of course be taken into account in the interpretation of the chromatograms, but they are of small practical significance because the quantities of material derived from the tissue culture samples are so much greater.

Viruses

Echo virus and a Rubella strain nonpathogenic for man (received from Dr P. Magnusson at the State Bacteriological laboratory in Sweden) were used.

Gas chromatographic techniques

A gas chromatograph (F&M model 400) with flame ionization detector (FID) designed to handle biological material was used. The temperature programming rates were 3°C per minute. The start delay was 0–20 minutes. The dimension of the glass columns was 3m 3mm I.D. and packed with carbowax, FFAP, OV-17, OY-1 or SE-30. The last two are especially suitable for mass spectrometry, as they exhibit low bleeding at higher temperatures. The column tem-

perature was at least 60°C lower than the flash heater and 30°C lower than in the detector. Nitrogen was used as the carrier gas with a constant flow of about 30 ml/min. Three to 5 μl of the dissolved samples were injected.

During the last 6 months a different analytical gas chromatograph (model PYE series 104 equipped with flame ionization detector) was used. This permits simultaneous operation of two columns, with two detectors and two injection ports. The same temperature regimen and columns as mentioned above were used.

Gas chromatography/mass spectrometry

Mass spectra were obtained by the combined GC–MS instrument LKB 9000. Two different columns were used, one of 1 per cent SE-30 on Gas Chrom P and one of 3 per cent OV–I on Gas Chrom W. The temperature of the column was programmed at 3°C/min from an initial value of 120°C. The temperature of the flash heater and the molecule separator was kept at about 250°C. Helium was used as a carrier gas with a constant flow of about 30 ml/min.

Mass spectra were recorded at a constant acceleration voltage of 3 500 V with an electron energy of 70 eV.

III. *Methods screened for extraction and separation of liquid fraction*

There are several publications concerned with the use of the gas chromatograph in the study of microbes and normal tissue culture metabolites extracted with organic solvents. The lipids can, in fact, be extracted with a variety of organic solvents: chloroform-methanol (2:1 v/v) [183], hot ethanol [178], ether-methanol (2:1 v/v) [184], chloroform ethanol (2:1 v/v) [185], and ethyl ether. [181] In our experiments ethyl ether was used.

Because some viruses contain lipids, and must consequently affect the lipid metabolism of the host cell, the present study was started with screening of such lipid compounds, which could easily be extracted with ether or chloroform, which permits a direct and simple approach. The difficulty of working with a gas chromatograph lies in the fact that it cannot handle high molecular lipids, only their simple constituents. This emphasizes the crucial importance of choosing suitable methods for the treatment and preparation of specimens, which might well require some type of breakdown.

Preparation of samples

The lipids were determined either in cells or in the nutrient fluid treated in the same way as cells destroyed in distilled water. The cell monolayer was trypsinized and centrifuged. When the trypsin had been removed the cells were resuspended in Hank's solution. After counting, the number of cells necessary for one sample was added to a 20 ml tube with a ground-in glass stopper. After a second centrifugation the salt solution was removed. Five ml of distilled water were then added. As an alternative, flasks with cell monolayers were washed twice with Hank's solution and then 5 ml of distilled water were added. In both cases the destruction of the cells was completed by freezing-thawing cycles. The extraction of lipids from tissue culture fluid was carried out by the method described by Henis and Gould. [186]

After the harvesting and disintegration mentioned, the pH of the fluid was adjusted to 2.0 with 1 N HCl. Five ml of ether were then added to each tube which was shaken for 10 minutes. Each sample was then saturated with 1.5 g of $Na_2SO_4 \times 10H_2O$, followed by another 15-minute period of shaking. Following this, the ether extract was placed in a 10 ml glass tube with a ground-in stopper and dehydrated by the addition of anhydrous Na_2SO_4 previously washed twice with ether. The ether extract was then evaporated under a stream of oxygen. This is a critical step because it appears that simultaneously with the evaporation of the ether, the lipids are oxidized yielding fragments which can be registered by the chromatograph.

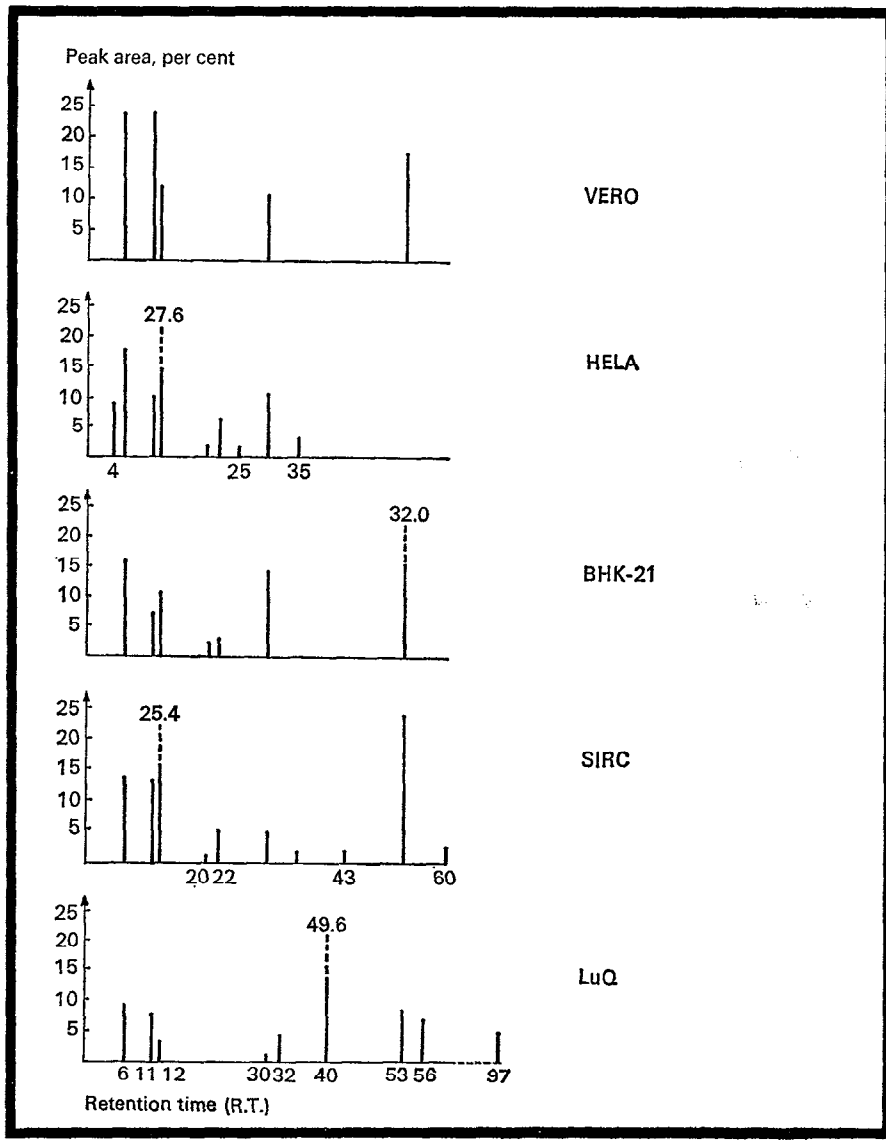
The samples prepared from 5 different cell lines have been studied (see chart 4A.2). Some differences were registered, for example, metabolites with R.T 32, 40, 56 and 97 minutes are specific for diploid lung cells and 35, 42 and 60 minutes for SIRC cells.

The treatment of the samples with oxygen is convenient, because of its speed and simplicity, but the oxidation is difficult to control and this limits the reproduceability of the method. In fact, 3–4 of 10 samples gave negative results.

Preparation of fatty acid methyl esters

Five ml tissue culture fluid containing 2.5 million Vero cells (cells destroyed by virus or by freezing-thawing cycles in maintenance medium) were extracted with a chloroform-ether mixture (2 : 1 v/v). After evaporation of the chloroform-ether extract under nitrogen (not to dryness) 3 ml 10 per cent KOH in methanol was added to the residue. The sample was then heated for 30 minutes at 60°C and cooled. The mixture was then extracted with petroleum ether and this was transferred to another tube. The remaining KOH-methanol lipid phase was then acidified to pH 2.0 with 5NHCl and extracted with diethyl

Chart 4A.2. Schematic representation of peak area—lipid compounds—registered in samples from 5 cell lines



ether, which was then added to the tube with petrol ether extract. The petrol ether and the diethyl ether were then removed by a stream of nitrogen. To the remaining substance was added 0.3 ml of diethyl ether, 0.1 ml of methanol and 0.2 ml of diazomethane. After 10 minutes at room temperature the solvents were removed by a stream of nitrogen until 0.1 ml were left. Three μl of this solution were injected into the gas chromatograph.

The samples studied by this method show a satisfactory esterification of

Chart 4A.3. Chromatogram of lipids prepared from the tissue culture fluid of Vero cells (the components were identified from mass spectra only)

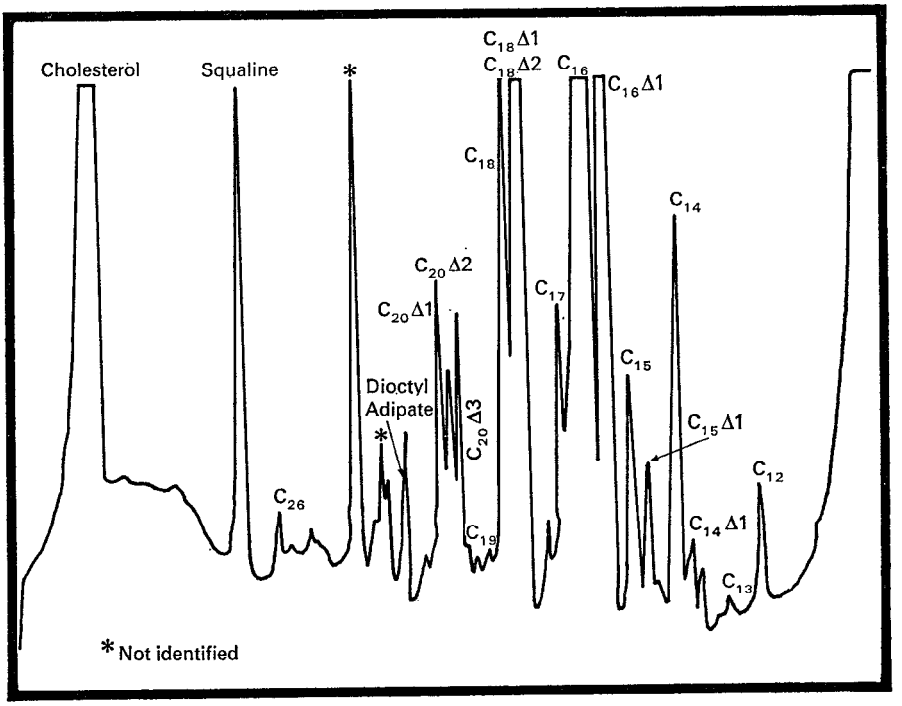


Chart 4A.4. Chromatogram of lipids prepared from the tissue culture fluid of Vero cells

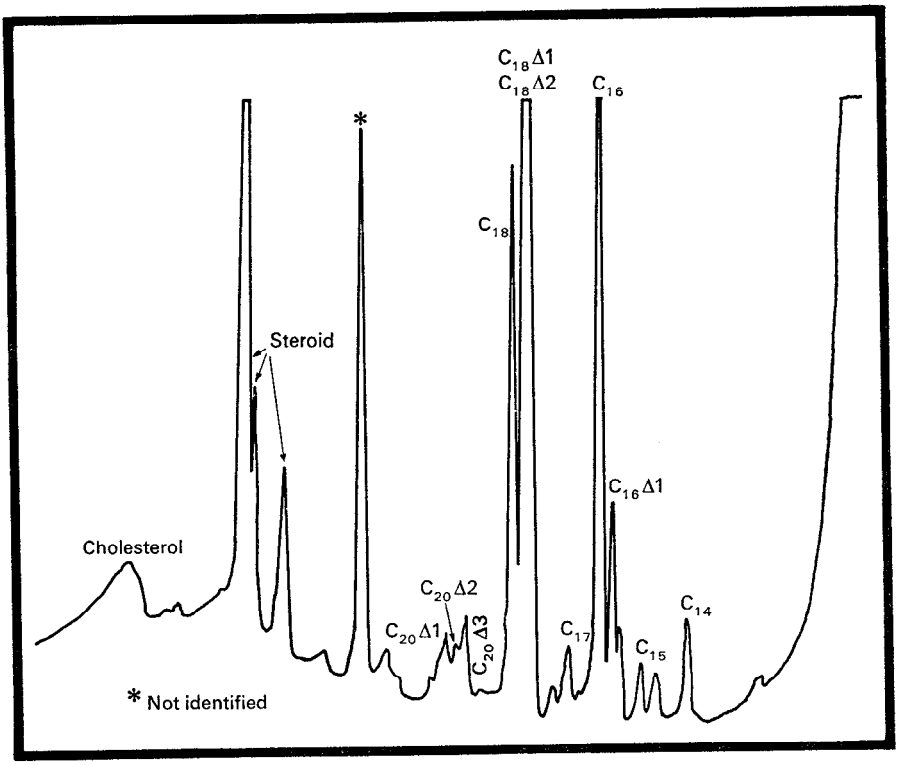
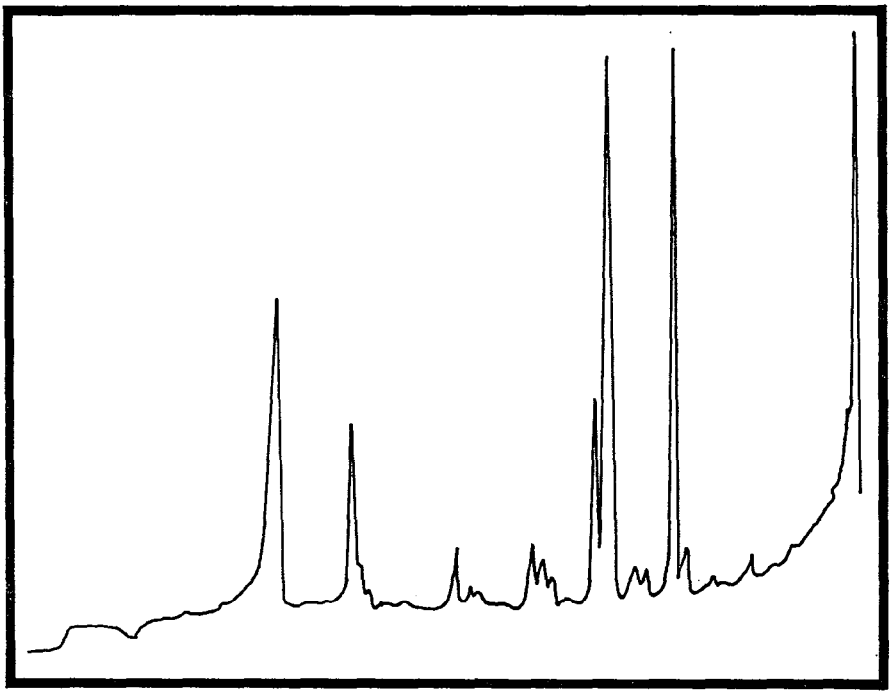


Chart 4A.5. Chromatogram of lipids prepared from the tissue culture fluid of Vero cells[a]



[a] The sample was esterificated directly with 1 per cent H_2SO_4 at 90°C for 10 minutes.

Chart 4A.6. Chromatogram of lipids prepared from the tissue culture fluid of Vero cells

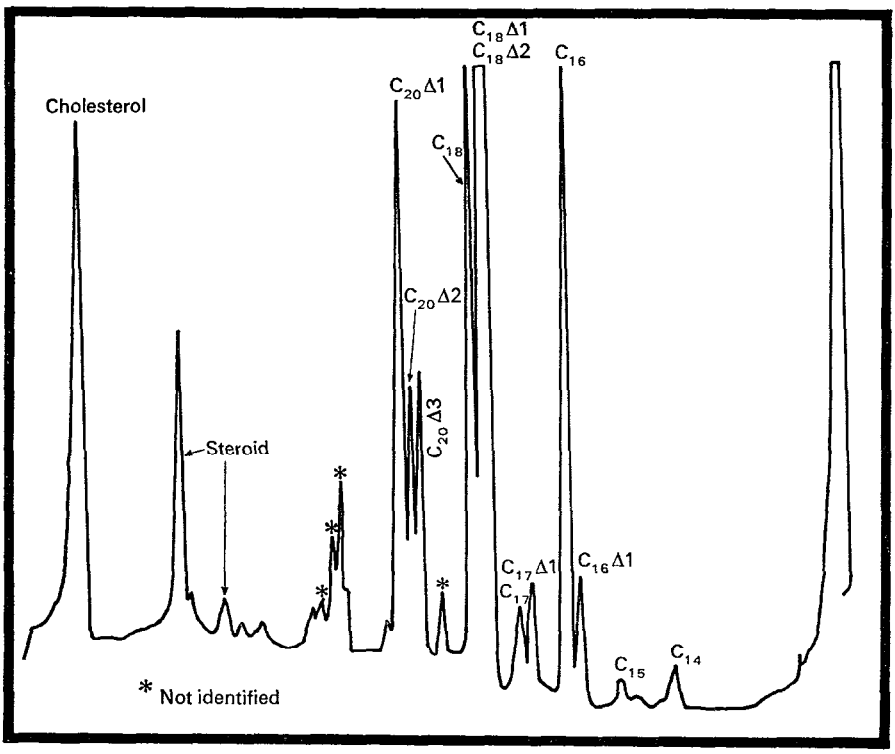


Table 4A.1. Peak area registered in samples of tissue culture fluid from Vero cells inoculated with Echo-8 virus and the corresponding controls

Retention time (min)	Days after inoculation 1		2		3		4	
	C[a]	I[b]	C	I	C	I	C	I
10.5	2.4	1.2	3.4	1.7	1.7	1.7	2.2	3.0
13	1.1	0.3	1.6	0.9	0.5	0.7	0.9	1.5
14	1.7	0.8	2.5	1.5	1.4	0.9	1.2	2.0
16.5	6.9	8.7	6.6	3.5	1.2	2.5	6.8	9.8
17.5	11.6	12.1	12.1	13.4	12.2	11.6	15.2	13.5
20	2.1	1.0	1.8	1.2	0.9	0.7	1.1	1.6
21	0.7	0.7	0.8	0.2	0.2	0.2	0.1	0.4
23	25.6	27.3	19.9	25.8	25.6	27.2	28.5	27.5
24	8.5	12.0	10.9	9.6	8.8	10.2	8.6	9.0
26.5	0.3	0.3	0.1	0.2	0.2	0.2	0.1	0.3
27.5	1.8	1.9	2.5	1.5	0.7	0.2	0.1	0.3
28.5	1.3	1.2	1.6	1.2	0.2	0.2	0.1	2.0
29	3.1	2.4	2.9	1.7	0.5	0.7	0.4	1.8
30	0.5	0.3	0.5	0.2	0.2	0.2	0.1	0.1
40.5	1.7	1.9	5.5	4.0	3.8	2.4	2.2	4.2
43	0.1	0.2	0.1	1.6	0.7	0.5	0.1	6.8
44.5	29.9	27.7	27.1	28.6	41.1	39.9	32.3	16.2
46.5	0.7		0.1	3.2				

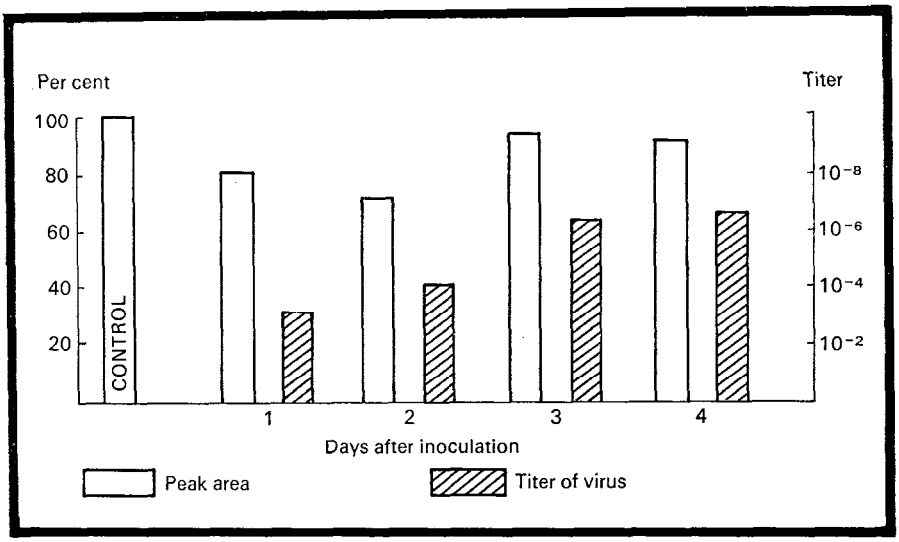
[a] C = control.
[b] I = infected.

Table 4A.2. Peak area registered in samples of tissue culture fluid from Vero cell inoculated with *Rubella* virus and the corresponding controls

Retention time (min)	Days after inoculation 1		2		3		4		6	
	C[a]	I[b]	C	I	C	I	C	I	C	I
10.5	2.4	1.3	2.8	2.8	2.8	2.5	2.0	2.0	4.3	5.8
13	0.4	0.2	0.3	0.3	2.0	2.0	1.3	1.8	1.9	1.8
14	1.4	0.9	0.2	0.6						
16.5	3.7	1.3	2.9	5.5	2.3	4.7	2.6	2.0	2.7	3.1
17.5	16.3	18.6	14.9	17.0	17.2	19.7	14.4	13.3	23.6	20.3
20	0.8	1.1	1.0	1.0	0.8	1.1	0.9	0.8	0.8	0.6
21	0.4	0.7	0.9	0.8	0.6	0.8	0.4	0.6	0.9	0.9
23	23.3	23.2	22.9	21.9	30.1	29.1	23.7	22.5	31.7	27.5
24	9.2	12.3	10.1	11.1	12.7	11.4	8.3	8.0	13.2	13.5
27.5	0.2	0.4	0.3	0.5	0.3	0.3	0.4	0.4	0.4	0.3
28.5	0.3	0.2	0.9	0.8	0.6	0.5	1.5	1.2	1.5	0.9
29	2.0	3.0	3.5	3.3	3.4	4.1	3.7	3.3	3.5	3.1
40.5	4.5	3.9	6.2	4.1	4.2	1.1	4.6	1.4	0.4	0.3
43	5.9	0.9	9.7	5.3	3.9	1.4	5.0	4.1	0.3	0.3
44.5	29.2	32.0	23.4	25.0	19.7	21.3	31.2	38.6	14.6	21.6

[a] C = control.
[b] I = infected.

Chart 4A.7. Titer of virus and total peak area in tissue culture fluid from Vero cells control and inoculated by ECHO-8 virus



lipid components, yielding distinct separations on the chromatogram (see chart 4A.3). The study of 19 samples has also illustrated the reproduceability of the method. However, the comparison of the results from samples prepared from infected and control material did not reveal any specific, constantly reproduceable differences. In a sense this is fortunate; since the esterification

Chart 4A.8. Titer of virus and total peak area in tissue culture fluid from Vero cells control and inoculated by *Rubella* virus

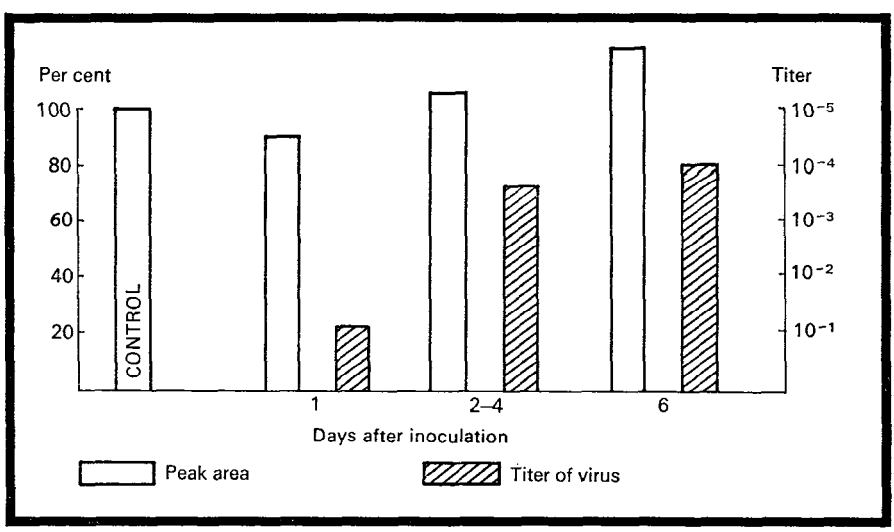
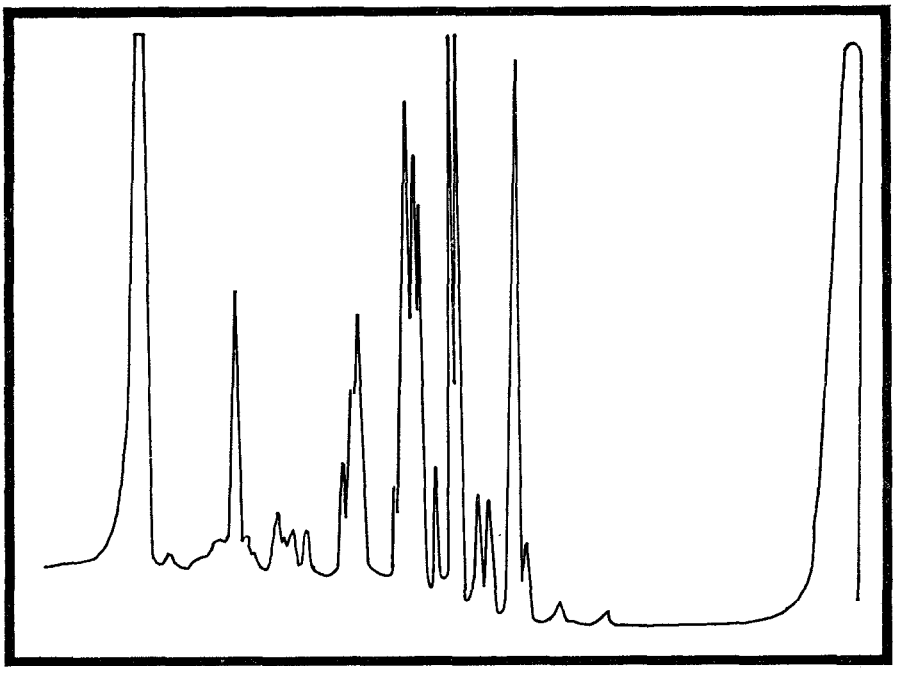


Chart 4A.9. Chromatogram of lipids prepared from the tissue culture of Vero cells



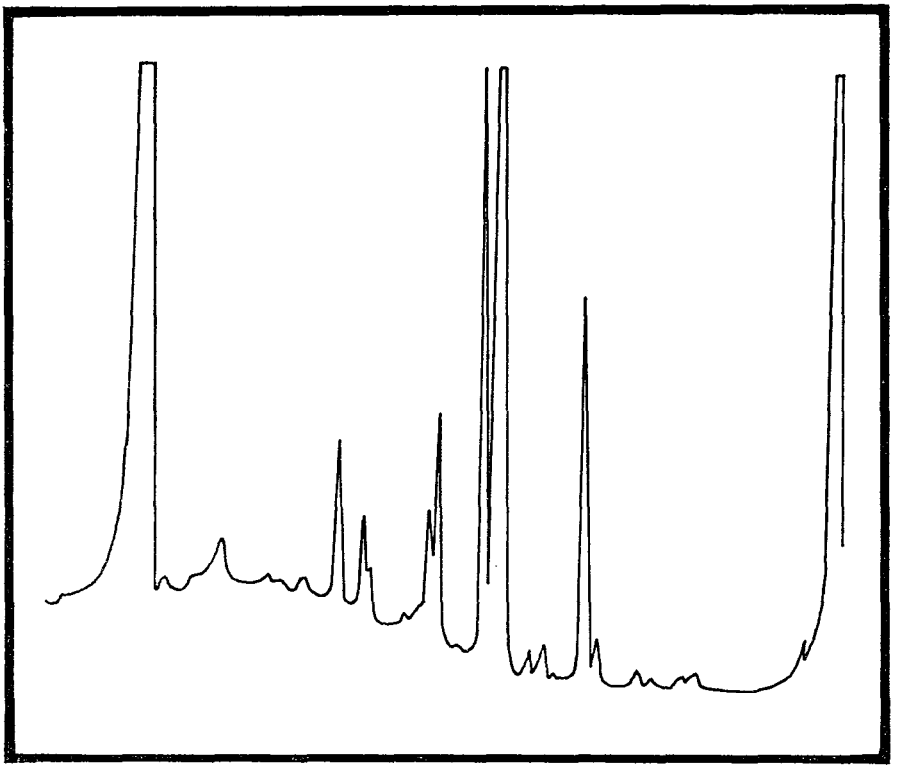
takes a long time, the repeated changes of glassware can lead to losses of material and also because diazomethane is a potentially dangerous chemical. For these reasons the method was discarded.

Direct hydrolysis of lipids with sulphuric acid

With small modifications the method described by Brian and Gardner, [183] was used. Five ml of tissue culture fluid containing about 1 million Vero cells were extracted twice with a mixture of chloroform and diethyl ether (2:1 v/v). The extract was evaporated under a stream of nitrogen (not to dryness). Five ml 1 per cent H_2SO_4 in methanol were then added. The esterification was completed by heating for 30 minutes at 70°C and this was followed by the addition of 3 ml distilled water. After cooling the sample, the fatty acids were extracted twice with a 5 and 2 ml mixture of hexan-ether (1:1 v/v). The extract was finally concentrated not quite to dryness by a stream of nitrogen. To the remainder was added a 0.1 ml mixture of hexane-ether (1:1 v/v) and 5 μl was then injected into the gas chromatograph.

A GC-MS study of samples where the lipids were esterificated directly with 1 per cent H_2SO_4 at 70°C for 30 minutes showed a distinct separation of 17

Chart 4A.10. Chromatogram of lipids prepared from the Eagle's medium with 2 per cent calf serum



fatty acids as well as steroids and cholesterol (see chart 4A.4). The method is reproduceable and it is convenient with regard to both speed and the simplicity of preparing samples. If necessary the required time for esterification can in fact be shortened. The same spectrum of fatty acids and steroids can, for instance, be obtained when the lipids are heated with 1 per cent H_2SO_4 at 90°C for only 10 minutes (see chart 4A.5). When the tissue culture fluid was treated twice with a mixture of chloroform and methanol (2:1 v/v) and a third time with hexane, the lipids, in particular the phospholipids, were extracted more completely, and the chromatogram showed a higher concentration of fatty acids, steroids and cholesterol (see chart 4A.6). But in this case the extracts must be hydrolyzed longer, for at least 1 hour.

IV. *Changes caused in Vero cell line by viruses*

A large number of samples prepared from Vero cells inoculated with Echo, *Rubella* viruses as well as the maintenance medium itself (Eagle's with 2 per

Table 4A.3. Fatty acids, steroids and cholesterol in different cell lines

Retention time (min)	Peak area in Vero (per cent)	Compounds identified by gas chromatograph/masspectrometer	Peak area with retention as in Vero (per cent)		
			HeLa	BHK-21	LnQ
10.5	*0.3*	Fatty acid methyl ester C_{14}	*1.2*	*1.0*	*0.5*
13	*0.1*	Fatty acid methyl ester C_{15} :1	*0.4*	*0.3*	*0.2*
14	*0.5*	Fatty acid methyl ester C_{15}	*1.9*	*0.9*	*0.8*
16.5	*1.4*	Fatty acid methyl ester C_{16} :1	*4.7*	*7.3*	*3.6*
17.5	*9.8*	Fatty acid methyl ester C_{16}	*11.2*	*11.8*	*10.2*
20	*1.5*	Fatty acid methyl ester C_{17}	*2.0*	*3.0*	*1.9*
21	*1.6*	Fatty acid methyl ester C_{17}	*1.6*	*3.4*	*2.2*
23	*8.0*	Fatty acid methyl ester C_{18}: 2:1	*22.5*	*19.7*	*18.9*
24	*8.5*	Fatty acid methyl ester C_{18}	*10.1*	*10.7*	*14.0*
26	*1.4*	Not identified	*0.5*	*0.2*	*0.2*
27	*4.7*	Fatty acid methyl ester C_{20} : 4, C_{20} : 3	*7.1*	*6.7*	*11.0*
27.5	*6.2*	Fatty acid methyl ester C_{20} : 3, C_{20} : 2	*4.1*	*2.5*	*2.8*
28.5	*8.7*	Fatty acid methyl ester C_{20} : 2, C_{20} : 1	*2.4*	*0.9*	*1.1*
29	*1.4*	Fatty acid methyl ester C_{20} : 1, C_{20} : 0	*0.3*	*0.3*	*0.2*
32	*5.2*	Not identified	*1.1*	*1.4*	*8.2*
32.5	*2.4*	Not identified	*3.9*	*1.7*	
34.5	*1.1*	Not identified	*0.3*	*0.1*	*1.3*
36	*0.8*	Not identified	*0.4*	*0.3*	*0.5*
40.5	*1.4*	Steroid	*0.5*	*0.9*	*0.3*
43	*0.8*	Steroid	*1.4*	*2.5*	
44.5	*5.2*	Steroid	*13.0*	*17.8*	*4.9*
50	*29.0*	Cholesterol	*9.0*	*6.5*	*17.5*

Chart 4A.11. Chromatograms of C_{20} fatty acids and nonidentified compounds in HeLa, Vero, BHK-21 and LuQ cells

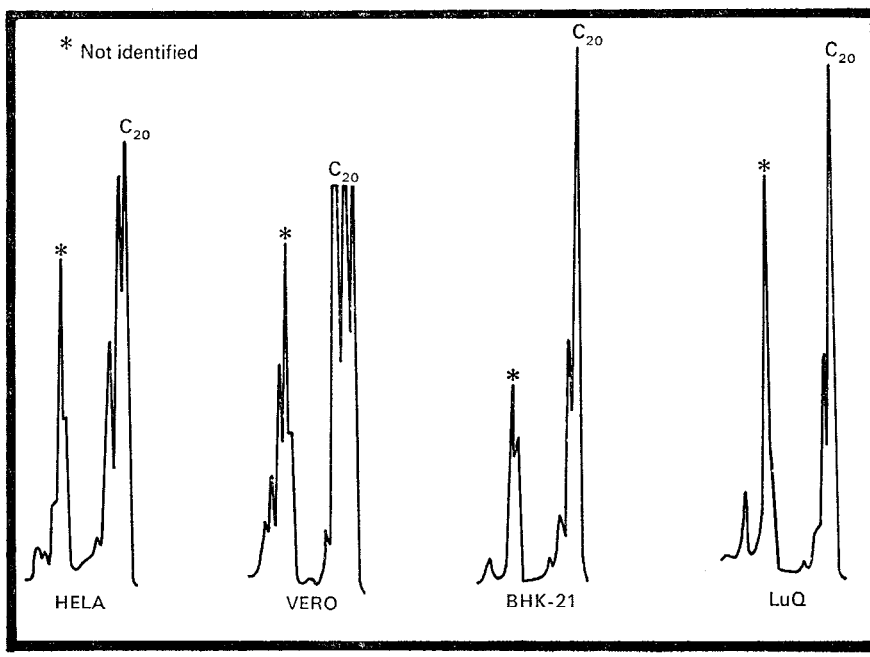


Chart 4A.12. Chromatogram of lipids prepared from the live polio-virus vaccine

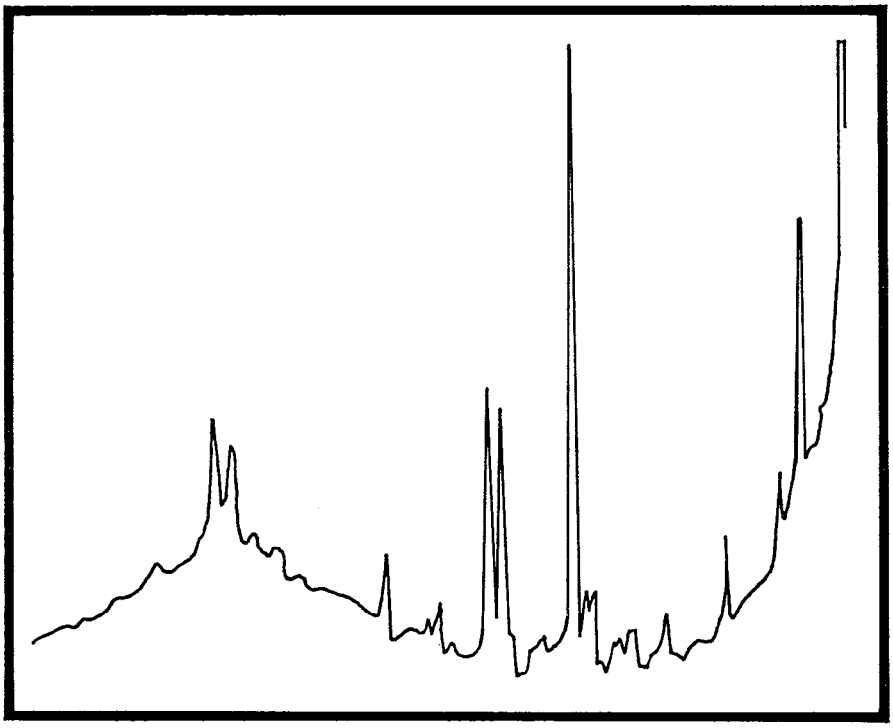


Chart 4A.13. Chromatogram of lipids prepared from yellow fever vaccine

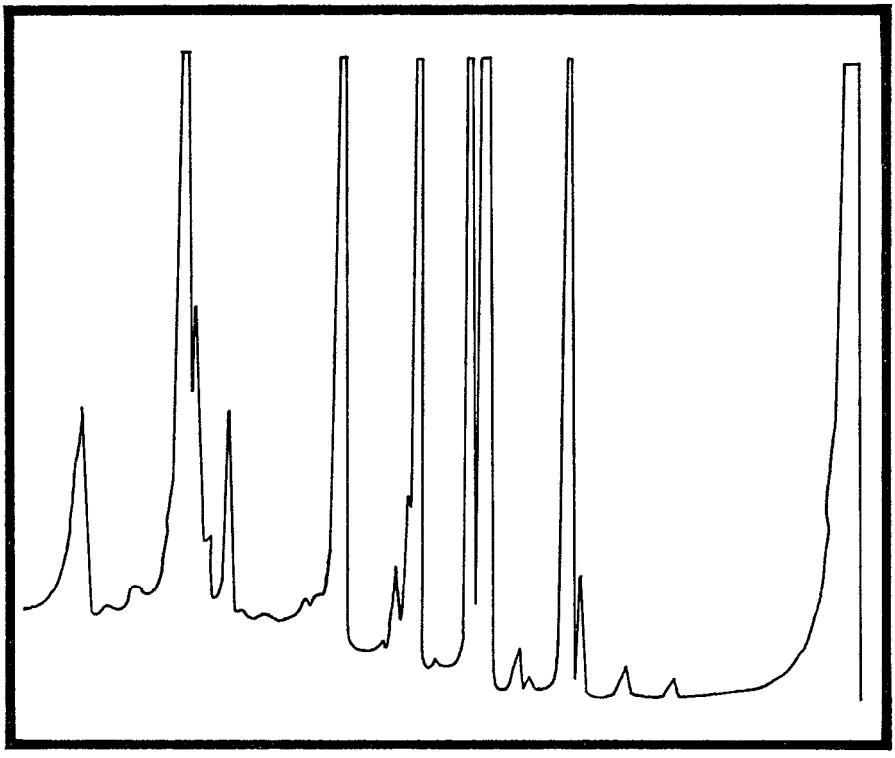
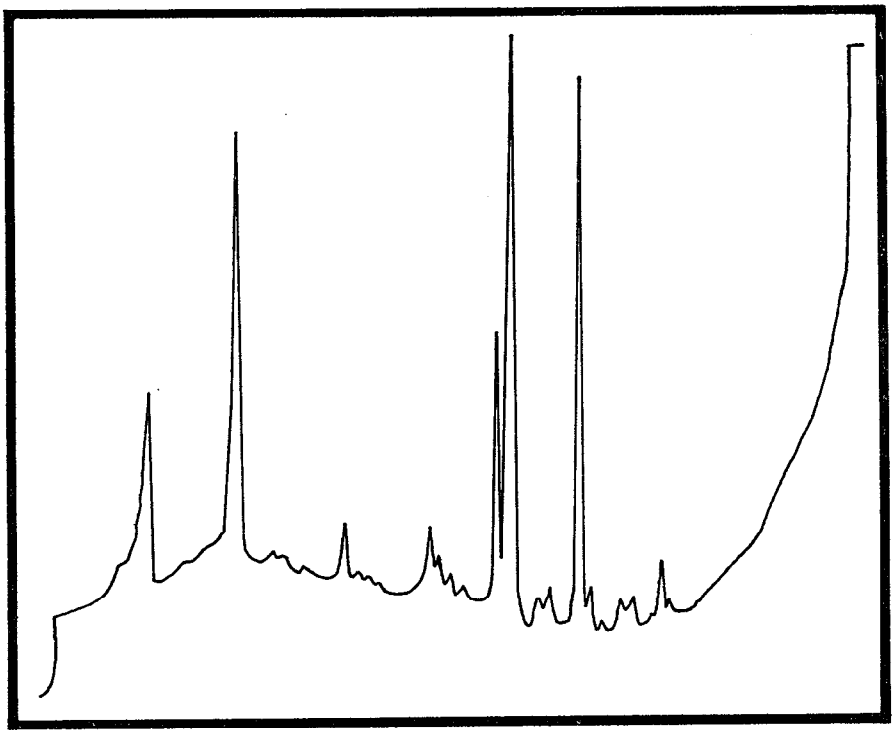


Chart 4A.14. Chromatogram of lipids prepared from 0.25 ml of the tissue culture fluid of Vero cells



cent calf serum) have been studied. Table 4A.1 illustrates the reproduceable presence of fatty acids, steroids and cholesterol as well as of unidentified substances present in small quantities. The peak area of the same fatty acid in samples prepared from the control and the infected material of different age is rather similar. The insignificant fluctuations can be attributed to errors in the method. Also no new peaks distinguishing infected from non-infected cells appear, irrespective of whether the virus is lipid-containing, (Echo-8) or not. Table 4A.2 illustrates the case of *Rubella* which belongs to the second category. However, if the normal background, in the form of medium components, is subtracted from the peak areas obtained for the tissue culture fluid from cells with infection at different levels of development, some differences are noted. As illustrated by chart 4A.7, the tissue culture fluid from Vero cells inoculated with Echo-8 virus, for instance, yields smaller quantities of fatty acids than the controls. In Vero cells inoculated with *Rubella* virus, on the other hand, the fatty acids are low on the first day but then increase (chart 4A.8). On the sixth day after inoculation, the highest titer of virus and the most pronounced degeneration of cells were observed. The most conspicuous differences in the fatty acids from the control and infected material were also noted.

In the course of the study it became evident that the spectrum of fatty acids, steroids and cholesterol is quite specific for the tissue culture (see chart 4A.9). The registration of fatty acids in the same cells destroyed by infection or freezing-thawing in the maintenance medium, chart 4A.9, (Eagle's medium + 2 per cent calf serum) shows no qualitative differences but only some changes in relative quantities, a result which is not astonishing considering the close similarity of the lipid spectra of the two materials (chart 4A.10). The spectrum of fatty acids is analogous for different cells (See table 4A.3). However, detailed analysis of the data of fatty acids indicates some quantitative differences; for instance, with regard to C_{20} fatty acid in Vero, HeLa, BHK-21 and diploid LuQ cells and to the character of the spectrum of C_{20} fatty acids (chart 4A.11). The specificity of the spectrum, the per cent composition and the character of fatty acid C_{20} are quite reproduceably detected. The typical spectrum of fatty acids for tissue culture fluid can even be found in purified commercial vaccines (see charts 4A.12 and 4A.13). If the samples are carefully prepared, a satisfactory spectrum can be obtained from 0.25 ml and smaller quantities of tissue culture fluid (chart 4A.14).

V. *Conclusions*

The sensitivity of the gas chromatograph method to study microbial metabolites was investigated in detail by Mitruca, et al. [182] They showed that the instrument could record some components (acetoin) on a picogram level.

The results presented in this paper also demonstrated a high sensitivity of the GC equipped with flame ionization detector for lipid compounds, especially for their products of hydrolysis, the fatty acids. These can be prepared in different ways, but direct esterification with 1 per cent H_2SO_4 in methanol is preferable because of its speed and simplicity. The method is reproduceable and by using the combined GC-MS instrument it was possible to separate, register and identify 17 fatty acids, steroids and cholesterol. Samples prepared from 4 cell lines and Vero cells on different days after inoculation by Echo-8 and *Rubella* viruses have been studied. The results obtained show that the GC-MS method does not demonstrate any new constantly reproduceable component in infected cells. However, some differences in samples prepared from control and infected tissue culture fluids were noted. Their value for identification purposes, however, probably rests on the possibility to integrate the differences with data on changes in carbohydrate and protein compounds. The results showed a high specificity in the spectrum of the hydrolysis products of the lipids extracted from cells and from tissue culture fluid. There are strong indications

that the spectrum of fatty acids can be used as a sign for detection of the tissue culture fluid associated with virus development.

The results show that some data in the spectrum of fatty acids is specific for definite cells. This is very important at least for preliminary identification of cells. If it is taken into account that the method does not require living cells, that results can be obtained after 40–50 minutes, and that it simplifies the preparation and study of samples, it must be concluded that the approach opens attractive possibilities as an adjunct to fluorescent antibody techniques used for rapid detection of virus aerosols.

Acknowledgements

We are grateful to Miss U. Darenius, Mrs Söderholm, Messrs R. Hjälm and D. Gooch for skilful technical assistance and to Miss S. Ericson and Mrs A. Israelson for their kind help in supplying needed tissue cultures.

Appendix 5. Background papers to a SIPRI symposium on possible techniques for inspection of production of organophosphorus compounds

List of participants and titles of background papers

Z. Binenfeld, Yugoslavia: Some thoughts on the problem of national and international control regarding organophosphorus compounds.

J. G. Kammüller, The Netherlands: Safety aspects in the production and formulation of pesticides of high mammalian toxicity.

S. J. Lundin (convener of symposium), Sweden: The inhibition of cholinesterase activity by organophosphorus compounds as a means in an inspection procedure.

N. N. Melnikov, Soviet Union: Future consumption patterns of elemental phosphorus in the USSR.

J. K. Miettinen, Finland: A mobile laboratory for verification of alleged manufacture of chemical warfare agents.

J. Moravec, Czechoslovakia: Some aspects of the problem of possible convertibility of organophosphorus plants.

N. V. Novikov, Soviet Union.

A. J. J. Ooms, The Netherlands: On the possibility to delimitate nerve gases within the field of organophosphorus compounds.

T. Osa (not present) and Y. Fukushima, Japan: Some aspects of organophosphorus industry in Japan.

J. P. Perry Robinson, United Kingdom.

A. R. Pittaway, United States: Production of organophosphorus chemical warfare agents.

I. Pochitalin, Soviet Union.

O. A. Reutov and N. N. Melnikov, Soviet Union: On the problem of the verification of the production of chemical weapons.

J. G. Riess, France: Some aspects of phosphorus chemistry related to the preparation and transformation of toxic esters.

R. E. Roberts, United States: Economic data monitoring for the production of organophosphorus compounds.

H. Scoville, Jr., United States: The objectives of inspection in a limitation on the production of CW agents.

T. Urbanski, Poland: Phosphorus pesticides—suggested principles for the control of their production.

R. Vilceanu, Romania: New developments in the chemistry of organophosphorus pesticides.

K. H. Vöpel, Federal Republic of Germany: The production of organophosphorus insecticides in the Federal Republic of Germany, and its control by government authorities and the Western European Union.

J. Vopršál, Czechoslovakia: Technology and possible control.

I. *Some thoughts on the problem of national and international control regarding organophosphorus compounds, by Z. Binenfeld*

Summary

An important aspect of the prohibition of chemical weapons is the national and international means of control or verification.

A commission comprising a few experts elected by the signatories to a treaty, re-elected every two years, should function under the disarmament committee. The commission could establish groups of experts, also approved by the treaty signatories, for solving specific problems. This group should recommend a decision in cases where other national and international safeguards have failed to solve the violation of the treaty. On-site inspection organized by the commission should be approved by the state possessing the object or territory to be investigated.

The organophosphorus products utilized as warfare agents can be identified as final products, intermediates and raw materials by different physico-chemical and biochemical methods.

The analysis of organophosphorus compounds based on the most modern detection methods, can provide the necessary qualitative and quantitative verification data, with one major limitation—the impossibility of maintaining long-distance supervision.

The questions concerning which types of organophosphorus compounds should be put under control and which raw material and intermediates should be controlled must be answered before a verification system can be proposed.

On the whole, some progress has been made during recent years in studying the problem of chemical weapons.

A considerable number of states have adhered to the 1925 Geneva Protocol, which in fact is evidence of the growing confidence in this international body and also demonstrates that the prohibition of chemical weapons has been accepted by the large majority of states, constituting a permanent element in international relations and a generally recognized rule of international law. It is evident, nevertheless, that its full meaning depends to a large extent on the future development of the situation in this connection.

The United Nations General Assembly resolution UNGA 2662 (XXV) (CCD/318) contains a recommendation which builds a solid and concrete basis for the proceeding of negotiations:

Requests the Conference of the Committee on Disarmament to continue its consideration of the problem of chemical and bacteriological (biological) methods of warfare, with a view to prohibiting urgently the development, production and stockpiling of those weapons and to their elimination from the arsenals of all States.

We would like to point out the fact that the UN General Assembly resolution cannot in any way be interpreted as meaning that the prohibition of the development, production and stockpiling of chemical weapons should be adjourned. Only real efforts, continuous and concrete, aimed at reaching tangible results could inspire confidence.

The prohibition of chemical weapons should first affect the development, production and stockpiling of chemical weapons, the prohibition of chemical weapons for war purposes, and finally, the prohibition of auxiliary equipment and vectors.

The next fundamental element following a prohibition, a logical consequence of the preceding, should be the compulsory destruction of all chemical weapon stockpiles. Further, there should follow the obligation to destroy or transform for peaceful purposes all auxiliary equipment and vectors, and all installations having been used for the production of chemical weapons. States should also be obliged to disband any military forces or other organizations intending to use chemical weapons and to undertake not to create new ones, nor to train any other forces, military, paramilitary or otherwise, in the use of chemical weapons.

To avoid misunderstanding, each treaty should contain appropriate definitions of the nature of chemical weapons and agents for war purposes. It seems to us that the definition proposed by Dr Lundin at the SIPRI Symposium on Chemical Weapons in Stockholm, 1968, very well fulfils this.

One of the important aspects of the prohibition of chemical weapons would be the exceptions, which should be clearly defined. These exceptions should apply to research, development, production and possession of chemical agents in quantities not exceeding scientific, prophylactic and protective needs, and other peaceful uses, as well as equipment necessary to implement these safeguards.

An important point in the prohibition of chemical weapons is the national and international means of control or verification. These safeguards can be divided into two groups: (*a*) legislative national means of renunciation and self-supervision by each country, and (*b*) means of international supervision.

The first obligation of every state, signatory to a treaty, would most probably be to put under civilian administration or civilian supervision (the Ministry

of Health or similar institutions), all installations for the development, production and stockpiling of chemical agents which could be put, either directly or after transformation, to purposes of war.

The aim of such a safeguard is to guarantee that at the coming into force of the prohibition, no installation mentioned in the protocol should be under military supervision. It should therefore become more difficult to manufacture secretly chemical weapons and chemical agents.

In the civilian sector secrecy cannot be ensured as strictly as in the military sector, and in one sense, supervision by the population of that particular country would be necessary.

The next measure could be the obligation of signatory states to publish all data relevant to weapons, agents, auxiliary equipment and vectors which should be destroyed or transformed, as well as the installations on territory under their jurisdiction and which are used or could be used for research, development, production and stockpiling of chemical agents, as defined in the protocol, as well as in the trial areas. The question of knowing which data should be published should be agreed upon beforehand.

Another national safeguard should be the obligation to present to an appropriate international body periodical reports on the nature and quantity of chemical weapons which should be allowed because of their civilian purposes, but which should anyway be supervised because of the danger of their being misused. These data should also include import and export statistics.

All these safeguards could be started through national legislation, which would help to create an atmosphere of confidence between the signatory states.

Concerning the international verification safeguards, it should be a duty for each state to destroy or transform, under the supervision of an international commission, all weapons, agents, military equipment and vectors, as well as factories or other complex installations, which should be destroyed or transformed for peaceful purposes.

It is important that a permanent commission of experts in the necessary fields should sit under the disarmament committee. Their election should be approved by the signatories of the convention and their task would consist of being kept up to date of all events connected with the implementation of the prohibition and adaptation of toxic war chemicals and to inform the committee. The members of this commission could be re-elected every two years.

Such a commission should have a relatively limited membership because it should not check details; its work should instead devolve on large quantities of official statistics published by the host country which should organize and supervise the implementation of the obligation to destroy or transform. In its work the commission would not only act on request, but also on approved suspicion of violation of the treaty.

The commission should have the power to request from the signatory powers all relevant technical and economic data which would render supervision more efficient.

Considering the possibilities of economic supervision of raw materials, intermediates and final production on the one hand, and detection and analysis on the other, it seems improbable that a country would supply false information by avoiding the fulfilment of the obligations it had undertaken.

Each on-site inspection should only be made on request by the committee and should be approved by the state possessing the object or territory to be investigated.

The invitation to an on-site inspection should not necessarily imply a formal obligation. If it had the character of a political responsibility of the states, it would exert great pressure on the states because the consequences deriving from not giving satisfactory replies would, in case of suspicion, always take a serious political turn.

In this connection, it would be appropriate for the elected international body to carry out the enquiry so as to avoid unnecessary political incidents.

For the solution of specific problems one could create groups of experts also approved by the treaty signatories. Their task would be complete when the defined problems were solved.

On the whole, in the verification system, the analytical group would represent only one of the groups working in the body supervising the enforcing of the treaty. Its duty would be to make decisions in cases where other national and international safeguards have failed to solve the violation of the treaty.

The *sine qua non* condition of a successful mission of this group is the independence of its task. This group should own a central analytical laboratory fully equipped with the most modern apparatus and mobile laboratories with their own transport facilities (ground, sea and air). In this way the two main prerequisites for performing a task successfully are accomplished; accuracy and speed.

This group must have complete freedom while testing. In carrying out its investigations, it must be able to make all the necessary enquiries; to check completely the area or object; to question the inhabitants, and to take samples of the object (apparatus, chemicals, storage, clothes, etc.), of the terrain (vegetation, sewage, earth, water, etc.), and of the inhabitants (blood, urine, clothes, etc.). The preliminary analyses should be made in the mobile laboratories and the final tests in the central laboratory.

The group should choose its own methods of analysis, because the problem is too delicate to be solved by general rules. This would, moreover, prevent the use of other means and substances to deceive a fixed analytical method.

The analysis of organophosphorus war poisons, based on physico-chemical

apparatus, with the physically, chemically and biologically most modern detection methods, can provide us with the necessary qualitative and quantitative data (contents and characteristics) to identify a component or a mixture. Moreover, in most cases it is possible to obtain information concerning intermediates or raw materials.

To our knowledge, the main limitation of all recent methods of analysis or detection was the impossibility, up to now, of maintaining long-distance supervision, that is, without coming into direct or close contact with the area or object to be checked. Even in the near future, we do not foresee a practical solution to this problem, even if the development of long-range technology (satellites, laser, etc.) is taken into consideration. But one cannot exclude the fact that the problem could soon be solved in a satisfactory way which would render it more simple.

Our present knowledge about the analysis and detection possibilities of the organophosphorus warfare agents proves that:

1. Detection which cannot reach the object or the area to be checked is obviously inefficient.

2. Given that the commission of experts has the means of reaching the object or the territory to be controlled, it would be extremely difficult, if not impossible to conceal the fact that fixed organophosphorus compounds had been used.

The organophosphorus products utilized as warfare agents can be identified as follows: (1) finished products (2) intermediates and (3) raw materials.

1. Tabun, sarin, soman, CHFP and the VX agents represent the finished products. They consist of derivatives of phosphonic acid distinguished by the alcoxy or alkyl group or by the acid part of the molecule. Apart from these, we also consider derivatives of phosphoric or phosphonic acids which possess, in the acid part of the molecule, the w.w. dithioethyl (or derivates) group or the p-nitrophenyl group. By adding the fluorophosphorylcholines to these compounds, one obtains almost the complete range of possible organophosphorus war poisons.

The best method of analysing these compounds would be a combination of physico-chemical and biochemical methods. The measure of the inhibition of cholinesterase *in vitro* or *in vivo* aiming at the per cent of the inhibition in relation to the quantity of the poison followed by the oxyme reactivation could offer qualitative data as well as preliminary quantitative results.

One should obtain clear results by following up the analysis with UV & IR spectrophotometric methods as well as fluorometry.

The ultimate analysis should be made with improved gas chromatography methods combined with mass spectrography enabling the identification of each organophosphorus compound present.

2. As intermediates in the synthesis of the named compounds could be in-

cluded all organophosphorus products synthetized by different means, which, reacting with the acid part of the organophosphorus war gases, produce the corresponding warfare agent. These include the alkyl derivatives of phosphoric or phosphonic acid, of thiophosphoric or thiophosphonic acid. Other compounds which through different transformations produce products able to serve as intermediates in these syntheses, for example, dimethylphosphite, methylphosphonic dichloride or difluoride, etc., are also intermediates.

These compounds can be identified through UV & IR spectrophotometry, gas or thin-layer chromatography and NMR. The functional groups analysis represents the basis of the analysis of these intermediates.

3. The raw materials concerned are yellow phosporus, phosphorus trichloride, oxychloride, pentasulphide and pentachloride, various aliphatic acids, choline, thiocholine, hydrofluoric acid, hydrocyanic acid, p-nitrophenol, dithioethane, dialkylamines, etc.

The methods of identifying all these products are well known and easy.

International exchange of scientific information in the field of chemistry connected with the prohibition could play an important part in the verification system, such as the setting up of an international body, more technical than political, which will assemble and distribute statistical data.

While not diverging from the main aim of this symposium, "Possible techniques for inspection of production of organophosphorus compounds", it appears to us that this conference of experts should give its views on some problems of primary importance for the success of negotiations on the prohibition of the manufacture and use of warfare agents.

In this connection it is extremely important to mention the remarkable work presented three years ago by Messrs Pittaway and Perry Robinson, which is still very up-to-date.

From the military point of view, organophosphorus warfare agents are: tabun, sarin, soman, CHFP and VX agents (it is still unclear whether this refers to a definite thiocholine or its tertiary analogue or another compound of this type).

Does verification only apply to known organophosphorus compounds such as nerve gases, or will it be enlarged so as to include all organophosphorus compounds which, under specific conditions, could act as warfare agents?

We would like to point out to this symposium that by discussing organophosphorus warfare agents, one does not pay enough attention to fluorophosphorylcholines and organophosphorus compounds, the molecule acid part of which is made up of 1.2-dithioethane derivatives. These compounds possess an important toxicity and cannot be excluded from the range of possible warfare agents.

We also wonder what the criteria are, according to which phosphoryl or phos-

phonyl p-nitrophenolates are not to be considered as potential organophosphorus warfare agents.

The toxicity of all these products, the economic problems of synthesis, and their qualities in relation to military demands meet precisely the requirements of a warfare agent.

Instead of listing the organophosphorus compounds by name, one should perhaps list them by type and accordingly specify their destruction capability and the raw and intermediate materials necessary for their production which should be watched and checked.

As well as coming to conclusions concerning the technical problems of verification and considering the large range of possible organophosphorus warfare agents, the symposium should clearly discuss:

(*a*) The possibility and the means that are available of transforming pesticides and other organophosphorus manufacture into production of warfare agents.

(*b*) The types of pesticides and other organophosphorus compounds which should be put under control or even forbidden.

(*c*) The raw materials and intermediates—the overproduction of which could lead to an enquiry—that should be controlled.

Only after discussing these points will it be possible to make a preliminary proposal as to the working of the verification system—possible methods, necessary equipment, etc.—which is the ultimate aim of this symposium.

II. *Safety aspects in the production and formulation of pesticides of high mammalian toxicity, by J. G. Kammüller*

Summary

In a pesticidal product, good quality and performance may be coupled with high acute toxicity to mammals. This is certainly a disadvantage for the product, but is not necessarily a reason for not commercializing it. Many such pesticides belong to the organophosphorus esters.

Their industrial production and formulation into various application forms, as well as their agricultural or public health use, naturally pose a number of special problems not common to other chemical operations.

Today's standards for an industrial operation of this kind are such that there must not be any possibility of an uncontrolled escape of products or intermediates into the environment. These precautions run parallel with adequate safeguarding measures for the operating personnel, who obviously must never be exposed to unacceptable hazards.

There seem to be no essential differences in the safety precautions to be taken whether the products in question are of very high or of only moderate toxicity.

The approach to such ventures must be an integral one. To some extent toxicity hazards can be greatly reduced by selecting a low-risk synthetic route. All possible emergency situations must be considered when the plant is designed and adequate waste-disposal methods and facilities must be provided.

One of the most important contributions for carrying out such hazardous operations comes from the intelligence and devotion of well-trained and highly qualified operating personnel. Very stringent and frequent medical control must be complemented by a whole range of appropriate extra precautions in the area of personal and plant hygiene.

In summary, in the production of a highly toxic product the aim has always to be a fully or at least maximally contained operation. Where this aim cannot be fulfilled, because of unacceptably high costs or simply for technical reasons, then such a venture should not be launched.

Introduction

There are few chemical industrial operations where safety in a broad sense of the word is of such great importance as in the production and formulation of pesticides of high mammalian toxicity. These are mostly but not necessarily insecticides and often belong to the group of organophosphorus compounds.

A great deal of research and development is being done by industry and governments in order to meet the demand for newer and more sophisticated crop-protection products and methods without which sufficient agricultural production of food and fibres for the ever growing world population could not be guaranteed, nor public health maintained in many areas. This effort is not directed exclusively to finding new active compounds and new modes of action, but also to finding novel application methods for already established agents, which often necessitates the introduction of new formulation techniques and recipes.

Many of the desired performance and quality requirements of pesticides are controversial. For example, the often-heard aim of ideal selectivity, that is, toxicity only towards a target pest and inactivity against useful organisms of any kind, can hardly be expected on purely biological grounds; it is moreover likely to be impractical from the users' point of view, quite apart from economic considerations. Moreover, to be economical, a product should have reasonable persistance to provide sufficiently long protection and it should have a reasonably broad spectrum of activity. It should also be cheap per unit weight and/or it should be highly active in order to achieve effect with only minute quantities per unit surface. There should be no potentially harmful residues of the active substance itself or its breakdown products in the crop, in the soil, in ground water, or in the air.

There is, in reality, no perfect selectivity and sometimes, unfortunately, high pesticidal activity, with consequent low dosage rate, and short persistence

are coupled with high mammalian toxicity. This does not in itself need to be a reason for discarding a product if such a product possesses highly desirable properties and performance in other respects.

Many of these are insecticides of the organophosphorus type though there are also certain newer highly toxic insecticides of as yet little commercial importance, which do not contain phosphorus. On the other hand, there are also organophosphorus insecticides of remarkably low mammalian toxicity.

The industrial production of such products, their formulation into emulsifiable concentrates, wettable powders, granules or newer application forms, as well as their agricultural or public health use, naturally pose a number of special problems, some of which are further discussed in this paper.

It may be stated straightaway that in the production of toxic substances on a scale typical for modern pesticides, varying from a few hundred to some thousands of tons per year, there is no greater or essential difference in the safety precautions to be taken, whether the products in question are of very high or of only moderate toxicity.

Today's standards for an industrial operation of this kind are such that there must not be any possibility of an uncontrolled escape of products or intermediates into the environment, be it water or air. Adherence to this condition is far from easy to achieve and is certainly extremely costly. The precautions for the protection of the environment naturally run parallel with adequate safeguard measures for the operating personnel who obviously must never be exposed to unacceptable hazards.

Process considerations

When talking about processes it is useful to distinguish between the synthesis of intermediates, the so-called toxification reactions, and the formulation and blending operations.

There are often several possible synthetic routes and a great deal of thought must always be devoted to the making of a proper choice. Obviously economics are important, as are availability and reliability of supply of base materials. But not the least consideration is operating safety and potential hazards to the environment.

If there is a choice, one prefers to have the formation of the most toxic compounds at the end of the process sequence. Processing of a solid toxic product is often considerably more hazardous than that of a liquid. Even nastier may be the problems with a semi-solid product such as isomer mixtures.

The isolation of a crystalline toxic product by centrifuging or filtration and, even more so, the subsequent drying, can be extremely hazardous operations for the simple reason that it is technically more difficult to prevent dusts from escaping than liquids or vapours.

The same obviously holds for the blending and milling of powder formulations of solid or liquid toxic agents. In such an operation even a comparatively innocent toxic liquid may give rise to severe toxic dust problems. The additional possibilities of dust explosions may further aggravate the problems.

One of the basic prerequisites to safely and successfully manufacturing highly toxic products is the working out of a complete mass balance for every single process step, not only for normal but also for extreme operating conditions. This may be a time-consuming effort and certainly involves a great deal of both process development and analytical work.

Usually the main process streams are well known but there are always a fair number of ancillary streams to sewers, vents or through vacuum lines, which, if they escaped attention, may become sources of trouble, if not during normal operations then perhaps under abnormal operating conditions. Full elucidation of all these streams under a range of conditions can often be obtained from well-designed bench scale experiments; larger-scale pilot plant operations are only necessary in exceptional cases.

The importance of having adequate analytical methods and techniques cannot be over-emphasized. Their development must form an integral part of the whole process development. Depending on the complexity of the process, there may well be dozens of co-products and impurities to be investigated.

An operational preparation carried out in this careful manner will also provide full information about the nature and quantities of the waste products to be expected and will enable methods for their disposal to be developed.

Such waste products may comprise normal process side streams, off-specification products, mother liquors, light and heavy fractions from distillation, accidental spills, excess sample material left over after analysis, etc., and their disposal is certainly one of the more difficult tasks.

The most radical method of waste disposal, and often the most appropriate one, is incineration. A modern pesticide production and formulation plant should therefore preferably have its own specialized incineration facilities suitable for handling both liquid and solid toxic wastes including halogen and phosphorus-containing materials. There may be cases where, with special precautions, use can be made of a third-party incinerator. Sometimes, for example, with most organophosphorus products, it is possible to detoxify waste by hydrolysis to less hazardous compounds.

Contaminated water streams may be extracted and the extract incinerated. Alternatively they may be treated with flocculants and then passed over a precoat filter and subsequently through activated charcoal before they are dispersed to public waters, after final and preferably continuous analytical control. Also contaminated metal, such as empty drums, is best deoxified in an oven.

As a general policy, toxic or even potentially toxic wastes should not be

unnecessarily stockpiled but disposed of as they come free or at least as soon as practicable. The necessary handling and transport should also be minimal.

Type of plant

Well-established organophosphorus insecticides may be used at a rate of several thousand tons of active ingredient per year and will usually be produced in specific plants. Before the market has reached that scale, however, it is not uncommon that they have been produced in semi-commercial facilities of high flexibility in which other products of medium annual tonnage are also made.

As noted in the introduction, there is, in the pesticide field, a trend towards a large number of quite different and more specific products. The manufacture of more products of lower but still substantial annual tonnage, on the one hand, and the emergence of new products, on the other, has been the reason for the creation by most enterprises engaged in this field of highly flexible and fairly sizeable production facilities (multipurpose plants) which make it easier to remain economical and competitive, by enabling new products to be taken into production quickly without having to erect new facilities first.

To achieve greater operational flexibility and often for process chemistry considerations such as yields, such plants are usually batch operated. The earlier problem of such plants being prohibitively labour-intensive has been largely overcome by the use of modern automation techniques.

As mentioned before, a modern plant of this type is bound to require from the outset a fair amount of general safety measures, emergency blowdown, waste disposal and environmental protection facilities, which can be relatively easily extended, should the need arise when a new product is to be introduced. Obviously such a plant cannot be cheap and to be economical it must have a fairly large production capacity to reduce the cost per unit weight of product; at the same time it should be sufficiently flexible to enable a reasonable loading with a number of products to be achieved throughout a business year.

It goes without saying that a given product may outgrow the capacity of such a multipurpose plant—in which case a specific and optimally designed plant would be built.

While in chemical process plants for pesticides the main toxicity hazards may be concentrated in part of the plant only, formulation plants handling toxic agents must be considered dangerous as a whole, and comprehensive safety precautions are an absolute requirement. The more equipment involved, the more elaborate the safety measures, and therefore plants producing solid formulations are disproportionately more complex and expensive than facilities for liquid formulations.

Protection of plant personnel and environment

In order to ensure that the risk of the production of a toxic product is acceptable both for the operating and for the maintenance personnel, a whole range of precautions and measures must be taken. The highest possible standards of plant hygiene must be maintained at all times. This means first of all, proper design, layout and accessibility of the technical equipment, if possible a built-in system for cleaning and decontamination of equipment to enable maintenance work to be carried out, and quick-action emergency shut-down and run-down systems, where necessary with distant control from a safe area. Products entrained through blowdown lines must never escape into the atmosphere and must be collected in at least one sufficiently sized collecting vessel, which in turn may have to be equipped with a condenser and perhaps a scrubber.

If the equipment is located in a wholly or partly closed building, source exhaust ventilation is required, for example, at drum-filling stations and at sampling points, in order to carry away any toxic vapours. In addition, forced ventilation will have to be provided for the whole working space. Naturally in many cases the exhaust air will have to be treated appropriately.

The design and operation of sampling points merit particular attention since these are notorious sources of possible intoxications in normal operation.

It is good practice to prescribe for all operating and maintenance personnel in such plants a special and strict régime with regard to personal hygiene and working circumstances. This may include a full change of clothes daily, including underwear, handkerchiefs, towels, socks and shoes, operation of a laundry within the respective department, "no-touch techniques" of handtools and the like, and the special technique of washing and discarding gloves after use.

In addition to this, whenever direct contact and handling of a toxic product or of contaminated equipment cannot be avoided, special and carefully selected protective clothes, shoes, gloves and full respirating masks with independent air supply should be used. Depending on the climatic conditions, working under these especially severe circumstances may not be continued for too long and there should always be at least a second man around during such a job in order to watch the first. Drumming-off technical or formulated products or taking samples are examples of where these extra precautions may be essential.

It is imperative that the operating personnel be kept under stringent and regular medical control at a frequency and in a manner which is adapted to the situation. This frequency may vary from once each month to once a day. In this way many minor technical weaknesses and also negligent behaviour of staff can be spotted in time to take appropriate measures.

Last but not least, the importance of selecting, including the pre-placement

medical examination, and training the personnel in the most careful way need hardly be stressed. Besides regular training and refresher courses, full analysis of accidents and near misses are particularly instructive. Creating and maintaining safety-mindedness among all the personnel involved must be a permanent effort.

The approach to such ventures must be an integral one and protection of the plant personnel must never be sought at the expense of polluting the greater environment or *vice versa*. With a responsible approach, the aims must be directed towards a fully or at least maximally contained operation. Where this aim cannot be fulfilled, be it for reasons of unacceptably high costs or simply for technical reasons, then such a venture should not be launched. However, with the right mentality and the proper means, the manufacture of highly toxic products is far from an unjustifiable venture.

III. *The inhibition of cholinesterase activity by organophosphorus compounds as a means in an inspection procedure, by S. J. Lundin*

Summary

Very low concentrations of organophosphorus compounds inhibit the activity of cholinesterase, an enzyme responsible for important physiological processes in the nervous system. Cholinesterase also occurs in both the plasma and the red cells of the blood. The inhibitory effect of the organophosphorus compounds is the basis for their use both as insecticides and as chemical warfare agents. In the latter context, they are often denoted as nerve agents.

Cholinesterase inhibition can also be utilized biochemically to detect the presence of an organophosphorus compound in the environment or in an organism. Such detection methods may be applied in procedures for limited on-site inspection of assumed production and storage facilities for organophosphorus compounds. This paper gives the technical background for these methods and discusses their possible use.

The method may be used to detect and measure an abnormal occurrence of organophosphorus compounds in the environment of a plant—for example, in the surrounding air or in the waste liquid—in some cases in concentrations as low as 1 gramme of substance in 100 000 m^3 of water or 0.1 microgram in 1 m^3 of air. The method can detect and measure deviations from the normal levels of cholinesterase activity in the blood of the workers in a plant, and thus indicate possible exposure to an organophosphorus compound.

It is necessary to investigate and evaluate the application of these methods for inspection purposes.

Introduction

Very low concentrations of organophosphorus compounds inhibit the activity of cholinesterase. This enzyme is responsible for important physiological pro-

cesses in the nervous system. It also occurs both in the plasma and in the red cells of the blood. The inhibitory effect of the organophosphorus compounds underlies their use both as insecticides and as chemical warfare agents. In the latter connection they are often denoted as nerve agents.

Cholinesterase inhibition can also be utilized biochemically to detect the presence of an organophosphorus compound in the environment or in an organism. These ideas have been expressed and worked on by several persons. [187–195]

Such detection methods may be applied in procedures for limited on-site inspection of assumed production and storage of organophosphorus compounds. The aim of this paper is to give their technical background and to discuss their possible use:

1. To detect and measure an abnormal appearance of organophosphorus compounds in the environment, for example, in the surrounding air or in the waste water of an industrial plant and

2. To detect and measure deviations from the normal levels of cholinesterase activity in the blood of the workers at a plant.

Short theoretical background [196]

An enzyme, like cholinesterase, facilitates (calalyzes) a particular chemical reaction in a living organism or in an isolated biochemical system. Without the presence of the enzyme, which itself is not consumed, the reaction would not have taken place or only very much slower. The rate of the chemical reaction can be taken as a measure of the activity of the enzyme. It is expressed as the rate at which the chemical substances taking part in the reaction appear or disappear.

The rate is usually proportional to the concentration of the enzyme and of the reacting chemicals, the so-called substrates, and the reaction products.

The catalyzing activity of an enzyme can be inhibited by chemical substances, different for different enzymes, which, for example, attach themselves to the active part of the enzyme molecule. Such inhibition can be registered as a diminished rate of the chemical reaction that is normally facilitated by the enzyme. The law-bound relationships between enzyme activity and the concentrations of the reacting substances, including an inhibitor, make it possible to calculate within certain limits the amounts of a known inhibitor that is present in a sample.

Accordingly, if we know the concentrations of a particular inhibitor, such as an organophosphorus compound, which gives different degrees of inhibition of an enzyme, e.g., a particular type of cholinesterase, we can construct a curve describing these relationships. If, in a sample containing the same inhibitor, we

Table III.A. pI_{50} for some organophosphorus compounds and eserine with different types of cholinesterase

Inhibitor	Source of cholinesterase		
	Red blood cells	Blood plasma	Plaice muscle
Tabun	8.6	8.1	9.8
Sarin	8.8	8.4	9.5
Soman	9.3	9.3	10.4
DPP	6.6	8.7	9.4
Methyl-iso-propoxy-phosphoryl thiocholine iodide	8.4	7.1	10.4
Eserine	7.0	6.9	8.0

Source: Lundin, S. J. Properties of a cholinesterase from body muscles of plaice (Pleuronectes platessa). *Acta Chemica Scandinavica* 22: 2183, 1968.

determine the degree of inhibition of the enzyme to which an unknown concentration gives rise, it is possible to find the actual concentration from the curve.

The conditions governing such measurements usually make it convenient to determine that concentration of an inhibitor which causes the reduction of an enzyme activity to 50 per cent of the normal. This inhibitor concentration is called the I_{50} concentration. It is usually expressed as the negative logarithm of the molar concentration of the inhibitor, or the pI_{50} value for the particular inhibitor and enzyme.

Detection of organophosphorus compounds and determination of their concentrations

As indicated, the relationships between the degree of inhibition and the concentration depend, among other things, upon the type of enzyme as well as of inhibitor. Regarding the different types of cholinesterase, their sensitivity towards the organophosphorus compounds may vary as much as about 1 000 times, which is obvious from table III.A. Thus it is important which type of enzyme preparation one chooses for the purpose of sensitive detection methods for these compounds.

From the figures given in table III. A., it can be calculated that, using the most sensitive type of cholinesterase, it would be possible to detect with good confidence as little as around 10^{-11} grammes of the organophosphorus compounds mentioned in a sample of 1 millilitre. Expressed otherwise, this would correspond to an amount of about 1 gramme of the substances in 100 000 m^3 of water.

Possible occurrence in air of any of the listed organophosphorus compounds could also be detected assuming, for example, that it was feasible to take up

the content in 1 litre of air in 10 millilitres of water. With the given figures this would mean that as little as about 0.1 microgram per m^3 of such a substance would be detected.

Certain methodological difficulties have not been taken into account in the two examples given. They do not generally influence the order of magnitude. However, substances which are rapidly decomposed in water [197] must be sampled soon after their dissemination in water.

Methods for determination of cholinesterase activities for different purposes have been thoroughly studied [198]. They are now availabe both in highly automated systems with large capacities, usable in mobile transportable laboratory facilities [199] as well as in simple field test methods utilizing colour reactions easily visible to the unaided eye. [190] There would be small technical difficulties involved in applying such methods for some sort of limited on-site inspection. However, this does not mean that the results of the determinations would be easy to interpret.

It is also clear from what has already been said that a great number of factors influence the measurements. Thus, even if on-site sampling were allowed, a thorough scheme would have to be worked out for the testing routine to make it possible to make qualified statements about the presence of an organophosphorus substance. On the other hand, the enzyme method is not only one of the most sensitive methods, it is also highly specific for organophosphorus compounds. It is thus very dependable once an inhibition of the enzyme activity is found as a result of incubating a test sample with the enzyme and the reaction mixture. The main difficulties would arise from certain carbamates, which are also relatively powerful cholinesterase inhibitors. [200] These would not, however, contain phosphorus. This fact might be utilized for discriminatory purposes if sensitive enough gas chromatography analysis [201] could be performed simultaneously. Another possibility would be to differentiate between different types of inhibitors by means of their reaction rates using certain types of so-called reactivating substances. [e.g., 202]

Lowered blood cholinesterase levels as indications of exposure to organophosphorus compounds

Cholinesterase occurs in nervous tissues and fulfils an important step in the transmission of nerve impulses from one nerve to another, or to a muscle. As mentioned, the enzyme also occurs in blood, both in the plasma and in the red blood cells. Its function in blood is not known.

When a man or an animal is exposed to organophosphorus concentrations, it is not only the colinesterase in the nerves that is inhibited with the actual poisoning as a result; the blood cholinesterases are also inhibited. Most of the

enzyme activity has to be inhibited before the symptoms of poisoning appear. This fact has been utilized as a control measure to ensure that health hazards do not prevail in the production of, or in some uses of, organophosphorus compounds as insecticides. [194–195]

The measurements are made on blood samples, which are easily obtained from the finger tip. The measured value of the cholinesterase activity must then be compared with known normal values, preferably with earlier values from the same person or with a set of normal values from several other persons. If many people in a suspected area are tested, the normal variations can be ruled out statistically and qualified statements can be made concerning deviations or lack of deviations from a normal value.

As mentioned, several methods have been worked out for routine health control of workers occupied with the production, formulation and other handling of organophosphorus compounds, for example, spraying of organophosphorus insecticides for agricultural purposes. Accordingly, a very wide range of experience exists concerning normal blood cholinesterase values and the effects of different types of exposition. These experiences should be applicable also for inspection purposes.

One extra advantage of the blood determinations, which is of great importance in connection with the inspection of assumed production of organophosphorus compounds, is the fact that blood samples may be sent from the place one wants to investigate to another place within or outside a country. The samples need not even be frozen or be specially rapidly treated. It is sufficient to let blood drops form spots and dry on filter paper, which can then be sent away even by ordinary mail. This was demonstrated very clearly in an investigation by the World Health Organization (WHO). [195] The sampling may actually be performed by very simple means and by relatively unqualified personnel.

Another important feature of this method is that by measuring the cholinesterase activity, especially in red blood cells, it is possible to demonstrate a decrease in the activity a rather long time after exposure to an accidental or chronical exposition. The explanation is that once inhibited, and provided certain treatment has not been given, the cholinesterase in the individual red blood cell generally does not recover its activity but remains on the level of inhibition it has once achieved. The cholinesterase activity is not totally recovered until all red cells have been replaced by newly formed ones. This takes about three weeks in human beings. [203]

It is clear that determinations of the cholinesterase activity in the blood must always be carried out, when suspicions concerning the actual use of nerve agents or other organophosphorus compounds as warfare agents have been aroused. Samples should and could be taken both from dead as well as from still living victims, even if some time had passed since an alleged attack.

Conclusions

The question of inspection of an assumed production of organophosphorus compounds can only be discussed in the context of the conditions allowed for the inspection. The prospects for an inspection expand with the permitted degree of access to the installation to be inspected. It is thus important that the access-conditions are stated when any particular inspection method is proposed or discussed, and before any conclusions concerning the value of the method are drawn.

The political difficulties in arranging on-site inspections for different purposes are often stressed. It falls outside the scope of this paper to discuss these, however. Nevertheless, it must be remembered that there may also exist legitimate production secrets, which limit the access to a plant or to a part of it.

Bearing these remarks in mind, the following conclusions can be drawn with respect to the use of cholinesterase inhibition as an inspection means.

Depending on the inhibiting capacity of the assumed substance and on the sensitivity of the cholinesterase preparation, the simple biochemical detection method utilizing the inhibition of cholinesterase activity by organophosphorus compounds seems feasible for inspection of the nature of a production or a field test. Inhibition by the product itself or by samples from the immediate vicinity, such as waste water or air, constitutes strong evidence that the product is an organophosphorus compound. An unknown factor concerning industrial plants is the extent of leakage, if any, that might occur during production of an organophosphorus compound. A leakage which may not constitute a health hazard may still be detected by the method. The sensitivity is comparable only to that of gas chromatography.

The method of measuring the level of cholinesterase in blood and comparing it with normal levels is also dependent on access to the vicinity of the place, that is, only to the workers of a production unit. However, besides the actual blood sampling, further determinations need not be performed at the site, as explained earlier. Advantage can also be taken of the time lapse between the exposition and the reappearance of the red blood cell cholinesterase.

If no inhibition of the cholinesterase activity is obtained, the results obtained by means of cholinesterase determinations tell nothing about an assumed production of organophosphorus compounds. Such production may or may not then occur. The value of the method depends on the confidence of the nature of the tested substance that can be felt once an inhibition really is demonstrated.

The methods for performing the measurements are available and well known. However, their application for inspection procedures have not been studied. Such work would also be of value for monitoring the health hazards arising

from the peaceful production and use of highly toxic compounds such as the organophosphorus pesticides.

The inhibition of cholinesterase should be used together with other methods in on-site inspection procedures.

IV. *Future consumption patterns of elemental phosphorus in the USSR, by N. N. Melnikov*

Summary

Data were presented on the production of elemental phosphorus and phosphorus-based products in the USSR during the past 4 years. The production of elemental phosphorus in 1967 totalled around 100 000 tons. Production grew by approximately 25 000 tons annually during the following 3 years. In 1971 the amount of elemental phosphorus produced will be about 218 000 tons. The target for 1975 is more than double this amount.

The major consumer of elemental phosphorus in the USSR is the manufacture of phosphoric acid (over 80 per cent of elemental phosphorus produced in 1971).

The consumption of phosphorus in 1971 is distributed roughly as follows: fertilizers and fodder phosphates (over 50 per cent), detergents (over 20 per cent), plasticizers (12 per cent), phosphoric acid salts (over 7 per cent), insecticides and flotation reagents (less than 4 per cent), and metal processing (less than 3 per cent).

It is well known that during recent years the manufacture and comsumption of elemental phosphorus has been steadily growing [204–205], as phosphorus is the basic raw material for the production of a large number of valuable chemical compounds used in various branches of the national economy.

The growth of the production of elemental phosphorus can be seen in table IV.A., containing data on the production of phosphorus in various countries during 1939–1968. [204–206] The expected growth of the manufacture of el-

Table IV.A. The manufacture of elemental phosphorus (yellow) in various countries, 1939–1968

thousands of tons

Country	1939	1952	1960	1965	1968
USA	40.0	177.0	371.0	500.2	533
FR Germany	19.0	—	40.0	70.0	90.0
United Kingdom	4.7	10.0	18.0	27.0	—
Canada	5.0	8.0	10.0	18.0	—
Japan	1.3	1.1	7.6	15.7	25.0
France	2.0	4.0	12.5	14.0	18.0
Italy	0.1	0.3	1.4	10.5	—
Australia	—	2.0	5.0	6.0	—
Sweden	0.1	2.0	0.5	0.5	—
Other countries	2.3	8.7	12.0	3.1	—

Table IV.B. The manufacture of elemental (yellow) phosphorus in the USSR, 1971–1975

thousands of tons

Year	Production
1971	218.3
1972	247.0
1973	309.0
1974	366.0
1975	500.0

emental phosphorus is contained in tables IV.D–IV.G. Hence in 1969 the capacity of plants manufacturing elemental phosphorus in the USA was 621 300 tons [201], and by 1975 it should exceed 800 000 tons. An analogous picture can be observed in Canada [208], Japan [209] and other countries.

In the Soviet Union the manufacture of elemental phosphorus from 1971 till 1975 should also grow substantially, as can be seen in table IV.B.

The spread of the manufacture of phosphorus in the USSR over various plants in different parts of the country is connected with the regions of its use because the major part of it is directed to the production of fertilizers and fodder phosphates necessary for animal husbandry. The consumption of elemental phosphorus in various regions differs and depends upon the trends of development of the corresponding branches of industry and the methods of the production of phosphorus compounds. However, the basic consumer of elemental phosphorus in the majority of countries seems to be the manufacture of thermal phosphoric acid. [210] Thus in 1968 in the USA, the manufacture of thermal phosphoric acid consumed 76.8 percent of the yellow phosphorus; in the UK and in the Federal Republic of Germany in 1965, 77 and 90 per cent, respectively.

Table IV.C. The manufacture and consumption of thermal phosphoric acid in the USSR, 1971–1975

thousands of tons

	Production (phosphorus equivalent)				
Product	1971	1972	1973	1974	1975
Phosphoric acid	183.2	219.0	256.0	312.0	444.0
Including:					
Fertilizers and fodder phosphates	115.4	136.0	163.6	211.0	325.0
Washing powders (sodium tripolyphosphates)	44.7	58.0	65.0	70.0	85.0
Phosphoric acid salts	16.5	16.7	19.2	21.7	24.4
Metal processing (and other processes) and ore-phosphatizing	6.1	8.0	10.0	10.0	10.0

Table IV.D. Production of red phosphorus in the USSR, 1971–1975

thousands of tons

Year	Production
1971	1.1
1972	1.1
1973	1.2
1974	1.3
1975	1.5

In the Soviet Union 183 200 tons of yellow phosphorus or 83.9 per cent were consumed in 1971 for the manufacture of thermal phosphoric acid.

The planned production and consumption of thermal phosphoric acid in the USSR during 1971–1975 is given in table IV.C.

Among inorganic substances it is necessary also to mention the manufacture of red phosphorus used in the production of matches and in other branches of industry. The manufacture of red phosphorus in the USSR during 1971–1975 is given in table IV.D.

During recent years the manufacture of phosphorus pentasulphide [211], phosphorus trichloride and phosphorus oxychloride has proceeded on a large scale. [212–213] Thus the manufacturing output in 1968 of phosphorus pentasulphide in the USA was about 62 500 tons [211], and phosphorus trichloride around 50 000 tons. [212] Also in the future a considerable increase in the manufacture of these products is envisaged.

It should be noted that the main consumer of phosphorus pentasulphide and halogen compounds of phosphorus is the synthetic organic chemical industry, primarily the production of plasticizers, insecticides, fungicides, herbicides, flotation agents and others.

First, as far as the scale is concerned, is apparently the consumption of phosphorus compounds for the manufacture of plasticizers, particularly triarylphosphates. Second is the consumption of phosphorus for the manufacture of pesticides.

Table IV.E. Manufacture of phosphorus pentasulphide in the USSR, 1971–1975

thousands of tons

Year	Production (phosphorus equivalent)
1971	1.0
1972	1.2
1973	2.2
1974	3.7
1975	4.4

Table IV.F. The consumption of phosphorus in the USSR for the production of pesticides and flotation agents, 1971–1975

thousands of tons

Year	Production (phosphorus equivalent)
1971	8.1
1972	10.0
1973	10.0
1974	12.0
1975	12.0

The Soviet Union produces and applies on a large scale such pesticides as, for example, Carbophos (Malathion), Metaphos (Methyl-parathion), Trichlormetaphos-3 (O-methyl O-ethyl 0–2,4,5-trichlorphenyl thiophosphate), Chlorophos (trichlorfon), DDVF (Dichlorphos), Butiphos (tributyl trithiophoshate), Methylmercaptophos (Demeton-methyl) and a number of others. Most of the thiophosphates are derived from thiophosphoryl chloride rather than from phosphorus pentasulphide, as is the practice in the USA, where 50 per cent of the phosphorus pentasulphide is used for the manufacture of pesticides. [211] In the USSR phosphorus pentasulphide is used for the manufacture of Malathion and flotation agents, among which the most frequently used is potassium dibutyl dithiophosphate, which at the same time appears to be an active desiccant for the pre-harvest drying of the leaves of a number of plants.

Data about the manufacture of phosphorus pentasulphide are given in table IV.E, above.

As flotation agents for rare metals, the use has been started recently not only of by-products of dithiophosphoric acid, but also ethers of phosphoric acid and some other phosphorus compounds. Likewise, organic compounds of phosphorus have found application as chelates and medicinal preparations in medical care.

Table IV.G. The structure of the consumption of phosphorus in the USSR, 1971–1975

thousands of tons

Product	1971	1972	1973	1974	1975
Fertilizers and fodder phosphates	115.4	136.0	163.6	211.0	325.0
Washing powders	44.7	58.0	65.0	70.0	85.0
Phosphoric acid salts	16.5	16.7	19.2	21.7	24.4
Processing of metals	6.1	8.0	10.0	10.0	10.0
Match industry	1.1	1.1	1.2	1.3	1.5
Manufacture of insecticides and flotation agents	8.1	10.0	10.0	12.0	12.0
Manufacture of plasticizers and export	26.4	17.2	40.0	40.0	42.1
Total	**218.3**	**247.0**	**309.0**	**366.0**	**500.0**

Table IV.F. contains data on the use of phosphorus for the production of pesticides and flotation agents. Among this group of compounds, the production of insecticides, particularly trichlorfon, occupies first place as far as the scale of the consumption of phosphorus is concerned.

The production of pesticides is also attaining a considerable magnitude.

No data are given on the production of phosphorus chlorides, since these do not constitute final products. They are utilized immediately at the plant for the production of the above-mentioned groups of compounds. Halogen compounds of phosphorus are only manufactured for laboratory purposes, and as reactants for scientific research organizations. The amounts are negligible and cannot influence the general balance of the consumption of phosphorus. The overall balance of phosphorus in the USSR is given in table IV.G, above.

V. *A mobile laboratory for verification of alleged manufacture of chemical warfare agents, by J. K. Miettinen*

Summary

A large organophosphorus plant producing a wide range of products may be able to justify the need for all the processing units required for producing active nerve agents.

It would be essential to identify exactly every compound produced, because the precursors of binary agents are relatively nontoxic, and large, obvious safety measures are not needed in the production plants. Nor would there be large stocks of the agent in storage.

Rigorous verification of nonproduction of nerve agents in such a plant would require identification of raw materials, intermediates, end-products and wastes, preferably even after-waste outlets, which in many cases would require collection and analysis of samples. If these could be analysed overnight, on the spot, questions could be put to the technical personnel next day, thereby revealing any clandestine production.

The number of *bulk waste products* from organophosphorus production (for example, NaCl, $AlCl_3$, HCl) is not very large and their identification could probably be carried out by specific reagents which take little space (for example Feigl's spot tests).

A method of identification applicable to nearly all *phosphorus esters* would be thin-layer chromatography. The main difficulties of this method would be the preconcentration of minor constituents to a sufficiently concentrated sample, suitable for thin-layer chromatography, and the procurement of a sufficiently large reference standard collection. Devices for extraction, concentration, thin-layer chromatography and spot development can be miniaturized to such an extent that they are transportable in suitcases.

The presence of even traces of *pesticides and active nerve agents* can be shown by the anticholinesterase method, which can also be miniaturized. The identity of the

agent cannot yet be elucidated by this technique, but prompt analyses could help guide the search for leaks or for more representative samples. If reference samples are available, identification of the agents can be performed by gas chromatography; but this instrument has not yet been miniaturized, and its use would require a laboratory van.

The possibility of carrying out analyses overnight and of asking surprise questions based on the results would give the investigation such intimacy and depth that clandestine activities could probably be revealed.

Introduction

Organophosphate nerve agents are so closely related to legitimate, peaceful organophosphates, such as plasticizers, surface active agents, lubricating oil additives and pesticides, that it is not possible to verify the end product of an organophosphorus plant by only external observation techniques like air- or satellite-photography, extramural observation or photography. Although there exist indirect verification techniques other than those mentioned, intramural inspection may also come in question in some cases, although it is a relatively intrusive verification technique.

If the inspection team is allowed to perform an on-the-spot inspection, then sampling and analysis become feasible. (In some cases, for example, in the case of an unexplainable accident, even extramural samples may be useful.) If the plant lacks essential facilities to produce active nerve agents, this can be verified by experts by just walking through, but even then production of binary agents may be possible and identifiable only by analysis. However, a large organophosphorus plant, producing a wide scale of end products, some of them only temporarily, may be able to justify as legitimate needs, the presence of all processing units needed for production of active nerve agents. Analysis would then be quite essential, because the identity of a chemical compound can usually only be rigorously verified by chemical analysis.

The exact identification is even more essential in the case of precursors of binary agents, because they are relatively non-toxic and large and revealing safety measures are not needed in the production plants. Also, there would be no large stocks of the agent in storage, but there might be some small samples left over from earlier pilot production.

We must assume that the inspection team probably cannot extend the plant visit to more than about 3 days (for instance, first day—presentations of plant management, interviews; second day—walk-through; third day—questioning, interviews). If analytical results are wanted immediately, in order to be able to pose further questions on the basis of them, 3 days do not allow the samples to be sent abroad for analysis. One solution would then be a mobile laboratory.

What samples would require prompt analysis?

The need for eventual prompt analyses depends on what samples could be obtained. The plant management could probably not easily justify a refusal to allow sampling of raw materials or end products. These samples are not likely to require prompt analysis, however. On the basis of a need for commercial secrecy, the firm would probably be able to justify refusal of sampling of intermediate products. A better yield or less harmful side products in some production phase may be economically decisive if the margin of profit is small. Refusal of sampling of *wastes* probably would not be so easily justifiable and any reluctance on the part of the plant management in this respect would already arouse suspicion. The waste analyses can be divided into two groups: identification of the bulk waste products and identification of minor impurities in them or in the contents of a deep disposal well. In both cases it would be useful if the result could be made available overnight. If samples were taken during the first day and answers were ready on the second day, new, more penetrating questions could be posed already on the second day.

What kind of mobile laboratory would be needed?

The mobile laboratory ought to be miniaturized as far as possible. A laboratory van, for example, could be psychologically disturbing. Three large suitcases would probably be the maximal volume that could be transported by the inspection team without attracting attention and this sets limits to the analytical methods applicable.

The number of *bulk waste* products from organophosphorus production (e.g., NaCl, $AlCl_3$, HCl) is not very large and their identification could probably be carried out by specific reagents which take little space (e.g., Feigl's spot tests). A universal method of identification applicable to nearly all *phosphorus esters* would be thin-layer chromatography. This permits only those compounds for which reference standards are available to be identified as a rule. Several hundred reference samples could be carried in a suitcase in small vials. Two-dimensional runs by two solvent pairs are usually sufficient for identification with nearly 100 per cent certainty and they can be carried out overnight. The standard reagent for spot development is ammonium phosphomolybdate. This reaction requires UV-light but can be carried out in a hotel room. The main difficulties of the method are the preconcentration of minor constituents to a sufficiently concentrated sample, suitable for thin-layer chromatography, and the procurement of a sufficiently large reference standard collection. Devices for extraction, concentration, thin-layer chromatography and spot development can be miniaturized to such an extent that they are transportable in suitcases.

The presence of even traces of *pesticides and active nerve agents* can be shown by the anticholinesterase method, which can be miniaturized. The identity of the agent cannot be elucidated by this technique, but prompt analyses could give guidance in searching for leaks or for more representative samples. If reference samples are available, identification of the agents can be performed by gas chromatography, but this instrument is not yet miniaturizable to a quite small size and its use would require a laboratory van.

Conclusions

Rigorous verification of nonproduction of nerve agents in an organophosphorus plant requires identification of raw materials, intermediates, end products and wastes, preferably even past wastes, which in many cases requires collection and analysis of samples. If these could be analysed overnight, on the spot, surprising questions could be placed to the technical personnel next day, which might reveal any past temporary clandestine production. Methods do exist which can be miniaturized to a suitcase-size laboratory, operable in a hotel room. Procurement of all necessary standard compounds would be a major project and possibly require the cooperation of specialized laboratories of several countries.

The possibility of carrying out analyses overnight and basing surprise questions on the results would give the investigation such intimacy and depth that clandestine activities could probably be revealed.

VI. *Some aspects of the problem of possible convertibility of organophosphorus plants, by J. Moravec*

Summary

The chemical production of organophosphorus compounds today is dictated by peaceful needs, but, at the same time, it represents a potential basis for their possible use for military purposes. This potential basis rests with:

(i) obtaining raw material resources;
(ii) the development of the organophosphorus industry; and
(iii) obtaining personnel with experience in handling toxic material.

The convertibility of organophosphorus plants into CW agent plants seems to be difficult but not impossible. Some processes, used in producing organophosphorus compounds in the plastics industry and in producing organophosphorus insecticides, approach those for producing esters used as half-products in the production of CW agents. It would be necessary to disclose additional changes during inspection.

The idea of a conversion of chemical production into the production of chemical warfare agents is connected with the history of the origin and development of chemical warfare.

The first chemical warfare agents of the then developing chemical industry (chlorine and phosgene) were used during World War I. During the war, processes and equipment pertaining to different branches of the chemical industry were used for the production of further agents.

The rapidly developing chemical industry furnishes a basis for a possible production of chemical warfare agents. To the potential agents, which still have not lost their significance, belong both phosgene and hydrocyanic acid as well as chlorcyane, which are produced in many states on large scale for peaceful purposes. A stormy development has been experienced in recent times also in the field of ethylene chemistry, which can form a basis for the production of vesicants.

In this report, I shall deal with some commercially and technically important organophosphorus materials which, through their production processes, are close to the group of organophosphorus CW agents.

At the outset I would like to say that, apart from brief reports on the production of tabun from World War II, information is lacking on the production processes of sarin [GB] and the V agents, although publications have reported that these products were manufactured in the United States, and maybe they are produced there even today. [214]

Detailed data on the technological production of individual insecticides are very brief and in practice are treated as economic secrets of the plants producing them.

But in both cases scientific publications are available dealing with syntheses as well as publications about patents which, in a way, define the trends applicable to industrial production. Therefore, in my further deliberations I shall present general technological presumptions deduced from theoretical properties of compounds and processes.

It is possible to place the beginning of the huge growth of industrial production of organophosphorus compounds at the period of World War II when the use of two groups of organophosphorus esters was prolific. The groups comprised insecticides and chemical warfare agents. Their growth continued after World War II with the expanding need for esters of the phosphoric acid in the production of plastic materials.

A result of the great effort in research in organophosphorus chemistry is the constantly widening assortment of insecticides which have become indispensable in the fight against harmful insects;[1] extending the assortment of plasticizers, stabilizers of plastic materials, additives for lubricants, etc.

[1] In the year 1960 the production of organophosphorus insecticides in the USA amounted to 17 300 tons, in 1966 to 54 000 tons (out of 43 500 in 1965, 25 100 tons went to parathion and methylparathion).

The increase in the production of organophosphorus compounds is accompanied by the growing production of elementary phosphorus. For example, the annual production of elementary phosphorus in the Federal Republic of Germany amounted to 70 000 tons in 1965, and in 1968 another plant was started with a production capacity of 30 000 tons per year. The share of West Germany (Knapsack Company) according to Wegler [215] amounts to 10 per cent of world production.

The development of chemical production of organophosphorus compounds today is dictated by peaceful needs; but at the same time it represents a potential basis for their possible use for military purposes.

This potential basis depends on (*a*) obtaining raw material resources, that is, elementary phosphorus; (*b*) the development of the organophosphorus industry, in producing phosphorus chlorides and phosphoric acid esters—in some cases it is the development of products which after further processing can be transformed into chemical warfare agents—and (*c*) obtaining personnel with experience in handling toxic material, as in the case of insecticides.

When considering the possibilities of transforming the production of organophosphorus compounds serving peaceful causes to a production of chemical warfare agents, it is necessary to consider these principles as a whole.

Possible processes leading to the production of CW agents

Bearing in mind the data published at the present time, it is possible to list the organophosphorus chemical-warfare agents in the following way:

1. *Tabun* (GA): manufactured in Germany during World War II;
2. *Sarin* (GB): prepared during World War II and manufactured in the United States after World War II; and
3. *V agents:* developed and produced in the United States.

1. *Tabun* (GA) [216]

Step I—amination—$POCl_3 + NH(CH_3)_2 \xrightarrow[\text{heating}]{\text{cooling}} (CH_3)_2NP(O)Cl_2 + HCl$ distillation

Step II—esterification, substitution CN

$(CH_3)_2NP(O)Cl_2 + 2NaCN + C_2H_5OH \rightarrow$
$(CH_3)_2NP(O)(OC_2H_5)CN + HCN + 2NaCl$ separation

2. *Sarin* (GB) [217–218]

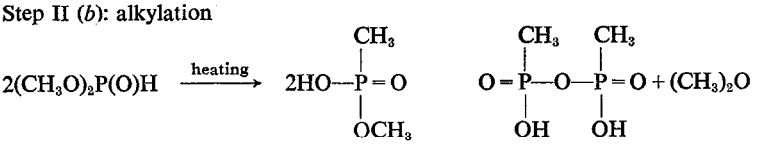
Step I: esterification

$PCl_3 + 3CH_3OH \xrightarrow{\text{cooling}} (CH_3O)_2P(O)H + CH_3Cl + 2HCl$

Step II (*a*): alkylation

$(CH_3O)_2P(O)Na + CH_3Cl \rightarrow (CH_3O)_2P(O)CH_3 + NaCl$ separation

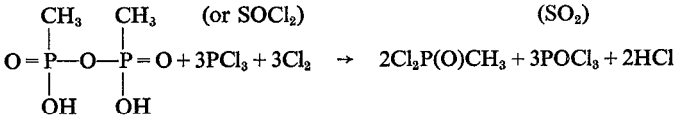
Step II (*b*): alkylation



Step III (*a*): chlorination

$$(CH_3O)_2P(O)CH_3 + 2PCl_3 + 2Cl_2 \rightarrow Cl_2P(O)CH_3 + 2POCl_3 + 2CH_3Cl$$

Step III (*b*): chlorination



Step IV: fluorination

$$2Cl_2P(O)CH_3 + 2HF \rightarrow Cl_2P(O)CH_3 + F_2P(O)CH_3 + 2HCl$$

Step V(*a*): esterification

$$F_2P(O)CH_3 + ROH \rightarrow RO(F)P(O)CH_3 + HF$$

Step V(*b*): esterification

$$Cl_2P(O)CH_3 + F_2P(O)CH_3 + 2ROH \rightarrow 2RO(F)P(O)CH_3 + 2HCl$$

Apart from these processes, direct alkylation of PCl_3 by means of CH_3Cl in the presence of a catalyst and decomposition of the complex thus obtained by means of water, SO_2 or aluminium are known. [219–220] By this reaction it is possible to obtain directly CH_3POCl_2 or CH_3PCl_2

The survey given above is not complete.

3. *V agents* [221–222]

According to the methods of laboratory preparation, as they have been published, a description is given for methylphosphonic dichloride which is also used in the production of sarin.

Step I(*a*): esterification

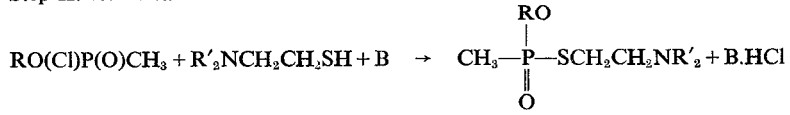
$$Cl_2P(O)CH_3 + ROH \rightarrow RO(Cl)P(O)CH_3 + HCl$$

Step I(*b*): esterification

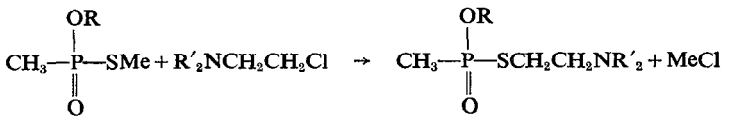
$$Cl_2P(O)CH_3 + 2ROH + 2B \rightarrow (RO)_2P(O)CH_3 + 2B.HCl$$

$$(RO)_2P(O)CH_3 + COCl_2 \rightarrow RO(Cl)P(O)CH_3 + RCl + CO_2$$

Step II: esterification



or



The given reactions are only a part of possible accesses to the organophosphorus chemical warfare agents as seen from the general schemes of access in the paper by Dr Pittaway. [223]

From the above examples in this paper it follows that, from the point of view of inspection, the following main raw materials and half-products come into consideration:

1. Elementary Phosphorus
2. Phosphorus trichloride
3. Phosphorus oxychloride
4. Alcohols
5. Dialkylaminoalcohols
6. Hydrogenfluoride
7. Metal fluorides
8. Thionylchloride
9. Phosgene
10. Dimethylamine
11. Sodium cyanide
12. Aluminiumtrichloride
13. Methylchloride
14. Dimethylphosphite
15. Methylphosphonic acid esters
16. Methylphosphonyldichloride
17. Dialkylaminothiols
18. Dialkylaminoalkylchlorides

Compounds listed under 1–14 are currently produced for peaceful purposes. Further, the following unit processes come under consideration:

1. esterification
2. alkylation
3. chlorination
4. fluorination
5. amination
6. oxydation

All the given processes are used during industrial production of either organophosphorus insecticides or plastic materials, with the exception of alkylation which appears with some insecticides only.

Organophosphorus compounds in the plastics industry

In this sphere we most often meet with the compounds of the esters of phosphoric acid, namely with triarylphosphates, alkylarylphosphates and trialkylphosphates. They are produced from phosphorus oxychloride and phenols or alcohols. When constructing equipment for their production, problems of the highly corrosive environment have to be solved (hydrochloric and phosphoric acids) and in view of their further application high demands are very often placed on purity, which in many cases has to be achieved by vacuum distillation.

Consequently, the nature of the process approaches that for esters used as half-products in the production of CW agents. It would be worthwhile to consider the possibility of their potential misuse for the production of these half-products.

In contrast to the CW agents, these are all non-toxic products. Therefore, during the production no protection of personnel on the premises, no extra ventilation and no special measures (equipment) for disposing of gaseous or liquid wastes are needed. The lack of security measures prevents such equipment from being used for production of highly toxic compounds; and their presence during an inspection would signal a change in the production programme.

In recent years, however, news has appeared about the preparatory use of the so-called binary systems for the construction of chemical weapons. This kind of construction does not assume the filling of ammunition with ready toxic CW agents. [224]

The realization of binary systems could mean an increased possibility of production of compounds designed for chemical weapons by exploitation of processes and facilities in the industry of plastics or other branches of the chemical industry. At the same time, this would involve considerable complications in surveillance of the production of this kind of weapon, as in this case the main important accompanying characteristic feature of the production of chemical warfare agents, that is, the necessary security precautions during their production and the necessary equipment serving to ensure security, would be lacking. Also, analytical methods based on direct proof of the existance of such agents (for example, a very sensitive biochemical reaction) would only be applicable with considerable difficulty.

In the plastic materials industry the following compounds are most often used:[2]

Tricresylphosphate, Triphenylphosphate, Diphenylcresylphosphate, Cresyldiphenylphosphate, Xylenyldiphenylphosphate, Diphenyloctylphosphate, Tri-

[2] Among their producers can be named, e.g. Monsanto, Bayer, Geigy (Manchester) and Farbwerke Hoechst A. G.

2-ethylhexylphosphate, Diphenyl-2-ethylhexylphosphate, Tributylphosphate and Tri-2-chlorethylphosphate.

Examples of esterification:

$POCl_3 + 3ArOH \rightarrow PO(OAr)_3 + 3HCl$
$POCl_3 + ArOH \rightarrow PO(OAr)Cl_2 + HCl$
$PO(OAr)Cl_2 + 2ROH + 2B \rightarrow P(O)(OAr)(OR)_2 + 2B.HCl$
(B = amine, ammoniac or natriumcarbonate)
$POCl_3 + 3ROH + 3B \rightarrow P(O)(OR)_3 + 3B.HCl$
$POCl_3 + 3(CH_2)_2O \rightarrow PO(OCH_2CH_2Cl)_3$

Recently, importance has been attributed also to phosphites used as stabilizers in the plastics and rubber industries and also as half-products, i.e. dialkylphosphites and diarylphosphites prepared from phosphorus trichloride and alcohols.

$PCl_3 + 3ROH \rightarrow HP(O)(OR)_2 + RCl + 2HCl$

Dimethylphosphite which is used as a half-product during the production of insecticides and as a lubricant additive is continually produced in this way. [225]

Triphenylphosphite, Trisnonylphenylphosphite, Di-decylphenylphosphite, Di-n-octylphosphite, Tris-2-chlorethylphosphite and Bis-2-chlorethylphosphite.[3]

The production of phosphites as stabilizers has not until now reached the same extent as the production of insecticides and plasticizers. But, from the aspect of control, it might be significant in the sense that phosphites with lower alkoxyl groups (CH_3O or C_2H_5O) can serve directly as half-products for the production of chemical warfare agents; aryl phosphites and phosphites with higher alkoxy groups are produced by means of esterification in equipment similar to that used to produce the esters of phosphoric acid.

Organophosphorus insecticides

Organophosphorus insecticides are often the subject of deliberations during which fears are expressed that the processes and the sites for their production may also be used for the production of CW agents. These deliberations stem from the fact that these compounds are toxic, some of them highly so.

At the outset of their industrial production, insecticides did not differ too much from chemical warfare agents in their toxicity, for example, tetraethylpyrophosphate and tabun. In the course of time the agents became more toxic to warmblooded animals while insecticides, on the other hand, became less toxic.

In their composition, the organophosphorus chemical warfare agents and

[3] Products of, for example, Deutsche Advance Production GmbH.

insecticides are cognate. They both have a central atom of phosphorus surrounded by four substituents, one of which is either sulphur or oxygen. In contrast to agents of the type of sarin and V, for which the carbon-phosphorus bond is characteristic, this structure is very rare with insecticides (pesticides).

The following compounds can serve as examples:

O,O-Dimethyl 2,2,2-trichloro 1-hydroxyethylphosphonate (Dylox)[4]
O-Ethyl O-p-nitrophenyl phenylphosphonothionate (EPN)[5]
O-Ethyl 2,4,5-Trichlorophenyl ethylphosphonothionate (Trichloronate)[6]
O-Ethyl S-phenyl ethylphosphonothiolothionate[7]

In recent years, in view of some unsuitable properties of chlororganic insecticides, significant attention has been paid to research on organophosphorus insecticides, the proof of which is a great number of scientific reports and patents. These frequently include compounds with the C–P bond [226].

In spite of lower toxicity as compared with CW agents, certain security precautions and equipment are necessary for their production which are not necessary for the production of other compounds. It is also necessary to detoxicate the waste water. The personnel must be trained to abide by security precautions. Possible production of chemical warfare agents would thus be relatively less conspicuous compared with other plants.

In the production of insecticides, it is possible to start from phosphorus trichloride, its esterification to dialkylphosphite or trialkylphosphite, and in further reaction procedures to prepare an active compound. From the insecticides thus produced, I should like to mention the following:[8]

Dipterex (Dylox)	O,O-Dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate
Vapona (DDVP)	O,O-Dimethyl 2,2-dichlorovinylphosphate
Phosdrin	O,O-Dimethyl 2, carbomethoxy-1-methylvinyl phosphate
Phosphamidon	O,O-Dimethyl-2-chloro-2-diethylcarbamoyl-1-methylvinyl phosphate
Bildrin	Dimethyl 2-dimethylcarbamoyl-1-methylvinylphosphate
Axodrin	Dimethyl 2-methylcarbamoyl-1-methylvinyl phosphate
Bomyl	1,3-*Bis*(methoxycarbonyl)-1-propen-2-yl dimethylphosphate
Bislane	2-Chloro-1-(2,4-dichlorophenyl)-vinyl diethylphosphate

[4] Introduced in 1952 by Bayer.
[5] Introduced in 1949 by E. I. duPont de Nemours.
[6] Introduced in 1960 by Bayer.
[7] Introduced in 1967 by Stauffer Chemical Company.
[8] Lists of insecticides (pesticides) together with further trade names and producers are given by Wegler [215] and appear in the "Lists of Approved Products for Farmers and Growers" issued yearly by Ministries of Agriculture in various countries.

Processes for preparing these compounds include esterification, and phosphites are used which can form a basis for the production of CW agents. To use trimethylphosphite for the production of chemical warfare agents would be possible but not economical.

It is also possible to obtain phosphorus oxychloride by oxidation of phosphorus trichloride and by esterification, amination or halogenic exchange to carry out further reactions.

TEPP	Tetraethylpyrophosphate
Schradan (OMPA)	Tetrabisdimethylamidopyrophosphate
Dimefox	Bis(dimethylamido)phosphorofluoridate.

The production processes of this small group of insecticides include esterification, amination and fluorination. The group contains compounds with relatively high toxicity for mammals. Therefore it is assumed that the amount now produced is small.

A much bigger group is formed by compounds that can be prepared both from P_2S_5 (phosphorus pentasulphide) or PCl_3 (phosphorus trichloride):

(A) $P_2S_5 + 4ROH \rightarrow 2(RO)_2P(S)SH + H_2S$
$2(RO)_2P(S)SH + 2Cl_2 \rightarrow 2(RO)_2P(S)Cl + 2HCl + S_2Cl_2$

(B) $PCl_3 + S \rightarrow PSCl_3$
$PSCl_3 + 2ROH \rightarrow (RO)_2P(S)Cl + 2HCl$

Systox	O,O-Diethyl-O-2-(ethylthio) ethyl phosphorothioate O,O-Diethyl-S-2-(ethylthio) ethyl phosphorothioate (isomer)
Metasystox	O,O-Dimethyl-O-2-(ethylthio) ethyl phosphorothioate O,O-Dimethyl-S-2-(ethylthio) ethyl phosphorothioate (isomer)
Parathion	O,O-Diethyl-O-p-nitrophenyl phosphorothioate
Methylparathion	O,O-Dimethyl-O-p-nitrophenyl phosphorothioate
Baytex	O,O-Dimethyl O-/4(methylthio)-m-tolyl/phosphorothioate
Chlorthion	O,O-Dimethyl-O-(3-chloro-4-nitrophenyl) phosphorothioate
Co–R al	O,O-Diethyl-O-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate
Diazinon	O,O-Diethyl-O-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate
Folimat	Dimethyl S-(N-methylcarbamoylmethyl) phosphorothioate

The production process of this group of insecticides basically includes esterification. It comprises also Amiton, which is not important commercially, but has a very similar composition to V agents and its production is closest to the production of chemical warfare agents.

The last group is formed by the insecticides which are produced directly from phosphorus pentasulphide *via* dialkylphosphorodithioic acid.

Di-Syston	O,O-Diethyl-S-2-(ethylthio) ethyl phosphorodithioate
Ethion	O,O,O′,O′-Tetraethyl S,S′-methylene bisphosphorodithioate
Guthion	O,O-Dimethyl-S-4-oxo-1,2,3 benzotriazin-3 (4 H)-ylmethyl phosphorodithioate
Malathion	S-(1,2 Dicarbethoxyethyl) O,O-dimethyl phosphorodithioate
Methyl Trithion	O,O-Dimethyl-S-p-chlorophenylthiomethyl phosphorothiate
Phencapten	O,O-Diethyl-S-(2,5-dichlorophenylmerkapto-methyl) dithiophosphate
Thimet	O,O-Diethyl-S-(ethylthiomethyl) phosphorodithioate
Trithion	S-(p-Chlorophenylthiomethyl)-O,O-diethylphosphorodithioate

The production of this group of insecticides includes esterification mostly in a non-corrosive environment and will thus differ most from the preparation of CW agents.

In conclusion, it is possible to state that the conversion of production of organophosphorus compounds destined for peaceful purposes would require modification and complementation of equipment, which it would be necessary to disclose during a commission's inspection.

Technical control would affect the establishment in which secret production is being carried out; in such a case, the work of the inspection commission would be easier because it would have a guideline in the material supplies—it could judge from the raw materials introduced into the plant *via* production data and according to the products leaving the plant; and at the same time, the commission would have at its disposal information on the purpose, necessity and parameters of the equipment and production processes.

Or control could be considered in a plant where CW agents are not produced but where preparations are being made for conversion to their production. This case is more complex because direct proof will always be missing. Nevertheless, it is necessary to take into account that, in contrast to waging war by biological warfare, waging war by CW agents requires a greater amount of these agents and therefore their production would be commenced at a much earlier stage before the beginning of a chemical attack.

From the point of view of the plant, it can be assumed that conversion of production of organophosphorus compounds to the production of CW agents would be easier in big plants which produce a wide assortment of organophosphorus compounds.

The inspection control within these big plants will be much more difficult

than within the plants producing only one single product, which work only with half-products brought from other plants.

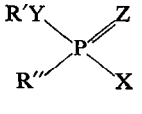
I think that specifically the problem of technical verification is not so complicated as the problem of the extent of control with respect to the number and the size of plants which come into consideration for control. In the case of a model inspection control, it will be necessary to devote attention to both these aspects.

Even though organophosphorus compounds produced at present, which include very toxic chemical warfare agents, were the object of my deliberations, recent experience, especially, for example, the use of chemical warfare agents and phytotoxic materials by the United States in Viet-Nam, shows how important it is to solve the problem of the abolition of use and production of all toxic chemical weapons. From this broader point of view, the problem of inspection becomes more complicated because the number of plants capable of CW agent production would become so large that every chemical plant would come into consideration for inspection. To prevent this complexity, which will certainly be reflected in the feasibility of some sort of international control, from defeating its own purpose, that is, a possible agreement on the abolition of use and production of chemical warfare agents, it would be purposeful to consider possibilities of control on a national level.

VII. *On the possibility to delimitate nerve gases within the field of organophosphorus compounds, by A. J. J. Ooms*

Summary

This paper deals with the problem of establishing some general rules according to which an organophosphorus compound behaves as a potent inhibitor of cholinesterase activity. As with most structure-activity relations, these rules are rather tentative, and no firm predictions are possible (although some quantitative predictions have turned out surprisingly well, according to published investigations). It is, therefore, impossible to delimitate the potential nerve agents using a general formula only. These formulae have to be used in conjunction with a toxicity criterion, which should be established by toxicological experts. For the general formula the following is proposed:



where

Y = O or S
Z = O or S

X = F, CN, N_3, $S-(CH_2)_n-SR'''$.
$S-(CH_2)_n-NR'''_2$, $S-(CH_2)_n-\overset{\oplus}{S}R'''_2$
or $S-(CH_2)_n-\overset{\oplus}{N}R'''_3$
R′ = (substituted) alkyl, cycloalkyl or hydrogen
R″ = Alkyl or dialkylamino
R‴ = Alkyl

In combination with a carefully selected toxicity criterion, the delimited group of compounds includes very few compounds which are used as pesticides and the majority of compounds which can be used as chemical warfare agents. This second group could therefore be subject to an unconditional prohibition on production and stockpiling, or be subjected to special verification.

Introduction

The Conference of the Committee on Disarmament (CCD) has among other problems on its programme, the prohibition of chemical and biological warfare. One of the aims of the discussions is to reach agreement on a treaty prohibiting the production and stockpiling of chemical warfare agents. According to a number of nations, a treaty like this can only be reached if proper and effective methods of verification can be established. The aim of this SIPRI symposium is to explore this problem.

Both from the Report of the Secretary-General of the United Nations [155] and from the Report of the World Health Organization [156], it is clear that the nerve agents constitute by far the greatest threat in chemical warfare. The decision to focus the discussions on these agents seems therefore a proper one.

Nerve agents belong to the group of organophosphorus compounds which also contains very useful compounds such as pesticides, polymers, flame retardents and plasticizers. This immediately brings up the problem of the possibility of distinguishing between organophosphorus compounds which have, and compounds which do not have, legitimate uses for civilian purposes. In other words, is it possible to delimitate nerve agents within the field of organophosphorus compounds?

In order to be considered as a potential chemical warfare agent a chemical compound should meet certain requirements. [227] Some of these can be listed as follows: a considerable toxicity for mammals, chemical stability in the presence of air and water, stability at explosion and a certain rate of penetration through skin and materials. This list can easily be extended. In this paper we have limited ourselves to the most important property of a potential CW agent, namely that of toxicity, taking the view that this property, more than any of the others mentioned, might be used in the delineation mentioned above.

In order to be classified as a potential pesticide, an organophosphorus com-

pound also has to meet certain requirements. Here, too, the most important will be a considerable toxicity for the pest species it is intended for, such as insects or spiders. Ideally a good pesticide should possess a very low toxicity for mammals in general and for man in particular: in other words it should possess selective toxicity.

Unfortunately, in the field of organophosphorus compounds the molecular basis of the toxicity is the same in both mammals and insects. Toxic action is mainly based on the inhibition of the enzyme acetylcholinesterase in positions where acetylcholine acts as a neurotransmitter. [228–229] However, there are a number of cases where organophosphorus compounds have a high toxicity for insects and a low toxicity for mammals (for example, the insecticide Malathion). In these cases the selectivity can be attributed to secondary effects such as differences in the rate of detoxification or to differences in the rate of penetration through membranes.

We are thus faced with the problem of whether we can draw a borderline between organophosphorus compounds that have an exclusive (potential) use as nerve agents, and related compounds that can be used as pesticides. Such a borderline could be based on a certain toxicity level. Whereas it is theoretically possible to use a nerve agent such as Sarin as an insecticide, this is highly improbable due to the very great hazards this procedure would cause to humans.

Thus, the delegation from Japan at the CCD [230] has proposed a toxicity level of 0.5 mg/kg subcutaneously as a borderline between compounds having exclusive use as CW agents and other compounds. In the same way, the Swedish delegation at the CCD [231] proposed a level of 1.0 mg/kg orally. These proposals seem to be very reasonable indeed, provided that sufficiently standardized methods for the determination of the toxicity could be worked out internationally by toxicological experts.

We do, however, feel that if this criterion can be backed up by a kind of general chemical formula we will at least have a lead in a possible verification process.

Toxicity of organophosphorus compounds related to chemical structure

In a way, the position with organophosphorus compounds is a favourable one in that we know a good deal about the mechanism of the toxicity on the molecular level: the aforementioned inhibition of the enzyme acetylcholinesterase. There seems to be a reasonable relationship between toxicity and the anti-acetylcholinesterase potency. [232] As toxicity depends not only on the intrinsic pharmacological effect but also on factors such as permeability through membranes, rate of excretion and rate of metabolism, a better-than-reasonable relationship cannot be expected. As the dependence of cholinesterase inhibition

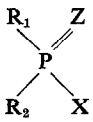
on chemical structure is more clear-cut than the dependence of toxicity, we have in the following limited ourselves to a survey of the first-mentioned relationship, bearing in mind that some organophosphorus compounds are not cholinesterase inhibitors *per se* but are metabolized into potent inhibitors in the organism.

All the nerve agents mentioned in the literature [233] are powerful inhibitors of acetylcholinesterase, in the majority of cases much more powerful than compounds used as insecticides. The problem is thus limited to that of predicting chemical structures giving rise to potent anti-acetylcholinesterases.

The relationship between chemical structure and the inhibition of acetylcholinesterase

A large amount of literature is available on this problem. It is not our intention to review these publications. We will instead give some summarizing results with the emphasis on measurements carried out in our laboratory, not that these are better than those obtained elsewhere but for comparative reasons.

The general formula of an organophosphorus compound that can inhibit acetylcholinesterase may be represented by



In this formula R_1 and R_2 are alkyl, alkoxy or amino groups; Z is oxygen or sulphur and X is a group that is split off in the reaction with the enzyme and is therefore called the "leaving group". In the process of inhibition the active site of the enzyme is phosphorylated in an irreversible way: recovery of enzyme activity does not occur or does so only at a very slow rate. The process of inhibition can be described by the following equation. [234]

$$E + I \underset{k_2}{\overset{k_1}{\rightleftharpoons}} [EI]_R \xrightarrow{k_p} [EI]_I$$

in which E is the enzyme, I the inhibitor, $[EI]_R$ an enzyme-inhibitor complex and $[EI]_I$ the irreversibly phosphorylated enzyme. The reversible step of the reaction depends on the affinity of the inhibitor for the active site of the enzyme and is determined by the dissociation constant $K_d = k_2/k_1$. The phosphorylation constant k_p is a measure for the rate of phosphorylation.

The most relevant data to obtain are obviously K_d and k_p. Unfortunately, however, only few data are available and fewer still for powerful anticholinesterases. Main [235–236] in a number of publications gives some data and we also obtained both constants for the reaction of stereoisomers of a V-type compound.

However, for the problem with which we are concerned, we may also use the bimolecular rate constant of the reaction

$$E + I \xrightarrow{k_i} [EI]_I$$

for which the relation $k_i = k_p/K_d$ in the case of a powerful inhibitor can be shown. [237] It is reasonably easy to determine k_i (for methods, see *inter alia* [238]).

In the following, we will use this rate constant as a measure for anti-acetylcholinesterase effects. In general, the rate of enzyme inhibition depends on a number of factors that can be grouped in two categories: (*a*) the strength of the P–X bond and (*b*) the interactions of the different parts of the organophosphorus compound with sites of the enzyme. The factors are of course interdependent.

For reasons of simplicity we will discuss the influence of the structure of group X and of groups R_1 and R_2 successively.

The influence of the structure of the "leaving group" X

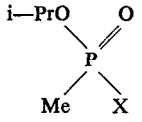
Some data on the rate of reaction of isopropyl methylphosphonates are shown in the table (taken from [238–239]). Only the rate constant of the faster reacting stereoisomer has been shown.

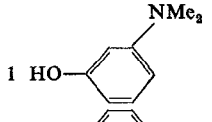
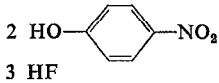
As indicated above, two factors can be distinguished: (*a*) the strength of the P–X bond, and (*b*) the interaction of group X with the enzyme.

(*a*) In general, the greater the strength of the P–X bond, the less reactive a compound will be in regard to reactions involving the breaking of this bond. This is a general effect which can be observed in both the hydrolysis and the rate of reaction with esterases. The strength of the P–X bond is related to the pK of the conjugated acid HX; the lower the pK_a of HX, the more reactive the organophosphorus compound will be. Thus we find that fluoridates are very reactive towards acetylcholinesterase whereas M-dimethylaminophenyl compounds are very unreactive. [238] The few experiments we carried out with azidates ($X = N_3$, pK_a $HN_3 = 4.7$) point in the same direction. A number of p-nitrophenyl compounds also show a reasonable rate of enzyme inactivation (pK_a p-nitrophenol = 7.0).

(*b*) The interaction of group X with certain sites of the enzyme is, of course, much more specific and will vary from enzyme to enzyme. Limiting ourselves to acetylcholinesterase, it is well known that this enzyme contains an anionic site which interacts with the cationic ammonium head of the substrate acetylcholine. If one introduces such a cationic head in the leaving group, a very high rate of inhibition of acetylcholinesterase is obtained [240] with a far more specific effect than with the compounds mentioned under (*a*). This rate is not in agreement with the above-mentioned dependence on the pK_a of the conjugated acid (see table VII.A.) and is therefore attributed to a favourable interaction of

Table VII.A. Bimolecular rate constants (M^{-1} $min.^{-1}$) of the inhibition of acetylcholinesterase by a number of compounds with the general formula:

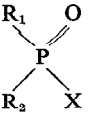


HX	pK_a of HX	k_I (25°·pH = 7.7)
1 	11.8	1.0×10^3
2 	7.0	7.0×10^5
3 HF	3.5	1.4×10^7
4 HS—CH_2—CH_3	10.4	5.4×10^1
5 HS—CH_2—CH_2—$CHMe_2$	10.6	7.1×10^2
6 HS—CH_2—CH_2—S—Me	9.6	1.5×10^4
7 HS—CH_2—CH_2—$\overset{\oplus}{S}$—Me_2	8.2	3.7×10^7
8 HS—CH_2—CH_2—NMe_2	8.0	1.0×10^7
9 HS—CH_2—CH_2—$\overset{\oplus}{N}Me_3$	8.2	5.3×10^7

this leaving group with the enzyme, probably the so-called anionic site. The importance of the charge can be seen by comparing the rates of compounds 4,5 and 6 on the one hand and compounds 7 and 9 on the other hand. Studies on the pH dependence have shown [238] that in the case of compound 8, only the protonated (charged) form reacts with acetylcholinesterase. That there is still a dependence on the strength of the P–X bond stems from the fact that the corresponding P–O–C-compounds do not show any anti-acetylcholinesterase effect whatsoever. Concerning the size of the groups on the nitrogen or the sulphur atom, we observed no great changes in the rate constants if the alkyl groups do not exceed a certain size. Concerning the number of carbon atoms between the thiol sulphur and the cationic head there seems to be an optimum between 1 and 4.

The cyano group as leaving group takes a special position. In phosphates and phosphonates the cyano group gives rise to extremely unstable compounds, but together with amido groups linked to the phosphorus atom, compounds with a fairly high anticholinesterase effect (Tabun) are obtained.

Summarizing the results discussed we may conclude that in the formula



compounds with

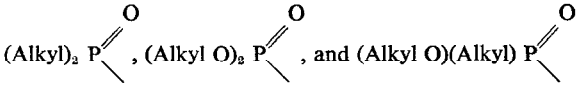
X = F, N_3, CN, S—$(CH_2)_n$—$\overset{\oplus}{S}R_2$, S—$(CH_2)_n$—NR_2 and S—$(CH_2)_n$—$\overset{\oplus}{N}R_3$

in general are very powerful anticholinesterases. The corresponding toxicity is in most cases also very high. These compounds have therefore to be regarded as potential CW agents with merely limited nonmilitary use.

The influence of the structure of the groups R_1 and R_2

If the influence of the structure of the leaving group X on the acetylcholinesterase inhibition rate is rather clear-cut, that of the structure of the groups R_1 and R_2, which remain bound to the central phosphorus atom in the process of the inhibition (we will not discuss here the subsequent process of ageing whereby one of these groups can be split off), is much more complex. The general outcome of our investigation, together with other results available, will be presented here.

First of all, we will distinguish among the following three groups



called phosphinates, phosphates and phosphonates, respectively.

The *phosphinates* are in general rather poor inhibitors of acetylcholinesterase (some of them, however, do inhibit other enzymes rather well) and fairly unstable.

The *phosphates* among them, for example, DFP give rise to rather good inhibitors with rate constants in the order of $10^5 M^{-1}$ min.$^{-1}$.

For CW agents, their potency seems to be too low, however.

The *phosphonates* comprise the group containing the most dangerous nerve agents, so a somewhat more detailed consideration seems to be in place.

Concerning the alkyl group directly bound to the phosphorus atom in the phosphonates, it seems that maximum rates are obtained with methyl groups and fairly high rates with ethyl groups. With larger alkyl groups the reaction rates drop off very rapidly. [238]

The structural requirements for the alkoxy group seem to be less stringent. There seems to be a maximum in the C_4–C_6 range. Very high rates are obtained with alkoxy groups containing a dialkylamino or a trialkylammonio group (so-called Tammelin compounds) [240] and with cycloalkyl groups. Rate constants of cycloalkyl methylphosphonofluoridates are all in the 10^8 range (M^{-1} min.$^{-1}$) from cyclopropyl to cyclooctyl. Also unsaturated alkoxy groups mostly give very effective cholinesterase inhibitors.

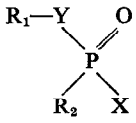
In general the substitution of thiols for the alcohols, giving phosphonothiolates, give somewhat less, but still some very potent inhibitors. [241]

The dialkylamido group has a somewhat peculiar position. In combination with an alkoxy group and the cyanogen group as the leaving groups, it gives rise

to compounds reacting rather rapidly with acetylcholinesterase and showing a correspondingly high toxicity (e.g., Tabun). [242] In some other combinations, rather unreactive compounds are obtained. The situation is certainly less clear than in the cases discussed earlier.

Finally, we have to consider the OH group. In the literature [243] it is stated that O-desalkylaction normally reduces anticholinesterase activity more than 100 000-fold but with certain tertiary amino-containing organophosphates the activity is only reduced 100-fold. As the tertiary amine compounds are the most active anticholinesterases known, the corresponding OH-containing compounds are still very active, a fact that was confirmed in our own studies.

Summarizing the results discussed, we may conclude that in the formula



compounds with Y = O or S; R_1 is alkyl, cycloalkyl, substituted alkyl or hydrogen and R_2 is alkyl or dialkylamino can give rise to compounds with high anticholinesterase rates and corresponding high toxicity, although not in every combination.

The compounds mentioned (with the X groups discussed earlier) have thus to be regarded as potential CW agents.

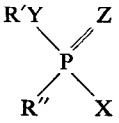
The influence of the P = Z group

Up till now we have discussed compounds containing a P = O group. It is, however, known that a number of compounds containing the P = S group are also toxic. Some of the compounds, virtually the P = S analogues of the nerve gas soman, are as potent inhibitors as the corresponding P = O compounds. [244] In other cases, the P = S compounds show a much lower inhibition rate than the corresponding P = O compounds but are still rather toxic because of a bio-oxydation to the corresponding P = O compounds. [e.g., 245] For this reason, we believe that certain organophosphorus compounds, containing the P = S group, may have applications as CW agents.

Conclusions

In the preceding paragraphs we have tried to establish some general rules according to which an organophosphorus compound behaves as a potent inhibitor of acetylcholinesterase. As with most structure-activity relationships, these rules should be regarded rather as tendencies and no firm predictions are possible (although some quantitative predictions turned out surprisingly well). [238] It is therefore impossible, using a general formula only, to delimitate

the potential nerve agents. This formula must be used in conjunction with a toxicity criterium which should be established by toxicological experts. For the general formula we propose:



in which

Y = O or S
Z = O or S
X = F, CN, N_3, $S-(CH_2)_2-\overset{\oplus}{S}R'''_2$, $S-(CH_2)_n-NR'''_2$
or $S-(CH_2)_n-\overset{\oplus}{N}R'''_3$
R′ = (substituted) alkyl, cycloalkyl or hydrogen
R″ = Alkyl
R‴ = Alkyl

In combination with a carefully selected toxicity criterion, we consider the delimitated group of compounds to include very few compounds which are used as pesticides, whereas the majority of compounds may be used as CW agents. This group could therefore be liable to an unconditional prohibition of production and stockpiling, or be subjected to special verification.

VIII. *Some aspects of the organophosphorus industry in Japan, by T. Osa and Y. Fukushima*

Summary

The paper includes a flowsheet of the consumption pattern of elemental phosphorus in the organophosphorus industry in Japan based on the newest data available. In 1969, production of yellow phosphorus amounted to 26 600 tons, and that of phosphorous chlorides to 10 300 tons (sum of the production of PCl_3, PCl_5 and $POCl_3$). The total capacity of three companies which produce yellow phosphorus amounts to 38 000 tons per year. The intermediates to organophosphorus compounds produced by eight companies in Japan are: PCl_3 by four companies; PCl_5, three companies; $POCl_3$, five companies; red phosphorus, two companies; P_2S_5, three companies; and H_3PO_4, four companies.

Thirteen organophosphorus compounds (8 700 tons in total), derived from phosphorus trichloride, phosphorus oxychloride and phosphorus pentachloride, are produced for agricultural use by nine companies. According to the tendency to require organophosphorus compounds of lower toxicity, the production and sales of Parathion and Tepp have ceased since the end of 1969. Twenty-five hundred tons of tricresyl phosphate (TCP) and 450 tons of a similar phosphate were produced as plasticizers for polyvinyl chloride. The amount of phosphorus oxychloride consumed was 570

tons. TNP is the most common phosphate type of stabilizer and is produced by seven companies (2 500 tons). Four hundred tons of phosphorus trichloride were consumed in preparing approximately 2 000 tons of an inorganic stabilizer, dibasic lead phosphite. Phosphorus trichloride (100 tons) and phosphorus sulphide (90 tons), phosphorus oxychloride (1 290 tons) and phosphorus sulphide (not estimated) were consumed for medical uses. Six hundred and forty tons of phosphorus trichloride were consumed to synthesize dyestuffs of Napthol AS (950 tons), BS (100 tons) and SW (80 tons). In addition to these compounds many other organophosphorus compounds are utilized, but commercial competition among the companies makes it difficult to get statistical data.

There are many types of equipment treating the organophosphorus compounds. The plants producing agricultural chemicals are usually equipped with air-tight (sealed), continuous and automatic systems. On the other hand, the plants producing plasticizers, stabilizers or additives for lubricating oil and gasoline only need equipment used by ordinary chemical plants. In some cases they adopt the batchwise processing system and, in such plants, special precautions must be taken to avoid exposing personnel to the chemical agent.

In the plants producing organophosphorus compounds in Japan, precautionary measures are taken to prevent possible accidents and especially to maintain the health of the personnel. Some medical criteria are recommended: in particular, that a criterion be adopted of a 30 per cent drop of the cholinesterase activity in a worker's plasma.

Introduction

The phosphorus industry in Japan, which began in 1913 with the manufacture of yellow phosphorus in electric furnaces, has since developed remarkably, producing various phosphorus compounds such as sodium tripolyphosphate for synthetic detergents, phosphoric acid for metal surface treatment or phosphorus halides and phosphorus sulphides for organophosphorus products. The direct raw materials in the organophosphorus industry are usually phosphorus trichloride, phosphorus pentachloride, phosphorus oxychloride and phosphorus pentasulphide derived from yellow phosphorus, and these compounds are transformed into final products after many reaction steps with various organic reagents. Final products in this industry are so-called "fine chemicals" such as agricultural chemicals, plasticizers, stabilizers for plastics, medicines, dyestuffs, additives for lubricating oil and gasoline and non-flammable or fire-retardable polymers. *In Japan, no organophosphorus compounds for chemical weapons have ever been produced.* Table VIII.A shows the transition of production amounts of yellow phosphorus and phosphorus chlorides.

Clearly, many kinds of organophosphorus products are now available and, furthermore, many new products are being produced commercially every year. The structure or composition as well as the manufacturing process of a new product is usually kept secret because enterprises in the organophosphorus industry are often not large in Japan and compete strongly with each other.

Table VIII.A. Production amounts of yellow phosphorus and phosphorus chlorides

tons

Year	Yellow phosphorus	Phosphorus chlorides[a]
1959	4 500	3 100
1962	12 600	4 300
1965	14 700	8 100
1969	26 500	10 300

[a] Figure shows the summation of production amounts of PCl_3, PCl_5 and $POCl_3$.

Therefore, a grasp of accurate information is very difficult. Chart VIII.A. shows the intermediates derived from elemental phosphorus and the application of each intermediate together with the amounts consumed and the percentages based on yellow phosphorus in 1969. The amounts consumed based on each intermediate are also shown in this chart. Utilization of red phosphorus, pentaoxide and phosphoric acid for inorganic chemicals are omitted from the chart.

Production and stockpiles of phosphorus chlorides of trichloride, pentachloride, oxychloride and phosphorus pentasulphide have been reported to the government every year. Production and shipping statistics of agricultural powders and liquids containing organophosphorus chemicals are also reported, because every company which produces and sells these commercial goods is required to submit to the law stipulating registration of agricultural materials. Thus, statistical data of organophosphorus compounds for agricultural uses can be estimated from the above amounts. Some published data on uses of organophosphorus compounds other than in agriculture have been collected by industrial associations. This category covers products for which the manufacturing processes employed by each company are well established and have been published. Examples are tricresyl phosphate (TCP), napthol dyestuffs, and so on.

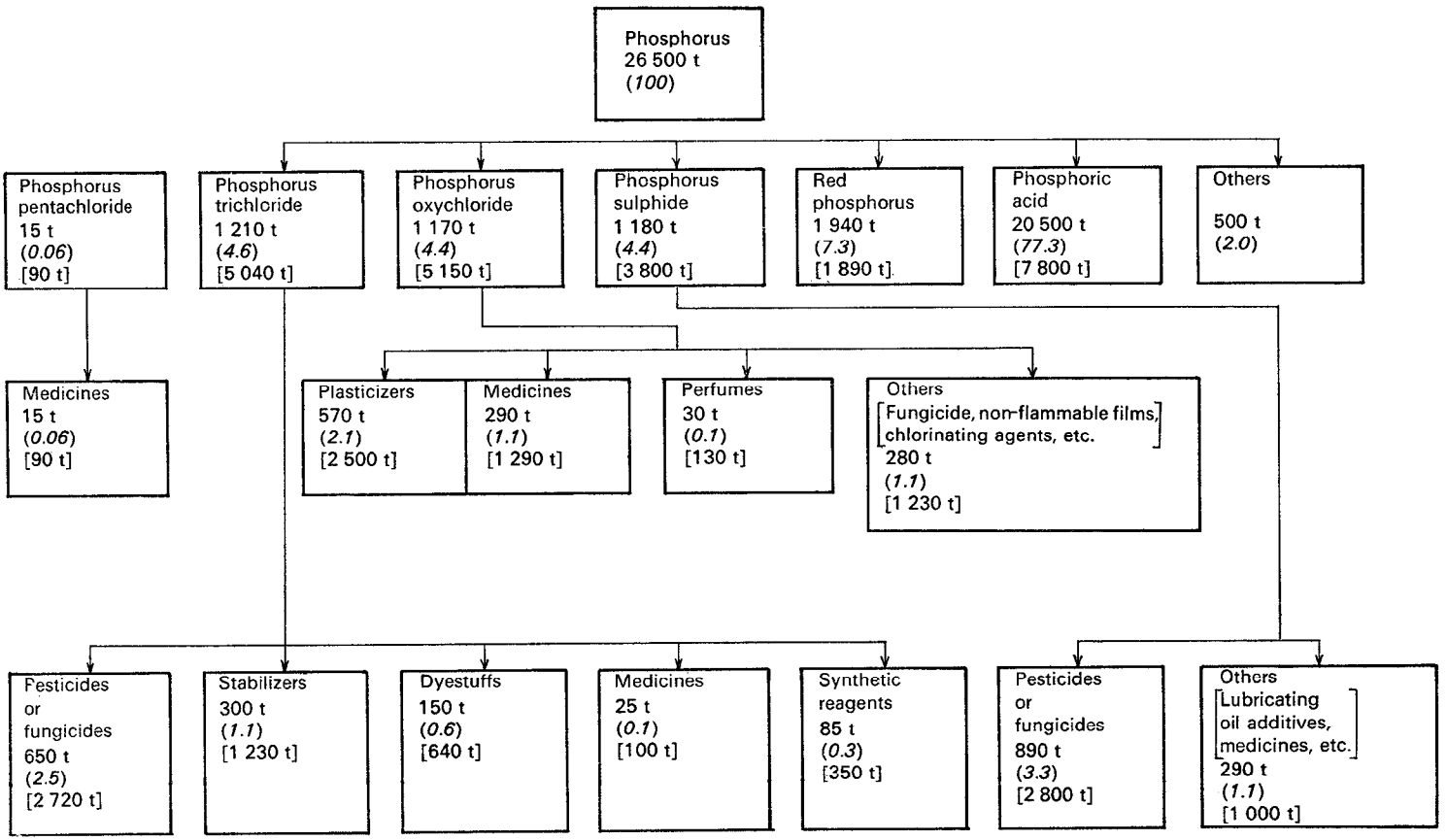
Production of intermediates to organophosphorus compounds

Seven companies manufacture phosphorus trichloride, phosphorus pentachloride, phosphorus oxychloride and phosphorus pentasulphide from yellow phosphorus as shown below:

Phosphorite rock → Yellow phosphorus A, B, C,
- → PCl_3 : A, D, E, F
- → PCl_5 : A, D, F
- → $POCl_3$: A, D, E, F, G
- → Red phosporus : A, B
- → P_2S_5 : A, B, C
- → H_3PO_4 : A, B, C, H

The name of each company is given here alphabetically from A to H. The production capacity for a group of three companies of yellow phosphorus by

Chart VIII.A. Consumption pattern of elemental phosphorus for organophosphorus industry, 1969[a]



[a] Items in parentheses are the per cent of elemental phosphorus consumed in each application; items in square brackets are the amount of each intermediate consumed in each application.

the electric furnace method from phosphorite rock is 38 000 tons a year for 1969 (unless otherwise stated, the table shows the amount for 1969).

Phosphorus trichloride is produced by the reaction of yellow phosphorus and chloride. The production capacity of each company is *A: 200, D: 700, E: 200, F: 200* tons/year, respectively. Phosphorus pentachloride is produced by the chlorination of phosphorus trichloride. Phosphorus oxychloride is produced mainly by the oxidation of phosphorus trichloride and partly by the reaction between phosphorus pentachloride and phosphorus pentaoxide. Capacities of phosphorus pentachloride, phosphorus oxychloride and phosphorus sulphide are not published. Phosphorylation (esterification), transesterification, halogenation, alkylation, oxidation and redistribution are sometimes used as basic reactions of industrial organophosphorus chemistry. Phosphorus trichloride is most reactive in the above-mentioned intermediates and can be changed to the subsequent important intermediates such as phosphorus trichloride, dialkyl phosphite, dialkylphosphoric chloride, lower trialkyl phosphite and phosphonitrile chloride. Some of these intermediates may be available commercially; however, statistical data concerning the production and the consumption of these compounds have not been published. Mainly, lower diakyl phosphites of dimethyl, diethyl, diisopropyl and dibutyl are produced and the production amount was about 2 000 tons calculated from the consumption of phosphorus trichloride. Dimethyl phosphite is used for DDVP as pesticide and diisopropyl phosphite is used for IBP as fungicide in large quantities (DDVP and IBP will be shown later). Higher triakyl phosphites or triaryl phosphites are not toxic and become final products. This (nonylphenyl) phosphite (TNP) is produced in a large quantity as a stabilizer of polymers. The consumption of phosphorus trichloride was estimated to be 850 tons for TNP.

Industrial organophosphorus chemicals

The following classification is possible for uses of industrial organophosphorus products.

Agricultural organophosphorus chemicals

The organophosphorus chemicals for agricultural use which were produced or imported in 1969 are shown in table VIII.B. Thirteen organophosphorus compounds (8 700 tons in total) derived from phosphorus trichloride, phosphorus oxychloride and phosphorus pentasulphide are produced for agricultural use by nine companies and become marketable goods in the form of solution, emulsion, smoke or dust diluted by liquid or solid, or mixed with other agricultural materials. There are more than nine companies making the marketable goods. As a great deal of social attention has been paid to the harm caused

Table VIII.B. Agricultural chemicals in organophosphorus compounds produced and imported in 1969

tons

Agricultural chemicals		Amounts		
Name	Structure	Production	Import	Remarks
	Raw material of PCl_3			
EPN	0-ethyl-0-nitrophenyl-phenyl-phosphonothioate	724	171	P+1, 2+3
CYP (Surecide)	p-cyanophenyl-ethyl-phenyl-phosphonothioate	43		P, 1
Salithion	2-methoxy-4H-1,3,2-benzodioxa-phosphorin-2-sulfide	63		P, 1
DEP (Dipterex)	0,0-dimethyl-1-hydroxy-2,2,2-trichloroethylphosphonate		300	P, 2
DDVP (Dichlorvos)	0,0-dimethyl-0-2,2-dichloro-vinylphosphate	622	21	P, 3
ESBP (inezine)	S-benzyl-0-ethylphenylphospho-nothiolate	46		F+2, 1
IBP (Kitazin)	0,0-diisopropyl-S-benzylphos-phate	1 660		F, 1
	Raw material of $POCl_3$			
EDDP (Hinozan)	0-ethyl-S,S-diphenylphosphate	700		F, 1
	Raw material of P_2S_5			
MEP (sumithion)	0,0-dimethyl-0-(3-methyl-4-nitro-phenyl)-phosphorothioate	3 106		P, 1
Diazinon	0,0-dimethyl-0-(2-isopropyl-6-methyl-4-pyrimidinyl)phosphorothioate	579	301	P, 1
MPP (Fenthion)	0,0-dimethyl-0-(3-methyl-4-mer-captophenyl) phosphorothioate		640	P,
Malathion	0,0-dimethyl-S-(1,2-dicarbethoxy) ethylphosphorodithioate	270		P, 1
i-System	0,0-diethyl-S-(2-ethylthioethyl) phosphorodithioate		280	P,
Dimethoate	0,0-dimethyl-S-(N-methylcarbamyl-methyl) phosphorodithioate	340		P, 1
PAP (Cidial)	0,0-dimethyl-S-[α-(ethoxycarbonyl) benzyl] phosphorodithioate	533		P, 1
PMP (Imidan)	0,0-dimethyl-S-(phthalimidomethyl) phosphorodithioate	35		P, 1

+1: P = pesticide.
+2: F = fungicide.
+3: Number of companies producing the chemicals.

to man and livestock and the problem of poisonous materials residued in agricultural products resulting from the use of chemicals, only organophosphorus compounds of lower toxicity tend to be used. *Thus, higher poisonous pesticides of Parathion and TEPP have been eliminated from production and sales since the end of 1969.*

Recently, chemical companies manufacturing versatile products or companies mainly concerned with blending agricultural chemicals for marketable

goods, have been stimulated by the expansion of fine chemicals to branch out into the synthesis of pesticides or fungicides.

Plasticizers

Twenty-five hundred tons of tricresyl phosphate (TCP) and 450 tons of similar phosphates were produced as plasticizers for polyvinyl chloride. The amount of phosphorus oxychloride consumed was 570 tons.

Stabilizers

Phosphites substituted by triaryl, higher trialkyl or a mixture of aryl and higher alkyl are used as stabilizers for polyvinyl chloride or polyolefins. The synthetic films of polymer containing these stabilizers are employed as wrappings for foodstuffs because of their non-toxicity. TNP, mentioned before, is the most common phosphite type of stabilizer and is produced by seven companies (2 500 tons). An inorganic stabilizer of dibasic lead, phosphite is mainly produced from phosphoric acid. One of seven companies produces it from phosphorus trichloride. Four hundred tons of phosphorus trichloride were consumed in preparing about 2 000 tons of dibasic lead phosphite.

Medicines

Phosphorus trichloride (100 tons), phosphorus pentachloride (90 tons), phosphorus oxychloride (1 290 tons) and phosphorus sulphide (not estimated) were consumed for medical uses. Medicines such as Vitamin B_1, Procaine hydrochloride, Acrinol, Cinnipirine, Bisatin, Quinophan and Bromovalerylurea do not contain a phosphorus element in their structures but are obtained *via* intermediates in the reaction with phosphorus chlorides, such as chlorination. On the other hand, there are some medicines formulated with calcium glycerophosphate containing phosphorus in the structure.

Dyestuffs

Six hundred and forty tons of phosphorus trichloride were consumed to synthesize Naphthol AS (950 tons), Naphthol BS (100 tons) and Naphthol SW (80 tons). However, these naphthol dyes show a decreasing tendency since there is no production increase of natural fibres or replacement of them into other reactive dyes.

Miscellaneous organophosphorus products

As well as the above mentioned, many organophosphorus compounds are being produced for wide utilization. Metal dithiophosphates (usually zinc) and metal phosphites of the alkyl or aryl group are used as lubricating oil additives, and various organic phosphates are used as gasoline additives since they act as

deposit modifiers in engine cylinders. Several companies producing these compounds have not published the detailed figures.

In the field of polymers, the treatment of polymers with organophosphorus compounds improves properties of non-flammability or fire retardation. Synthetic resins and fibres reacted with organophosphorus compounds and woods treated with organophosphorus liquid are commercialized for this purpose. Strong competition in this field has given rise to secrecy noticeable in publications relating to the actual situation, but this field is expected to develop considerably in the near future. Various kinds of organophosphorus intermediates show great promise, for instance, lower dialkyl phosphite and methylphosphonic dichloride which are also potential intermediates for nerve agents of V-type.

Equipment used in the organophosphorus industry

The most careful attention has been paid to toxicity in the production and treatment of pesticides and fungicides because, in the present organophosphorus industry, pesticides or fungicides are usually toxic for mammals, although they are much less toxic than the earlier pesticides or nerve agents. The plants producing agricultural chemicals are usually equipped with air-tight (sealed), continuous and automatic systems; a typical plant is one producing Sumithion. However, such an operation does not take place with a lower internal pressure in the reaction apparatus than atmospheric pressure, or directed by completely remote control as is assumed to be the case in plants producing nerve agents. Purification treatment is usually carried out in the liquid phase of distillation or extraction. Poisonous solid products of agricultural chemicals are isolated from the crude reaction products only by filtration or are directly commercialized by the dilution of crude reaction products with water, solvents, powders, and so on.

On the other hand, only the raw materials of phosphorus trichloride, phosphorus pentachloride, phosphorus oxychloride and phosphorus pentasulphide are poisonous and corrosive in the plants producing plasticizers, stabilizers or additives for lubricating oil and gasoline. These plants therefore do not need any different equipment for operations other than that used by ordinary chemical plants.

Generally speaking, however, the organophosphorus industry belongs to the field of fine chemistry. The fine chemical industry is characterized by its multiple and complex reaction processes and by its smaller scale of production. Thus, in some cases batchwise processing has to be adopted and a reactor has to be used for the production of several kinds of compounds. In such a plant, special precautions have to be taken to avoid exposing personnel to the chemical agent.

Precautionary measures adopted to prevent possible accidents in the organophosphorus industry

Plants producing organophosphorus compounds in Japan have made great efforts to maintain the health of the personnel. The government has also adopted a guidance policy for the health of workers in factories producing or using organophosphorus compounds. Under this policy a method of medical examination is proposed which is used as a criterium for diagnosing such symptoms as a decline in the level of activity of the cholinesterase in the blood, sudoresis or excessive perspiration, myosis or contraction of the pupil and muscular fibrillation of the eyelids and face. In particular, in cases where the activity level of the cholinesterase in the plasma of a worker is found to have dropped by 30 per cent or more, he should be transferred to another job or given a certain period of rest until the level of activity of the cholinesterase in his plasma returns to normal. The recent survey shows that there are few workers who exhibit decreases in their level of cholinesterase activity in the plasma greater than the criteria described above allow.

In the autumn of 1970, all chemical factories were subjected to a governmental enquiry. This concerned production capacity and production results of compounds composed of fire-hazard chemicals, gases in pressure tanks, toxicants and explosive materials together with plant disposition manufacturing processes. It was undertaken in order to prevent accidents at chemical factories and to further the sound development of the chemical industry. The results of the investigation have not been analysed. This kind of investigation is very useful but the publication of the results is hampered owing to secrecy imposed by manufacturers.

Conclusions

The organophosphorus industry in Japan will develop further in the future with increases in production quantities and types of organophosphorus compounds. However, knowledge of the exact aspects of the phosphorus industry is no easy matter apart from those aspects which the government requires to be made public, that is, statistical data.

The close attention paid to toxicity applies to the production and treatment of organophosphorus compounds for agricultural use but not for other uses, since such organophosphorus compounds are usually not toxic. In the latter case, only those raw materials used for manufacturing phosphorus chlorides and phosphorus sulphides that are designated as toxicants are subjected to consideration. There are no particular differences between industries producing

these substances for non-agricultural purposes and chemical industries producing dyestuffs, medicines, perfumes or surface-active agents.

In protecting the health of the workers from toxicity, it is customary to pay greater attention to the equipment used in plants producing poisonous substances such as hydrogen cyanide, phosgene, ethyleneimine, etc.

IX. *Production of organophosphorus chemical warfare agents, by A. R. Pittaway*

Summary

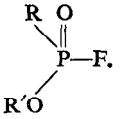
The primary production process routes for Sarin-type nerve agents are presented. To make other members of the Sarin family, it is necessary only to change the input alcohols and metal alkyls. To make other basic structures, which might require amination, thio-esters or oxidation by sulphur, it is usually necessary to re-arrange the order of the processes as well as change the input chemicals. Of course, a few additional types of process reactions are required when other families of agents are to be produced.

However, the set of production process methods is finite for any basic agent structure or any specific agent. An inspector's handbook could be developed based on current production technology to serve as a checklist for on-site inspections. The most advantageous production process route is a function of the quantity of material to be produced and the availability of required raw materials. A combined knowledge of the processing requirements and of the chemical economy of the country involved, will serve to focus attention on that set of processes which are most probably involved in any given country's production activity at any one time.

Introduction

A general discussion of the problem of inspecting production facilities was presented in my paper at the 1968 SIPRI Symposium. Subsequent discussions have disclosed that it would be useful to have more information on the production processes themselves to illustrate the scope of the inspection problem. In this paper I will attempt to present some additional information on this subject.

In the earlier paper it was stated that the total number of production routes for any given agent structural type could be theoretically specified, as well as the general chemical structure of all intermediates. A chart of production routes can be generated for each general agent structural type and the chart will be specific for production of that type material. For example, the general structure of Sarin type agents is:



To produce any agent belonging to this family requires execution of at least four unit processes, oxidation, halogenation, alkylation and esterification. Since four processes are involved and they can theoretically be performed in any order, there is a total of 24 different sequential paths to production of any agent in this family. At this point, if we apply the limits imposed by known types of chemical reactions, the number of reasonable paths is reduced to about 10.

We can construct such a set of paths for each structural type, with the number of theoretical and possible paths depending on the number and type of unique groups to be attached to the phosphorus atom.

There are at this point two remaining problems: (*a*) determination of the number of basic structures of interest and (*b*) conversion of the predicted routes into some concept of the actual process chemistry. I will not attempt to solve this first problem. Two basic structures (the Tabun and Sarin family structures) are discussed in the literature and several more can be projected from toxicity data available on isolated compounds published in the open literature. I offer the personal opinion that the problem may solve itself if agreements between governments are achieved to limit CW agent production. My opinion is based on the observation that one country would normally seek to obtain prohibitions against production in a second country of agent types it itself knew were important, and surely this release of information would at least extend to identification of the primary bonds around the phosphorus atom. The second problem, conversion of predicted routes into some concept of the actual process chemistry will be the subject of the remainder of this presentation. Manufacture of structures similar to Sarin will be used to illustrate both the chemistry and the methodology.

However, let me make one further point before I present some of the process chemistry. It is useful to make a distinction between possible and practical production routes. A practical route is dependent on the quantity of material to be produced and the resources available. Available resources are defined in terms of: availability of raw materials in sufficient quantity; availability of equipment; cost of production; and waste disposal problems. These practical considerations further constrain the number of routes which might be used by a given country at a given time. Chemical economics and the quantity required to achieve a national capability will play a large part in predicting the most probable routes a given country will use as a production process.

The known chemistry for conduct of the five unit processes required to produce any currently identified phosphorus poison is finite and can be specified. My knowledge in this particular field of chemical processing may not be current and I request the assistance of all who are interested to fill in my omissions. However, I will make an attempt to discuss a number of key reactions. My plan is to present known chemistry for each unit process of interest followed by

a listing of how the chemistry of a given unit process affects sequential linkages to other unit processes. I will conclude with a few examples of linked processes which produce overall production sequences.

Process chemistry

Alkylations

Alkylation is one of the most important processes associated with agent production. For present purposes alkylation is defined to mean the attachment of an unsubstituted alkyl carbon group to a phosphorus atom. Some of the principal methods are: Michaelis-Arbuzov reactions involving alkyl chlorides; reactions involving aluminium chloride; reactions with metal alkyls (Grignard); thermal rearrangement; and high temperature reactions with aliphatic hydrocarbons.

Michaelis-Arbuzov reactions involving alkyl chlorides

There are three noteworthy considerations about these reactions: (*a*) they are conducted on trivalent phosphorus following esterification with aliphatic alcohols; (*b*) they combine two of the required unit processes, alkylation and oxidation; and (*c*) the esterification reaction which precedes this process may or may not be one of the required primary unit processes in the agent production route.

There are two types of reactions with alkyl halides involved. Triesters react in this manner:

$$P(OR)_3 + R'X \rightarrow R'\overset{\overset{O}{\|}}{P}(OR)_2 + RX$$

where X is a halogen and R′ is an alkyl group. Esters of alkylphosphonic acids can be alkylated with alkyl halides if first converted to their sodium salt:

$$(RO)_2POH + Na \rightarrow (RO)_2PONa + 1/2H_2$$

$$R'X + (RO)_2PONa \rightarrow R'\overset{\overset{O}{\|}}{P}(OR)_2 + NaX$$

when X can be a halogen atom or a sulphate group.

In a typical process the diester or mixtures of the di- and triphosphorus esters are reacted with molten sodium to form the sodium salt of the diester. The triester is unaffected. This mixture is mixed with the appropriate alkyl chloride and reacted under pressure to produce product dialkyl methyl phosphonate. Reducing the pressure allows the alkyl halide to flash off and the product-salt slurry is separated by distillation.

This type reaction is one of the best available from a manufacturing viewpoint if sufficient quantities of the proper alcohols and sodium metal can be made available.

Reactions involving aluminium chloride

Phosphorus trichloride will react with methyl chloride in the presence of aluminium trichloride:

$$PCl_3 + CH_3Cl + AlCl_3 \rightarrow CH_3PCl_4 \cdot AlCl_3$$

in which the product is obtained as a complex salt. The complex can be broken with the addition of water forming methyl phosphonic dichloride:

$$Cl_3PCH_3 \cdot AlCl_3 + H_2O \rightarrow Cl_2\overset{\overset{O}{\|}}{P}CH_3 + AlCl_3 \cdot H_2O + 2HCl$$

The product can be removed by filtration and purified by distillation. Breaking the complex with other materials in the absence of water will produce methyl phosphonous dichloride (CH_3PCl_2).

Reactions involving metal alkyls

The typical Grignard reaction can be used to alkylate pentavalent phosphorus compounds such as phosphorus oxychloride ($POCl_3$).

$$3CH_3MgCl + POCl_3 \rightarrow Cl_2P(O)CH_3 \cdot MgCl_2$$

The complex formed can be broken by high temperature distillation at low pressure.

Also of interest are reactions with methyl aluminium chlorides and phosphorus trichloride. The reaction of aluminium with methyl chloride produces:

$$3CH_3Cl + 2Al \rightarrow CH_3AlCl_2 + (CH_3)_2AlCl.$$

Reacting the product mixture with PCl_3 produces:

$$CH_3AlCl_2 + (CH_3)_2AlCl + 3PCl_3 \rightarrow CH_3PCl_2 + 2CH_3PCl_2 \cdot AlCl_3$$

The complex with $AlCl_3$ can be broken with sodium chloride.

Thermal rearrangement

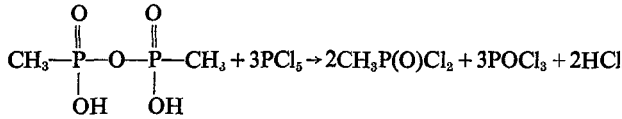
Heating an organophosphorus ester causes condensation and thermal rearrangement as follows:

$$(CH_3O)_2POH + \text{heat} \rightarrow CH_3P(O)(CH_3O)OH$$

$$2CH_3P(O)(CH_3O)OH \rightarrow CH_3\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}O\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}CH_3 + (CH_3)_2O$$

The reaction product is a mixture of the two materials. Examination will show that this reaction completes two of the required processes, alkylation and oxidation. Chlorination with PCl_5 renders the compound available for further processing:

$$CH_3P(O)(CH_3O)OH + PCl_5 \rightarrow CH_3P(O)Cl_2 + CH_3Cl + 2POCl_3 + HCl$$



$$CH_3{-}P(O)(OH){-}O{-}P(O)(OH){-}CH_3 + 3PCl_5 \rightarrow 2CH_3P(O)Cl_2 + 3POCl_3 + 2HCl.$$

High temperature reactions with aliphatic hydrocarbons

According to patents of the Shell Oil Company, aliphatic hydrocarbons react with PCl_3 at moderate pressure, at temperatures of the order of 1 000°F, in this manner:

$$PCl_3 + CH_4 \rightarrow CH_3PCl_2 + HCl.$$

The yields are not quantitative and the separation of excess PCl_3 from CH_3PCl_2 would require an extensive fractional distillation system due to the similarity in boiling points.

Esterification

All phosphorus poisons contain at least one ester group. The reactions to produce an oxygen ester are uniformly similar regardless of the phosphorus chloride intermediate used: the reactions vary principally in their rate. Consequently the types of reactions discussed can generally be extended to other alcohols.

If the first principal process in the agent production sequence is to be esterification, then the alcohol used will be the one needed to produce the proper ester in the agent. However, if the alkylation reaction is to be by thermal rearrangement or reaction with an alkyl halide, then the esterification will probably be with cheap, readily available methyl alcohol, if large quantities are to be produced. If the agent requires an ester of a higher alcohol than isopropyl, it is improbable that esterification with that alcohol will be the first principal process because of cost and material availability.

The greatest savings in alcohol occur when esterification is the final process in the sequence, or the last process prior to oxidation. Consequently, as the quantities to be produced and the size of the ester group increase, the probability of the principal esterification being the last step in the sequence also increases. It also follows that if oxidation is the last step under these conditions, the reaction sequence will be alkylation, fluorination, esterification, oxidation, and the alkylation will not be by either the Michaelis-Arbuzov or the thermal rearrangement methods.

Reactions with PCl_3

Aliphatic alcohols react practically instantaneously with PCl_3 to form the triester. Unfortunately, the presence of HCl causes the product to degrade quickly

to the diester and ultimately to the monoester, producing the alkyl chloride as a by-product. Since the triester is usually the most desirable product in order to reduce processing costs, the production problem becomes a trade-off in production economics.

There are three methods for process control: low pressure operations, conversion of the alcohol to a metal-alcoholate to prevent HCl formation, and use of a scavenger such as sodium metal or an amine to react with the HCl as it is formed. Significant yield of the triester cannot be obtained with the low-pressure approach. Use of the metal-alcoholate is expensive and only produces about 70 per cent triester yields with a 15 per cent pyro-phosphite by-product. Use of amine bases allows high yields of triesters.

Reactions of methyl phosphorus dichloride with alcohol

The reactions between CH_3PCl_2 and alcohols are similar to the PCl_3 reactions except that the esters formed are more stable and less subject to degradation by the by-product HCl produced.

Reactions of methyl phosphonic dihalide and alcohols

Reactions with $CH_3P(O)Cl_2$ occur at a slower rate and are easier to control. Under the proper operating conditions it seems probable that selective replacement of the chlorine could be accomplished, thus producing an intermediate ready for the final process of fluorination.

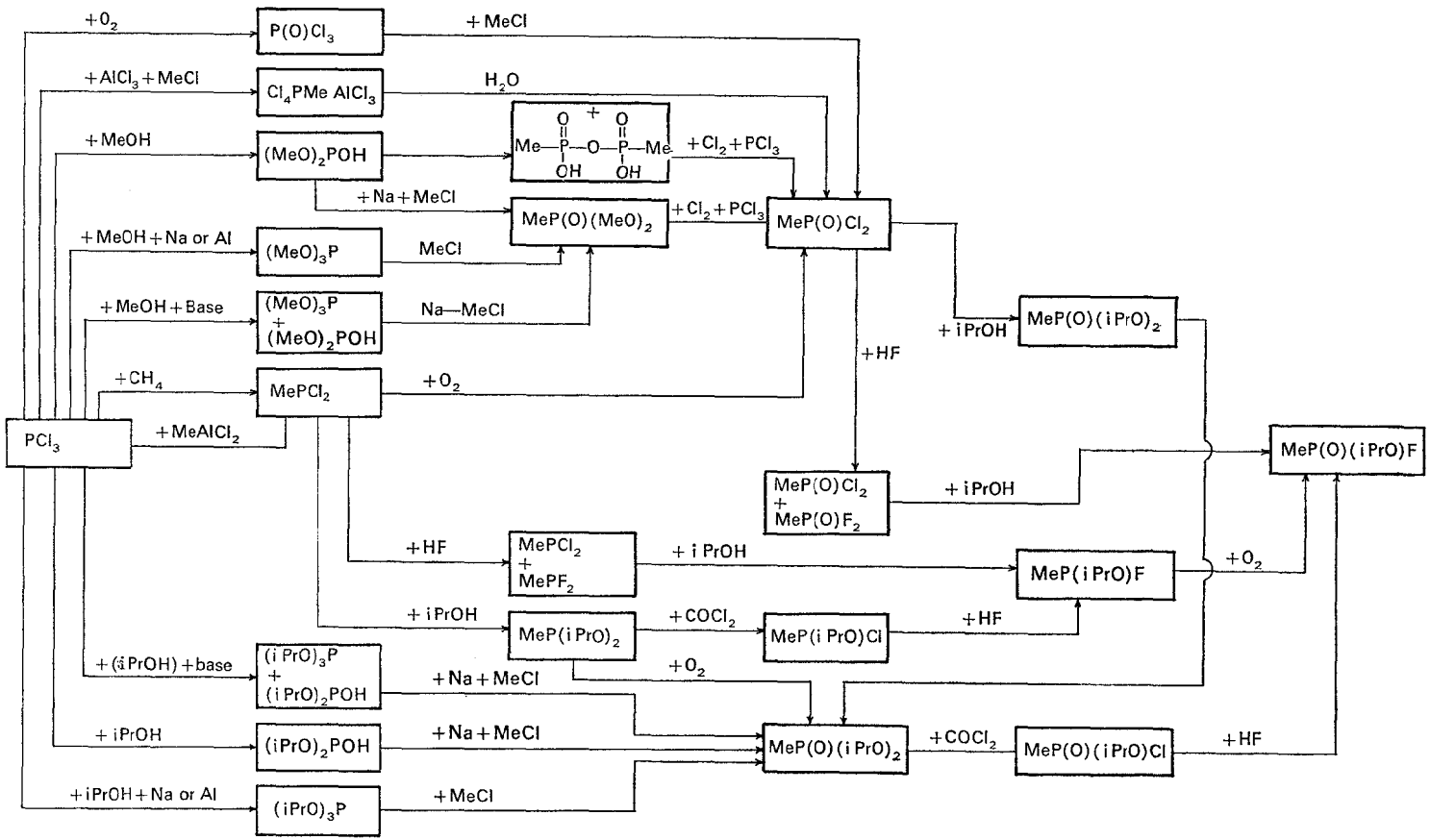
Oxidations

Oxidations of phosphorus intermediates are conducted in two ways: (*a*) as part of an alkylation process and (*b*) directly with oxygen gas. Oxidations in combination with alkylations have already been discussed. When necessary, a trivalent phosphorus compound can be oxidized by direct exposure to oxygen gas when proper precautions are taken to control the temperature rise. High dilutions are recommended.

Fluorinations

Fluorine will replace chlorine attached to phosphorus spontaneously. In addition, with a dichloride the replacement of one chlorine atom increases the ease of the second replacement, the result being that unless sufficient fluorine is added to remove all chlorine, the resultant product will be a mixture of difluorides and dichlorides. However, when an alcohol is introduced into such a mixture, the system reapportions itself, eliminating the chlorine as HCl, and distributing the fluorine among all the organophosphorus molecules.

Chart IX.A. Production process schematic



Production sequences

Chart IX.A illustrates how the individual processes discussed in the previous section can be linked together to form production schemes. Ten sequences of the four principal processes are represented. A word of explanation is now in order to define the difference between a principal process to attach a molecular structure which is a component of the final agent structure, and an auxiliary process. An auxiliary process is one which enables a principal process to be carried out in a subsequent step. In chart IX.A, these are the chlorinations and the methyl alcohol esterifications.

While the number of paths are limited, the number of methods to execute reactions required by those paths are numerous. A given sequence of these reactions creates a production process characterized by its own set of economics, equipment requirements, waste and by-product streams. The differences in chemical raw material requirements and overall product costs can be quite large.

It is possible to define the items of equipment that would be needed to conduct any one of these processes, as well as to identify the input and output streams to and from the equipment, including the waste products. Production of each one of these intermediates requires a particular set of apparatus to conduct the reactions as well as to separate the by-products from the products. Consequently, the set of equipment in terms of reactors, type distillation columns, filters, internal and external cooling requirements, operating temperatures and pressures, as well as stream line contents can be used to establish a fingerprint for the reaction.

By-products and wastes

1. Alkylations

There are significant differences in the quantities and types of by-products and wastes produced in the various forms of alkylation reactions. The thermal rearrangement produces dimethyl ether, hydrogen chloride and approximately 3 1/2 pounds of phosphorus oxychloride per pound of Sarin.

The aluminium trichloride catalyzed reaction with methyl chloride produces about 5 pounds of hydrate cake ($AlCl_3 \cdot H_2O$) per pound of $CH_3P(O)Cl_2$ produced. Also lost is about a pound of methylene dichloride process coolant per pound of product.

The methyl aluminium chloride process produces about 2 pounds of salt mixture per pound of CH_3PCl_2 intermediate. It also results in loss of all the input aluminium and one-third of the input chloride in unrecovered waste.

The alkylations involving either the Michaelis-Arbuzov or thermal rearrange-

ment eventually result in the production of 2 moles of alkyl chlorides per mole of final product.

The high temperature alkylation should not produce significant quantities of by-products or wastes.

2. Esterifications

The use of metal alcoholates or ammonia as a base to reduce ester degradation by HCl produces large quantities of salts. In many esterifications HCl is produced in large quantities. When esterification is conducted early in the production sequence, subsequent processing will result in an alkyl chloride by-product whose alkyl group is identical to the ester group on the agent.

3. Oxidation and fluorination

Direct oxidations with O_2 do not produce significant amounts of by-products. Fluorinations are usually conducted to replace chlorine which usually produces an HCl waste stream.

Materials of construction

Handling HCl off-gases, particularly if heated, requires highly corrosion-resistant equipment. The direct alkylation processes have more severe corrosion problems than the processes which combine alkylation with oxidation.

Esterification reactions frequently involve the HCl corrosion problem. However, when a scavenger is used to tie up the chlorine, the processing can for the most part be conducted in ordinary steel equipment.

Oxidation reactions with oxygen do not of themselves create a corrosion problem. Fluorination reactions involve HCl contaminated with HF which creates one of the most severe corrosion problems in the process sequence and requires the most resistant material available to handle.

Conclusions

In concluding this discussion of agent production, I would like to point out that the principles discussed here apply to most processing associated with agents. To make other members of the Sarin family, it is only necessary to change the input alcohols and metal alkyls. To make other basic structures which might require amination, thio-esters or oxidation by sulphur, it is for the most part only necessary to rearrange the order of the process and to change the input chemicals. A few more types of reactions will be added, of course.

However, the set of methods is finite for any basic structure or any specific agent. An inspector's handbook could be developed to serve as a checklist for

any on-site inspections. Combining the knowledge of processing requirements with knowledge of the chemical economy of the country involved, will serve to focus inspection attention on that set of processes which are most probably involved in any given country's production activity at any given time.

X. *On the problem of verification of the production of chemical weapons, by O. A. Reutov and N. N. Melnikov*

Summary

Although they have lower acute toxicity than organophosphorus compounds, many other classes of chemical compounds may be significant CW agents. A few of these are: mustard gas and its nitrogen analogues, phosgene, and so on. Raw materials for them are produced in millions of tons annually. Thus, control of their production and stockpiling becomes very difficult. In the opinion of the authors, national verification, including the participation of public organizations as well as governmental bodies, would be the most effective means of control.

Due to circumstances beyond our control we were unable to take part in last year's SIPRI conference to discuss the question of control over production and accumulation of chemical agents in various countries. We were informed later that the basis for discussions at that conference was the premise that the most important subjects for such control are organophosphorus toxic agents, the substances with the highest toxicity of all the numerous chemicals known at present and available for industrial production. In our paper we would like to share certain ideas on possible ways and means of control over production and accumulation of chemical agents. While discussing this problem we want to make it clear from the beginning that the views expressed below serve only as our personal point of view and in no way reflect either the official position of the Soviet Government or the position of one of its departments.

While discussing the numerous aspects of the approach to control and the methods of control, one question emerges: will it be enough to confine ourselves to the control over the production and accumulation of organophosphorus substances? And whether there is likely to arise in connection with this, a possibility of uncontrolled accumulation of toxic substances belonging to other chemical classes? In my opinion these questions require a comprehensive discussion and analysis.

At present, a great number of toxic substances are known that do not contain phosphorus but the utilization of which as chemical weapons is very likely on various grounds—including tactical grounds. Among those substan-

ces, I can mention first of all such well-known agents as mustard gas—an example of a blistering agent, as well as other nitrogen analogues of it.

The production of such substances is simple and they can easily be produced in very large quantities. It will be enough to remember that the world output of ethylene oxide considerably exceeds the million ton mark at present—the quantity sufficient for the production of about two million tons of mustard gas or its nitrogen analogues. The above quantity of mustard gas is enough to contaminate extensive areas and to cause innumerable sufferings to mankind. More than that, if we take into account the fact that mustard gas can be produced not only on the basis of ethylene oxide but also from ethylene and sulphur chloride it would mean that mustard gas can be used for military purposes on a practically unlimited scale.

One can argue that the toxicity of mustard gas and its nitrogen analogues is several times less than that of modern organophosphorus compounds, but then, as is seen from the simple example above, mustard gas and its analogues can be produced in tremendous quantities. Besides, the production costs for mustard gas and its nitrogen analogues are considerably lower than for organophosphorus substances. We could also add that protection from vesicants—particularly for the civil population—is sufficiently complex and unwieldy. Remember also that mustard gas casualties have to undergo extensive and prolonged treatment, and the treatment is rather difficult as, up to now, sufficiently effective preventive and medical substances do not exist. To say nothing of the fact that a considerable portion of the medical personnel will have to attend to the casualties.

It is a well-known fact that despite the development of much more toxic agents of the organophosphorus group, mustard gas and its nitrogen analogues belong to weaponry of a number of armies—among others, to the US Army as an ND agent.

From the above mentioned, it follows that vesicants constitute a very real danger and the problem of their production and accumulation is still topical.

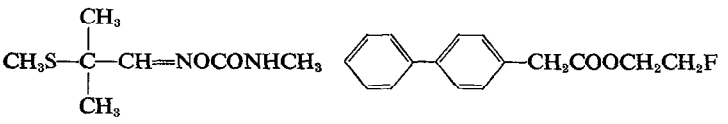
When speaking about systematic poisons one should remember hydrogen cyanide and its derivatives. It is well known that the production capabilities for hydrogen cyanide in various countries are rather high, being measured by hundreds of thousands of tons per annum. This is connected with the fact that hydrogen cyanide is very important as an intermediate product in the production of a number of industrial compounds. Hydrogen cyanide is extensively used in the chemical industry for the production of such substances as acrylonitrile, cyanurchloride, acetone-cyanhydrine, etc. The scale of their production is sufficiently large to create a potential possibility of their utilization in considerable quantities as chemical warfare agents.

The same can be said about agents of phosgene type which serve as inter-

mediates for the production of a great variety of chemical substances, among them semifabricates and dyes, herbicides and insecticides, polymers and pharmaceutical preparations—products produced by the peacetime industry which are rather hard to control.

In the opinion of many specialists, the existence of considerable production capabilities for hydrogen cyanide and phosgene determine their significance and place as potential chemical warfare agents.

The last few years have seen the start of a practical application in agriculture of certain oxims and derivatives, the toxicity of which is close to the toxicity of war agents, though the utilization of elementary means of protection made it possible to use them in agriculture without any harmful consequences. The example of such compounds is the "temic" preparation, the acute oral toxicity of which (LD_{50}) for laboratory animals is 0.9 mg/kg, and, for instance, 2-fluoroethyldiphenylacetate (LD_{50}=0.9 mg/kg).



Temic

2-fluoroethyldiphenylacetate

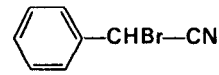
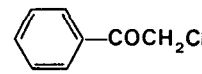
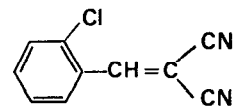
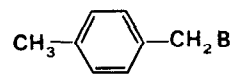
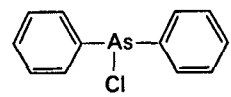
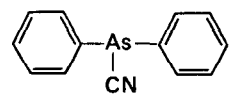
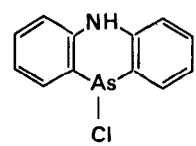
The literature gives a large number of compounds with similar toxicity for warm-blooded animals and some of these compounds can therefore be considered as potential war agents.

A large group of war agents is represented by lachrimators and substances irritating the throat and interfering with breathing (sternutators), some of them are listed in table X.A. below.

It should be noted that some of the substances listed above often belong to relatively safe exhausting agents, but, depending upon the concentrations while breathing, the vapours or aerosols of the above substances can result in serious poisoning, often leading to lethal outcome. Thus, these types of warfare agents should be considered as dangerous agents and their production and storage should be kept under necessary control. Particularly dangerous are arsenic compounds of adamsite, diphenylchloroarsine and diphenylcyanoarsine type which interfere with breathing and are capable of causing heavy poisoning even at low concentrations. Serious poisoning causing harm to the central nervous system can be achieved by certain organic compounds containing lead which, though less irritating in comparison with organic compounds of arsenic, still have high toxicity.

It is well known that, during the last few years, intensive research has been conducted on the study of toxicity and other biological properties of various classes of organic compounds. As a result, highly dangerous substances which can be considered as potential warfare agents have been discovered. Out of

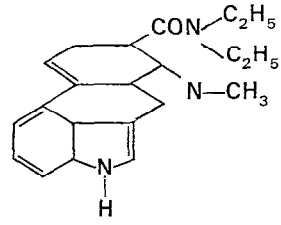
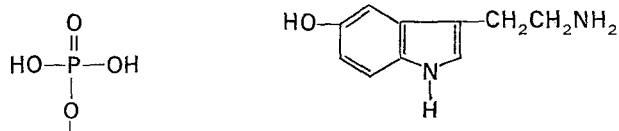
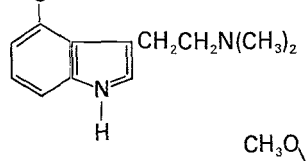
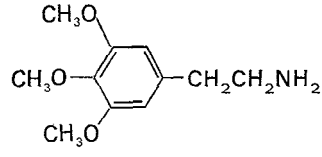
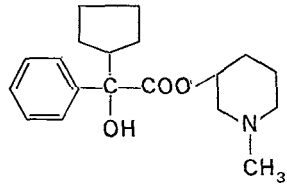
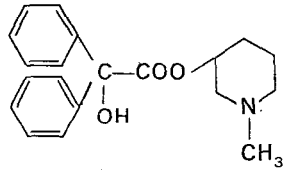
Table X.A. Certain irritant warfare agents

Agent	Formula	Minimal concentration causing irritation *mg/l*
Bromobenzil cyanide		$1.5.10^{-4}$
Chloroacetophenone		3.10^{-4}
2-chlorobenzalmalonodinitril (CS)		1.10^{-3}
Xylylbromide		$3.8.10^{-3}$
Diphenylchloroarsine		2.10^{-4}
Diphenylcyanoarsine		5.10^{-5}
Adamsite		2.10^{-3}

this type of compounds, a group of substances should be singled out—those having psychotomimetic action, the vapours or aerosols of which, when taken in, cause disturbances in normal mental activities and, in a number of cases, have had serious consequences influencing the activities of the human brain. Science knows at present a number of such compounds, some of which are listed in table X.B. LSD when taken in in a small quantity of 0.001 mg/kg weight results in psychosis that continues for more or less long periods, sometimes up to 24 hours. Many other substances have similar effects.

One should also note the high toxicity of many natural compounds—the products of life activities of micro-organisms, which can be lethal in very

Table X.B. Certain substances disturbing normal psychic activities

Substance	Formula
Diethylamid of lysergic acid (LSD)	
Serotonin	
Psylocybine	
Mescaline	
N-methyl-3-piperidyl cyclopentylphenyl-glycolate	
N-methyl-3-piperidyl benzilate	

small doses. The examples are toxins—botulinum toxin, salmonella and many others.

It is known from literature that such toxins can be produced under artificial conditions which provide grounds for believing that in future the possibility of their use for military purposes cannot be ruled out.

During the last few years, the USA has used chemical weapons on a wide scale for the elimination of vegetation in South Vietnam. This results in a grave violation of ecosystems and in many instances has serious consequences for man. The wide utilization of the orange mixture, the main component of which is butyl ether 2,4,5-T produced from technical 2,4,5-T, and containing considerable amounts of tetrachlorodibenzodioxine, resulted in a large number of mutant births. It is well known that tetrachlorodibenzodioxine has a strong teratogenic action. The USA widely utilizes, as warfare herbicides in Indochina, such preparations as 2,4-D, picloram and others that are currently used in agriculture for fighting weeds. It is only natural that it is very difficult to control the production and storage of such products. Even such a preparation as cacodylic acid could be used not only for military purposes for the destruction of agricultural vegetation on enemy territory but also in peaceful agriculture for the destruction of undesirable vegetation. The use of such substances in considerable amounts per area unit could result in poisoning the soil with arsenic for long periods, as well as having undesirable consequences for people eating agricultural produce from these contaminated areas.

The use of so-called defoliants to get rid of leaves on tropical trees causes their destruction and causes great damage to the economy of the attacked country.

It is clear from this brief review that not only organophosphorus compounds but also many other classes of organic compounds, including a number of substances of biological origin, can be used as warfare agents. Besides being widely used in agriculture, herbicides can be used as phytotoxicants.

Vast possibilities of utilization for military purposes of many classes of organic compounds make the task of international control over their production and storage extremely difficult as this control must embrace, practically speaking, all branches of the chemical industry, which is hardly possible. In connection with this, it would seem to me that the most rational system of control over the production and storage of chemical weapons should be based upon national control using the services of specialists of those countries where the control is carried out. Such a system of control is much less expensive and more effective as the citizens of the country being checked are familiar with the industry in their country and they are in a better position to visit industrial enterprises than foreign observers. Of course, such a system would require a necessary level of trust between partners. The question of trust, for instance, would include the necessity of ratifying the existing treaties, including the Geneva Protocol prohibiting chemical and biological weapons which is, unfortunately, still not ratified by the United States.

In conclusion, we would like to stress again that this paper expresses our own personal opinion and in no way determines the policy of the USSR in the field of chemical disarmament.

XI. *Some aspects of phosphorus chemistry related to the preparation and transformation of toxic esters, by J. G. Riess*

Summary

The paper focuses attention on the close structural and preparative relationships which exist between the most useful and commercially developed phosphorus compounds on the one hand, and the most toxic ones on the other. The same basic principles of phosphorus chemistry apply to both categories; thus, they may often be derived from the same reactive, industrially significant intermediates and may in principle be produced by using only slightly modified industrial equipment. Moreover, the interconversion of compounds from both categories may be possible.

Tables are presented which stress the characteristic structural features to be found in the principal phosphorus-based CW agents and other potent cholinesterase inhibitors, as well as the most important synthetic routes which can account for these structural features.

Possible instrumental verification techniques are briefly discussed. These will differ greatly depending on whether the problem is to detect a possible concealed production of nerve agents, or to check, on a routine basis, the alleged production of known phosphorus compounds or intermediates in freely accessible industrial facilities, or to establish the structure of an hitherto unknown agent. A complete, graduated array of methods will be needed in order to face all the possible situations. Special attention is given to nuclear magnetic resonance spectroscopy for the structural identification of phosphorus compounds.

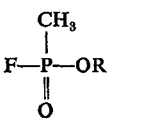
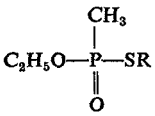
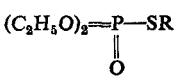
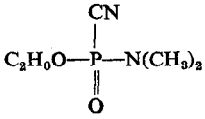
Finally, the common synthetic routes and potential precursors for obtaining the toxic phosphorus esters are discussed and summarized in the tables.

Introduction

A considerable number of phosphorus derivatives belonging to widely different structural classes have been prepared. Of these, the most useful and commercially developed compounds, as well as the most toxic nerve gases, unfortunately belong to closely related classes. Thus, the same basic principles of phosphorus chemistry apply to the preparation of both categories. They may often be derived from the same reactive, industrially available intermediates and may probably be obtained by using the same or only slightly modified industrial equipment. Moreover, the interconversion of certain members of these two categories —useful and harmful—should in some cases be straightforward.

This short report is intended to point out some of the structural and preparative similarities and possible means of interconversion between (i) commercially available, widely produced and widely used, phosphorus derivatives or intermediates, and (ii) some of the most toxic war gases. Thus, the structural characteristics of the most potent cholinesterase inhibitors will be recalled along

Table XI.A. Structure of some phosphorus-based cholinesterase inhibitors

Structure		Common name	LD_{50} mice (mg/kg)
	R = —$CH(CH_3)_2$	Sarin GB)	0.4
	—$CH(CH_3)C(CH_3)_3$	Soman (GD)	0.6
	—$CH_2CH_2CH_2\overset{+}{N}(CH_3)_3$ I^-	Tammelin ester	0.05
	—$CH_2CH_2N[CH(CH_3)_2]_2$	VX	0.01
	—$CH_2CH_2N(CH_3)_2$		0.02
	—$CH_2CH_2SC_2H_5$		0.4
	—$CG_2CH_2\overset{+}{S}(CH_3)C_2H_5$		0.03
	—$CH_2CH_2N(C_2H_5$	Amiton	
		Tabun (GA)	0.6

with some efficient methods for their identification. A brief summary will then cover the most fundamental reactions of phosphorus chemistry as applied to the key industrial intermediates. Finally, some special attention will be given to redistribution reactions, which may be of significance, as well as transesterification reactions, with respect to the preparation and transformation of phosphorus esters.

Structural features found in the phosphorus-based cholinesterase inhibitors and how they may be identified

Table XI.A lists the principal known phosphorus-based chemical warfare agents and other potent cholinesterase inhibitors of comparable toxicity. They are all derived from four-coordinate phosphorus atoms and are esters, thio-esters or amides of phosphoric or phosphonic acid. The substituents on phosphorus may be varied to some extent, but it is nevertheless striking that two out of three of these substituents (apart from the phosphoryl oxygen) are limited to a few, very simple and common groups or atoms: methyl (ethyl), ethoxy (methoxy, isopropoxy), dimethylamino, fluorine and cyano. This may be seen from the

partial structures shown on the left of table XI.A. The third substituent is a somewhat more complex oxy or'thio-ester residue of which the toxic effectiveness seems to depend on a close structural and steric relationship with acetylcholine. A strong inhibitor appears to have to possess a cationic group which can be associated with the anionic site of the enzyme whereas the phosphoryl end phosphorylates the esteratic site.

Nuclear magnetic resonance (NMR) spectroscopy is presently the best physical method available for the unambiguous identification of these structural features provided a mg-size sample is available. It is found that the conditions in this respect are particulary favourable since all of the phosphorus derivatives under discussion contain at least the ^{1}H and ^{31}P, and often the ^{19}F nuclei, that is, the three active nuclei for which NMR measurements are most straightforward. ^{31}P resonance itself is quite sensitive to the environment of the phosphorus atom, with chemical shifts being spread over a wide range of frequencies, especially when derivatives of three- and four coordinate phosphorus are compared. The presence of fluorine atoms bonded to phosphorus atoms results in large coupling constants (700-1400 Hz), whereas proton-containing groups give further characteristic coupling patterns with smaller coupling constants (< 18Hz). Thus, CH_3, OCH_3, OCH_2, OCH, $N(CH_3)_2$ . . . groups linked to phosphorus are immediately recognizable. Phosphorus NMR is considerably less sensitive than ^{1}H NMR but the signal-to-noise ratio may be considerably improved by Fourrier-Transform data accumulation techniques. Proton NMR spectra, are generally easier to carry out in routine analysis. The various sets of equivalent protons present in the molecule will exhibit different chemical shifts. The first order coupling patterns with the phosphorus and fluorine nuclei may be considerably complicated by additional splitting when non-equivalent protons are present in a substituent. Further information is then furnished on the structural nature of the organic residue. The method is non-destructive and permits quantitative measurements through direct integration of the recorded signals. In the past decade NMR spectroscopy has become an essential, well-documented tool in phosphorus chemistry for the identification of products, determination of structures, reaction control, and quantitative analysis of relatively complex mixtures. Thus it may also provide an efficient way of monitoring the industrially-produced organophosphorus derivatives with respect to a suspected development of chemical warfare agents or precursors thereof.

Structural analysis of phosphorus derivatives may be further complemented by infrared spectroscopy, especially for the detection of the P = 0, P = S, $P–CH_3$. . . groups which exhibit characteristic absorption frequencies. Mass spectrometry will also be very efficient for both detection and identifiction of phosphorus compounds.

On the other hand gas-phase chromatography is presently the most widely

used and most rapid procedure available for routine control of known reaction processes and for the detection of known compounds in complex mixtures. It allows for the separation and quantitative evaluation of organophosphorus compounds in trace amounts as in residues, especially since the development of flame photometric detectors which are specific for phosphorus.

Other methods for trace analysis of phosphorus derivatives related to those of table XI.A include paper, thin-layer, ion exchange and gel chromatography, liquid chromatography, colorimetry, oscillopolarography and the very sensitive and increasingly attractive enzyme inhibition determination procedures.

The analytical techniques to be chosen will differ greatly depending on whether the problem is to detect a possible ***concealed*** production of nerve agent, or to check, on a routine basis, the identity of known phosphorus compounds or intermediates in freely accessible industrial facilities, or to establish the structure of a hitherto unknown agent. A complete, graduated array of methods will be needed in order to face all the possible situations. The present concern with the problems of pollution and pesticide residues has contributed to the fast development of automatic trace analysis and verification.

Some common synthetic routes for the preparation of toxic phosphorus esters

Table XI.B. summarizes the main routes by which one may obtain the structural features indicated above (table XI.A) in phosphorus compounds. These represent indeed the most basic and best known reactions of all organophosphorus chemistry. First of all, phosphorylation may be considered the most important since the largest class of phosphorus derivatives, both from the point of view of number and applications, consists of esters of four-coordinate phosphorus. Phosphorylation is often the first step in the preparation of organic derivatives from the inorganic chlorides (PCl_3 and to a lesser extent $OPCl_3$) or from oxygen or sulphur-bridged phosphorus atoms in P_2O_5 or P_2S_5. Although the P-Cl bond is generally initially present in the inorganic raw material, it may also be introduced or reintroduced when necessary by the action of such common reagents as Cl_2, SO_2Cl_2 or $COCl_2$ on acid residues such as > P(O)OH or >P(O)SH, or from the lower oxydated phosphorus derivatives such as >P(O)H. Another fundamental reaction is the oxydation of triply to quadruply connected phosphorus compounds. In fact the formation of a phosphoryl group is one of the strongest driving forces of all phosphorus chemistry. The establishment of a phosphorus-carbon bond may be realized by several routes, of which the Arbuzov-Michaelis rearrangement reaction of an ester of trivalent phosphorus with an alkyl halide, is the most versatile and straightforward. Mixed esters or ester-thioesters may be obtained by stepwise phosphorylation, or by transesterification where a larger alcoxy group replaces a smaller, more

Table XI.B. Basic reactions of industrial organophosphorus chemistry

Structural feature to be introduced	Reactions	Reactants	Examples
P–OR	Phosphorylation	1) >P–Cl + ROH,	$PCl_3 + C_2H_5OH \rightarrow (C_2H_5O)_2\overset{O}{P}H$
P–SR	(alcoolysis) and	>P–Cl + RONa,	$POCl_3 + 2HN(CH_3)_2 \rightarrow PO[N(CH_3)_2]_2Cl$
$P–NR_2$	related reactions	O RSNa	
		(s) R_2NH	
		2) $>P-O-P< + ROH$	$PSCl_3 + 2C_2H_5ONa \rightarrow (C_2H_5O)_2\overset{S}{P}Cl$
		O (S) O	$P_2S_5 + 2C_2H_5OH \rightarrow (C_2H_5O)_2\overset{S}{P}SH$
		(S) (S)	
		3) >PNa + RX	$(C_2H_5O)_2\overset{O}{P}Na + NCSCH_2CH_2N(C_2H_5)_2 \rightarrow$ Phosphorylthiocholines
		O (Cl, NCS)	
		>PSK	
		S	
P–Cl	Chloration	P–OH	$(C_2H_5O)_2\overset{O}{P}H + SO_2Cl_2 \rightarrow (RO)_2\overset{O}{P}Cl$
intermediates	(or from PCl_3, $OPCl_3$)	P–SH + Cl_2, SO_2 Cl_2	$(C_2H_5O)_2\overset{S}{P}SH + Cl_2 \rightarrow (C_2H_5O)_2\overset{S}{P}Cl$
		→ >PH >PH	
		O S	
P–F	Fluoration	P–Cl + HFNaF, KF, ZuF_2	$[(CH_3)_2CHO]_2\overset{O}{P}Cl \xrightarrow{NaF}$ DFP
P=O (S)	Oxydation (or from P_2O_5, P_2S_5)	→P + O_2, S	$PCl_3 \rightarrow OPCl_3$
P–S– O	Oxydation thiono-thiolotautomerism	>P Na + S	$(C_2H_5)_2\overset{O}{P}Na \longrightarrow (C_2H_5)_2\overset{O}{P}SNa$
		O	
		>POR⇄PSR	$(C_2H_5)_2\overset{S}{P}OCH_2CH_2N(CH_3)_2 \rightleftarrows (C_2H_5)_2\overset{O}{P}SCH_2CH_2CH_2N(CH_3)$ tetram +
		S O	
P–C	Grignard	P–X + RMgX	$(CH_3O)_2\overset{O}{P}H + RX \rightarrow (CH_3O)_2\overset{O}{P}R$
	Arbuzov–Michaelis other alkylations	$(RO)_3P \xrightarrow[RX]{\Delta} (RO)_2\overset{O}{P}H$	$PCl_3 + AlCl_3 + RCl \rightarrow \overset{O}{RPCl_2}$ / $\overset{O}{RPCl_2}$
Mixed esters	Transesterification	RO–P + R'OH	$(RO)_3PO + R'OH \rightarrow (RO)_2\overset{O}{P}OR'$
Ester chlorides, Ester amides	Redistributions (stepwise phosphorylation)	By mixing more symmetric esters, halides	$2(RO)_3P + PCl_3 \rightarrow 3(RO)_2PCl$
			$P_2O_5 + 4OP(OC_2H_5)_2 \rightarrow 3$ TEPP

volatile one, as in simple methyl or ethyl esters. Redistribution of substituents is an important potential route to a variety of mixed-substituted derivatives. Of importance is also the thiono-thiolo tautomerism for the access to the phosphorylthiocholine type agents. This transformation has generally been observed to increase the anticholinesterase inhibitor activity. Transalkylation such as in Systox analogues:

$$2(RO)_2P(O)SCH_2CH_2SC_2H_5 \xrightarrow{H_2O} RO(HO)P(O)SCH_2CH_2SC_2H_5$$

$$+ (RO)_2P(O)CH_2CH_2\overset{+}{S}R \quad (C_2H_5)$$

may also increase toxicity considerably. The latter reactions are likely to occur readily on heating and therefore commonly arise during distillation or during prolonged storage.

Redistribution reactions result in an exchange of substituents or fragments, such as F, Cl, OR, SR, NR_2, –O–, –S–, between molecules. Such behaviour may be exemplified by the fast exchange of halogen atoms in mixtures of methyldichlorophosphine and methyldibromophosphine which results in random distribution of the halogen atoms.

Equilibrium distributions are not always random. Thus, for example the redistribution of fluorine with chlorine between $CH_3P(O)F_2$ and $CH_3P(O)Cl_2$ leads to lesser amounts of the mixed fluorochloride than expected from the random sorting of the halogen atoms on the methylphosphonyl centre, whereas $CH_3P(O)F_2$ and $CH_3P(O)\,[N(CH_3)_2]_2$, when heated together in stoechiometric amounts at 120° for 4 hours, furnishes the methylphosphonyl fluoroamide in quantitative yields. Thus, in some cases and when the reactants are mixed in definite proportions, the reaction may be directed toward the almost exclusive synthesis of a single product. A well known example is the reaction of phosphorus pentachloride with phosphorus pentoxide to give the oxychloride $OPCl_3$. When industrially performed, the pentachloride is produced *in situ* from phosphorus trichloride and chlorine. A further example is the preparation of tetraethylpyrophosphate (TEPP) from phosphorus pentoxide and triethylphosphate. This method may find additional applications in the preparation of mixed ester-halides and amide-halides of phosphorus.

Commercial products which are potential precursors of phosphorus-based warfare agents

Let us now consider the organophosphorus derivatives and intermediates which have found wide-scale industrial development. Table XI.C represents an at-

tempt to summarize some of the synthetic routes from inorganic phosphorus raw material to commercially important products, although some of these routes may have no industrial significance at present.

Phosphorus trichloride is probably the most generally used starting material. By way of the dialkylphosphites, which are extremely versatile intermediates, it may serve in the preparation of almost all classes of phosphorus compounds. Next, dialkylphosphate chlorides are very important intermediates in the manufacture of mixed phosphorus esters and thio-esters. Trialkyl phosphates or alkylarylphosphates (lubricants, solvents, plasticizers, functional fluids, gasoline additives) may be further converted by transesterification or redistribution reactions. Mono- and dialkyl acid phosphates are also useful for flame-proofing.

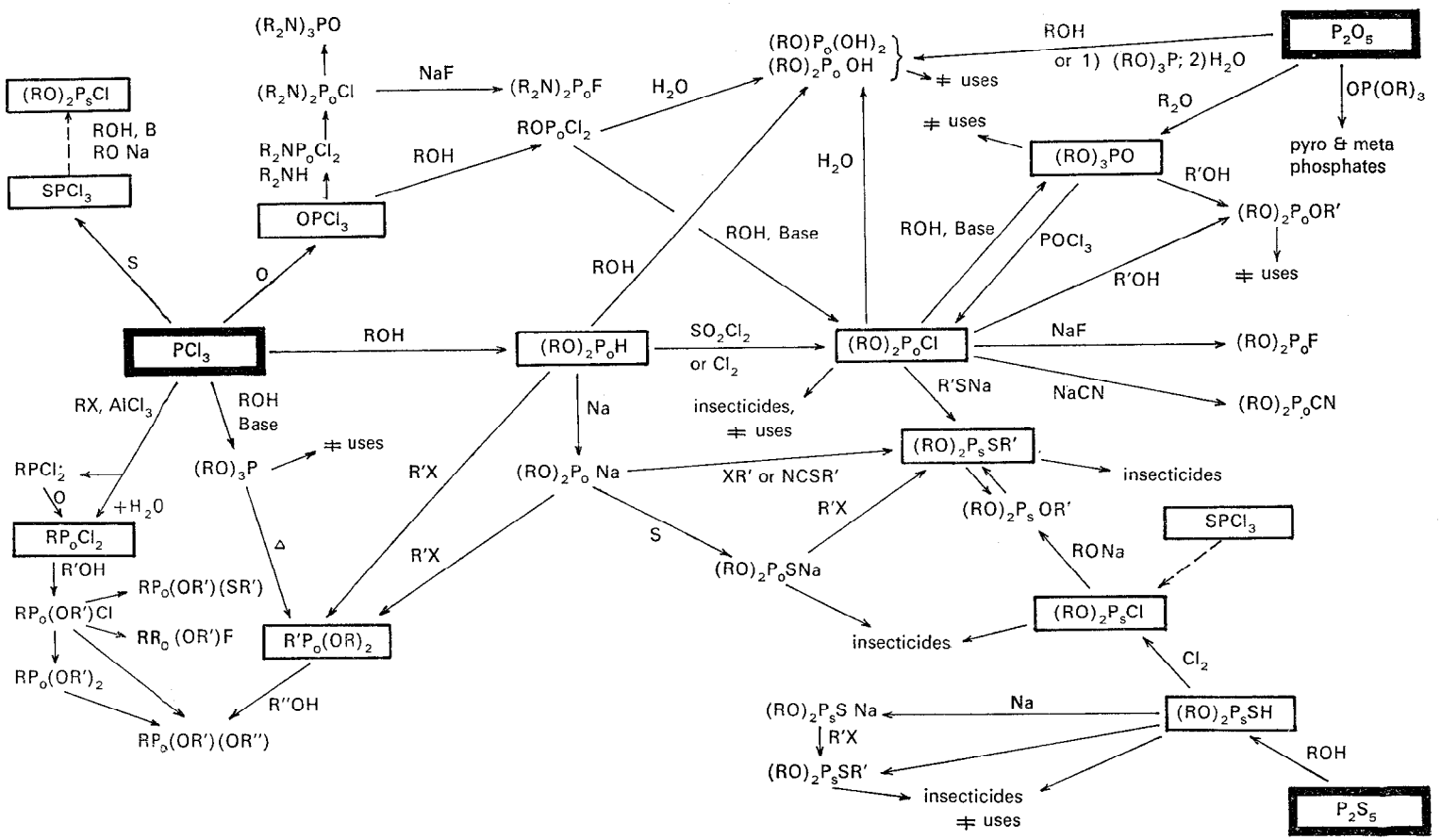
The important thiophosphorus-based insecticides are most conveniently obtained from P_2S_5. The dialkyldithiophosphates have found further application as flotation agents and as lubricants. Dimethyl and diethyl thiophosphoryl monochlorides are obtained by chlorination of the corresponding dialkyldithiophosphates and open the route toward numerous mixed thiophosphorus insecticides; they may also serve as a primary route to phosphorylthiocholine toxics.

Of the low molecular weight trialkylphosphites, $P(OCH_3)_3$ is of commercial interest as an antioxydant stabilizer. Mixed arylalkylphosphites can be prepared by ester interchange reactions. Phosphonates and, generally speaking, phosphorus-carbon-containing products have as yet undergone much less commercial development, but new trends seem to favour their development in the near future. They may be directly derived from the dialkylphosphites, or from $CH_3P(O)Cl_2$ which is obtained through alkylation of phosphorus trichloride.

Thus we are obliged to realize that the few most important industrially produced organophosphorus derivatives and intermediates are very closely related to the most potent cholinesterase inhibitors and may be used as their precursors. Indeed, the partial structures shown on the left of table XI.A are present or potentially present in the major products or intermediates of table XI.C. A final oxidation by sulphur on the introduction of the remaining organic residue is then merely a simple phosphorylation which could be performed in a separate, concealed plant, or even in the bombs or shells while being shot (the so-called binary agents). These close structural and chemical similarities are a matter of great concern when the problem of detecting possible industrial production of organophosphorus warfare agents is considered.

Despite the complicated nature of the problem, it is the opinion of the author that adequate analytical methods are now available which could be successfully adapted to the verification of the production of phosphorus-based warfare agents, inasmuch as such a verification is agreed upon.

Table XI.C. Some reactions involving common phosphorus intermediates



XII. *Economic data monitoring for production of organophosphorus compounds, by R. Roberts*

Summary

This paper examines three roles which economic data monitoring can play in verifying agreements banning organophosphorus agent production.

First, national chemical economies can be examined to identify resource differences and other characteristics. The findings of such analyses can act as guides for on-site and related physical inspections.

Second, a statistical analysis technique which can be applied to national economic data is illustrated. Regression analysis of sets of five input materials can produce quite precise indicators of agreement compliance or violation, if appropriate data are adequate.

Third, an economic component of an inspection system is defined in some detail. Documentation responsibilities and flows, as well as operating procedures of the system, are set forth in general terms. The administration of the system incorporates a mix of national and international controls. The economic component of the system is not foolproof. It must be supplemented by physical inspection techniques.

The system is predicated on the designation of elemental phosphorus and phosphorus compounds as controlled items. A standard system of licensing accounting, reporting and auditing is applied to all nations. All controlled-item production and imports must be accounted for by legitimate, non-agent consumption or export, otherwise a nation will be judged to have violated the agreement. Thus, the philosophy is to deter agent production by denying the allocation of a key ingredient to agent production.

General

This paper is concerned with the economic aspects of inspecting for compliance with an arms-control agreement banning the production of organophosphorus agents. If unrestricted access to all chemical production facilities were possible, there would be no reason to examine the economics of organophosphorus agent production. On-site technical inspection is a much more precise mode of inspection. However, it is clear that few nations, if any, would submit to an unlimited number of unannounced on-site inspections. Thus, to move toward verifiable bans, we must examine the potential of less-than-perfect inspection procedures, the hope being that these can be combined to produce reasonable assurances of compliance at minimum levels of intrusion.

This brings us to a brief examination of why nations enter into arms-control agreements. In simplest terms, they do so, I presume, to improve their national security. For nations to achieve more security with a treaty than without, the

Table XII.A. Estimated chemical production, 1968

	US $ billion	Per cent
USA and Canada	54.8	*36.5*
Western Europe	47.4	*31.6*
USSR and Eastern Europe	24.8	*16.5*
Japan	12.0	*8.0*
Latin America	4.4	*2.9*
Asia	1.9	*1.3*
Africa	0.7	*0.5*
Rest of world	4.0	*2.7*
Total	**150.0**	**100.0**

Source: Chemische Industrie International, April 1969.

inspection system must: (*a*) provide each with the confidence that others are not violating, and (*b*) do so in a manner which provides each with assurance that its vital national and proprietary interests will not be compromised during the inspections. Unless this balance is achieved, verifiable argeements will not be achieved.

There appear to be two major schools of thought on what constitutes adequate inspection. One emphasizes the assurance that others are not violating the agreement; it insists upon absolute verification. This implies some form of international inspection which can *detect* any and all violations of the ban. The other view emphasizes the protection of national and proprietary interests. It suggests self-inspection by each nation. Neither view satisfies the joint criteria for improved national security. Some middle ground must be found. I suggest that the criteria for judging an inspection system might be *deterrence* of agent production rather than either absolute assurance of *detection* or complete reliance upon national *trustworthiness*.

If deterrence is set as the objective, the task of the inspection system is to alter the cost-benefit ratio associated with the accumulation of organophosphorus agents. This would be achieved by inspection procedures which increase the expense of subverting the agreement so that the cost or penalties of evading detection are not worth the benefits of having agents. This view of inspection seems particularly appropriate to organophosphorus agents. Such agents add only marginally to the military capability of the nuclear powers. Few reductions in strategic wherewithal would accrue to the nuclear powers if organophosphorus production were renounced. Thus, inspection systems which impose relatively small additional costs may deter violations. On the other hand, accumulation of organophosphorus agents by non-nuclear nations could pose real threats to their neighbours. Fortunately, the chemical production capability of "Nth" countries is quite limited. (See tables XII.A and XII.B.)

Table XII.B. World chemicals, 1967

US $ mn

Country	Total production	−Exports	+Imports	Apparent consumption	Per capita consumption
United States[a]	42 470.2	2 802.6	963.3	40 630.9	204.05
FR Germany[a]	8 685.0	2 704.7	940.8	6 921.1	115.60
Japan[a]	8 375.0	684.3	610.6	8 301.3	83.01
United Kingdom[a]	7 580.0	1 360.0	906.7	7 126.7	129.42
France[a]	6 460.0	1 244.1	962.4	6 178.3	121.95
Italy[a]	5 855.0	681.4	669.5	5 843.1	109.28
Canada[a]	2 100.0	372.0	566.2	2 294.2	110.35
Spain[a]	1 950.0	87.2	320.1	2 182.9	67.92
Netherlands[a]	1 780.0	892.8	657.8	1 545.0	122.65
Brazil[b]	1 412.0	29.6	236.9	1 619.3	18.90
Mexico[b]	1 372.0	67.2	215.6	1 520.4	33.29
India[b]	1 095.0	21.0	362.5	1 436.5	2.81
Belgium[a]	1 030.0	476.7	517.1	1 070.4	111.72
Switzerland[a]	900.0	703.0	402.7	599.7	98.78
Sweden[a]	775.0	171.6	395.4	998.8	127.28
Argentina[b]	714.0	44.3	159.5	829.2	36.00
UAR[b]	478.0	7.0	85.4	556.4	18.00
Austria[a]	455.0	106.0	225.9	574.9	78.51
Venezuela[b]	325.0	0.9	142.2	466.3	48.57
Norway[a]	320.0	138.5	206.7	388.2	102.59
Denmark[a]	350.0	148.3	284.5	486.2	100.48
Finland[a]	295.0	33.5	174.5	436.0	93.20
Taiwan[b]	230.0	32.2	111.5	309.3	23.54
Portugal[a]	200.0	46.3	95.5	249.2	26.40
Philippines[b]	200.0	5.0	106.9	301.9	8.71
Australia[b]	878.8	49.5	303.0	1 132.3	94.92
Colombia[b]	171.0	5.6	89.2	254.6	13.19
Africa (ex. UAR and SA)[b]	170.0	43.0	512.1	639.1	2.56
Peru[b]	160.0	2.8	68.0	225.2	18.18
South America (all other)[b]	131.0	93.0	319.4	357.4	8.90
Ireland[a]	120.0	23.3	98.5	195.2	67.33
South Korea[b]	111.2	2.4	164.6	273.4	9.18
Greece[c]	100.0	15.9	120.6	204.7	23.49
Chile[b]	91.0	26.8	71.0	135.2	15.13
Pakistan[b]	80.0	5.8	121.6	195.8	1.83
Asia (all other)[b]	69.0	39.1	255.0	284.9	2.60
Indonesia[b]	63.0	—	8.5	71.5	0.65
Iran[b]	41.0	5.8	166.2	201.4	7.66
South Vietnam[b]	39.0	—	45.0	84.0	4.95
Thailand[b]	22.0	0.9	126.4	147.5	4.51
Burma[b]	14.0	0.1	12.3	26.2	1.02
Turkey	19.0	3.4	114.1	129.7	4.13
Subtotal	97 686.2	13 177.6	12 915.7	97 424.3	
Rest of world	31 198.8				
Total	**128 885.0**				

Sources:
[a] *The Chemical Industry*, 1967–1968, OECD Publications, Paris, 1969.
All export and import data are for 1967. Figures for production data are for 1967, except for Ireland, Denmark, and Norway, where 1966 data were used.
[b] *Chemische Industrie International*, Verlag Handelsblatt GmbH, Düsseldorf, April 1969, Chemische, February 1970, (Exports and Imports) Australia, Production 1966–1967. Africa exports $50 million allocated, 14% UAR on same basis as imports, 86% Africa.
[c] Greece: BDSA Quarterly Industrial Report, Chemicals, June 1969.

As a result, inspection systems which moderately increase the cost of agent production may also exert significant deterrent forces upon smaller nations.

Given the preceding, what are the tools an inspection system designer has to work with to achieve *acceptance* of inspection and *deterrence* of violations? Two modes of inspection are available: technical and economic. Technical inspection is defined as those procedures which directly measure *physical* facts associated with agent production. The mode focuses on detection of agent production by the identification and measurement of the agent's physical characteristics or those of the plant and equipment used in agent production. The economic inspection mode relies on second-order measures of agent production, such as, accounting records. It focuses on *records* generated in providing the resources for agent production.

This paper examines three specific roles that economic monitoring can play in the inspection process. The first two are keyed to inspection schemes which have the mission of detecting violations. In both, economics plays a supporting role to technical inspection. The third is predicted upon inspection geared to *deter* violations. This approach assigns the dominant role to economics, with technical inspection performing a validation function.

Prior economic analysis—a guide for technical inspection

In this instance, the view is: what can prior economic analysis do to minimize the amount of technical inspection required?

Any organophosphorus agent is composed of a phosphorus atom bound to four elements or compounds. If a nation does not produce or import all five of these inputs, it cannot produce an agent. Therefore a nation-by-nation analysis of the capability to supply each input material would be quite useful in the design of inspection systems. Technical inspection efforts could be tailored to the peculiar resource characteristics of each nation.

A typical set of analyses might include the following. First, the ratio of input production to required quantity for agent would be examined. Then capacity and production might be compared. If there is substantial excess capacity, the input would most logically be supplied for agents by increased production. If unutilized capacity is not available, the agent production requirement would have to be met by construction of new capacity, diversion from existing uses, or imports. Analyses of this type can provide a shopping list of the most probable economic distortions which would precede or accompany agent production.

Location analysis can provide similar insights. Production of some inputs have characteristic locational patterns: near raw materials' sources, near mar-

Table XII.C. Annual production of 90 chemicals vs. quantities required to produce 30 000 tons of agent

		Number of input chemical possibilities					
		Phosphorus	Oxidation	Amination Fluorination	Esterification	Alkylation	Total
Input production greater than agent requirement							
Input production as percentage of agent requirement	Less than 1 per cent		2		3	4	**9**
	1–10 per cent			1	3	3	**7**
	10–35 per cent			1	2	2	**5**
	35–100 per cent	1			2	1	**4**
	Sub-total	1	2	2	10	10	**25**
Input production less than agent requirement							
Number of times agent requirement greater than input production	1 to 5			2	5		**7**
	5 to 15			5	9	1	**15**
	15 to 100			2	1	4	**7**
	over 100			10	12	14	**36**
	Sub-total			19	27	19	**65**
	Total input possibilities	**1**	**2**	**21**	**37**	**29**	**90**

kets, concentrated in certain geographical areas, etc. Deviations are suspect from an inspection viewpoint. Identification of deviations can trigger technical inspection activities. An example of a characteristic locational pattern can be found in the United States where the production of PCl_3 and $POCl_3$ is closely interlocked. Four of the five PCl_3 production sites further process the trichloride into oxychloride. Identification of an additional PCl_3 production site which does not also produce $POCl_3$ would disclose an atypical situation. An explanation is worthy of additional inspection effort because PCl_3 is required in agent production but $POCl_3$ is not (for most agents). In fact, $POCl_3$ is often a by-product of agent production. [246–248] Thus, the availability of $POCl_3$ from a non-PCl_3 production site is also suspect. Neither of these situations provides conclusive evidence that agent production is underway. Further inspection may disclose harmless reasons for the deviations, but they do serve an *alert* function.

To illustrate that all chemical economics have deficiencies which can be exposed by economic analysis, table XII.C summarizes the ability of the United States to supply inputs for agent production during a recent year. Table XII.C. indicates that the United States produced some 90 materials which might be used in agent production. Had the United States chosen to produce 30 000 tons of agent in that year, production of 65 of the possible input ma-

terials would not have been sufficient to support the postulated quantity of agent production. In fact, had the nation chosen to produce 30 000 tons of the one agent which made the least distortion in its chemical economy, it would have had to allocate to that end in excess of 35 per cent of the output of one input, between 1 and 10 per cent of another input, and less than 1 per cent of the other three inputs.

Since the US chemical industry is by far the largest in the world, much greater dislocations would be required in the chemical economies of other nations—even if the quantity of agent under consideration were substantially reduced. Some idea of the scale of US industry *vis-à-vis* the rest of the world is provided by the facts that: US chemical output is three times that of the Soviet Union, the second largest producer; the 1967 output of only five non-socialist nations in the world exceeded the sales of the largest US chemical company, DuPont; and the output of only ten western nations exceeded the 1967 sales of Monsanto, the second largest US chemical producer.

Economic statistical analysis—a component of a violation detection system

I now want to turn to an example of how economic analysis can participate in an operational inspection system aimed at violation detection. The technique is to apply relatively simple statistical analysis to national production data on inputs usable in agent production. The rationale underlying the technique is that commodities are only produced to satisfy demand. This is true whether an economy is of the market price or administered variety. Major departures from production trends are caused by major changes in the demand pattern.[1]

The analytical procedure has two parts: the first is addressed to production of individual input materials. By regression analysis, we examine production trends to identify departures, if any, from the historical pattern. The probability that production in the most recent period is explained by the trend, is determined for each potential agent input material. In step two, we examine the probability statements for sets of five inputs which might be combined to produce an agent. Very precise indicators of the presence or absence of a production ban violation can be derived thereby. This is because the probability that a set of events (simultaneous departures from historical production trends) will occur is the product of the individual event probabilities. When the tech-

[1] It should be understood that this example deals only with the supply of inputs for agent production by increased input production. It does not consider other input supply options available to a country. These are: increased imports of the inputs: decreased exports of the inputs, diversion of existing input production from civilian uses to agent production, or some combination. This example is only intended to demonstrate that some of the problems associated with economic data monitoring can be surmounted by the use of statistical techniques.

Table XII.D. Probability that observed variations are due to random forces when:

Input material	Agent produced 50 tons/day
Phosphorus trichloride	0.05
Oxygen (air)	—
Hydrofluoric acid	0.45
Isopropyl alcohol	0.50
Methyl chloride	0.35
Combined probability	0.004

nique discloses that random variations *do not* account for observed production increases in a set of five inputs, then some new demand force, perhaps agent production, is at work.

To illustrate, assume that the United States had decided to produce an agent at a rate of 50 tons per day during a recent year. Further assume that the production of each of five necessary inputs was increased over and above normal levels to support the agent production. Table XII.D shows the individual probabilities that departures of these magnitudes would be explained by random forces. The table also shows the combined probability—only four chances in a thousand—that all these changes would be taking place without the introduction of a new demand force. Thus, the power of the combined analysis is greater by a factor of at least ten over that of individual input examinations. The point is that seemingly innocuous information can be analysed so as to produce valuable contributions to an inspection effort, and that these contributions can be achieved at relatively low levels of intrusiveness.

Data criteria for economic monitoring

The preceding examples of economic contributions to inspection systems have been role- or technique-oriented. Data problems have been largely refined. The availability of appropriate data is, however, a fundamental requirement for any economic monitoring system. The criteria for appropriate data include the following:

1. *Definitions:* A standard set for both data collection and reporting.
2. *Coverage:* Data on all relevant facets of each economy.
3. *Scope:* Data from each nation participating in the agreement.
4. *Source:* Data collected and reported by an authoritative source, such as the national government.
5. *Continuity:* Data published or reported on a periodic basis.
6. *Time Lags:* Minimum time lapses between data collection and reporting.

7. *Units of Measure:* Compatible with the objectives of the monitoring system. In general, physical units are preferable to financial units.
8. *Aggregation:* Individual data series for each commodity important to the monitoring system. Reporting which lumps statistics on several commodities into one series is unacceptable.
9. *Accuracy and reliability:* The data should be accurate (error-free) and reliable (true representations of the facts).

Given these stringent criteria, it can be speedily concluded that the open data now available on the world's chemical economies are totally inadequate for effective economic monitoring.

Violation deterrence *via* a controlled item records monitoring system

A third approach to economic monitoring which surmounts many problems with existing data and capitalizes upon the inherent non-intrusiveness of economic monitoring is as follows:

The suggested economic inspection component (hereafter referred to as the CIRM system) of a complete verification system to police a ban on the production of nerve agents would be based on the designation of elemental phosphorus as a controlled item.

Blend of arms-control problem requirements and economic inspection capabilities

Before presenting a description of the organizational and procedural aspects of the CIRM system, its overall characteristics will be examined as they relate to the problems of arms-control agreement verification.

First, the system is limited to a key ingredient in nerve-agent production. All of the, perhaps 100, other possible ingredients are ignored, the philosophy being that extremely tight control over one essential input can deter agent production almost as well as control over all inputs. This can be done with much less intrusion and much lower cost than control over all inputs.

Second, a standard system of controls, accounting, and reporting is applied to all nations, thereby circumventing the enormous problems present in the existing data systems of the world's nations.

Third, the designation of phosphorus as a controlled item—and the imposition of the requirement that all its production be accounted for by legitimate, non-agent consumption—shifts the burden of verification proof from the inspection system to the inspected nations, that is, any imbalance in the CIRM accounts must be justified by the nation in question or a treaty violation will

Table XII.E. World production of elemental phosphorus

metric tons

Country	Output 1967	1969
United States	495 000	566 000
Soviet Union	100 000	142 000
Canada	30 000	100 000
FR Germany	70 000	70 000
Netherlands	0	60 000
Japan	26 000	26 000
United Kingdom	31 000	18 000
China	18 000	18 000
DR Germany	15 000	15 000
France	14 000	14 000
Italy	10 000	10 000
South Africa	0	6 000
Australia	5 000	5 000
Yugoslavia	1 500	1 500
Sweden	300	300
Total	**815 800**	**1 051 800**

Sources:
Phosphorus and Potassium (British Sulphur Corporation, Ltd) 42, July/August 1969; 25, October/November 1966; 36, July/August 1968.
Chemische Industrie International, Düsseldorf, December 1966.
Kirk-Othmer, *Encyclopedia of Chemical Technology*, Second Edition, Vol. 16, New York, p. 286.
Summaries of Trade and Tariff Information, Schedule 4, U.S. Tariff Commission, Washington, D.C. 1968.

have occurred. In effect, the economics portion of the inspection effort inspects for compliance with the provisions of the CIRM system as a proxy for compliance with the ban on agent production. The role of technical inspection is to validate that the records and reports of the CIRM system are accurate representations of *physical* distribution and consumption of phosphorus. The CIRM system is a sustaining, omnipresent component of the total inspection system and more intrusive technical inspection is retained for *ad hoc* validations and challenge investigations.

Fourth, the scale of elemental phosphorus production throughout the world is relatively small. Total production is currently about 1 million tons. This production is accomplished at about 35 production sites in 15 nations. The 12 US production plants account for over half of world production. (See table XII.E.) The limited number of production sites means that the system can be operated within reasonable cost, intrusion, and administrative limits.

Scope of control by the CIRM system

The procedures of the system are modelled after those in use in the United States to control tax-free alcohol. [249] There are also international precedents for a control system of this type, the most recent, comprehensive, and syste-

matic being the Safeguards System of the International Atomic Energy Agency. In addition there is the system recommended by the United Nations Conference for the Adoption of a Protocol on Psychotropic Substances. [250–251]

Entries into the CIRM accounts of a nation would begin at the elemental phosphorus production plant, with the importation of elemental phosphorus or phosphorus compounds, and/or with the recovery of phosphorus from processing wastes or any phosphorus compound.[2] Accounting and control would continue to be exercised until final consumption or export.

Control within the system would include: regulation of those who are authorized to import, produce, process, consume, recover, transport and export phosphorus and its compounds; authorization of specific quantities allowed each producer, processor etc., periodic reports from each enterprise participating in the system; special reports providing notice of losses and justifications therefore; plus a comprehensive system of routine and challenge audits.

CIRM organization and administration

Three operating levels are envisaged. They are: an International Control Agency (ICA) to oversee the entire system, National Control Agencies (NCA) to be primarily responsible for the system within their borders, and an industry level including all enterprises—within each nation—that handle phosphorus and phosphorus compounds.

The National Control Agency is included for a number of practical reasons: first, all economic data generated within a nation are under the control—to some extent—of the national government. It is, therefore, within the power of most nations to withhold, alter, or suppress certain data. The question faced by an economic inspection system designer is—how to deal with this fact? The approach I have chosen is to institutionalize governments into the system. Assigning to each a responsible role in the functioning of the data collection and analysis process commits each government to the operation of the system. A nation intent on violation of the treaty ban could still alter its records but must do so within the constraints of maintaining its portion of the system in operation and in balance; it must do so while subject to ICA audits.

Second, interposing an NCA between the international inspectors and each nations's industrial enterprise protects justifiable national and proprietary interests from disclosure outside the country. Disclosure of information relating to many such interests—not essential for inspection—might otherwise occur.

[2] As the technology of phosphorus industries advances it may become possible to produce nerve agent inputs (such as phosphorus trichloride) by direct reactions with phosphate rock and thus bypass the present intermediate step of converting phosphate rock to elemental phosphorus. Should this occur the starting point for the CIRM system could be moved back to phosphate rock production. If this adjustment became necessary, the scope of the system would be dramatically expanded.

Third, bringing national governments into the system permits the employment of their legal powers in the institution, operation, and enforcement of the controls within their borders. The implementation procedure recommended is to incorporate in the treaty a model statute setting forth the full particulars for the CIRM systems's operation. Each nation would then adopt and operate the model statute's standard data acquisition, reporting, and enforcement procedures.

Fourth, the National Control Agencies would be responsible for the major administrative portion of the system, thus, the costs of system operation would be distributed in a manner roughly proportional to the level of controlled item production and consumption within each nation.

Fifth, the socialist states at the 1970 Conference Committee on Disarmament meeting insisted that verification of any ban on chemical warfare agents should rely *primarily* upon national self-policing. To implement national enforcement, these nations suggested their willingness to establish elaborate legal, procedural and administrative controls. [252–254] The CIRM system is my suggestion for effecting national enforcement with minimized international oversight.

A display of the information flows in the system and the interactions of the three administrative levels is presented in chart XII.A.

Operating procedures of a CIRM system

The procedures for exercising control, accumulating data and reporting within the CIRM system are:

1. *Plant registration:* All facilities handling the controlled item or its derivatives must be registered with the NCA.

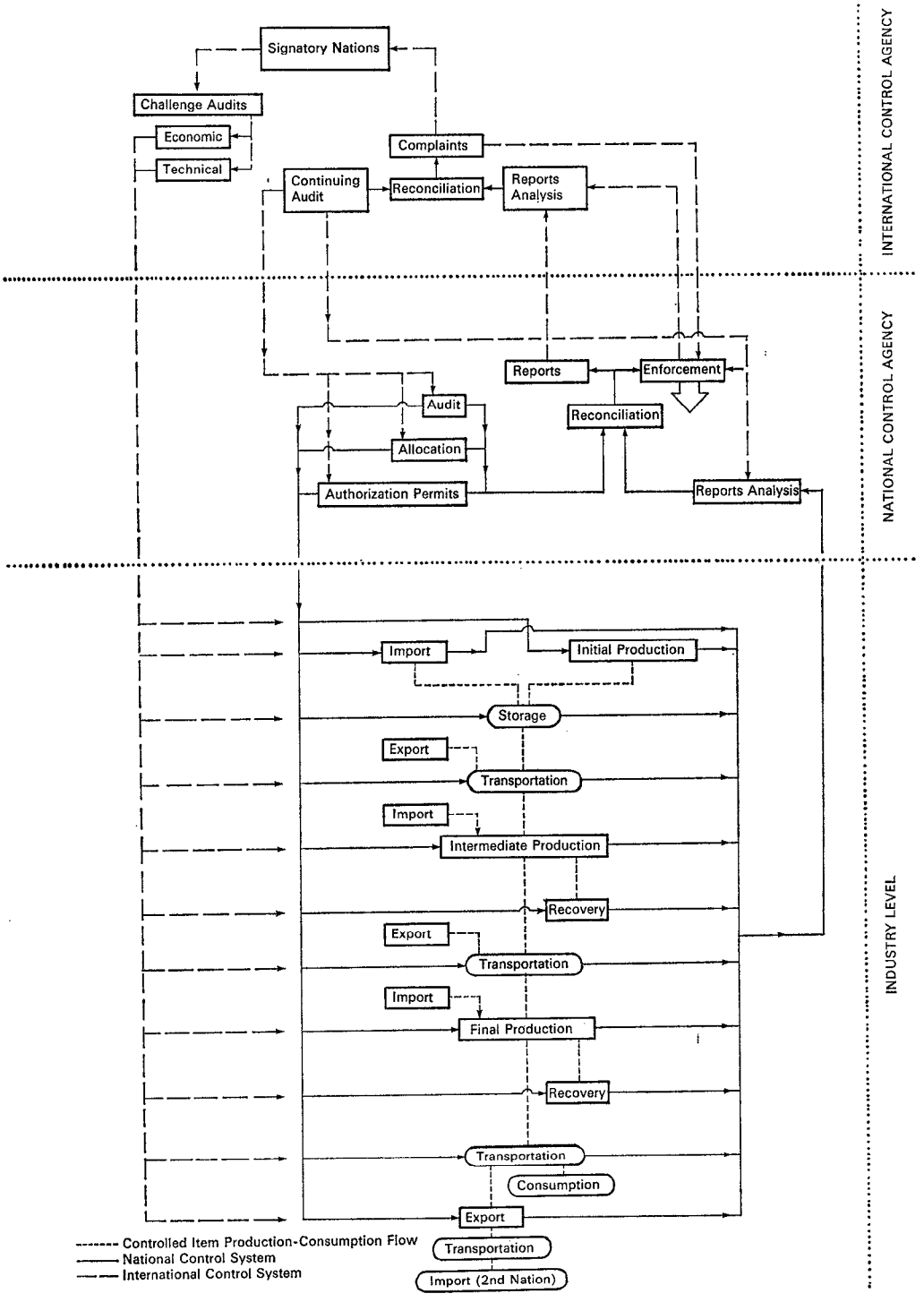
2. *Industrial use permit:* These would be issued by the NCA to each approved plant. They would designate permitted and prohibited uses of the controlled item.

3. *Withdrawal permits:* These provide a second echelon of control and are the means by which specific annual ceilings for the controlled item and its derivatives are fixed. Quantities authorized may not be greater than those sufficient to meet the bona fide needs of the applicant as specified on the industrial use permit.

4. *Withdrawals under permit:* When a permitee desires to procure quantities of the controlled item, the permit is forwarded to the supplier of the controlled item or its derivative. The supplier may make shipments only after the permit is in his possession.

Units of measure, where appropriate, will include both actual quantities and "equivalent" controlled item quantities, for example, the units of measurement

Chart XII.A. Schematic of a national/international economic data reporting and monitoring system



on a withdrawal permit for a plant purchasing phosphorus trichloride would be expressed both in units of PCl_3 and in equivalent units of *phosphorus contained in* the compound, PCl_3.

5. *Shipment and receipt:* Shipment will be made by shipping document. Upon receipt of the shipment, the consignee will acknowledge intact receipt of the shipment or file a loss report. In either event, copies of appropriate documentation will be retained for the files of all parties, and will be dispatched to the NCA.

6. *In-plant storage and records:* An appropriate running balance inventory accounting system for controlled items will be maintained. Issue from stock will only be made upon receipt of requisitions for authorized uses. In-plant losses will be reported to the NCA, as detected.

The plant will also maintain records of output and processing losses in sufficient detail to trace the usage of the controlled item or derivatives to specific lots, and to enable the reconciliation of the controlled item or derivatives which enter and leave the plant.

7. *Losses:* Loss reports will be filed with the NCA for all disappearances of the controlled item or derivatives. Permissible rates may be established for certain types of losses, for example, shipping and processing losses. But, for all others, the loss reports must describe the quantities lost and the circumstances of the incident.

All losses are subject to investigation by the NCA which, in turn, must account to the ICA.

8. *Recovery:* Recovery of the controlled item from its derivatives (e.g. recovery of phosphorus from phosphoric acid) is permissible only upon approval of the NCA.

9. *Reports:* Periodic reports of activities will be made by each holder of an industrial use permit. The reports will summarize transactions during the period. All entries on the report will be supported by and derived from documentation for individual transactions conducted during the reporting period.

10. *Audits:* The NCA is responsible for record and physical audits to verify compliance with the control system. Each participant in the system will be subject to unannounced audits. The NCA is also responsible for conducting a programme of activities to ensure that no production, use, import or export of the controlled item occurs outside the system.

11. *Reports to the International Control Agency:* Although each participating nation is primarily responsible for the operation of the system within its bor-

ders, the NCA will supply certain types of information to the ICA. This will include: (*a*) Permit terms and lists of plants to which industrial use permits have been issued; (*b*) Permit terms and lists of plants to which withdrawal permits have been issued; (*c*) A monthly consolidation of loss reports including disposition of major cases; (*d*) A similar consolidated report of procedural violations and the disposition of enforcement proceedings; (*e*) a consolidated annual report of transactions and (*f*) Copies of the shipping documents for each import or export transaction.

12. *International Control Agency Audits:* All records maintained by the individual National Control Agencies are subject to routine, unannounced audit by the ICA. Under challenge proceedings, some number and form of audits at the industry level would be incorporated into the International Control Agency's mandate.

13. *International Trade:* Procedures for operating of the international trade portion of the CIRM system are analogous to those applied within a nation, except that the ICA is an active participant in the process.

Annual export and import quotas are established for each nation upon application by individual exporters and importers to National Control Agencies. Each NCA reports the authorized quantities to the ICA.

Losses occurring during international movements are reported and justified in the same manner as losses occurring within a nation, except that losses occurring during transoceanic movements—outside the borders of any nation—are the investigatory responsibility of the ICA. Unjustified losses constitute discrepancies in the accounts of both the exporting and importing nations.

CIRM system effectiveness assessment

In all probability there is no set of inspection procedures which can provide absolute assurance that violations of treaties banning chemical warfare agent production will be detected. Even if such a foolproof inspection system were designed, there is almost no chance that it would be adopted. It would be much too intrusive and enormously costly.

The controlled item records monitoring system is but one component of the total inspection system and, as such, it is inherently fallible. The test of the CIRM system's effectiveness is, therefore, the magnitude and intricacy of record and data alternations which would be required to avoid detection by the system. If a potential violator finds that the costs of avoiding detection exceed the benefits of adding nerve agents to its arsenal, then the system will have served its purpose.

XIII. *The objectives of inspection in a limitation on the production of CW agents, by H. Scoville, Jr.*

Summary

The objective of verification of an arms-control agreement is to provide all parties confidence that a violation is not jeopardizing their security. Suspicions created by legitimate activities could, unless allayed, become intolerable when vital security issues are at stake.

The verification procedures must be directed toward realistic military threats and need not be designed to have a high probability of detecting every violation, no matter how minor. The verification system should be designed to employ unilateral means, that is, those not requiring access into another country, to the maximum extent possible and only use inspection when other methods are inadequate. Information made available publicly by parties will greatly facilitate this objective.

For verifying a production ban, CW agents can be separated into three classes: (*a*) those agents for which there is no legitimate peaceful use, (*b*) those normally produced in large quantities for industrial purposes, and intermediate between these, (*c*) those agents which as such have no peaceful use but employ processes or intermediates which do. The organophosphorus agents fall in this third class.

In this case, unilateral verification will probably have to be supplemented by inspection to obtain satisfactory assurance. These would have two major goals: (*a*) the determination that a plant is not producing a CW agent or (*b*) that a raw material or intermediate chemical is not being diverted from legitimate to agent production, perhaps in another plant or directly in a weapon itself. Such inspection should be directed at critical points in the process to avoid unnecessary disclosure of commercial secrets. The procedures may be different in a large nation where the production of small quantities of organophosphorus agent would have little military significance from that in an underdeveloped country where even relatively small amounts could upset the military balance in the area. The accuracy and detail of the information sought by either unilateral and national means or by inspections must be consistent with the quantity of agent which would have to be concealed to be of security significance.

General principles

Verification of an arms-control agreement is necessary in order to provide all parties with confidence that another nation is not acquiring a significant military advantage through failure to abide by its terms. If such fears cannot be allayed, then the agreement is likely to be unstable and could, in the long run, create tensions which would defeat the purpose of the agreement. In most cases, an actual violation will not be involved; however, natural events or programmes carried out for perfectly legitimate purposes can give rise to suspicions which could become intolerable when vital national security interests are

at stake. In other words, verification will in most cases be needed to provide explanations of normal activities rather than to detect actual violations.

It is also important to keep in mind that verification does not have to be 100 per cent effective in order to be quite adequate for serving the purposes of the arms limitation agreement. It is only necessary to be able to be confident of detecting those violations which provide a realistic threat to security. Only those normal activities which might be misidentified as ones which might provide an important security risk need be explained. For example, in the case of the comprehensive test ban, a single earthquake in the United States or the Soviet Union which gave seismic signals indistinguishable from those of an explosion should not be of any security concern. Since both these nations have already conducted a large number of nuclear tests, one or even several clandestine tests could not upset the security balance. In the US-USSR case of an agreed ceiling on the number of ICBMs, which might be negotiated at SALT (Strategic Arms Limitation Talks), it would not be necessary to detect every new ICBM launcher. When 1 000 or more launchers are already available, the addition of a few more would be of no consequence. If each side had only 25 launchers, then a small clandestine programme could have important military consequences. For a ban on the production of biological warfare agents, verification can be minimal and yet satisfy US security requirements, since the United States has made the unilateral decision to forego all biological warfare programmes. If a nation believes its security is not threatened by such a one-sided action, it certainly does not need much additional information to allay any fears in a treaty situation in which other countries reciprocate. In this case, verification might be limited to some types of international mechanisms for requesting clarification of suspicious activities.

In some fields the verification requirements will vary from nation to nation. Thus, an agreement which banned the production of fissionable materials for weapons purposes between the US and the USSR, would not require any stringent verification procedures since the clandestine production of a few kilograms or even hundreds of kilograms of fissionable material by either of these countries, which already have thousands of kilograms in weapons stockpiles, would be of no consequence. On the other hand, the diversion of even a small amount of fissionable material, that is, that sufficient to make only one or a few bombs, would be extremely serious in some areas of the world. The introduction of a single nuclear weapon on either side of the Middle Eastern confrontation could have catastrophic effects not only for that area but, because of the risks of escalation, for the world as a whole.

Fortunately, in most cases where small violations could be most serious, the concealment of clandestine programmes would be most difficult. For example, to have confidence that the diversion of 10 kilograms of fissionable material did

not occur in a country like the United States or the Soviet Union, with their large nuclear power programmes, would be virtually impossible. A diversion of greater than 1 per cent of output might be difficult to conceal from IAEA Safeguards inspection, but few nations have peaceful power programmes producing a 1 000 kilograms of plutonium in a reasonable time period. The same type of logic can be applied to the chemical industry and CW agents. In summary, the verification of every arms-control agreement must be analysed not only in terms of the nature of limitation itself but also in terms of the nation and situation in which the verification is to be carried out.

The general term "verification" has been used to encompass all techniques which might be available to ensure that an arms-control agreement is complied with. In general, such verification can be divided into two categories: the so-called unilateral[1] techniques, and "on-site inspections". The unilateral systems are those which do not require any intrusion into another nation. This is the information which would be readily available to foreign countries in many cases from the open literature, newspapers or trade journals, or from phenomena observable outside a nations' borders. The on-site inspections, however, do require permission of the host country either to visit that country to obtain the necessary information or to station inspectors at key installations in order to observe allowed operations which might be used as a cover or a means for diversion. The verification against the diversion of fissionable material under the Non-Proliferation Treaty is an example of the latter, while the verification of the Limited Test Ban Treaty by unilateral systems capable of detecting nuclear explosions at long distance and outside borders is an example of the former.

Insofar as possible, every attempt should be made to satisfy the verification requirements by unilateral means. These avoid intrusion into and interference with, the affairs of other nations. Thus, in designing verification procedures for any arms-control agreement, one should first look carefully at the unilateral capabilities and then supplement these with agreed on-site inspections only where unilateral means are inadequate. Even then the information available from unilateral means should be used as a background for the inspection in order to minimize its intrusiveness. Great care should be exercised to avoid seeking inspection for inspection's sake. One should carefully analyse the situation and decide the mimimum information which is necessary to satisfy security concerns and avoid creations of unnecessary suspicions, and then develop inspection techniques which will provide that information. Data should not be sought just because it would be nice to have it. The inspection techniques must

[1] In the United States this is frequently referred to as "national" means of verification. However, this should not be confused with the "national" verification systems used by the Soviet Union and others.

be designed to avoid the unnecessary disclosure of facts which might be of commercial or national security interest and which do not contribute toward the objectives of the arms-limitation agreement. It was the concern about disclosure of commercial data which was one of the major stumbling blocks in negotiating the Non-Proliferation Treaty. Agreement in this area was only achieved after reasonable assurances were provided that sensitive design information on nuclear-power processes would not be disclosed and after the US and the UK offered to allow inspection of their facilities to demonstrate their belief that the IAEA procedure would not reveal industrial secrets.

Verification of limitation on CW agent production

A ban on the production of chemicals for military purposes presents very complicated problems which are probably unlike those in any other field of arms control. A wide variety of potential CW agents exists, and the verification problems are different from one agent to another. For the purposes of discussion, however, they can probably be divided into three general classes.

First are those chemicals whose only function would be for use as chemical-warfare agents. An example of this type would be the chemical developed and used in World War I, mustard gas, for which there is no important industrial use and for which any large production would be unquestionably military in purpose. For such agents all production would be banned, and the verification task would be the detection of any secret plants for the production of such chemicals. Unilateral verification methods would probably be the major source of information in such a situation, but since such chemical plants might be easily confused with those producing other chemicals, some arrangement might be required for visits or inspection of suspicious facilities. Since the number of potential plants might be large, this might be very difficult unless the security implications of a violation were not great. If many plants were required to provide a significant military capability, the probability of a violation being detected would be greatly increased. However it is not this kind of inspection that is the subject of this symposium.

Second, on the other end of the spectrum are those chemicals which might be used for chemical warfare purposes but which have very large, justifiable peaceful applications. Examples of these are the common industrial chemicals, hydrogen cyanide and phosgene, both of which are produced on an extremely large scale for industrial purposes. However, they are very toxic compounds and have been used in the past for chemical warfare purposes. A limitation on the production of CW agents clearly cannot ban the production of such chemicals, and in this case the verification problem becomes one of preventing their diversion from peaceful to military purposes. In some respects the problem

here is similar to that which exists with fissionable material, but, unfortunately, the prevention of diversion would be far more difficult than in the aforementioned case. Maintaining accountability on all the hydrogen cyanide and phosgene produced and used would be a herculean task and probably impossible. Export controls might be of some, but probably limited, value for less developed countries. On the other hand, neither hydrogen cyanide nor phosgene are considered very practical chemical agents, and their control would not be very critical, at least in the larger nations. In general, the best CW agents have few civil applications, perhaps because their toxicity reduces their usefulness.

The third category of CW agents, which is intermediate between the first two, includes those chemicals which by themselves do not have any widespread industrial use but whose production involves processes and intermediates which are frequently employed for peaceful purposes. In some cases it may only be the final chemical process in the manufacture of the agent which may be different from that used in perfectly legitimate chemical production. Examples of this are the organophosphorus nerve agents which are the primary subject of discussion at the present symposium. The processes and raw materials for manufacturing such agents are not radically different from those for producing some insecticides which are used in large quantities. Therefore, very close observation might be required in order to determine the true purpose of a given plant or to ensure that part of the plant production is not being diverted to military purposes. This is further complicated by the fact that the final chemical reaction producing the agent could be carried out in the weapon itself.

In such a situation, unilateral means of verification are probably quite unreliable and would provide little confidence that clandestine production was not occurring. Published industrial information on chemical manufacture and usage might be valuable as background, but suspicions would be likely to develop that legitimate production was only being used as a screen to conceal a violation of an agreed limitation. It is in such a situation that on-site inspection can be of greatest value.

Such inspection could have two major goals: first, the determination that a plant is not actually producing a toxic chemical warfare agent, or secondly, that some of the intermediate chemical that might be used for either an insecticide or a CW agent is not being diverted from the legitimate production to some other use. This might occur either in the same plant or in another facility where the final agent might be produced.

In designing the inspection system, one should keep clearly in mind the general principles which have been previously discussed. Only that part of the production cycle which is critical to detect forbidden activity should be inspected. Every attempt should be made to avoid inadvertent disclosure of

industrial secrets. Inspection of the plant should be limited to those areas in which diversion of the critical intermediates might occur or in which secret production of the agent might be taking place. A similar situation exists in safeguarding fissionable material. Inspection of the reactor can in most cases be minimal as long as the fuel input and output are known. Instead inspection can be concentrated on the chemical reprocessing and the enriched fuel fabrication parts of the cycle where the risks of, and opportunities for, diversion are the greatest.

Details of chemical engineering techniques need only be sought for those parts of the plant where clandestine production might occur. The inspectors should require only that production data which is needed to establish a material balance and ensure that some chemicals are not being used for forbidden purposes. The accuracy and detail of the information required should be consistent with the quantity of agent production which would have security significance. For example, in a large industrial nation with a big military establishment, the clandestine production of tens and even hundreds of tons of chemical agents might have very little security significance. Therefore, closing a material balance to a few tons is unnecessary. On the other hand, in a small and relatively underdeveloped country, a limited production of a chemical agent could be of great military significance and potentially destabilizing if available for use against a small neighbouring country. In such a situation, however, the inspection may not have to be radically different since the industrial base of the small nation will be much more limited and the opportunities for clandestine production consequently restricted.

All these factors must be kept in mind as one attempts to design inspection systems to verify a CW agent production limitation. The techniques used must be sufficiently detailed and unequivocal so as to provide the necessary confidence that violations are not occuring and the required deterrence to any nation considering a violation. On the other hand, inspection techniques which are so intrusive as to be unacceptable or which cannot be soundly justified as necessary to avoid significant violations will only defeat their own purpose and eventually perhaps the purpose of the agreement itself. The development of these techniques requires a careful balancing of the interests of both the inspected nation and the inspecting organization in order to ensure the achievement of a viable agreement.

XIV. *Phosphorus pesticides—suggested principles for the control of their production, by T. Urbánski*

Summary

It is obvious that any control, particularly international control of industrial production, is very difficult in most countries.

It is therefore suggested that the control of phosphorus pesticides should start at the users, that is, in *agriculture*, which could form a *partial* solution of the problem.

Thus the use of phosphorus pesticides of a toxicity higher than LD50 = 100 mg/kg (by oral administration in rats) should not be permitted. The only exception should be for those substances which can readily be hydrolyzed: for them a toxicity limit of LD 50 = 50 mg/kg may be accepted.

The figures used here refer to agricultural production in a moderate (for example, European) climate, and they certainly should differ in sub-tropical and tropical agricultural production. However, as long as highly toxic pesticides are prohibited, they will not be produced.

It is further suggested that international bodies such as FAO and WHO in cooperation with IUPAC and IUFOST, should be consulted in working out the principles of international control, based on the above suggestions.

Nevertheless, a national control system should be created in every country. It should aim at the fulfilment of internationally accepted principles.

Introduction

It is generally known that the use of phosphorus pesticides is one of the important factors governing the present development of the modern and national agricultural production and animal-husbandry.

It is estimated by FAO experts that if we were to eliminate chemicals in food production, about one-third of the world's population would not eat at all: "millions would starve without chemical defences against insects".

Among pesticides, phosphoric acid derivatives are gaining considerable importance. It is known that over 100 phosphorus compounds are in use as pesticides and 50 000 tons/year are being used in the United States alone.

I. *Advantages*

The advantages of phosphorus pesticides are well known.

1. They have a wide spectrum of activities as insecticides, acaricides, nematocides, fungicides, herbicides and defoliants, etc.

2. They have fast action.

3. A relatively low quantity is required to cover a surface area under control with most of the compounds.

4. A relatively fast and sufficient hydrolysis into less-toxic or non-toxic compounds occurs when exposed to the action of water and sunlight, thus reducing or completely removing the toxic substances from agricultural products.

5. Some of the substances are characterized by relatively high systemic activity, a highly appreciated property of non-persistent pesticides. They enter the vascular system of the plant, spread their protection throughout, and then metabolize into harmless degradation products. Most of the substances are also transformed by a relatively fast metabolism in the organs of higher animals and men into non-toxic substances.

However, the metabolites are not always less toxic than the original substances (see below).

II. *Disadvantages*

1. The main *disadvantage* of most phosphorus pesticides is their more or less strong anticholinesterase activity. This creates the major danger for warm-blooded animals, particularly in the instances when an irreversible process has been induced.

2. In some cases metabolites (mentioned above in 1–5) can be more toxic than the original substances and this creates an additional danger.

3. Many phosphorus substances can be absorbed through the skin and show their toxicity that way, and this makes them much more dangerous.

Conclusive comment to the introduction

The toxicity range is extremely wide, the LD 50 (in experimental animals, usually rats) being from less than 1 mg/kg up to 5 000 mg/kg. This is an important feature which forms the basis for the suggestions given below.

Suggestions for control of the use and production of phosphorus pesticides

It is suggested by the author of the paper that

1. *Only low toxicity phosporus compounds should be permitted for use as pesticides.* This requirement should have another bearing:

2. *Only those phosphorus pesticides should be produced which are permitted for use as pesticides.* Forcibly, *the production of* phosphorus pesticides will be limited to *low toxicity products only.*

3. The toxicity index I_t as a criterion for admission of phosphorus pesticide could be introduced. The numerical value of I_t should be that of the LD50 in mg of the substance given to rats per kg weight of the animal. Various routes of administration of the substance should be taken into account: oral, intraperitoneal and dermal (by absorption through the skin).

For the time being and as the first approximation, the LD50 determined by oral administration can be taken, but the LD50 with different ways of administration should be considered for a more detailed estimation of the compounds. I_t values given below are related to the LD50 when the substance is given *orally* to rats. They are referred to the agricultural production in a moderate (e.g., European) climate.

It is suggested that only two groups of phosphorus pesticides should be permitted for use; those with a relatively slow hydrolysis, and those hydrolyzing rapidly. Those which resist hydrolysis *should not* be permitted for use or production.

4. *It is suggested that for the first group the value of the index* $I_t = 100$ (i.e. LD50 = 100 mg/kg) *should be accepted,* and the phosphorus pesticides with a lower value of I_t should not be permitted.

5. For the latter group of pesticides with a faster hydrolysis, *the value of the index* $I_t = 50$ (i.e. LD50 = 50 mg/kg) *could be accepted.* Subsequently, all highly toxic substances having an LD50 below 50 mg/kg and some having an LD50 between 50 and 100 mg/kg should be withdrawn from practical use. This in turn should have a negative impact upon the production of phosphorus pesticides of high toxicity, leading eventually to complete stoppage of their production.

There are at least 20 phosphorus pesticides which should be *immediately* withdrawn from use and a further 10 which should be put on a list of *doubtful compounds* allowed only where they have been found readily hydrolyzed.

Proposals

It is obvious that any control of an industrial production in most countries is very difficult, if at all possible. The action should therefore start with the users, that is, agricultural bodies, which are much easier to control and which can be induced to follow instructions imposed upon them by such international bodies as FAO and WHO.

1. It is suggested that FAO and WHO should be given the job of compiling a list of phosphorus compounds which should not be permitted for use and subsequently for production on the basis of their toxicity. Substances of a toxicity higher than LD50 = 100 mg/kg, i.e., $I_t < 100$ and in exceptional cases (as described above) higher than LD50 = 50 mg/kg, i.e. $I_t \leq 50$ should be banned from use and their production prohibited.

2. It is suggested that IUPAC (International Union of Pure and Applied Chemistry) should be approached through its Applied Chemistry Division, Section on Pesticides. Here, the Commission on Terminal Pesticide Residues with Dr H. Hurtig as Chairman and Dr K. R. Hill as Secretary, and the Com-

mission on Pesticide Residue Analysis with Dr R. A. E. Galley as Chairman and Dr D. C. Abbott as Secretary, are very active and have published reports on their meetings in 1970 and 1971. Dr Galley recently became the Secretary General of the IUPAC. The International Congresses of Plant Protection and International Symposia on Pesticides which are IUPAC-sponsored meetings should be asked to help establish the permitted limits of toxicity and should be involved in the task of enforcing the practical application of the rules.

Again, the IUFOST (International Union of Food Science and Technology) should be approached. The Union sponsors International Congresses of Food Science and Technology. Furthermore, attention should be paid to the work of the symposia on "Food Additives and Residues of Pesticides in Foods" which are also sponsored by the IUPAC Division of Applied Chemistry. National bodies, such as the Pesticides Group of the Society of Chemical Industry, London, should also be involved in the action.

It seems advisable to create a special "Inter-Union Committee" embodying the activities of the IUPAC Pesticides Section and IUFOST, with the assistance of FAO and WHO, to regulate the prohibition of the use and production of high toxicity phosphorus pesticides.

3. Appropriate publicity should be given in existing specialized journals such as the Journal of Agricultural and Food Chemistry (organ of the American Chemical Society), Journal of the Science of Food and Agriculture and Pesticide Science (United Kingdom), and IUPAC, Pesticides Section, Newsletter.

4. It is suggested that governmental bodies of the member countries to FAO and WHO should be given the task of ensuring that the pesticides mentioned above (I: 4 & 5) are neither used nor being produced.

Regulations limiting the use of substances of higher toxicity should be promulgated. Similar regulations exist in some countries, such as the Union of Soviet Socialist Republics and the United Kingdom (the latter through the Ministry of Agriculture and Fisheries).

5. One of the basic prerequisites for the realization of the suggested principles of the control of the production of phosphorus pesticides is the universal adherence to the 1925 Geneva Protocol prohibiting the use in war of asphyxiating, poisonous or other gases, and of bacteriological methods of warfare, the importance of which was duly reflected in the United Nations resolution of December 1969 inviting all states which had not yet done so to accede or ratify the Geneva Protocol.

Acknowledgement

The author of the present paper is much indebted to Professor Z. Eckstein for his valuable expert advice and suggestions.

XV. *New developments in the chemistry of organophosphorus pesticides, by R. Vilceanu*

Summary

The efforts of scientific investigation today seem to be directed towards the synthesis of some products differing from the classical organophosphorus insecticides; the new products are less toxic and of a stronger effect, and spare the natural equilibrium. The main groups of organophosphorus compounds selected and applied as chemosterilants are shown in the report. Some esters of the sulphonic acids, analogues of purine and pyrimidine, derivatives of S-triazine, and so on, also have chemosterilant properties.

The use of these compounds, together with the use of hormonal pesticides (sex attractants, pheromones), will stop the intensive race for new, more and more toxic, organophosphorus products in order to solve the problem posed by the insects' increasing resistance to the compounds.

It is logical to prefer those products that do not entail a development of the insects' resistance.

Increasing interest in this new field of investigation will certainly lead to many useful discoveries in entomology, biochemistry and other allied sciences. It is assumed that the progress to be reached with chemosterilants will have a decisive role in the further orientation of pest control. The possibilities of strengthening pest control by means of sterilization are quite enough to justify a substantial effort in this field of investigation.

Introduction

Investigations concerning the relationship between the structure and the reactivity of organophosphorus compounds show that, for the time being, even the few available rules of wider applicability are based on some empirical or semi-empirical considerations. Initially, most of the respective studies were restricted to the qualitative aspect of the problem. Since 1953, articles dealing with the quantitative aspect of this relationship have been appearing in the literature. [255–258] However, even some purely chemical properties, such as saponification rates, are predictable only within very narrow limits.

Systematic investigations have lately been centered on logical correlations between the physiological action and chemical structure of the organophosphorus compounds. The common mechanism according to which the majority of organophosphorus insecticides act—namely the mechanism of cholinesterase inhibition—has been described. [228, 259–260] In actual fact, the essential phenomenon is complex and, even today, different variants are still being proposed concerning the respective mechanism for the product in question. [261]

Recently, O'Brien [262] showed that the latest studies in this direction lead

to the conclusion that the cholinesterase active site contains, besides a "catalytical" (active) site, at least three binding sites. One of them is hydrophobic and large enough to accept six methyl groups. The other two are in all probability, similar to the Wilson "anionic sites". [263] In the light of these results, methods can be formulated which permit the easy recognition of phosphoric esters that can become strongly linked to the active site of the enzyme; these methods take into account, mainly, the stereo- and the electronic factors of the system.

The existence of at least three kinds of binding sites grouped around the catalytic site shows the complexity of the system: every amino acid is potentially a site for $\pi-\pi$-binding; every long alkyl group is potentially a site for hydrophobic bonding; every free carboxyl or amino is potentially an ionic site; every OH or SH—is a potential hydrogen-bonding site. Recently, O'Brien has studied the effect of cholinesterase alkylation by means of an aziridine derivative and the site where complete acetylcholine blocking takes place.

Toxicity of organophosphorus compounds related to chemical structure

The discovery of the insecticidal properties of the organophosphorus compounds has initiated an intensive search to find new compounds, the great majority of which are formulated with phosphate, phosphonate esters and their thioanalogues [259]. Parallel to the interest in substituting the chlorohydrocarbons, the stimulation of the synthesis of new organophosphorus insecticides and the study of their biological action must be correlated with the increasing resistance of insects to the respective compounds. As a consequence, compounds with high toxicity have been synthesized.

Concerning the development of organophosphorus compounds, several marked stages can be noted differing both in activity (toxicity) and the criterium for their selection:

Compounds containing phosphorus according to a classical formula of the Schrader type (in phosphates, phosphonates, etc.)

G. Schrader [264–265] has elaborated a criterium of selection for organophosphoric esters used as insecticides, embodied in a classical formulation where the four-coordinated phosphorus is linked to an organic or inorganic acid radical; the other substituents may be varied considerably. Different variants have been attempted to modify the respective structures by a proper substitution of the molecule in order to lessen the toxicity, the insecticidal activity being maintained unchanged (for example, by modifying the methylparathion, the chlorthion, and the paraoxon molecule). [266] A series of structural conditions of the radicals directly attached to phosphorus groups, necessary for a high anticholinesterase activity, has been elucidated. [267]

The Schrader formula offers the perspective of an infinite amount of com-

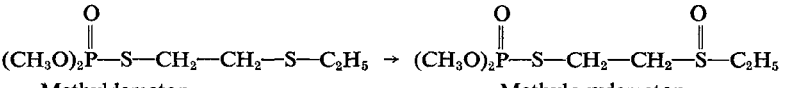
pounds with potential insecticidal properties but its accuracy is not sufficient and it does not permit quantitative estimation of the respective compound toxicity.

More complex correlations between the measurable properties of molecules (with a variable number of substituents) with different constants of these substituents, allow an *à priori* appreciation of the biological activity of the respective compounds. [258] A graduated scale of organophosphorus compound toxicity could be defined by interpreting the structural conditions that can ensure the minimum toxicity for such compounds. [258]

The enolphosphates have also constituted a field of intensive investigation [268–269], since these substances offer many possibilities of structural diversification.

Attention has also been focused on compounds having different radicals linked via sulphur.

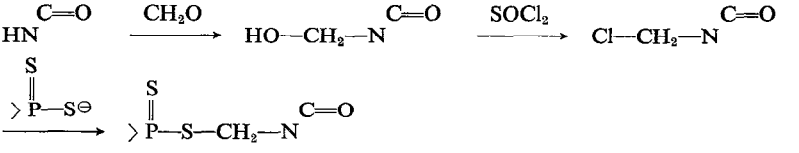
Concerning the pesticidal activity, it is of considerable interest that some esters are constructed on the principle of "phosphoryl-sulphur-polar group spaced at suitable distances", for example:



Oxidation to sulphoxide may be effected here by the insect or plant. The distance between phosphorus and the polar group can be varied. Sulphones may also be used instead of sulphoxides.

A large number of products in this series have been presented but only a few of them have become commercialized. [261]

The compound group of "Imidan", "Azinphos", "Supracide" and "Phosolene" type, where the carbonyl group is part of an aromatic ring:



The development of this group of fungicides was greatly promoted following prohibition of the use of mercurial fungicides in Japan.

The mechanism of action must be different from that of cholinesterase-inhibiting insecticides.

Low toxic compounds having phosphorus-nitrogen bonds

Despite the stimulation aroused by the synthesis of new organophosphorus pesticides and the study of their biological action, as well as the increasing

resistance shown by the insects to these compounds, the investigation did not lead to any outstandingly original ideas, apart from the structures mentioned.

The only original finding in recent years seems to be the discovery by Interchemical and Dow, USA, of the insecticidal properties of aziridinyl-phosphine oxides, for example, the tris-(2-methyl-1-aziridinyl)-phosphine oxide, [266] which is a good sterilant against flies and gnats.

A series of new organophosphorus compounds, characterized by the presence of phosphorus-nitrogen bonds in the molecule having, besides acid radicals, alkyl- or dialkylamino groups linked with the phosphorus are quite different from the classical conception represented by Schrader's formula, in that they have a very low toxicity.

Due to their sterilizing effect on insects, such compounds are of great current interest.

The chemosterilants can be defined as chemical substances which reduce or totally inhibit the reproductive capacity of the animal they are administered to. They can act upon one of the sexes of an animal with sexual reproduction or upon both sexes. Their effect can be temporary or permanent; action can be immediate or tardy.

The term "chemosterilant" appears in the literature for the first time in 1960 [270] in connection with trials concerning pest control. The investigations in this field were started under the guidance of Knipling [271] who suggested a complex program of new and revolutionary investigations for pest control; the first experiments were carried out by gamma ray irritation of *Cochliomya hominivorax Coquerel.* The population of this insect was eradicated by modification of the ratio of normal: sterile insects.

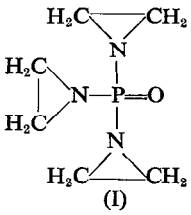
The theoretical basis for the use of chemosterilants as a means of reducing or sexually eradicating organisms, was established for the first time by Knipling. [272] Since the use of these substances does not imply the direct destruction of the insects, they can be considered insecticides only indirectly. From the theoretical point of view, this method of pest control by sterilization constitutes a considerable advance in the phytopharmaceutical field.

Studies aimed at the systematic selection of some chemical and structural characteristics specific to chemosterilization [273] led to the conclusion that the aziridine compounds form the most numerous group of agents with biological alkylation. The most efficient chemosterilants contain substituents which accept electrons, thereby lessening the basic character of the nitrogenous cycle.

The most efficient functional groups in this respect, which adhere to the nitrogen cycle, contain double bonds of a high polarity, as, for example, $C=O$, $P=O$, $C=N$, $P=S$, $S=O$, SO_2. The substitutions on the carbon atoms from the chemosterilant cycles influence the reactions of the cycle opening (i.e. its alkylating action), thus reducing their sterilizing action towards the insects.

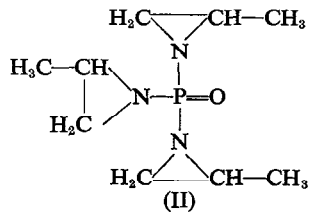
A class of such compounds, having a sterilizing action on male insects, is formed from organophosphorus compounds with aziridinyl radicals linked directly with the phosphorus atom. [274]

In this series, the following commercialized compounds can be specified:



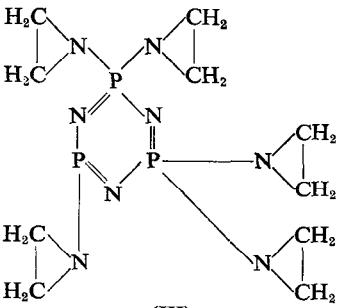
(I)

Tepa (APO, Aphoxide)



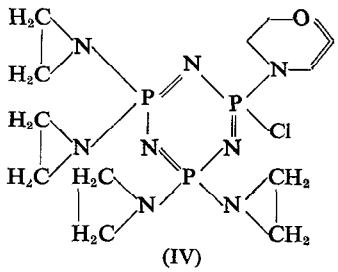
(II)

Metepa (MAPO or Metaphoxide)



(III)

Apholate

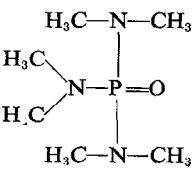


(IV)

All these substances are very unstable at temperatures above 100°, and in the presence of acid catalysts they are easily decomposed, their degradation products being inactive as sterilants. [275] "Metepa" (II) and (IV) also have well-marked sterilizing properties. The former has a very low toxicity.

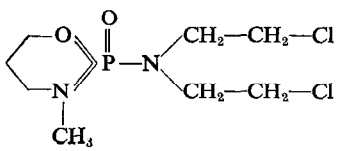
The derivatives of the phosphonic triamide (V), without possessing alkylizing properties, have a definite sterilizing effect on male insects, similar to the effect of the aziridine compounds. In this class of substances, good results have been obtained with "Hempa" (HMPA) and its sulphur analogue "Thiohempa".

The derivatives with amides containing only radicals of strongly basic aliphatic amines have similar effects.



(V)

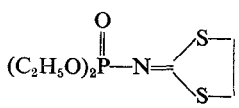
Hempa



(VI)

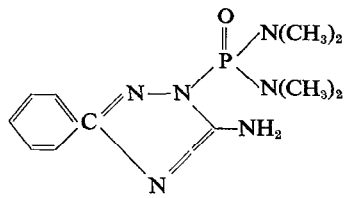
Endoxan

The (VII) and (VIII) derivatives, having a pesticidal action, are N-phosphorylated heterocycles.



(VII)

(Derivative of 2-iminothioran)

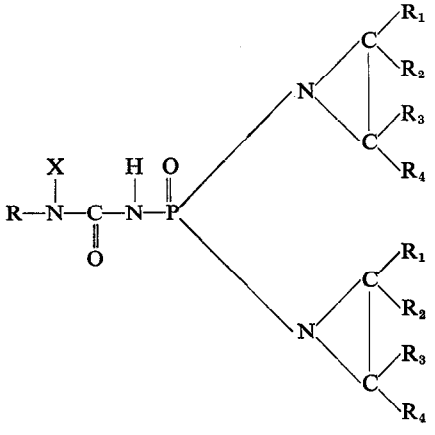


(VIII)

Triamphos

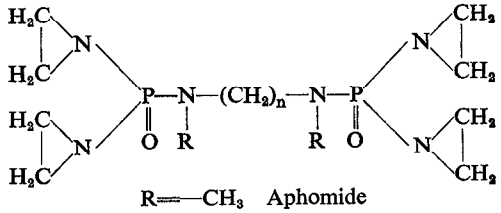
The alkylating effect of aziridine is claimed to be responsible for the activity in this case; the P=O group supports this alkylation by its electron-pulling effect.

Well-defined chemosterilizing effects, even when very low concentrations are used, are also shown by some organophosphorus derivatives having the structure of ureides of N-substituted phosphine-oxides.



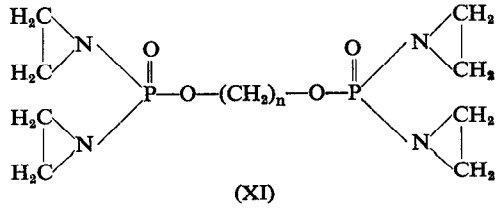
(IX)

These derivatives are obtained from the respective phosphorus isocyanates. The phosphorus-aziridine chemosterilants can also be obtained on the basis of the X, XI and XII structures.

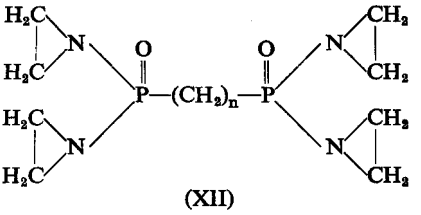


R=—CH_3 Aphomide

(X)



(XI)



(XII)

What are the advantages of these classes of compounds in comparison with those of the classical Schrader structure?

1. They are specific for insects, particularly for a certain category of insects that must be destroyed (selectivity).

2. They are not toxic or active for higher animals; do not cause problems of pollution, toxic residues, residual persistance, etc.

3. The principle of their activity is similar to the principle formulated by Paul Ehrlich as the basis of chemotherapy: to find in the insect a biochemical pathway or a compound essential to the insect but which is not found in higher animals-and to inhibit this pathway or interfere with the activity of this compound by means of a certain product or medicine.

Further, by using methods of sterilization, either by ionizing radiation or by chemosterilants, male insects can be sterilized in parallel with the females. Some of the sterilants (for example "Aphoxide") have a sterilizing action on both sexes.

Knipling has calculated that if there is a natural population of 1 million virgin females, the release of 2 million sterile males in each of four successive generations will theoretically reduce the female population to zero. Nevertheless, as it has been recently shown, the sterilization of both sexes is sometimes even superior to the release of the sterile males alone.

It can be assumed that chemosterilization has a biochemical mechanism-the cross linking of DNA chains which causes dominant lethal mutations.

Raw materials and intermediates in obtaining compounds with P-N bonds, having the above-mentioned structures

The intermediate products and the raw materials are quite different from those used for synthesis of classical organophosphorus insecticides (PCl_5, dichlorethan, phosphorus isocyanates, diphosphonates, aziridines).

The equipment for the preparation of these classes of compounds differs considerably compared to the classical equipment necessary for manufacturing classical organophosphorus insecticides. Secondly, since the products are not toxic, protection measures for workers and packers are normal. Furthermore, the intermediates offer the most varied applications, economy-wise.

Conclusions

1. The efforts of scientific investigations seem to be directed today towards the synthesis of some products differing from the classical organophosphorus insecticides, less toxic and of a stronger effect, that do not disrupt the natural equilibrium. The latest congresses dealing with these problems (Paris 1969, Tel-Aviv 1971) support this statement. The main groups of organophosphorus compounds selected and employed as chemosterilants are described in the present report. Similar actions are also achieved with some esters of the sulphonic acids, analogues of purine and pyrimidine, derivatives of S-triazine, etc.

2. The use of these compounds in conjunction with the use of hormonal pesticides (sex attractants, pheromones) will stop the intensive search for new and increasingly more toxic organophosphorus products purported to be necessary for solving the problems posed by the development of resistance in insects.

I believe that those products which do not lead to the development of resistance in insects are logically to be regarded as both modern and preferable.

The increasing interest in this new field of investigation will certainly lead to many useful discoveries in entomology, biochemistry and other allied sciences. It is assumed [276] that the progress achieved with chemosterilants will play a decisive role in further developments in pest control. The opportunities for improving pest control by sterilization are quite sufficient to justify a substantial effort in this particular field of investigation which is of great current interest.

XVI. *The production of organophosphorus insecticides in the Federal Republic of Germany, and their control by government authorities and the Western European Union, by K. H. Vöpel*

Summary

For both humanitarian and economic reasons, it is not possible to do without the production of organophosphorus insecticides. At present, there is no effective alternative to chemical pest control for preventing serious losses of world crop production from insects. Many research groups are working on the development of low-mammalian-toxic insecticides, that is, selective insecticides. So far, however, it has usually been found that compounds which are less toxic to mammals are also less effective against pests. Fresh problems, such as the development of insect resistance or residue problems brought about by the use of high dosages, may then arise.

The national control of production facilities by government officers is described.

This control is preceded by a licensing procedure both for new production plants and for changes in the use of existing facilities.

The applications for licenses filed by the industry are publicly discussed. All existing production facilities are registered. The authorities are fully informed on the capacities of the production facilities and on the individual products made in them. The authorities make controls to ensure that the licensed production fully conforms with the practised production.

The international control by the Arms Control Agency of the WEU is specially concerned with inspection for the nonproduction of chemical and biological warfare agents, although this paper refers only to organophosphorus compounds, in keeping with the theme of the symposium. In the revised Brussels Treaty of 1954 the German Federal Republic has clearly undertaken, with respect to organophosphorus chemicals, to permit only the production of such compounds which do not constitute chemical warfare agents. The control for nonproduction of chemical warfare agents extends also to the use of so-called "characteristic substances".

The procedures practised in the German Federal Republic therefore undoubtedly guarantee a most adequate measure of control. As far as the author is aware, the control provided for in the WEU Treaty is the only international control currently practised in the chemical sector. The experiences gained from this control procedure may provide very useful suggestions for future steps in obtaining a world-wide solution to this problem.

Introduction

The Federal Republic of Germany, one of the world's largest producers of organophosphorus insecticides, undertook on 23 October 1954, within the framework of the Brussels Treaty, not to manufacture chemical warfare agents of any kind. The German Federal Republic submitted, as the only country, to the effective control of the nonproduction of chemical warfare agents, including those based on organophosphates, by an international authority, the Arms Control Agency of the Western European Union (WEU). This paper will therefore report on the production of organophosphorus insecticides in the German Federal Republic, together with details of the control procedures governing such compounds and quantities of organophosphates, which enter into consideration as chemical weapons.

Production of organophosphorus insecticides in the German Federal Republic

Supplying the world population with ample quantities of high-quality foods is definitely one of the chief problems that must be solved in the coming decades. It is known that out of the present world population of about 3 500 million, some 2 000 million suffer from hunger or malnutrition. [277-278]

The results of surveys show that by 1975 the world's food supplies will have to be increased by more than 35 per cent merely to sustain the world's

population at its present unsatisfactory level of diet. If a reasonable improvement in the level of nutrition is to be achieved, then the world food supply will have to be increased by more than 50 per cent by 1975.

What measures can be adopted to remedy this situation? There are six factors which are of decisive importance to the solution of this problem, namely:

1. Agricultural structure, i.e. its improvement;
2. Crop acreages, i.e. their expansion;
3. Crop protection, i.e. consistent and expert use of appropriate measures;
4. Fertilisation, i.e. its intensification;
5. Plant breeding, i.e. exploitation of advances made in this field, and
6. Soil cultivation, i.e. its improvement.

This list of factors has purposely been drawn up in alphabetical order because an assessment of their priority will greatly differ from one country to another, one growing area to another, and from one crop to another.

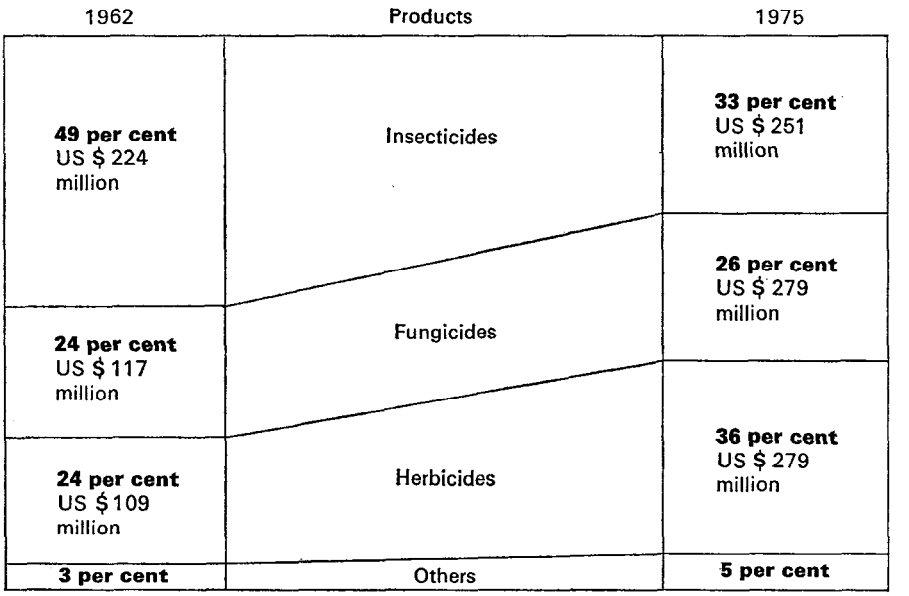
It is, however, an undisputed fact that crop protection, despite the complex of problems with which it is confronted, has an established place within this group and is thus an indispensable aid in programmes directed towards overcoming hunger and starvation in the world.

Abandonment of modern crop protection practices would not cast us back amidst conditions of the kind described in the Bible (Book of Haggai, Chapter 2, verse 16-17: "Since those days were, when one came to an heap of twenty measures, there were but ten: when one came to the pressfat for to draw out fifty vessels out of the press, there were but twenty. I smote you with blasting and with mildew and with hail in all the labours of your hands . . .") but it would have catastrophic consequences as regards keeping the world population supplied with sufficient food, which is a difficult undertaking anyway.

It has been estimated [279] that 35 per cent of the total world harvest is still destroyed by insect pests, plant diseases and weeds which is equivalent to a financial loss of approximately US $75 000 million. Insect pests account for about 14 per cent of this crop production loss, plant diseases for 12 per cent and weeds for 9 per cent.

Apart from these aspects of food production, crop protection gives two other impulses which are of national economic importance. Firstly, there is the agricultural economic factor which likewise continues to gain in importance year after year for in those countries which go in for intensive agriculture there is usually also big industrial development resulting in a shortage of labour. Hence chemical methods become absolutely necessary for achieving optimization of agriculture, especially with far-reaching mechanization in mind. (In the German Federal Republic, 10 per cent of the national labour force are foreign workers).

Chart XVI.A. Forecast of production of crop protection chemicals in the USA



And, last but not least, the industrial economic importance of the active ingredient productions and the associated auxiliary goods industries is of considerable national economic interest.

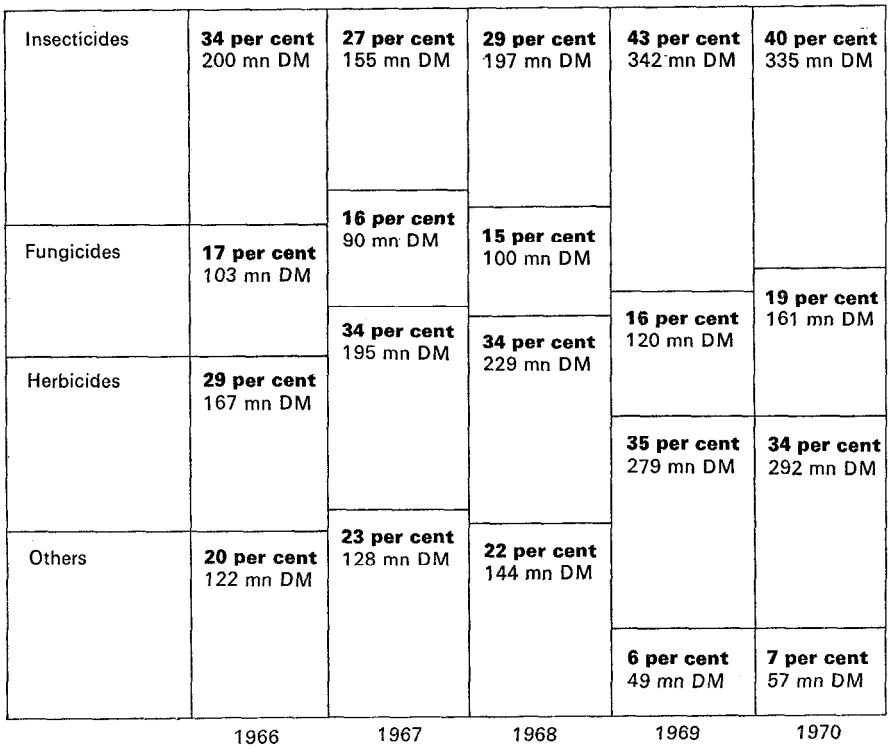
A study of the three principal groups of crop protection chemicals, namely insecticides, fungicides, and herbicides, giving consideration to a forecast by Johnson, Krog and Poland [280–281], shows that in the United States the share of insecticides in the crop protection chemical market will drop, relatively speaking, whilst that of fungicides will remain about constant and that of herbicides will increase considerably. This forecast holds for the United States which, however, is estimated to account for approximately 50 to 60 per cent of the total world consumption of crop protection chemicals (chart XVI.A.).

In 1969, 500 000 tons of crop protection chemicals were produced in the United States. Of this volume 178 400 tons were herbicides, equivalent to 35.6 per cent; 63 800 tons were fungicides, equivalent to 12.7 per cent; and 258 000 tons were insecticides and rodenticides, equivalent to 51.7 per cent. 41 900 tons of organophosphates were produced, equivalent to 8 per cent of the insecticide share of total production.

The total sales value was $851.17 million, of which herbicides accounted for 58.2 per cent, fungicides for 7.1 per cent and insecticides for 34.6 per cent. Organophosphates had an 8.5 per cent share of the total sales. [282]

The trend in the German Federal Republic, illustrated in chart XVI.B., is somewhat different. One of the reasons for this is that more than 50 per cent

Chart XVI.B. Production of crop protection chemicals in the Federal Republic of Germany



of the production of crop protection chemicals in the United States is for the home market and that the structure of agriculture in the USA is different from that of the countries to which the Federal Republic of Germany chiefly exports. The German industry, on the other hand, is able to sell no more than about 15 to 20 per cent of its active ingredient production on the home market. Its high proportion of exports is due, among other things, to the fact that the development of organophosphorus insecticides began in Germany.

The total world turnover of crop protection chemicals in 1969 is estimated at approximately US $2 000 million. Approximately 30 per cent of this turnover is manufactured by Western European companies.

Of the insecticides manufactured on a large industrial scale, there are three groups which together account for the main share of the market, these being: (*a*) chlorinated hydrocarbons; (*b*) organophosphates, and (*c*) carbamates.

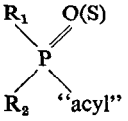
The most outstanding position among these compounds is held by the organophosphorus insecticides. Their importance will undoubtedly increase during the coming years since the use of a number of chlorinated hydrocarbons has been banned in many countries because of their persistence and the as-

sociated hazards, which recently have given cause of much discussion. The organophosphorus insecticides are currently the only practicable and economic alternative.

The annual world production of organophosphorus insecticides is estimated at 120 000 tons. The German Federal Republic's share of this production is estimated to be 30 per cent.

It was G. Schrader who, through his search for synthetic organic insecticides in the mid-thirties, paved the way for the development of a now almost countless range of organophosphorus active ingredients. Following the breakthrough made by Schrader, his team of research workers continued to make unceasing progress in the following years by consistently expanding on the findings achieved.

Schrader [283] empirically postulated, at a very early date, the following formula for the structure of biologically active phosphoric esters:



that is, in the molecule the pentavalent phosphorus must contain a semipolar-bound oxygen or sulphur atom. Two like or dissimilar groups R_1 and R_2 having a simple bond, e.g., alkyl, alkoxy or alkylamine groups, must bond the two other valences of the phosphorus whilst the fifth valence is bound by the remainder of an organic or inorganic acid.

On the basis of these findings, more than 23 000 compounds have so far been synthesized by Dr Schrader, his successor and their research group, and tested for their potential commercial use as insecticidally active ingredients.

Despite all endeavours, it is still not possible to make accurate statements on the toxicological properties of an active ingredient solely on the basis of its chemical structure. These properties must always be determined empirically after the synthesis of the active ingredient.

Therefore, no scientist will be surprised to learn that patent applications were filed for only about 10 per cent, in other words, 2 300 of the tested compounds. The other compounds either displayed insufficient effectiveness against pests or they proved to be unsuitable for commercial use due to their high order of mammalian toxicity.

The requirements that have to be met by a technically useful insecticide have grown from year to year. It will be noted from tables XVI.B, XVI.C and XVI.D that out of the 23 000 compounds, only about 0.1 per cent have met with economic interest.

What properties must an active ingredient possess if it is to have a chance of

entering the market or to compete successfully with other products already established on the market?

The properties that will now be listed are not given in order of priority because this differs from one use to another and from one country to another. These properties are as follows:

1. A high measure of effectiveness
2. Possibly a high degree of selectivity
3. Very low order of mammalian, fish and bird toxicity
4. Fast degradation *in vitro* and *in vivo*
5. Economical to manufacture
6. Satisfactory storage stability
7. Clarity regarding metabolites and residue problems
8. Possible effectiveness against resistant strains
9. Good formulating property and ease of application

The following tables will show that only few compounds succeeded in collecting enough significant points in their favour to be manufactured on a large industrial scale.

In the tables, the pathway is shown, starting from phosphorus, which leads through the different inorganic basic chemicals to the phosphoric ester intermediates and to the active ingredients.

The phosphorus derivation chart down to the organic intermediates is illustrated in table XVI.A. All these compounds still have no insecticidal activity.

It will be noted that there are only four inorganic and eight organic phosphorus compounds on which the entire industrial production of organophosphorus insecticides is based in the German Federal Republic.

From these intermediates, 26 insecticides are manufactured on a large industrial scale, as shown in tables XVI.B, XVI.C and XVI.D. In the tables, the first line on the right of each structural formula gives the trade name of the respective product, followed by the name of the manufacturer (FFB = Farbenfabriken Bayer AG; BASF = Badische Anilin- und Sodafabriken), and then by the year in which production was started. The second line gives the respective LD50 values, for a study (exposure) period of 7 days unless otherwise stated.

As already mentioned, only a very small share of the German Federal Republic's production of organophosphorus insecticides is sold on the home market, over 90 per cent being exported. About 80 per cent of the products exported are in the form of active ingredients, the balance consisting of several hundred different formulations and premixes.

The production plants of Farbenfabriken Bayer AG in the German Federal Republic are situated at Elberfeld and Dormagen, and those of BASF are located at Ludwigshafen.

Table XVI.A. Phosphorus derivation chart of the most important intermediates used for production of phosphorus insecticides

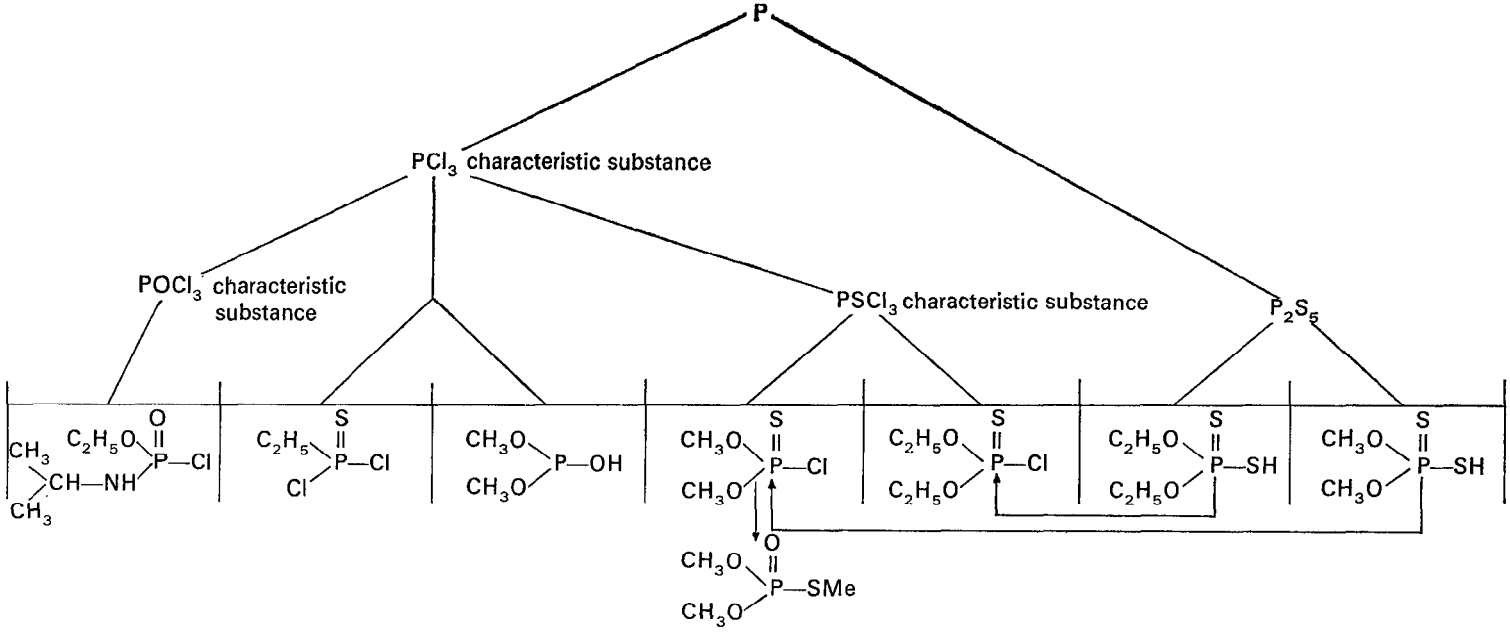


Table XVI.B. Phosphorus derivation chart of well-known phosphorus insecticides (commercial products)

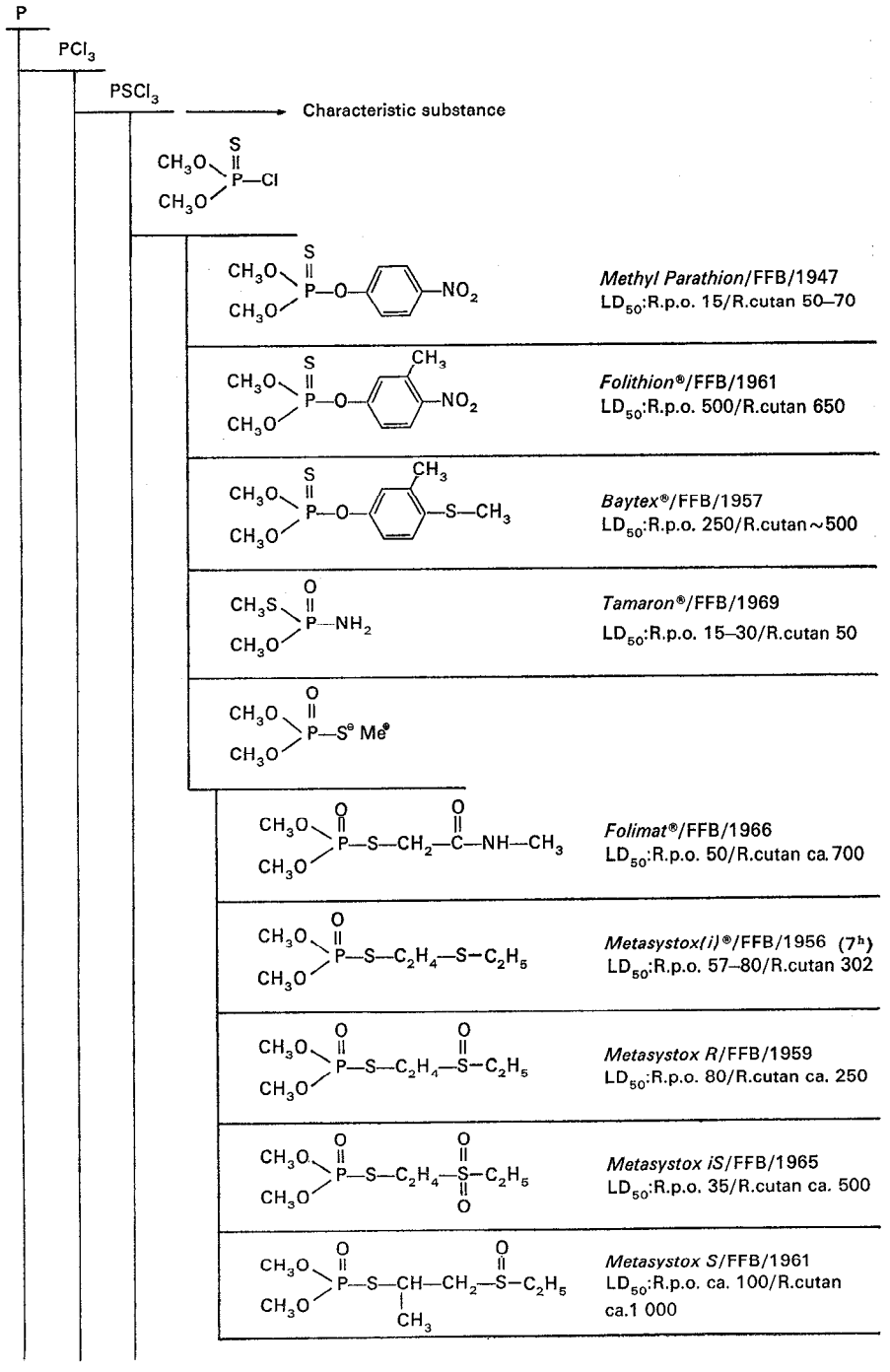


Table XVI.C. Phosphorus derivation chart of well-known phosphorus insecticides (commercial prodcts)

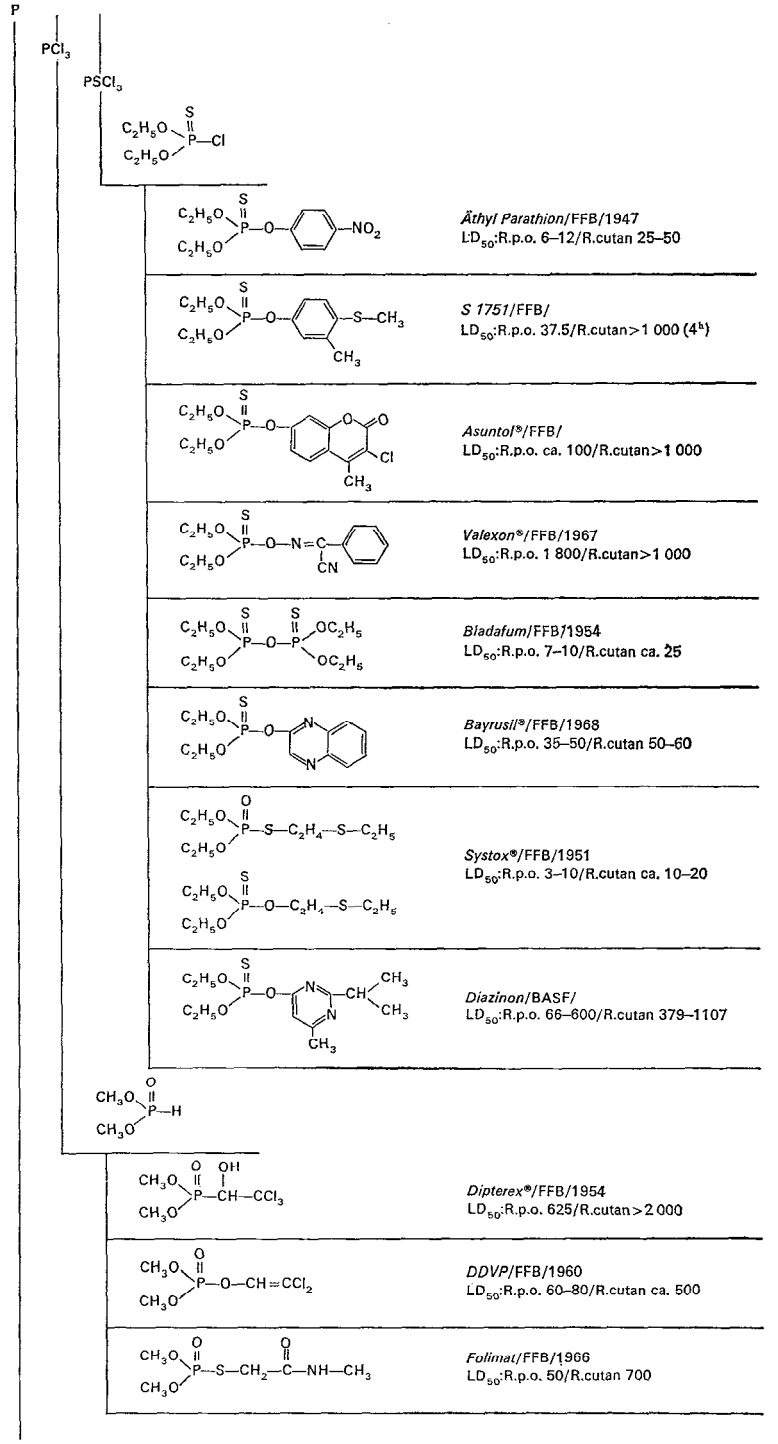
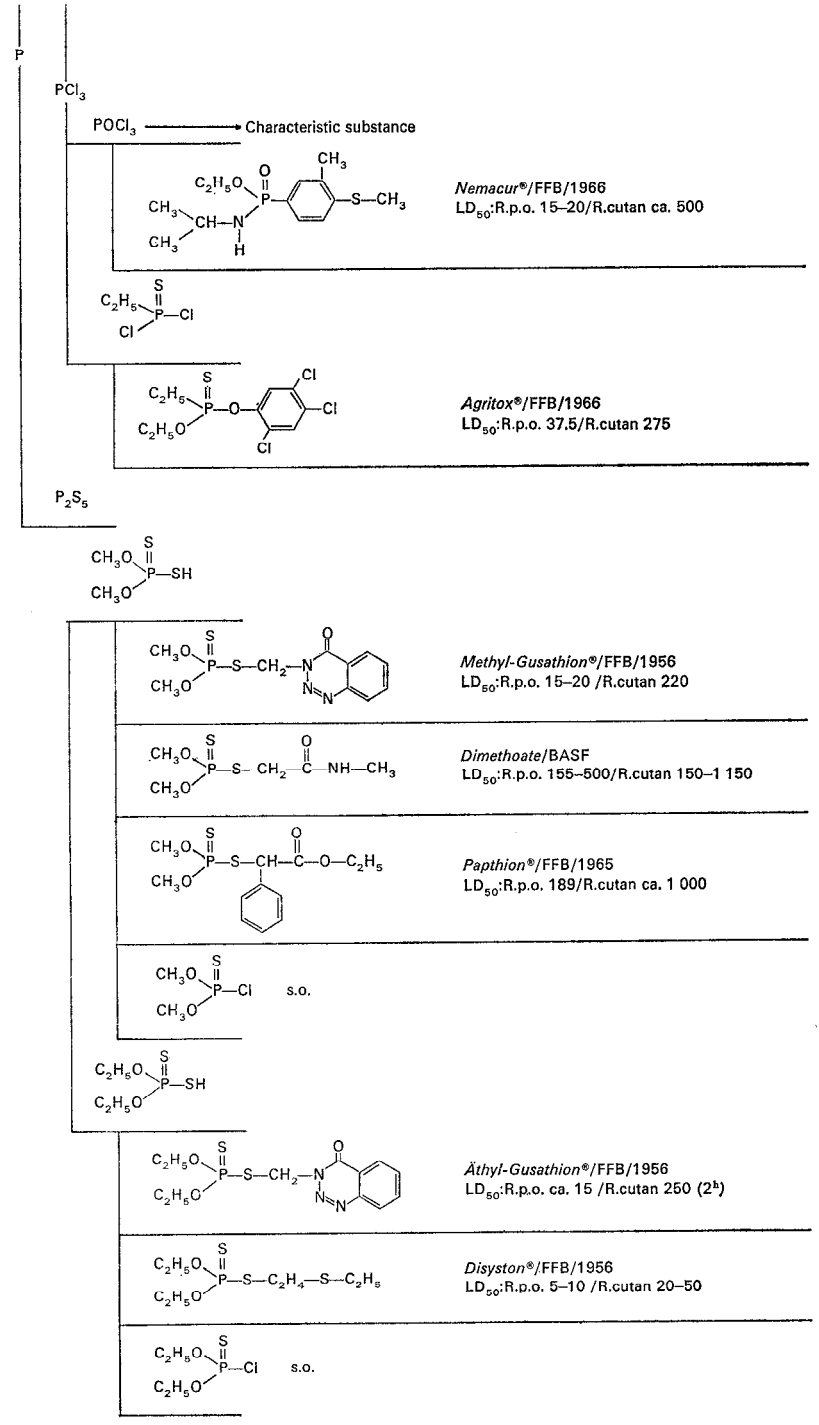


Table XVI.D. Phosphorus derivation chart of well-known phosphorus insecticides (commercial products)



Control of production plants by government authorities

The production plants for pesticide active ingredients may be divided into three groups with respect to capacity:

1. Plants producing less than 100 tons per year
2. Plants producing more than 100 tons per year
3. Plants producing more than 10 000 tons per year

Production of up to 100 tons per year

These productions are usually carried out in pilot plants. In most cases high production costs are involved and use is made of universal apparatus although this is not necessarily the best technical solution for the manufacture of the active ingredient. These plants are used for producing the quantities required for the introduction of the active ingredient on the market and for acquiring the know-how that is absolutely necessary for the design of the large-scale industrial production plants.

Production exceeding 100 tons per year

These productions are carried out in plants exactly matched to the peculiarities of the active ingredient. Such plants are so highly optimized technically that they also constitute an economic optimum. All processing steps which can be done continuously are fully monitored by appropriate instrumentation to ensure optimum economy and control of the production process, as well as to obtain the set standard of product quality and also for reasons of environmental protection, that is, to have constant streams of waste water and waste gases for which there are special treatment units in each plant.

Due to the degree of optimization needed to achieve economy as well as to meet the requirements of environmental protection, these plants are usually technological units in which only one active ingredient group can be manufactured.

To give but one example, Methyl Guthion(R) and Ethyl Guthion can be produced in one apparatus but other active ingredients (e.g. Perfekthion(R), Papthion(R), Di-Syston(R) made from the same phosphoric ester intermediates as Guthion cannot be manufactured in the Guthion plant.

Production exceeding 10 000 tons per year

For production exceeding 10 000 tons per year, specialization is still greater. These plants are so designed to obtain a technological and economic optimum for the manufacture of the one specific major product.

With such plants, it is often difficult to change the plant capacity as required because otherwise such equipment as extraction columns, distillation columns,

absorption columns, continuous reaction cascades, etc. may no longer produce the performance required.

Therefore, it may be generally said that as the size of a plant increases, the possibility of manufacturing other products in it becomes correspondingly less. This is because within the different steps of the manufacturing process all measures taken for internal safety, that is, for the plant and the people working in it, and for external safety, i.e., for environmental protection (waste gases and waste water), are specifically matched to the characteristics of the individual chemical product made in the plant.

All such plants must be approved by the responsible regional government. [284] To obtain this approval, it is necessary to submit detailed construction plans, drawings of apparatus, detailed descriptions of processes, flow sheets, and plans of systems for waste water and waste gas treatment and disposal.

Before approval is granted, the planned building project is published in the press. Objections or protests can then be lodged by the public or by communal institutions, which will be discussed in public hearings.

The government also has appraisals prepared by the following authorities and organizations:

1. Department of trade and industrial control
2. Department of health
3. Public works department
4. Water department
5. Employer's liability insurance association of the chemical industry
6. Fire service

Therefore, a very large section of the population is informed and can inspect documents before the permit to build a production facility is granted.

The use of the licensed production facility is subject to constant control by the Department of trade and industrial control. Inspections are made at irregular intervals, and the inspector from the department of trade and industrial control can visit the production facilities at any time without previously informing the management. The purpose of the inspection is to establish that only those products licensed for production in the permit are being manufactured and also only in the licensed quantities.

Each change of the use of a production facility requires a new permit, that is, the procedure described above must be carried through again. In this respect, an increase in capacity, without changing the product being manufactured, is also considered as a change in the use of the production facility.

In accordance with paragraph 141 of the Factory Act and paragraph 33 of the law relating to disregard of provisions and regulations, heavy penalties are

imposed upon persons who are responsible for management and operation if they depart from the terms of the licences.

Besides these laws and regulations on industrial production, compliance must be made in the German Federal Republic with the 1961 law relating to the control of war weapons, which in an appendix giving a list of war weapons, refers to the WEU definitions also of chemical weapons, thus prohibiting the production of such weapons and placing violators of the law under penalty. The structural formulas of the different chemical warfare agents not usable for peaceful civilian requirements are included in the list of war weapons. Compliance with the provisions of the war weapons control law is controlled by the Bundesamt für gewerbliche Wirtschaft in Frankfurt. [285]

WEU control of the nonproduction of organophosphorus warfare agents

It is generally known that the German Federal Republic has agreed, within the framework of the Brussels Treaty of 1954, not to produce chemical weapons. The amended Brussels Treaty (WEU Treaty), together with the Paris Protocols and other documents, came into effect for the Federal Republic on 6 May 1955. The member nations of the Western European Union are Belgium, The Federal Republic of Germany, France, Holland, Italy, Luxembourg and the United Kingdom. The Federal German law ratifying the amended Brussels Treaty was promulgated on 24 March 1955 (Bundesgesetzblatt Teil II, p. 256 and 630).

The Arms Control Agency of the WEU in Paris, acting as the responsible international authority, controls the fulfilment of the obligations undertaken by the German Federal Republic, also with respect to organophosphorus warfare agents. Production facilities, depots and the armed forces are regulated. The Arms Control Agency requests information every year, stating, among other things, what active ingredients, i.e., insecticides, are produced and in which production plants; also in which production plants the so-called characteristic substances are produced; furthermore, what quantities are being produced in the current year, and what quantities are planned to be produced in the coming year and for what purpose they are to be used.

This information provides the Agency with a survey of the production relevant to the control, and also provides the basis on which the Agency can take a decision as to which of the approximately 4 000 chemical plants should be controlled.

The annual requests of the Agency for such information are answered in writing by the responsible Federal German authorities. Three control measures are distinguished in the WEU:

1. The visit which is understood to mean the establishment of direct contact between the control agency and the management of the company involved.
2. Sondage is a spot check at specific points on the factory premises of the company involved.
3. Inspection is an exact control of all facilities and materials of the company subject to inspection.

The inspectors are given: (*a*) free access to the production facilities and depots, (*b*) insight into records and documents, and (*c*) information required for the control. Provision must be made, however, that business or production secrets are not violated in any way whatsoever by these controls. The controls take place at irregular intervals.

The following controls have so far been made at the factories of Farbenfabriken Bayer AG:

15 October, 1958	FFB Elberfeld
16 October, 1958	FFB Elberfeld
17 October, 1963	FFB Leverkusen
18 October, 1963	FFB Elberfeld
6 July, 1967	FFB Leverkusen
22 May, 1968	FFB Dormagen
16 June, 1969	FFB Elberfeld
17 May, 1970	FFB Leverkusen

An appropriate legal procedure for the protection of private interests during the course of inspections in factories and other nonmilitary installations and facilities is set out in an agreement dated 14 December, 1957. The Federal German law ratifying this agreement was promulgated on 10 April, 1961 (Bundesgesetzblatt Teil II, p. 384).

However, this agreement on legal protection has not yet come into effect because it still awaits ratification by France. Therefore, the situation at present is that the inspections and controls require the consent of the companies involved and the responsible Federal German authorities. However, this consent has always been given willingly.

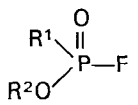
What is controlled?

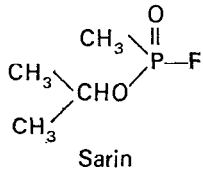
The answer to this question basically lies in the definition of chemical weapons given in Protocol No. III, Annex II, Section II of the WEU Treaty:

(*a*) A chemical weapon is defined as any equipment or apparatus expressly designed to use, for military purposes, the asphyxiating, toxic, irritant, paralysant, growth-regulating, anti-lubricating or catalyzing properties of any chemical substance.

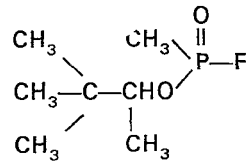
Table XVI.E. List of well-known phosphorus warfare chemicals

(1) Alkylphosphonic acid-alkylester-fluoride (e.g. Sarin, Soman)



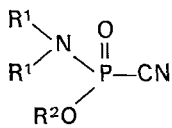


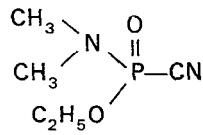
Sarin



Soman

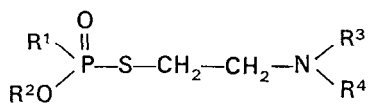
(2) Phosphoric acid-dialkylamid-cyanid-alkylester (e.g. Tabun)

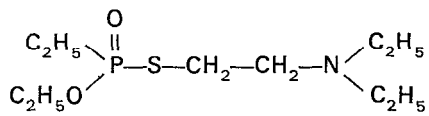




Tabun

(3) Alkylthiophosphonic acid-S-(2-dialkylaminoethyl)-alkylester





Phosphon-Amiton

(*b*) Subject to the provisions of paragraph (*c*), chemical substances having such properties and capable of being used in the equipment or apparatus referred to in paragraph (*a*), shall be deemed to be included in this definition.

(*c*) Such apparatus and such quantities of the chemical substances as are referred to in paragraphs (*a*) and (*b*) which do not exceed peaceful civilian requirements shall be deemed to be excluded from this definition.

This very general definition is interpreted more exactly, as already provided for in the Treaty, in a *list of chemical substances* approved in 1958 by the Council of the WEU, and which has since been repeatedly reviewed and supplemented.

Besides chemical substances which exceed peaceful civilian requirements (Section I of the list), chemicals of bivalent character (Section II of the list), that is, chemicals which are used for peaceful civilian requirements but which could also be used as chemical weapons (hydrocyanic acid, cyanogen chloride, p-chlorophenyl dimethyl urea, maleic hydrazide) are also controlled.

In accordance with the valid WEU list, the organophosphorus substances listed in table XVI.E, are to be regarded as chemical weapons because they exceed peaceful civilian requirements.

In practice, the inspection for the nonproduction of chemical weapons covers the so-called characteristic substances (see tables XVI.A, XVI.B, XVI.D) required for their production. These substances are not chemical weapons in themselves. They are starting substances or key products without which the chemical weapons in Section I of the list cannot be produced. Depending upon which other chemical substances are added, end products for peaceful civilian requirements or the chemical weapons as defined in Section I of the list can be produced from the characteristic substances. By making an inspection in the factory, the Agency wishes to make sure that the characteristic substances are not processed during the controllable phase to chemical weapons as set out in Section I of the list.

To characterize the intermediates required for the listed active ingredients, a phosphorus derivation chart is given in table XVI.F, analogous to tables XVI.B to XVI.D. The AO = acute oral LD50 values and the AD = acute dermal LD50 values have been taken from the Lehrbuch für Militärchemie, Band 2/1968, Deutscher Militärverlag, Berlin, DDR.

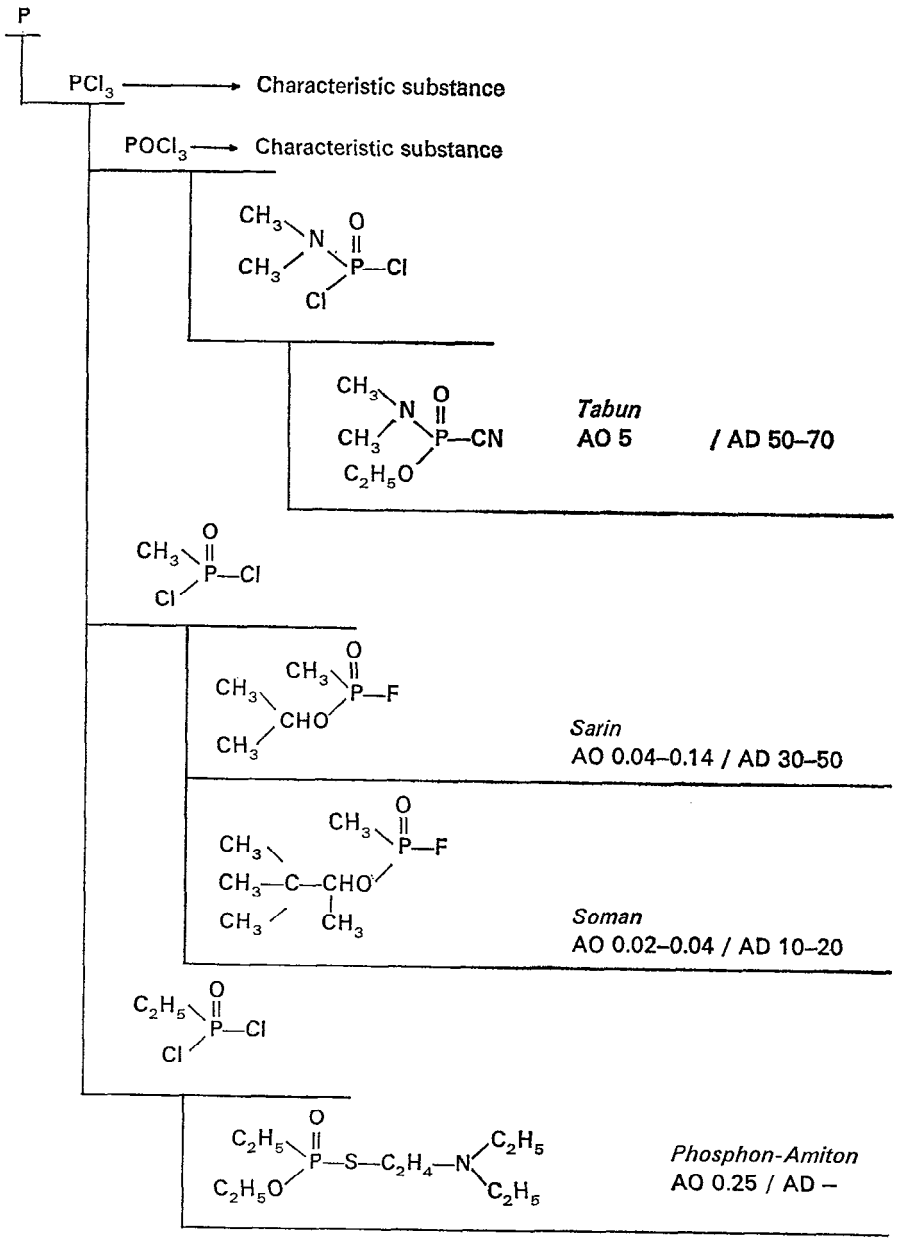
Conclusions

It is evident from what has been said in the first section, that for both humanitarian as well as economic reasons, one cannot do without the production of organophosphorus insecticides. At present there is no effective alternative to chemical pest control for preventing serious losses of world crop production from insects. Many research groups are working on the development of low-mammalian-toxic insecticides, i.e. selective insecticides. So far, however, it has usually been found that compounds which are less toxic to mammals are also less effective against pests. Fresh problems such as the development of insect resistance or residue problems brought about by the use of high dosages may then arise.

The national control of production facilities by government officers is described in the second section. This control is preceded by a licensing procedure both for new production plants as well as for changes in the use of existing facilities.

The applications for licenses filed by the industry are publicly discussed. All existing production facilities are registered. The authorities are fully informed on the capacities of the production facilities and on the individual products made in them. The authorities make controls to ensure that the licensed production fully conforms with the practised production.

Table XVI.F. Warfare chemicals derived from phosphorus



The international control by the Arms Control Agency of the WEU, described in the third section, is specially concerned with inspection for the nonproduction of chemical and biological warfare agents, whereas in this paper reference has been made only to organophosphorus compounds in keeping with the theme of the symposium. In the Brussels Treaty, the German Federal Republic has

clearly undertaken, with respect to organophosphorus chemicals, to permit only the production of such compounds which do not constitute chemical warfare agents. The control of nonproduction of chemical warfare agents extends also to the use of so-called "characteristic substances".

The procedures practised in the German Federal Republic therefore undoubtedly guarantee a most adequate measure of control. It would be interesting to learn in this connection whether and, if so, what controls exist or could be introduced in other countries. Perhaps we can come back to this question in the discussion.

As far as I am aware, the control provided for in the WEU Treaty is the only international control currently practised in the chemical sector. I believe that from the experiences gained from this control procedure very useful suggestions can be derived for future steps undertaken to obtain a world-wide solution to this problem.

XVII. *Technology and possible control, by J. Voprsál*

Summary

Technology and control of organophosphorus compounds are very intimately connected. The possible control may be realized as (*a*) national control, or (*b*) international control. The former type seems more convenient and acceptable. The results might be presented to the United Nations.

The technical items to be controlled comprise raw materials, technical equipment, safety measures and economic conditions for production. For technical reasons, it seems improbable that insecticide plants could be converted for production of nerve agents. It would probably be more convenient to erect new facilities.

The task of detecting suitable targets for inspection would be a difficult one. However, the nerve agents are, at present, the most important chemical warfare agents. Further, it appears that the technical possibilities for inspection have increased.

Introduction

The technology and control of organophosphorus compounds are connected very intimately.

Most states have accepted the Geneva Protocol of 1925. This Protocol embodies an important and generally recognized rule of international law prohibiting the use of chemical and bacteriological warfare methods. Since that time, evolution has become more complicated. The results of World War II indicated that it is very necessary to restrict or prohibit completely chemical and biological weapons. The United Nations General Assembly accepted resolutions in the problem of chemical and biological (bacteriological) weapons.

It seems to be very necessary to define exactly both groups of agents. The definition proposed at the SIPRI Symposium on Chemical Weapons, Stockholm 1968 by Dr Lundin fulfils this very well.

With the technical progress in this field, the problems of control also became urgent. Possible control may be realized as (*a*) national control, or (*b*) international control.

It seems probable that, in most cases, the national control (self-inspection) will be more convenient and acceptable. The self-inspection would be organized in a proper manner so as to give reliable results. These results can be presented to organs of the United Nations, if desired. Some points of view were discussed at the Conference of the Committee on Disarmament, 6 August 1970 and need not be presented now.

Technology

The process of synthesis of the chemical agent needs:

(*a*) adequate raw-materials
(*b*) technological schemes and technological equipment

Raw materials

For the production of an organophosphorus agent one needs as raw materials the following substances: yellow phosphorus, phosphorus trichloride, pentachloride, oxychloride, pentasulphide, chlorides of different aliphatic acids, derivatives of phenol, hydrofluoric and hydrocyanic acids, choline, thiocholine etc.

These raw materials make possible the first step in the control procedure. Methods for identification of all these compounds are well and generally known and also sufficiently easy to be carried out in the laboratory.

Naturally, the presence of all these materials or any part of them does not necessarily imply or ascertain any production of chemical agents.

Technology and technological equipment

Manufacturing equipment must be highly resistant to corrosion and the proper material must be considered and chosen with this in mind. Very often this circumstance may be significant at the time of the control. The construction of such equipment needs stainless steel, copper and its alloys, nickel, Monel and Hastelloy. This is important as one criterion of possible CW production; it is obvious that general equipment in the chemical industry is also made from such materials and therefore further items must be taken into account in forming a judgement of CW production.

Many other details of chemical engineering techniques may be taken into account if needed.

A plant manufacturing process requires the processes of oxidation, alkylation, esterification, amination and fluorination. The principles of these unit-processes are very well known in organic technology. During these operations one needs to heat or cool and this cannot be done with water-steam or water-cooling, as mentioned later.

Economy of the production

Also this aspect may be very valid in judging the total process of CW production. For example, a country which imports all the raw-materials needed, can probably produce CW on a limited scale, if at all. An analysis of the chemical import in respect to raw materials used seems therefore to be very important. The erection of a plant, the judging of its capacity and production, problems of transportation and many other viewpoints are valid and useful for purposes of control.

Agent production control

Inspection systems must be completely independent of the search for specific agents. Very important is how to solve the problem of detecting production when neither the method nor the agent is known. We must take into account the fact that agent production differs significantly from commercial operations. Pesticide structures differ from agent structures. High toxicity is associated with alkylated phosphorus compounds. In contrast, pesticide research develops materials which possess high toxicity towards insects and pests but low toxicity to mammals. Economical requirements dictate that the most applicable compounds will be those which are highly effective against pests and can be easily manufactured at low costs from readily available materials. Organophosphorus compounds for military purposes, on the other hand, must be highly toxic, which can only be achieved with some difficulty at acceptable cost. Usually one observes that highly toxic materials are more difficult to produce and often have higher prices.

Because there exist important differences in the structure of pesticides and agents, there are also substantial differences in the raw materials required, and in the kind and nature of the equipment involved. It is possible to say that large-scale production of agents cannot be made in standard pesticide facilities.

A. R. Pittaway [286] states a minimum of 24 possible combinations when any four unit-processes mentioned above are arranged in all their possible orders. Pittaway's chart is very important. It represents all the theoretically possible approaches to the production problem.

The practical methods of the production will vary with:

1. The quantity of material to produce,
2. The economic situation of the country,
3. The general level of technical development of the country and its military demands.

The specific agent being produced will be determined by:

1. The theoretical route,
2. Practical limitations in chemical technology,
3. The material resources of the country.

Conversion of pesticide plant

Practical comparison between a pesticide plant and an agent production plant indicates that the conversion of the former to the latter is difficult from the technological point of view. Commercial production of pesticides is realized in equipment or facilities which do not correspond and do not allow full synthesis of the agent required. It seems to be simpler and more convenient to erect new facilities, although exceptions could be made.

The equipment required for agent production is distinguishable from much of the equipment normally used in other synthesis of organophosphorus chemicals. Material requirements for construction and installation of equipment are exacting.

Waste disposal is a serious problem in agent plants. All chemical wastes are treated before they are permitted to reach the main disposal system. The method of handling waste liquids and also the handling of gaseous exhalations is not very easy, since special precautions must be taken. On the other hand, these precautions are distinctive and easily distinguishable.

One of the most distinguishing features is severe safety measures during all processes in agent production. The operations can be divided into (*a*) hazardous but non-toxic, and (*b*) hazardous and toxic.

The real danger that exists here calls for very unusual precautions. The level of safety precautions in manufacturing facilities would be a helpful criterion in ascertaining the nature of the raw materials and intermediates. Safety equipment such as gas masks are carried by all personnel and protective suits are readily available to all. Medical help must be up-to-date.

On the contrary, an organophosphorus pesticide plant does not need any of the safety features required in toxic agent manufacture.

The control of CW agent manufacturing installations

If an inspection team visits a CW agent factory, it would have little difficulty in ascertaining the production of CW agent. A much more difficult task would be that of detecting suitable targets for inspection in the first place.

One distinguishing feature of certain types of organophosphorus processing plants, including nerve-gas intermediate plants, as compared with most other types of chemical plants, is in the means of heating and cooling certain of the reaction vessels. Steam-heating and water-cooling must be avoided, because of the danger of explosion. [286] The absence of steam clouds that are a normal feature of chemical plants and a positive indication of the presence of heating appliances from infrared sensors, would be suggestive. [287]

Effluents from a chemical-weapons factory, both liquid and gaseous, obviously have to be carefully handled. Some sort of pre-discharge treatment would have to be applied to remove toxic materials. It is unlikely, however, that this treatment would destroy all indications of the types of processes from which the effluents were discharged. Analysis of chimney wastes or liquid wastes would almost certainly yield useful information.

Discharge of liquid wastes into river or sea water would not be particularly conspicuous in itself; it is a common method of waste disposal for chemical factories. The inspection team could, however, easily identify the waste as being from a nerve-gas plant. Whatever type of pre-discharge treatment was used, it would be most unlikely that all characteristic components would be destroyed; compounds containing phosphorusmethyl linkages, for example, would almost certainly persist and be identifiable for long periods after discharge.

A nerve-gas waste lagoon, because of its size and proximity to a chemical factory, and because it would represent an unusual method of waste disposal, would quickly attract the suspicions of an inspection system. It is likely that the microflora growing in it would also be distinctive to aerial reconnaissance. Having gained access to it, an inspection team should have little difficulty in identifying its function by chemical analysis. The indications are, however, that waste-lagoons are unlikely to be used for nerve-gas waste disposal except when no alternative method is possible.

Well-discharge, although expensive in terms of capital investment, and suited only to particular areas of the earth's surface, probably provides both the safest and the least conspicuous method of waste disposal.

All intermediates of chemical war gases were prepared by industies which manufacture compounds of public utility. As a rule, transformation of one or more harmless compounds into highly toxic compounds takes place in a single stage and if a country is interested in camouflaging its production of chemical war gases, it will attempt to perform this transformation in installations, built

for this single stage, in particularly remote localities, far away from urban areas.

At the present time, the nerve gases are the most important agents. Also, technical possibilities are now greater than they were before the Second World War. The technical possibilities of inspection therefore appear to have increased.

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