OPCW

Scientific Advisory Board

Ninth Session
12 – 14 February 2007

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REPORT OF THE NINTH SESSION OF THE SCIENTIFIC ADVISORY BOARD

1. INTRODUCTION

1.1 The Scientific Advisory Board (SAB) held its Ninth Session from 12 to 14 February 2007 at the OPCW headquarters in The Hague, the Netherlands. The Session was opened by the Chairperson of the SAB, Jiří Matoušek of the Czech Republic; Mahdi Balali-Mood of the Islamic Republic of Iran served as Vice-Chairperson. A list of participants appears as Annex 1 to this report.

1.2 The SAB adopted the following agenda for its Ninth Session:

1. Opening of the Session and adoption of the agenda
2. Welcoming address by the Director-General
3. Overview of developments at the OPCW since the Eighth Session
4. Work of the temporary working groups
5. Consideration of the report on biomedical samples:
   - (a) Discussion of the report and its recommendations
   - (b) Recommendations of the SAB
6. Consideration of first report of the temporary working group on sampling and analysis:
   - (a) Sampling and analysis: background briefing by the Secretariat on the current status, including recent developments and improvements, and problem areas
   - (b) Adoption of the terms of reference for this group
   - (c) Discussion of the report and its recommendations
   - (d) Initial SAB recommendations

7. International Union of Pure and Applied Chemistry (IUPAC) meeting on advances in science and technology:

   (a) Information on the status of preparations for the IUPAC meeting

   (b) SAB contributions to the IUPAC meeting in Zagreb

8. Chemical education and outreach: update on the latest IUPAC developments

9. Timetable and responsibilities for preparation of the provisional SAB report to be submitted by mid-June 2007

10. Briefing on the preparations for the commemoration of the tenth anniversary of the entry into force of the Chemical Weapons Convention:

    (a) Overview of events being organised

    (b) Briefing on the 2007 Academic Forum

11. Any other business

12. Adoption of the Report

13. Closure of the meeting

1.3 The Director-General made a statement in which he welcomed the members, and set out his views on the future work of the SAB; he particularly emphasised the contributions expected from this group during the preparations for the Second Special Session of the Conference of the States Parties to Review the Operation of the Chemical Weapons Convention (hereinafter "the Second Review Conference"). He also informed the members of the SAB that the Technical Secretariat (hereinafter "the Secretariat") has already sent a request to all Member States to support the Trust Fund that was established in April 2006, and hopes that positive responses will secure the funding that the SAB needs in order to prepare for the Second Review Conference.

1.4 The SAB was briefed on developments at the OPCW since its Eighth Session, which took place from 8 to 10 February 2006. An update on the progress being made in preparations for the Second Review Conference was also provided (the open-ended working group had begun its preparatory work for this Conference in July 2006).

2. CONSIDERATION OF THE REPORT OF THE TEMPORARY WORKING GROUP ON BIOMEDICAL SAMPLES

2.1 The SAB received the third and final report of the temporary working group on biomedical samples (see Annex 2). The report addressed issues of biomarkers, analytical methods, criteria for identification, sample handling and transport, and confidence-building exercises.

2.2 This temporary working group was of the view that it, as an advisory body, had met its terms of reference. Any further elaboration of sample handling, analytical methods, identification criteria, and reporting criteria should be undertaken by the

OPCW Laboratory in conjunction with experts from Member States, and should be tested and refined in confidence-building exercises. The provisional offer from TNO Defence, Security and Safety (subject to receipt of the required funding) to prepare samples for the first confidence-building exercise is providing the impetus for this process to begin.

2.3 The SAB accepted the following recommended progression in terms of its work on biomedical sampling: from dissemination of knowledge to confidence-building exercises, then to proficiency tests, and finally to designation. It recognised that this will be a slow and gradual process, as was the case for the designated laboratory system for environmental analysis.

2.4 The SAB discussed issues of cost, alternative methodologies, the use of biomedical sampling in the event of a terrorist attack involving chemical weapons, and the length of time that biomarkers are detectable after exposure. The Chairperson of the temporary working group advised that international agencies such as the World Anti-Doping Agency (WADA) would be consulted to ensure best practice. The SAB agreed to address issues relating to conducting post-mortems on human and animal samples at a later date.

2.5 The SAB endorsed the following key recommendations of the temporary working group:

(a) that the OPCW Laboratory, with assistance from experts from Member States, should now be moving forward on the issue of biomedical sampling and analysis;

(b) that the SAB request the Director-General to make sufficient resources available to the OPCW Laboratory to initiate and maintain this process;

(c) that the OPCW Laboratory, with the assistance of experts from Member States, should compile details of analytical methods, and of synthetic methods or commercial sources for analytical standards;

(d) that confidence-building exercises should commence as soon as is practically possible; and

(e) that proficiency tests should proceed only when a level of expertise has been achieved by a number of laboratories.

3. CONSIDERATION OF FIRST REPORT OF THE TEMPORARY WORKING GROUP ON SAMPLING AND ANALYSIS

3.1 The SAB reviewed and approved the provisional terms of reference for the sampling-and-analysis temporary working group (see Annex 3). The Chairperson of this group presented a report.

3.2 During its first meeting, this temporary working group addressed the following issues in relation to sampling and analysis:

(a) the status of verification procedures;

(b) chemicals (in connection with toxin analysis and proficiency testing);

(c) proficiency testing; and

(d) analytical methodology.

3.3 This temporary working group made the following recommendations to the SAB:

(a) that developments in the preparation of aqueous samples or extracts, which avoid the need to concentrate aqueous solutions to dryness, should be reviewed;

(b) that the OPCW Laboratory should obtain information on the capabilities of designated laboratories to perform toxin analyses;

(c) that the SAB should consider the issue of the designation of laboratories by the Director-General for Schedule 1 toxin analysis (in accordance with subparagraph 56(b) of Part II of the Verification Annex to the Convention), including consideration as to whether this designation would require a modification of a decision taken by the Conference of the States Parties at its First Session (C-I/DEC.61, dated 22 May 1997);

(d) that the following should be considered: the inclusion of riot control agents in the context of allegations of use, non-scheduled degradation products of scheduled chemicals considered relevant to verification, and chemicals related to old chemical weapons (OCW) and abandoned chemical weapons (ACW) in the OPCW Central Analytical Database (OCAD), and the selective use of data from the OCAD in accordance with the inspection aims as specified in the Convention; and

(e) that a new working group should be established to evaluate the potential for liquid chromatography-mass spectrometry (LC-MS) to be used during on-site analysis.

3.4 After considerable discussion, the SAB adopted the recommendations, but made the following comments:

(a) There was consensus about the adoption of the new format for proficiency testing. However, some SAB members were of the opinion that an additional trial exercise should be conducted amongst designated laboratories. This will involve additional costs and time.

(b) The existing temporary working group should undertake the evaluation of LC-MS as an on-site analytical technique.

4. THE INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY'S INTERNATIONAL SYMPOSIUM (The Impact of Advances in Science and Technology on the Chemical Weapons Convention, Zagreb, Croatia, 22 to 25 April 2007)

4.1 The SAB was informed about the state of play in relation to preparations for the above-mentioned meeting, which will be sponsored by the OPCW and by the North Atlantic Treaty Organisation (NATO).

4.2 This symposium will bring together expertise available within the IUPAC, the OPCW, the international academic community, and the global chemical industry to address scientific progress in the fields of chemistry and chemical industry, and will also address a number of issues relevant to the Convention, including:

(a) developments with regard to new chemicals, processes, and production equipment;

(b) technological advances in micro-reactors, nanotechnology and aerosol-dispersion techniques;

(c) verification technologies and equipment for the analysis of chemical, environmental, and biomedical samples; and

(d) research in rapidly expanding fields, such as drug development, synthetic biology, bio-informatics, and genomic/proteomics.

4.3 The report from the symposium will be widely disseminated amongst States Parties to the Convention, and will be considered at the next session of the SAB, which will take place in May 2007, with a view to the SAB's making recommendations in its report to the Second Review Conference (which will take place on 7 to 18 April 2008).

4.4 Three members of the SAB (Jiří Matoušek, Mahdi Balali-Mood, and Danko Škare) are members of the International Advisory Board of the Symposium, and more than 10 members of the SAB will also contribute.

5. OVERVIEW OF THE EVENTS RELATED TO THE TENTH ANNIVERSARY OF THE ENTRY INTO FORCE OF THE CONVENTION

The SAB heard an update, highlighting important dates and the venues (both in the Netherlands and around the world), on the events that were being planned in relation to the tenth anniversary of the entry into force of the Convention.

6. BRIEFING ON THE 2007 ACADEMIC FORUM

6.1 A presentation was given on the 2007 Academic Forum, which will be held on 18 and 19 September 2007 in The Hague, and is one of the events marking the tenth anniversary of the entry into force of the Convention.

6.2 The aims of this forum were explained, and it is hoped that it will become a permanent platform for communication and dialogue between the OPCW and other important institutions. Details were provided about a tentative programme, which will include a plenary session and four workshops on the following topics:

(a) destruction of chemical weapons agents;

(b) the verification regime;

(c) the impact of developments in science and technology on the Convention; and

(d) the future role of the OPCW after 2012 (the date when it is expected that destruction of all stockpiles of chemical weapons will have been completed).

6.3 Experts from around the globe will be invited to speak, as will members of the SAB. It was also announced that, in March 2007, a website would be created where information will be available on the Academic Forum.

7. CHEMICAL EDUCATION AND OUTREACH

7.1 The SAB heard an update on the progress that had been made on the joint project of the OPCW and the IUPAC following the international workshop held in Oxford in July 2005 and the International Seminar on Operating Aspects on the Joint IUPAC-OPCW Project on Chemical Education and Outreach, held in Bologna, Italy, in September 2006.

7.2 The group was informed that four sets of written material have been prepared on the issue of the multiple uses of chemicals and on the ethical issues arising from this; these materials included information on codes of conduct and sample case studies that could be used by chemistry teachers and students. Two further papers are being prepared. The SAB was informed that Professor Alistair Hay of the University of Leeds, leader of the joint OPCW-IUPAC project, had stated that successful pilot studies had been conducted at IUPAC meetings that had been held in October 2005 in Moscow, Russian Federation, and in August 2006 in Seoul, Republic of Korea. These studies have confirmed the validity of the educational concept and the usefulness of these materials, which have subsequently been improved. Further pilot studies are being considered during 2007; plans are also being made for the written materials to be translated into the six official UN languages and for them to be made available on the IUPAC website.

7.3 The SAB was also briefed on the progress being made in the development of codes of conduct. In this context, there had been a recognition that it is useful to think of codes of conduct as having a number of levels, including: (i) Guiding Principles (a "universal code"), (ii) Scientific Society Codes (codes of ethics), and (iii) institutional or workplace codes (codes of practice). These various codes are seen as complementary and mutually reinforcing. Copies of drafting elements for the Guiding Principles and Scientific Society Codes, and a draft Institutional or Workplace Code were presented to the SAB.

7.4 The SAB confirmed its continuing support for the joint OPCW-IUPAC project, and emphasised how important it was for National Authorities to become actively involved in education, outreach, and codes of conduct, in that they played an important role in generating and maintaining support amongst governments for the inclusion in school and university curricula of appropriate references to the Convention and its requirements, and of related information and ethical guidance that benefited students and teachers. The SAB also noted the important role played by the IUPAC and national chemical societies in promoting the development of codes of conduct that were germane to the Convention, and in incorporating Convention-relevant elements into existing codes.

7.5 The members of the SAB recognised that they had an individual and collective responsibility to promote an awareness and an understanding of the requirements of the Convention amongst the entire scientific community (in other words, that the outreach should not simply be to students, chemists and chemical engineers, but should also include, more broadly, scientists active in the life sciences, and the public).

7.6 The SAB was informed by one of its members that a draft Charter for Chemists had been presented at the international seminar that had been held in Bologna, Italy, on 22 September 2006. During subsequent discussions, the SAB came to the view that there were fundamental ethical issues raised in this Charter that went beyond the scope of the Convention (for example, in relation to matters involving public health and the environment), and suggested that the further development of this Charter would best be undertaken by other international and professional organisations, including the IUPAC.

8. FUTURE WORK OF THE SCIENTIFIC ADVISORY BOARD

8.1 The SAB agreed that it will continue to address the following:

(a) the practical aspects of biomedical sampling and analysis, as recommended in paragraph 2.5 of this report; and

(b) the technical aspects of toxin analysis (to be further considered by the temporary working group on sampling and analysis). It will also consider how toxin analysis can be included in the work of the current network of designated laboratories, or if additional provisions are required (see subparagraphs 3.3(b) and (c) above).

8.2 After the IUPAC symposium (to be held in Zagreb, Croatia, in April 2007), the SAB, utilising information that will be contained in an initial report from that meeting, will continue to address the issue of the impact that developments in science and technology are having on the implementation of the Convention. In this context, the following issues are of particular relevance:

(a) the advances in synthetic chemistry, such as computer-aided syntheses, combinatorial chemistry and like, which create opportunities for an

accelerated pace of design and synthesis of "tailored", biologically active chemicals, new drugs, and bio-regulators;

(b) advances in molecular biology (such as genomics and proteomics), which create opportunities for new biologically active chemicals to be designed, and for processes to be developed to synthesise them using enzymes and cell-based systems;

(c) advances in nanoscience and nanotechnology, leading to altered toxic properties of manufactured nano-sized chemicals, to new methods of dispersal, as well as to new opportunities in analysis, protection, decontamination, and medical treatment;

(d) advances in manufacturing technologies, leading to changes in the chemical industry, such as automated micro-reactors, multipurpose batch facilities (enabling rapid switching from one product to another), and the like; and

(e) advances in instrumental analytical chemistry, giving new opportunities for innovations in verification procedures and equipment.

8.3 The SAB will continue to address the whole complex of scientific, technological, and medical aspects of assistance and protection against chemical weapons, and the opportunities for the OPCW to further develop its international cooperation portfolio, which includes the promotion of the peaceful application of chemistry and education and outreach in the context of the Convention.

9. CONCLUSION OF THE MEETING

The SAB concluded its Ninth Session on 14 February 2007 at 17:45 with the adoption of this report.

Annexes:

Annex 1:
List of Participants of the Ninth Session of the Scientific Advisory Board

Annex 2 (English only):
Report of the Temporary Working Group on Biomedical Samples

- Appendix 1: List of Participants of the Temporary Working Group on Biomedical Samples
- Appendix 2: Presentation summarising the discussions of the 2nd meeting
- Appendix 3: List of cases where biomarkers had been detected in human exposures to CW agents
- Appendix 4: Discussion Paper: Points for consideration by TWG members prior to the third meeting
  - Attachment 1: EU System of IPs for analysis of banned/controlled residues in animal products
  - Attachment 2: Some Criteria used in OPCW Proficiency Tests for ppm levels

Annex 1

LIST OF PARTICIPANTS OF THE NINTH SESSION OF THE SCIENTIFIC ADVISORY BOARD

	Participant	Member State
1.	Rolando A Spanevello	Argentina
2.	Robert Mathews	Australia
3.	Herbert de Bisschop	Belgium
4.	Zhiqiang Xia	China
5.	Danko Škare	Croatia
6.	Jiří Matoušek	Czech Republic
7.	Jean-Claude Tabet	France
8.	Detlef Maennig	Germany
9.	László Halász	Hungary
10.	R Vijayaraghavan	India
11.	Mahdi Balali-Mood	Islamic Republic of Iran
12.	Alberto Breccia Fratadochi	Italy
13.	Koichi Mizuno	Japan
14.	Abdool Jackaria	Mauritius
15.	José Gonzáles Chávez	Mexico
16.	Godwin Ogbadu	Nigeria
17.	Bjørn-Arne Johnsen	Norway
18.	Titos Quibuyen	Philippines
19.	Young-chul Lee	Republic of Korea
20.	Philip Coleman	South Africa
21.	Miguel A Sierra	Spain
22.	Valery Kukhar	Ukraine
23.	Robin Black	United Kingdom of Great Britain and Northern Ireland
24.	James Robert Gibson	United States of America

Annex 2

REPORT OF THE TEMPORARY WORKING GROUP ON BIOMEDICAL SAMPLES THE HAGUE, 5 AND 6 FEBRUARY 2007

1. INTRODUCTION

1.1 The SAB Temporary Working Group on Biomedical Samples held its third meeting on 5th and 6th February 2007 in The Hague.

1.2 The meeting was chaired by Dr Robin Black on behalf of the SAB.

1.3 The list of participants in the meeting is given in Appendix 1.

1.4 The following agenda was adopted.

1. Welcome
2. Summary of 2nd meeting of the TWG
3. Current status of the OPCW laboratory
4. Review of recent advances in biomarkers and analytical methods
5. Criteria for analytical results
6. Sample handling and transportation
7. Validation of methods
8. Availability of standards
9. Confidence building exercises
10. Recommendations for the way forward

1.5 Mr Patrice Palanque welcomed members of the TWG on behalf of the Director General of the OPCW. This is an important year for the OPCW in its preparation for the Second Review Conference to be held 7 - 18 April 2008. The work of the TWG was seen as an important contribution to the report and recommendations of the SAB to the Director General prior to the Review Conference.

1.6 Mr Palanque reiterated the important role that biomedical sample analysis would play in investigations of allegations of CW use. At present, capabilities were limited to very few laboratories within the system of laboratories designated by the Director General for environmental analysis. It was recognised that biomedical sample analysis would be prohibitively expensive for some laboratories. The OPCW

laboratory currently has no appropriate facilities or experience to undertake biomedical sample analysis and therefore the DG welcomes the assistance of member states in this area. The TWG is asked to make recommendations for initiating a series of confidence building exercises, with the long term goal of a system of laboratories designated for biomedical sample analysis.

1.7 The presentation by Dr Robin Black to the 9th meeting of the SAB, summarising the discussions of the 2nd meeting of the TWG is given in Appendix 2.

2. CURRENT STATUS OF THE OPCW LABORATORY

2.1 Dr Gary Mallard, newly appointed head of the OPCW laboratory, summarised the current situation with regard to enhancing the resources of the OPCW laboratory. Proposals for additional staff and equipment for biomedical sample handling and analysis have not been approved. These will be proposed again for inclusion in the 2008 budget.

2.2 Confidence building exercises could still proceed, with the OPCW laboratory playing a coordinating role, provided that one or two laboratories from member states could prepare and distribute samples. Dr Daan Noort informed the TWG that TNO had requested funding from the Netherlands Ministry of Foreign Affairs to undertake sample preparation for the first exercise, and for the provision of technical advice to the OPCW laboratory.

2.3 Dr Mieczyslaw Sokolowski reported on the current equipment in the OPCW laboratory and its suitability for biomedical sample analysis. One GC-MS and one LC-MS-MS ion trap instrument is available, but the laboratory has no GC-MS-MS or research grade LC-MS-MS instruments capable of analysis at low or sub-ppb levels.

2.4 The OPCW laboratory had received more than 40 replies from member states in response to its questionnaire on current biomedical sample capabilities. These would be collated into a database that will be distributed to member states when available (estimated 3 months). It is also intended to compile a ‘manual’ of analytical methods, plus synthetic methods or commercial sources for analytical standards.

3. RECENT ADVANCES IN METHODOLOGY

3.1 Dr Marcel Van der Schans (TNO laboratory) reported initial results of a generic method for detecting nerve agent adducts with human butyrylcholinesterase. The method, based on LC-MS-MS (triple quadrupole instrument), employs precursor ion scanning of common non-phosphylated product ions derived from phosphylated peptic nonapeptides. The method can, in theory, detect adducts from all Schedule 1 nerve agents based on scanning for 42 precursor ions.

3.2 Dr John Barr (CDC) reported a sensitive LC-MS-MS method for detecting alkyl methylphosphonic acids in urine. This method complements existing GC-MS/GC-MS-MS methods. In combination these methods should provide identification to the required number of identification points (see section 5).

3.3 Analysis of plasma samples from the subject of an accidental laboratory exposure to sulphur mustard in the US had detected the alkylated tryptic tripeptide derived from the albumin 34-cysteine adduct up to 42 days post exposure. ß-Lyase metabolites were detected in the urine of this subject. In early urine samples, thiodiglycol and thiodiglycol sulphoxide were excreted in almost equal amounts, but with time thiodiglycol sulphoxide became the dominant excretion product derived from hydrolysis, as has been observed with CW casualties of sulphur mustard poisoning. The bis-N-acetylcysteine conjugate of mustard sulphone, a major urinary metabolite in the rat, was detected only at low levels. Thiodiglycol and the albumin adduct were detected in blister fluid.

3.4 A low level accidental human exposure to VX in the US was detectable using fluoride reactivation of butyrylcholinesterase up to 16 days post exposure after the subject had received oxime therapy, and by fluoride reactivation of acetylcholinesterase in red blood cells.

3.5 Work on nitrogen mustards is being undertaken in at least three laboratories (CDC, DSO Singapore and TNO) with regard to urinary metabolites and albumin adducts. All three nitrogen mustards have been shown to alkylate the 34-cysteine residue on albumin in vitro.

3.6 CDC has developed a new method, based on LC-MS-MS, for detecting saxitoxin in urine.

3.7 Dr Barr announced that a special issue of the Journal of Analytical Toxicology would address biomedical sample analysis for CW residues, to be published in the second half of 2007. Contributions were invited.

4. REVIEW OF RECOMMENDED BIOMARKERS

4.1 The status of biomarkers listed at the second meeting of the TWG was reviewed. The group discussed several issues.

4.2 Should pairs of metabolites, such as the two ß-lyase metabolites of sulphur mustard, and alkyl methylphosphonic acids and methylphosphonic acid from nerve agents, where one could arise from chemical oxidation or hydrolysis of the other, be regarded as one or two metabolites for identification purposes? The consensus of the TWG was that they should be regarded as two metabolites when identified as separate species. Thiodiglycol and its sulphoxide could also be regarded as two metabolites but, because of their occurrence at low levels in non-exposed subjects, their usefulness as biomarkers was limited and required careful consideration.

4.3 It was noted by the TWG that studies into background levels of biomarkers or interferents had been undertaken only on urine samples from US and European populations. Background levels in other populations are a knowledge gap. The TWG recommended that once expertise had been established in other geographical areas through confidence building exercises, these laboratories should be encouraged to study background levels in regional populations. Differences in the environment and diet may affect these background levels.

4.4 The status of methylphosphonate residues on cholinesterase resulting from ageing of nerve agent adducts was discussed. Although key structural information on the nerve agent is lost, Professor Matoušek noted that these did provide generic evidence of exposure to nerve agents and would support other evidence consistent with nerve agent exposure. Dr Mathews added a note of caution on methylphosphonic acid as a urinary metabolite because low levels of methylphosphonates may be present as impurities in OP pesticides, e.g. those used in sheep dips. This had resulted in incorrect preliminary conclusions being drawn by a forensic laboratory in a case in Australia concerning the Aum Shinrikyo sect.

4.5 The TWG agreed that there are no satisfactory urinary biomarkers for tabun. The primary hydrolysis products are labile, and the secondary hydrolysis product, ethyl phosphoric acid, occurs widely in urine from other sources. The phosphylated residue on butyrylcholinesterase is also labile under the conditions used for peptic digestion. However, fluoride reactivation to produce 'fluoro tabun' has been shown to be successful. The albumin tyrosine adduct provides a non-aged residue with tabun, although this is a less sensitive biomarker. Identification of VX also presented a problem with regard to the dialkylaminoethylthio moiety. Although a blood metabolite had been identified in a Japanese casualty, no urinary metabolites derived from the side chain have yet been identified.

4.6 Phosgene and cyanide remained a problem with regard to background levels of known biomarkers. Excessive levels might provide supporting evidence if consistent with medical diagnosis but could not be regarded as unequivocal. The fate of phosgene in the body remained a knowledge gap.

4.7 The chairman agreed to append a table to the minutes listing those cases where biomarkers had been detected in human exposures to CW agents (Appendix 3).

5. CRITERIA USED FOR IDENTIFICATION

5.1 A discussion paper on criteria circulated by the Chairman is given in Appendix 4. This paper discusses criteria used in other areas where results may have legal consequences.

5.2 Minimum criteria for identification should ensure that any results arising from investigations of allegations of use will stand up to international scrutiny.

5.3 A number of points arose from a lengthy discussion of criteria that might be adopted for the interpretation of results. It was noted by Dr Datta and Dr Verschraagen that there was considerable debate on the scientific soundness of criteria used in trace analysis.

5.4 The TWG agreed that a flexible system based on identification points would be appropriate to the requirements of the OPCW. This should be based on a system similar (but not identical) to that recommended by the European Community for residues in animal products.

5.5 The recommendations of the TWG should be regarded as a starting framework to facilitate confidence building exercises. They should also indicate to laboratories what level of equipment is likely to be required in order to undertake biomedical sample analysis. The finer details of criteria should evolve from confidence building exercises.

5.6 The group recommended that as a starting point the required level of identification points should be 4 (similar to the EC directive for residues in animal products) or 5 (more consistent in most cases with current OPCW proficiency test requirements). It was proposed that when details of analytical methods are compiled the number of identification points that could be obtained is included.

5.7 A feature of a flexible system is that the appropriate number of identification points can be obtained using a single analytical method with a high level of specificity, or using two or perhaps three analytical methods for a single biomarker, or single analytical methods for two or three biomarkers. However each analytical method would be required to meet a minimum number of identification points, suggested as 2 for consistency with other fields of trace analysis. For consistency with current OPCW requirements for environmental analysis, detection by two techniques may need to be considered.

5.8 As a starting point it was recommended that criteria for retention windows and signal to noise ratios ($\geq$ 5:1) should be the same as currently used in OPCW proficiency tests.

5.9 In allegations of CW use, the number of agents of concern is likely to be considerably smaller than the number of analytes that are used to test identification capabilities in OPCW proficiency tests. Results should therefore be compared to standards spiked into the appropriate matrix. The use of internal standards is strongly recommended by the TWG. The possibility of linking internal standards to identification points should be considered during confidence building exercises.

5.10 The issue of demonstrating lack of cross contamination and the importance of system blanks was also noted. This should be addressed in confidence building exercises.

6. SAMPLING, HANDLING AND TRANSPORTATION

6.1 Dr John Barr provided the TWG with the collection, packaging and transportation SOPs currently used by CDC Atlanta (Appendix 5). These would need some modification for OPCW requirements. It was noted that empty tubes and collection cups were included so that laboratories could check that these contained no chemicals that would interfere with analytical methods. Ideally, population control samples are desirable, collected on-site from individuals with no history of exposure to a suspected CW agent.

6.2 With regard to anticoagulants added to blood samples, EDTA was recommended rather than heparin, but analytical methods should be checked against interference from EDTA. This area should be addressed in confidence building exercises.

6.3 Blood is preferably separated into plasma or serum and red blood cells on-site and the plasma/serum frozen.

6.4 Samples collected by the OPCW team would need to be split into 5 aliquots for analysis. The TWG recommended that a minimum of 5 ml of blood or urine be available to each laboratory; 10 ml would be preferable to allow full duplicate analyses. This would require collection of 25-50 ml of sample.

6.5 An important issue discussed by the TWG was that of microbiological safety, particularly with respect to hepatitis, HIV and other viruses in blood. It was acknowledged that sterilisation on-site would be logistically difficult, and the OPCW laboratory does not have the capability. Issues of microbiological safety would therefore have to be dealt with by analytical laboratories, at least until a more satisfactory solution could be found. It was recognised that very few laboratories had access to a gamma ray facility, and work was required to ensure that gamma irradiation did not have an adverse effect on analytes. John Barr reported that CDC also used heating for 1 hour at 90°C but that this did have an adverse effect on some analytes. Chemical sterilants, e.g. DNA chelators, were currently under investigation.

6.6 The TWG recommended that the effects of different methods of sterilisation be addressed during confidence building exercises.

6.7 Dr Gibson noted that the CWC also makes provision for the collection of human post-mortem and animal samples. Most laboratories had little or no experience of handling or analysing such samples. It was recommended that the OPCW laboratory should take expert advice with regard to the collection and handling of animal samples, particularly with regard to microbiological contamination.

7. VALIDATION OF METHODS

7.1 The TWG reaffirmed its view that accreditation of methods, though desirable, would be prohibitively expensive.

7.2 John Barr outlined the validation procedures undertaken by the CDC laboratory. Guidelines on validation should be issued by the OPCW laboratory with input from

member states as part of confidence building exercises. In the context of OPCW analysis, the TWG recommended that laboratories should be required to demonstrate in house validation of methods. Laboratories should be asked to demonstrate the limits of detection and specificity of their methods by the analysis of samples spiked at low, medium and high concentrations of analyte. Suitable protocols for ensuring absence of cross contamination would also be required. It was recognised that some laboratories would need to be selective in the methods that they develop.

7.3 Further validation would be provided by proficiency testing.

7.4 In the initial confidence building exercises modifications of methods and problems should be discussed with the aim of providing robust and documented methods. Consideration should be given to the production of a 'Blue Book' for biomedical sample analysis, similar to those issued by Verifin on environmental analysis.

7.5 The TWG suggested that a workshop for interested member states would be useful if financial support was available.

8. AVAILABILITY OF STANDARDS

8.1 The availability of standards is a key issue with regard to the wider adoption of analytical methods. In particular, peptide adducts and labelled internal standards for some urinary metabolites are not easily synthesized.

8.2 Dr Noort proposed that the problem posed by peptide adducts is overcome by generating crude adducts by incubating blood, plasma or protein with agent, e.g. cholinesterase fully inhibited by addition of the respective nerve agent. Dr Noort agreed to provide details of how these should be prepared. It was noted that some laboratories may not have access to the agents and would need to request them from other laboratories.

8.3 Detailed methods for the synthesis of urinary metabolites will be made available, particularly those for the ß-lyase metabolites of sulphur mustard. All members of the TWG were again asked to provide details of synthetic methods and commercial sources to the OPCW through the chairman.

9. CONFIDENCE BUILDING EXERCISES

9.1 Dr Gary Mallard proposed the following strategy for development of confidence building exercises.

9.2 In consultation with members of the TWG and other experts, the OPCW laboratory will make available a table of methods broken down by instrumentation requirements, matrix and analyte. This will allow laboratories to examine what methods are possible with current equipment, and help them decide if they wish to participate in the first confidence building exercise. When available, full details of each method will be distributed together with references.

9.3 Confidence building exercises would commence with analytes that can be analysed by GC-MS using equipment available to most member states, and gradually progress to methods more demanding in terms of equipment (GC-MS-MS, LC-MS-MS) and analytical standards. It was noted that in the case of terrorist incidents, where initial samples may be collected within 24-48 h (as was the case with the sarin release in Tokyo), simple GC-MS methods may be sufficient to identify free metabolites in urine and blood.

9.4 After lengthy discussion the TWG agreed that the first exercise should be restricted to urine to avoid any problems with regard to the handling of blood. The recommended analytes were isopropyl methylphosphonic acid, thiodiglycol and its sulphoxide, and ß-lyase metabolites of sulphur mustard, all of which can be analysed by GC-MS. Participants will be informed of the spiking chemicals in advance of the exercise. Laboratories equipped to undertake other methods of analysis would be encouraged to apply these in order to demonstrate the level of sensitivity and specificity achievable. Spiking levels in this first exercise would be 50 ng/ml, a level that would require trace analytical techniques (selected ion monitoring etc.) but which should not be too demanding.

9.5 It was emphasised that the purposes of these exercises were to further develop and refine methods of analysis, and to help laboratories reach the appropriate level of technical competency through mutual help. There should be no ‘scoring’ of performance and laboratories would be allowed sufficient time.

9.6 The confidence building exercises should also help identify problems of transport and stability of samples.

9.7 The first exercise would be an important step forward in the progress of biomedical sample analysis.

9.8 The time scale for the first exercise was uncertain. Subject to funding by the Ministry of Foreign Affairs, TNO could prepare samples during 2007, but the necessary preparations and documentation may require the exercise to be held in early 2008. It was noted that such exercises should not coincide with OPCW proficiency tests for environmental analysis. April, May, October and November should be avoided.

10. THE WAY FORWARD

10.1 Further elaboration of sample handling, analytical methods, identification criteria and reporting criteria should be undertaken by the OPCW laboratory in cooperation with experts from member states. These should be exercised and refined in confidence building exercises.

10.2 The provisional offer from TNO, subject to funding, to prepare samples for the first confidence building exercise provides the impetus to commence the process. Offers should be invited by the OPCW laboratory from other member states for subsequent exercises.

10.3 The TWG suggests that the OPCW, through the International Cooperation and Assistance Branch, consider hosting a workshop aimed at capability building for laboratories seeking to participate in confidence building exercises.

10.4 The TWG recognise that the provision of a full capability for BMS analysis through a designated laboratory system will be a slow and gradual process, as has been the case with environmental analysis. The recommended progression is: dissemination of knowledge → confidence building exercises → validated methods → proficiency tests → designation.

10.5 The SAB is asked to address issues of post mortem human and animal samples, and the analysis of biomedical samples for toxins and riot control agents.

11. KEY RECOMMENDATIONS

11.1 The TWG recommends that BMS analysis is now taken forward by the OPCW laboratory with assistance from experts from member states.

11.2 The SAB requests the Director General to make sufficient resources available to the OPCW laboratory to initiate and maintain this process.

11.3 The OPCW laboratory, with assistance of experts from member states, should compile details of analytical methods, plus synthetic methods or commercial sources for analytical standards.

11.4 Confidence building exercises should commence as soon as is practically possible. These should gradually build in terms of instrumental requirements and technical difficulty. They should not be tests and should involve mutual help.

11.5 Proficiency tests should proceed only when a level of expertise has been achieved in a number of laboratories.

Appendix 1

LIST OF PARTICIPANTS OF THE TEMPORARY WORKING GROUP ON BIOMEDICAL SAMPLES

	Participant	Member State
1.	Robert Mathews	Australia
2.	Jiri Matoušek	Czech Republic
3.	Marja-Leena Kuitunen	Finland
4.	Anne Bossée Replacing Bruno Bellier	France
5.	Marianne Koller Replacing Franz Worek	Germany
6.	Ashok Datta	India
7.	Abbas Shafiee	Iran
8.	Daan Noort	Netherlands
9.	Miranda Verschraagen	Netherlands
10.	Marcel van der Schaans (observer)	Netherlands
11.	Stanislaw Witek	Poland
12.	Sng Mui Tiang	Singapore
13.	Philip Coleman	South Africa
14.	Sten-Ake Frederiksson	Sweden
15.	Robin Black, Chairman	United Kingdom of Great Britain and Northern Ireland
16.	Robert Read	United Kingdom of Great Britain and Northern Ireland
17.	John R. Barr	United States of America
18.	John R. Smith	United States of America
19.	James Robert Gibson	United States of American

Appendix 2
Presentation summarising the discussions of the 2nd meeting

SAB TWG on Biomedical Samples
3rd Meeting, 5-6 Feb 2007

Report to the 9th Meeting of the SAB, 12-14 Feb 2007

Robin Black

ORGANISATION FOR THE PROHIBITION OF CHEMICAL WEAPONS

The OPCW requirement for biomedical sample analysis

- The CWC provides for the collection and analysis of biomedical samples (BMS) from human & animal sources in investigations of the alleged use of chemical weapons
- DG requested the SAB to review the scientific aspects of BMS & consider how the OPCW could develop such a capability
- The Executive Council (March 2006) noted the TS intention to proceed with developing an OPCW capability for BMS

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Differences in requirements from OPCW analysis of chemical/environmental samples

- BMS in most cases require trace analysis (low ppb)
  - in complex matrices, primarily blood and urine
    - possibly others such as skin and saliva
  - analyte or class targeted analysis as opposed to generic
- Looking for biological markers rather than agents
  - free metabolites in urine and blood
  - covalent adducts with blood proteins and DNA
- Initial requirement is to identify key biomarkers
  - in vitro & in vivo studies required

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Terms of Reference (1)

The TWG was requested to report to the SAB on the following:

Technical aspects:

1. Specification of key biomarkers of exposure to scheduled CW agents
2. Appropriate analytical methods
   - for metabolites, adducts and other indicators
   - in urine, blood and other suitable matrices
3. Capability/knowledge gaps

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Terms of Reference (2)

Organisational aspects:

4. Suggest procedures to develop a network of laboratories
5. Suggest procedures for confidence building and proficiency assessment
6. Provide information on laboratories and capabilities

5

Terms of Reference (3)

Additional considerations:

- scenarios requiring BMS analysis
- collection, handling and transportation
- training and resources
- preparing the OPCW lab

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TOR 1: Specification of key biomarkers (1)

- The list of biomarkers was reviewed
- These are defined as:
  - Confirmative
    - demonstrated in human samples, no background levels
    - reported only for sulphur mustard, sarin and VX
  - Presumptive
    - demonstrated in animals, no background observed in human samples, no known non-CW source
  - Supportive
    - demonstrated in human/animal samples but trace levels occur in non-exposed subjects
  - Unacceptable
    - large and variable background levels in human samples

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TOR 1: Specification of key biomarkers (2)

Confirmative biomarkers: examples

- Sulphur mustard
  - ß-lyase metabolites, haemoglobin valine and histidine adducts, albumin cysteine adduct
- Sarin
  - isopropyl methylphosphonic acid (hydrolysis product), adduct with BuChE

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TOR 1: Specification of key biomarkers (3)

Presumptive biomarkers: examples

- Lewisite I
  - chlorovinylarsonous acid, CVAA (hydrolysis product)
- Soman
  - pinacolyl methylphosphonic acid (hydrolysis product), albumin tyrosine adduct

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TOR 1: Specification of key biomarkers (4)

Supportive biomarkers: examples

- Sulphur mustard
  - thiodiglycol, thiodiglycol sulphoxide
  - trace levels occur in non-exposed subjects
    - usually <1 and <10 ng/ml, but sometimes higher
- Nitrogen mustards HN-1, HN-2
  - N-methyl and N-ethyldiethanolamines
  - not detected in >100 US samples from non-exposed subjects but both have industrial uses
- Supportive biomarkers in isolation do not provide unequivocal evidence of exposure

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TOR 1: Specification of key biomarkers (5)

Unacceptable biomarkers: examples

- Tabun
  - ethyl phosphoric acid (hydrolysis product)
- Nitrogen mustard 3, HN-3
  - triethanolamine (hydrolysis product)
- Hydrogen cyanide
  - thiazoline-4-carboxylic acid
- All occur at variable, often high levels in non-exposed subjects

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TOR 1: Specification of key biomarkers (6)

Additional issues

- Should pairs of biomarkers, where one could be produced by simple chemical oxidation or hydrolysis of the other, be regarded as separate biomarkers for identification purposes?
  - e.g. two ß-lyase metabolites of sulphur mustard
  - isopropyl methylphosphonic acid & methylphosphonic acid
- Consensus of TWG was that they should, provided they were identified individually

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TOR 2: Recommendation of analytical methods (1)

- Currently used analytical methods are indicated in list of biomarkers appended to the report
- The TWG recommends that experimental details be compiled through the OPCW laboratory (with assistance from experts) prior to confidence building exercises
  - member states asked to provide copies of all relevant papers
- Refinement of analytical methods would be undertaken as part of confidence building exercises
- The compilation of a 'Blue Book' of recommended analytical methods should be considered

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TOR 2: Recommendation of analytical methods (2)

Identification criteria

- Analytical methods should provide identification to a level that would withstand international scrutiny
- The TWG recognised that criteria applied in OPCW proficiency tests, which assume levels (ppm) at which full scan spectra can be obtained, would not be appropriate
- A flexible system of identification points is recommended as a starting point
  - to be trialled/modified as part of confidence building exercises

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TOR 2: Recommendation of analytical methods (3)

Identification points (IPs) system

- Assigns IPs on the basis of specificity
  - based on schemes used by EC and other regulatory bodies
  - full scan > GC/LC-MS-MS > GC/LC-MS-MS
  - depending on number of ions/transitions
  - EC requires 4 IPs for banned substances in animal products
- The EC allows IPs to be acquired using up to 3 analytical methods
- For OPCW purposes the identification of up to 3 biomarkers should also be considered

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TOR 2: Recommendation of analytical methods (4)

Identification points (IPs) system

- The TWG recommends consistency with OPCW criteria for current proficiency tests where practical
  - retention window tolerances, signal to noise ratios $\geq$ 5:1
  - full scan data with 3/4 significant ions EI and one CI = 4/5 IPs
  - a requirement for 2 analytical techniques should also be considered
- Should consult other regulatory bodies
  - e.g World Anti-Doping Agency (WADA)

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TOR 2: Recommendation of analytical methods (4)

Internal standards

- The TWG strongly recommends use of internal standards
  - help avoid false negatives and false positives
- Possibly link to identification points system

Demonstrating lack of cross contamination

- Inclusion of system blanks before each analysis should be mandatory

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TOR 3: Knowledge/capability gaps (1)

Biomarkers

- Urinary biomarker for tabun
- Urinary biomarker identifying the side chain ($-SCH_2CH_2NR_2$) of V agents
- Urinary biomarker for nitrogen mustard 3 (HN-3)
  - and no demonstrated blood biomarkers for nitrogen mustards 1,2 and 3
- No satisfactory biomarkers for phosgene and HCN
  - because of background levels

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TOR 3: Knowledge/capability gaps (2)

Background levels

- In populations other than US and Western Europe
  - member states participating in confidence building exercises should be encouraged to address these

Specificity of methods

- Each analytical method should be assessed in terms of the level of identification it provides
  - to be incorporated into the compilation of analytical methods

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TOR 3: Knowledge/capability gaps (3)

Riot control agents

- Important in the context of allegations of use
- Which ones?
- Some biomarkers identified but no analytical methods

Toxins

- Some methods available for ricin and saxitoxin

- Views of the SAB requested

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TOR 4: Suggest procedures for building an OPCW capability

- The next stage in building a capability should be coordinated by the OPCW laboratory with assistance from member states
- A progression is recommended:
  collation/dissemination of knowledge
  ⇒ confidence building exercises
  ⇒ validated methods
  ⇒ proficiency tests
  ⇒ designation
- The TWG reaffirmed its view that a requirement for accreditation of methods would be prohibitively expensive
  - demonstration of in-house validation is recommended

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TOR 5: Suggest procedures for confidence building exercises (1)

- TWG recommend that the OPCW laboratory coordinate confidence building exercises as soon as is practically possible
  - TNO has requested funding from its Ministry of Foreign Affairs to prepare samples
  - a second laboratory may offer to supply analytical standards

- Exercises should commence with the simpler methods
  - for which basic GC-MS can be used
    - i.e. free metabolites and reversible protein adducts
  - at levels not too demanding but requiring trace analytical techniques
    - lower levels when a basic level of proficiency has been established

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TOR 5: Suggest procedures for confidence building exercises (2)

- For first exercise metabolites of sulphur mustard & sarin recommended, in urine at 50 ng/ml
  - ß-lyase metabolites, thiodiglycol, thiodiglycol sulphoxide
  - isopropyl methylphosphonic acid

- Blood at a later stage
  - when laboratories are used to handling BMS

- Participants will be informed of the spiking chemicals in the early exercises

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TOR 6: Provide information on laboratories and capabilities

- OPCW laboratory has received > 40 replies from member states in response to a questionnaire on BMS capabilities

- Will be compiled into a data base and distributed to member states

- OPCW laboratory with assistance from experts/member states will compile details of analytical methods plus synthetic methods or commercial sources for standards
  - availability of standards is a major obstacle to the broader adoption of some methods

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Additional considerations: scenarios

- BMS relevant only in the context of allegations of use
- Probably will be several days before an OPCW team would be on-site
  - levels of biomarkers are therefore likely to be low
  - unlike a terrorist release where initial samples may be collected within 24 h, e.g. Tokyo 1995
- Biomedical sample analysis should not be regarded as 'stand alone' evidence
  - it must be considered in conjunction with eye-witness accounts and reported symptoms
  - these may also guide the analysis

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Additional considerations: sample collection, handling and transport (1)

- The OPCW laboratory has documented procedures that were exercised in 2005
- Detailed protocols used by CDC Atlanta, US were supplied
- Fine details that need to be addressed in confidence building exercises include:
  - EDTA (CDC) vs heparin (OPCW) as anticoagulant for blood
  - separation of blood into serum/plasma & red cell
- Each sample must be split into 5 aliquots to meet OPCW requirements
  - 25 ml recommended as the minimum quantity, 50 ml preferable to allow full duplicate analyses

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Additional considerations: sample collection, handling and transport (2)

- The TWG recognised that safe handling of blood is an important issue
  - particularly with regard to viruses e.g. HIV, hepatitis etc
- Few laboratories have access to γ-ray sterilisation facilities
- Heating at 90°C for 1 hour being investigated but will degrade some analytes
- Chemical sterilants under investigation
- OPCW laboratory does not have facilities to sterilise samples prior to dispatch
- Each laboratory will individually have to address this issue

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Additional considerations: post-mortem and animal samples

- The CWC makes provision for the collection of post-mortem and animal samples
- This was considered outside the expertise and experience within the TWG
- Expert advice is required
  - SAB asked to advise

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Additional considerations: resources/training

- There will be significant resource/cost implications
- Member states will individually need to consider investment *vs* benefit
  - some may seek a BMS capability in support of counterterrorism
- The OPCW, through the International Cooperation and Assistance Branch, should consider hosting a workshop prior to confidence building exercises

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Additional considerations: preparing the OPCW laboratory

- The OPCW will need enhanced resources
  - additional personnel and equipment
  - not approved in 2007
  - SAB is asked to recommend that the DG supply these resources
- Experts from member states should assist OPCW laboratory
  - TNO has requested funding from Ministry of Foreign Affairs to provide technical advice

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Key recommendations (1)

- The TWG believes that it has met its TORs within the limitations of an advisory group
- It is recommended that BMS analysis now be taken forward by the OPCW laboratory with assistance from experts from member states
  - most of whom are members of the TWG

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Key recommendations (2)

- The OPCW laboratory with assistance from experts from member states should compile details of analytical methods, information on standards etc
- The SAB should request the Director General to make sufficient resources available to the OPCW laboratory

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Key recommendations (3)

- Confidence building exercises should commence as soon as practically possible
  - late 2007 or early 2008
- These would gradually build in terms of technical requirements and difficulty. They should not be tests and would involve mutual help
  - cf round-robin exercises for environmental samples 1990s
- Proficiency tests should proceed only when a level of expertise has been achieved in a number of laboratories

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- Members of the TWG
- Dr Tom Inch (chair for 1st meeting)
- Dr Ralf Trapp, Dr Brian Davey
- Dr Mieczyslaw Sokolowski, Dr Gary Mallard
- Mr Patrice Palanque and staff

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Appendix 3

List of cases where biomarkers had been detected in human exposures to CW agents

HUMAN SAMPLES IN WHICH BIOMARKERS HAVE BEEN DETECTED

TABLE 1: ANALYSES OF SAMPLES FROM HUMAN CASUALTIES OF DELIBERATE EXPOSURE TO SULPHUR MUSTARD

Analyte*	Casualties (number)	Time of sample collection	Approx. concn. (no. of samples)	Remarks
TDG	Iranian CW (25)	Up to ~10 days	1-100 ng/ml (24/25) 330 ng/ml (1/25)	Mean in control samples was 5 ng/ml, highest 20 ng/ml. TDG was converted to sulphur mustard with conc. HCl (Wils *et al.* 1985, 1988).
Albumin cysteine adduct			≡ 0.4-1.8 μM	Expressed as concentration of sulphur mustard that induced the same level of adduct on incubation with human blood (Noort *et al.* 1999).
TDG + TDGO	Iranian CW (5)	5-10 days	27-69 ng/ml (3/3)	Believed to include samples from some of the casualties above.
β-Lyase metabolites			0.5-5 ng/ml (4/5) 220 ng/ml (1/5)	Patient with 220 ng/ml died (Black and Read 1995a).
Hb valine adduct			0.3-0.8 ng/ml (4/4)	Calculated on the basis of released amino acid adduct. Control < 0.15 ng/ml.
Hb histidine adduct			0.7-2.5 ng/ml	Control < 0.3 ng/ml (Black et al. 1997).
β-Lyase metabolites	Kurdish CW (2)	13 days	0.3, 0.1 ng/ml	TDG + TDGO levels within those of control urine (Black and Read 1995a).
Hb valine adduct	Iranian CW (2)	22, 26 days	≡ 0.9, 0.9 μM	
DNA adduct			≡ 0.22, 0.16 μM ≡ 0.43, 0.25 μM	First figure refers to lymphocytes, second figure to granulocytes; determined by immunoassay (Benschop *et al.* 1997)

* metabolites in urine, protein and DNA adducts in blood

TABLE 2: ANALYSES OF SAMPLES FROM HUMAN CASUALTIES OF ACCIDENTAL EXPOSURE TO SULPHUR MUSTARD

Analyte*	Casualties (number)	Time of sample collection	Approx. concn	Remarks
TDG	Accidental (2)	2-3 days	2, 2 ng/ml	Exposed from a WWI munition. Control urine < 1 ng/ml.
TDG + TDGO			69, 44 ng/ml	Control urine ~11 ng/ml.
β-Lyase metabolites			42, 56 ng/ml	Control urine < 0.1 ng/ml (Black and Read, 1995b).
β-Lyase metabolite 1			15, 17 ng/ml	Analysed by LC-MS-MS after 15 years storage (Read and Black 2004a).
β-Lyase metabolite 2			30, 34 ng/ml	
Bis-N-acetylcysteine conjugate			1, 1 ng/ml	Analysed after 15 years storage; some degradation may have occurred (Read and Black, 2004b).
Hb valine adduct			0.3 ng/ml (1/1)	Calculated on basis of released amino acid adduct; control blood < 0.15 ng/ml.
Hb histidine adduct			2.5 ng/ml (1/1)	Control blood < 0.3 ng/ml (Black et al. 1997).
TDG	Accidental (1)	Up to 14 days	max. 20 μg/day, ~65 ng/ml	Laboratory exposure. Concentrations <10 ng/ml after day 7; 0.243 mg excreted over 14 days (Jakubowski *et al.* 2000).

- metabolites in urine, protein and DNA adducts in blood

TABLE 3: ANALYSIS OF SAMPLES FROM CASUALTIES OF TERRORIST INCIDENTS IN JAPAN, EXPOSED TO SARIN OR VX

Analyte/matrix	Casualties (number)	Time of sample collection	Approx. concn	Remarks
iPrMPA/urine	Matsumoto (1)	<1-7 days	760 ng.ml (day 1) 80 ng/ml (day 2) 10 ng/ml (day 7)	Severe casualty. Quantitation estimated on basis of response to MPA standard. Total excretion estimated as 2.1 mg.
MPA/urine			140 ng/ml (day 1) 20 ng/ml (day 3)	Total excretion estimated as 0.45 mg (Nakajima et al. 1998).
iPrMPA/urine	Tokyo (not given)	~1 h-7 days	see remarks	In one severely intoxicated patient, max excretion was at 12 h, expressed as 3.4 µl/g creatinine (Minami *et al.* 1997).
iPrMPA/MPA from blood AChE	Tokyo (4)	Few days, post mortem	not given	Full scan MS spectra obtained of TMS derivatives (Nagao et al. 1997).
MPA from brain AChE			not given	Post mortem tissue samples stored for ~2 years. No iPrMPA was detected (Matsuda *et al.* 1998).
iPrMPA/serum	Matsumoto, Tokyo (18)	1.5-2.5 h	<1-136 ng/ml	Analysis by LC-MS-MS (Noort *et al.* 1998).
Sarin from BuChE/serum			0.2-4.1 ng/ml (12/18)	Fluoride reactivation used (Polhuijs *et al.* 1997).
BuChE/serum			10-20 pM	Analysed as peptic nonapeptide (Fidder *et al.* 2002).
Ethyl MPA/serum	Assassination (1)	1 hour	1250 ng/ml	PC exposure on neck, died after 10 days, analysed after 6 months storage (Tsuchihashi *et al.* 1998).
VX metabolite/serum $MeSCH_2CH_2NiPr_2$			143 ng/ml	

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Appendix 4

Discussion paper: points for consideration by TWG members prior to the third meeting

1. Suggested criteria for interpretation of results

1.1 In order to assess the suitability of analytical methods and different biomarkers of exposure, criteria need to be defined for unequivocal identification in the context of trace analysis in allegations of CW use. Although the TWG at its 2nd meeting was of the view that criteria should be agreed in detail by member states in consultation with the OPCW laboratory, it would facilitate the process of implementing confidence building exercises if the TWG were to recommend criteria as a starting point. These could then be accepted, modified or rejected on the basis of experience gained in confidence building exercises.

1.2 Within the context of the CWC, it is likely that fully controlled and documented biomedical samples will be collected several days after the alleged CW incident (in contrast to homeland terrorist incidents where samples are likely to be collected much earlier). Analysis for biological markers of exposure will therefore be at trace (low or sub-ppb) levels, where full scan mass spectral data will either not be obtainable, or will be obtainable only on the most expensive research grade mass spectrometers. It is proposed that the criteria for interpretation of results should therefore be based on a flexible system of identification points (IPs) rather than on a rigid set of criteria as is currently applied in OPCW Proficiency Tests for environmental-type analysis at ppm levels. This approach would be in line with the EU Decision [1-3] for the identification of residues in live animals and animal products, and which is being adopted or recommended in other areas of regulatory/forensic trace analysis, e.g in forensic toxicology and doping [4]. Criteria adopted or recommended by different regulatory bodies are given in references [5-8]. Two examples are summarised in Annex 1; some differences are discussed in reference [8]. The general application of mass spectrometry in confirmatory analysis is discussed in references [9] and [10].

1.3 The system based on IPs, for banned substances in animal products, allows the accumulation of analytical evidence using up to three analytical methods (see Attachment 1). Each method is assigned IPs according to the degree of specificity, e.g. full scan GC-MS > GC-MS-MS- MRM > GC-MS-SIM, and the number of diagnostic ions/transitions available. Other aspects, such as retention times and ion ratios, are also taken into account. This system acknowledges that for certain analytes the acquisition of the appropriate number of IPs cannot be obtained by a single technique, e.g. in cases where < 4 ions are available in SIM or only one transition is available in MRM (e.g. see reference [11].

1.4 For the purposes of the OPCW, another factor needs to be considered, i.e. the accumulation of IPs, or evidence, based on different biomarkers of the same agent. In such a case, consideration will need to be given to the minimum number of IPs required for each analyte. The use of multiple markers does not appear to have been addressed in other areas of trace analysis (please provide details if they have).

1.5 In considering such a system for biomedical sample analysis, it is desirable that there should be a degree of consistency with the criteria currently used in OPCW Proficiency Tests for environmental-type analysis.

1.6 For example, the EU directive for residues in animal products requires a minimum of 4 IPs for banned substances. These can be achieved using one, two or three analytical methods. The criteria used by the OPCW for environmental analysis requires identification by two different techniques, that would be equivalent to at least 5 IPs for most analytes, e.g. based on the acquisition of full scan EI-MS (4 diagnostic ions) and CI-MS (1 diagnostic ion) data. For some biological markers, particularly protein adducts that are analysed using LC-MS-MS, only one analytical method may be available. In these cases, should a second biomarker be required?

1.7 If practical, it would be appropriate to adopt the same criteria for retention times in GC-MS and LC-MS analysis for biomedical samples as is used by the OPCW for environmental samples.

1.8 Reporting criteria, which are critical to the acceptability of data and success in OPCW Proficiency Tests, will need to be carefully considered in the context of trace analysis, where demonstration of absence of cross contamination is crucial. For example, it should be mandatory that a system blank be demonstrated immediately before a positive confirmatory analysis. Reporting criteria should evolve from initial confidence building exercises.

1.9 The recently reformed TWG on Sampling and Analysis is likely to address trace environmental analysis in the context of allegations of CW use, and again it is important that there is consistency between any systems recommended.

2. Some points for discussion

2.1 If a system based on IPs is recommended, what should be the minimum number of IPs required for OPCW purposes, i.e. has this subject been exposed to a CW agent?
- suggested 5
- in full scan mode this would equate to 4 diagnostic ions >10% relative abundance using EI (4 IPs) , plus a quasi-molecular ion in CI (1 IP);
- will need to demonstrate that 5 IPs is practical for Schedule 1 agents of main concern.

2.2 Is it acceptable to obtain 5 IPs using only one technique?
- or should a second technique or second biomarker be mandatory

2.3 What criteria for ion ratios should be accepted? - there is some variation in the requirements of different regulatory bodies [8].

2.4 If such a system allows accumulation of IPs based on different biological markers of exposure, what should be the minimum number of IPs required for each analyte?

- suggested 2, consistent with EU requirements that at least one ion ratio must be measured
- the latter effectively disqualifies single ion monitoring.

2.5 It is recommended that all confirmatory analyses should be preceded by a system blank, be followed by a system blank, and confirmed against a 'standard'.
- for protein adducts is a standard generated by incubation of agent with human blood acceptable? (it is probably the only practical solution in the near future).
- should confirmation be against pure standard or a blank matrix spiked with the standard?

2.6 Internal standards should be used where possible (mandatory would probably not be practical).

2.7 All MS data should be generated in combination with a chromatographic technique (flow injection analysis not acceptable). If practical, windows for retention data should be the same as for environmental analysis as applied in OPCW Proficiency Tests (see Attachment 2).

2.8 The generally accepted standard for chromatographic peaks in trace analysis is a minimum S/N ratio of 3:1. For Proficiency Tests the OPCW require a S/N ratio of 5.
- what should be acceptable?
- definition of 'noise'

2.9 What techniques (if any) other than MS should be acceptable?
- immunoassay
  - if so what criteria and how many IPs?
  - the TWG on Sampling & Analysis will probably address this issue for toxin analysis
- GC/LC with less specific detectors (GC-FPD, LC-UV, LC-fluorescence, LC-ECD etc)
  - suggestion is that MS is currently the only acceptable detector
- NMR may be applicable in very rare instances.

2.10 What should be the status of 'supportive biomarkers', e.g. thiodiglycol and its sulphoxide, that occur at trace levels in normal human urine?
- the problem with accepting them is that quantitation and threshold levels for reporting become issues that will need careful consideration
- or is there a simpler way of dealing with it?

2.11 Should there be any difference in acceptability between presumptive and confirmed biomarkers?
- probably not, although VX, soman and tabun could be a problem with regard to unequivocal identification based on current methodology
- soman and tabun age on ChE, no satisfactory urinary metabolites are known for tabun, only one biomarker (a blood metabolite) is known for identification of the side-chain of VX.

2.12 What is the question that the OPCW needs to answer:
- has the subject been exposed to a specific nerve agent, e.g. sarin, VX etc?
- or has the subject been exposed to a certain class of nerve agent?
- this is an issue with regard to protein adducts with nerve agents that rapidly age, and with VX if no evidence for the side chain is presented.

3. Review of acceptable biomarkers

3.1 It is proposed to briefly review the list of biomarkers recommended at the second meeting of the TWG, and to report any significant advances in the past year. Major knowledge gaps should be highlighted.

3.2 The status of metabolites that could arise at trace levels from other sources, e.g. thiodiglycol, its sulphoxide, and N-methyl- and ethyl-diethanolamines, needs further clarification.

3.3 The status of metabolites that could arise from simple chemical oxidation or hydrolysis of another should also be clarified, e.g. sulphides and sulphoxides, and alkyl methylphosphonic acids and methylphosphonic acid. Should these pairs be regarded as a single biomarker?

3.4 Ideally, at least two biomarkers would be useful for each agent in each matrix (blood, urine etc) to ensure identification to the required level, particularly if only one type of sample is available. In those cases where only one biomarker is available, e.g. nerve agent metabolites in urine, the availability of alternative analytical methods needs to be addressed (e.g. LC-MS), again in order to ensure confirmatory identification at trace levels.

4. Recommendation of analytical methods

4.1 It was proposed that a 'manual' of recommended analytical methods is compiled. Should these be outline methods, with references to full experimental details as reported in open publications or should they be fully detailed? They should include minor variations, e.g. in clean up or chemical reagent gas used in CI, that achieve similar levels of detection.

4.2 The OPCW laboratory has requested copies of all relevant publications on biomedical sample analysis from the past 10 years, preferably in PDF format if available.

4.3 Should the TWG recommend levels of detection that should be achieved for each analyte?

5. Confidence building exercises

5.1 The OPCW would like to commence a series of confidence building exercises as soon as is practically possible. Any provisional offers for sample preparation would be gratefully received.

5.2 The TWG is asked to recommend how exercises should be initiated. At the 2nd TWG meeting it was generally accepted that these should not be 'tests' (Proficiency Tests would be considered once a certain level of proficiency has been achieved). It is suggested that samples would initially be spiked with specified (?) biomarkers at concentrations that would not be too demanding, but would give laboratories a preliminary indication of the suitability of their methods (or should high and low concentration samples be provided as this would require little extra effort?). It is suggested that these exercises be undertaken along the lines of the 'round robin' exercises that preceded the entry into force of the CWC. There would be no scoring of performance and constructive help would be available to laboratories having difficulties.

5.3 The TWG is asked to recommend which analytes and matrices would be appropriate for the first exercise. It was recommended at the second meeting that these should involve simple metabolites in urine, and/or protein adducts in blood or plasma where residues were displaceable. A problem that would need to be considered for some protein adducts is 'ageing' (dealkylation) or slow hydrolysis.

5.4 A format for reporting results should evolve from these exercises.

6. Acquisition of analytical standards

6.1 The availability of standards is one of the major obstacles to the adoption of analytical methods in a broader number of laboratories. Preferably, most laboratories should prepare or purchase their own standards. At the second meeting of the TWG, laboratories currently undertaking biomedical sample analysis were asked to provide the Chairman with full details of synthesis or commercial sources of various metabolites. Please would you provide this information if available. In the case of protein adducts, it would be useful to have experimental details of how adducts are prepared in vitro by incubation with human plasma or whole blood.

Robin Black
Chair
SAB TWG on Biomedical Sampling

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Attachment 1

EU system of IPs for analysis of banned/controlled residues in animal products [1-3]

General principles for qualitative analysis:

The basic idea of IPs is that a laboratory can use any molecular spectrometric technique or combination of techniques (maximum 3) in order to obtain the minimum number of IPs required for a confirmatory identification. For banned substances the minimum number of IPs required is 4.

MS technique*	Identification points per ion
LR full scan or SIM	1
LR-MS^n precursor ion	1
LR-MS^n transition products	1.5
HR-full scan or SIM	2
HR-MS^n precursor ion	2
HR-MS^n transition products	2.5

LR, HR = low, high resolution
MS^n is usually MS-MS

Additional criteria:

Where full scan MS data is acquired, all diagnostic (structurally characteristic) ions > 10% in the reference spectrum must be present; a minimum of 4 such ions must be present.

For SIM/MRM, S/N ratios must be ≥ 3. For SIM, ions should be characteristic of the structure, e.g. M^+, structurally indicative fragment ions, and preferably not originating exclusively from the same part of the molecule.

Tolerances for relative ion intensities

Relative intensity (% of base peak)	EI-GC-MS (relative)	CI-GC-MS, GC-MS^n, LC-MS, LC-MS^n (relative)
> 50	± 10	± 20
> 20-50	± 15	± 25
> 10-20	± 20	± 30
≤ 10	± 50	± 50

Examples of IPs earned for techniques and combinations

Technique	Number of ions	IPs
GC-MS (EI or CI)	n	n
GC-MS (EI & CI)	2 (EI) + 2 (CI)	4
GC-MS (EI or CI) 2 derivatives	2 (deriv A) + 2 (deriv B)	4
LC-MS	n	n
GC-MS + LC-MS	2 + 2	4
GC-MS-MS LC-MS-MS	1 precursor 2 products	4
GC-MS-MS LC-MS-MS	2 precursors each with one product	5
GC/LC + HRMS	2 + 1	4

WADA identification criteria for qualitative assays

Some differences from above:

Although IPs are not used, the system does allow for a combination of techniques to achieve the required number of diagnostic ions.

For SIM, 3 diagnostic ions are required, or a second derivative can be used that yields different diagnostic ions, or a second ionization technique that also yields different diagnostic ions (but not utilizing a technique that changes only the relative abundance of the same ions). In all cases, at least one ion ratio must be measured, so a minimum of 2 diagnostic ions must be present.

These requirements disqualify single ion monitoring.

For MRM, the requirements state that 'a single precursor ion - product ion pair may be sufficiently unique to be definitive'. Otherwise a second derivative or second ionization technique should be used.

Tolerances for relative ion intensities

Relative intensity (% of base peak)	EI-GC-MS	CI-GC-MS, GC-MS^n, LC-MS, LC-MS^n (relative)
> 50	± 10 (absolute)	± 15 (absolute)
> 25-50	± 20 (relative)	± 25 (relative)
< 25	± 25 (absolute)	± 10 (absolute)

Attachment 2

Some criteria used in OPCW Proficiency Tests for ppm levels

Only a selection of criteria relevant to GC-MS and LC-MS are summarised.

Identification must be by two different techniques, at least one of which must be spectrometric. Although one identification technique can be by retention index (within strict guidelines), this would not be appropriate for trace analysis where the second technique is not full scanning.

With regard to two mass spectral techniques, these can be two ionization methods (EI and CI), possibly positive and negative ion (needs clarifying), two derivatives, or in combination with two chromatographic techniques (GC, LC, CE). GC-MS and GC-MS-MS are allowed as two techniques but LC-MS and LC-MS-MS are regarded as one (requires further clarification).

For all chromatograms, S/N ratios must be ≥ 5:1.

There are no rigid criteria for what is an acceptable full scan spectrum in comparison to a standard, but any spurious ions should be identified.

Retention time criteria:

For GC, retention times must fall within ± 0.1 min of the retention time of the reference chemical.

For LC, retention times must fall within ± 0.2 min of the retention time of the reference chemical.

Any peaks falling within the retention window in blank samples (S/N ≥ 5:1 or > 1% of the intensity of the analyte peak in the sample) must be shown not to be the analyte.

Appendix 5
Collection, packaging and transportation SOPs currently used by CDC Atlanta

Centers for Disease Control and Prevention
Shipping Instructions for Specimens Collected from People Who May Have Been Exposed to Chemical-Terrorism Agents

SECTION ONE: COLLECTING AND LABELING SPECIMENS

Required Specimens

Unless otherwise directed, collect the following specimens from each person who may have been exposed:

Whole blood

- Collect blood specimens from adults only unless you receive specific instruction from CDC to collect blood from pediatric patients.
- Collect a minimum of 12 mL of blood.
- Use three 4-mL or larger vacuum–fill only (unopened), non-gel, purple-top (EDTA) tubes; use four tubes if using 3-mL tubes.
- Using indelible ink, mark each purple-top tube of blood ***in the order collected*** (e.g., # 1, # 2, # 3, # 4 [if using 3-mL tubes]).
- In addition, collect another specimen using one 3-mL or larger, vacuum-fill only (unopened), non-gel, green- or gray-top tube. Allow the tube to fill to its stated capacity.

Urine

- Collect at least 25-50 mL from potentially exposed adults and children.
- Use a screw-cap plastic container; do not overfill.
- Freeze specimen as soon as possible (−70° C or dry ice preferred).
- If other than "clean catch", note method of collection on the specimen cup (e.g., obtained by catheterization).

Blanks

For each lot number of tubes and urine cups used for collection, provide the following to be used as blanks for measuring background contamination:

- Two (2) empty, unopened purple-top tubes.
- Two (2) empty, unopened green- or gray-top tubes.
- Two (2) empty, unopened urine cups.

Labeling Specimens

- Label specimens with labels generated by your facility and follow your facility's procedures for proper specimen labeling.
- In addition to unique patient identifiers (e.g., medical records number, specimen identification number) labels should convey the collector's initials, date and time of collection so that law enforcement officials may trace the specimen to the collector should investigations lead to legal action and the collector has to testify that he or she collected the specimen.
- If you use bar-coded labels, place the labels on blood tubes and urine cups so that when these containers are upright, the bar code looks like a ladder.
- Maintain a list of names with corresponding specimen identification numbers at the collection site so that results can be reported to patients. It is recommended that you record additional data for use in the interpretation of results. Additional data may include: time of potential exposure, method of urine collection if other than "clean-catch", indication if sample was collected post-mortem, and antidotes administered prior to sample collection.
- Information provided on labels and lists may prove helpful in correlating the results obtained from CDC's Rapid Toxic Screen and subsequent analysis with the people from whom the specimens were collected.

Shipping Instructions for Specimens Collected from People Who May Have Been Exposed to Chemical-Terrorism Agents

SECTION TWO: PACKAGING SPECIMENS

Packaging consists of the following components: primary receptacles (blood tubes or urine cups), secondary packaging (materials used to protect primary receptacles), and outer packaging (polystyrene foam-insulated, corrugated fiberboard shipper).

Secondary Packaging for Blood Tubes

- To facilitate processing, package all blood tubes from the same patient together.
- Place absorbent material between the blood tubes and the first layer of secondary packaging. Use enough absorbent material to absorb the entire contents of the blood tubes.
- Separate each tube of blood collected from other tubes, or wrap tubes to prevent tube-to-tube contact. Regardless of the method used, the first layer of secondary packaging must be secured with one continuous strip of evidence tape and initialed half on the tape and half on the first layer of secondary packaging by the person making the seal. Examples of some ways to do this are to—
  - Pack blood tubes in a gridded box lined with absorbent material. Seal the top half of the box to the bottom half with one continuous piece of evidence tape and write your initials half on the tape and half on the box.
  - Pack a sealable polystyrene foam container or blood tube shipment sleeve and transport tube with individually wrapped tubes. Seal the polystyrene foam container or transport tube with one continuous piece of evidence tape and write your initials half on the tape and half on the container.
- Wrap and seal the first layer of secondary packaging (e.g., gridded box) with absorbent material.
- Seal one wrapped gridded box or alternative container inside a clear, leak-proof biohazard polybag equivalent to Saf-T-Pak product STP-701, STP-711 or STP-731.
- Place this bag inside a white Tyvek® outer envelope (or equivalent) and seal the opening with a continuous strip of evidence tape initialed half on the packaging and half on the evidence tape by the individual making the seal.
- According to 49 CFR 173.199(b), if specimens are to be transported by air, either the primary receptacle or the secondary packaging used must be capable of withstanding, without leaking, an internal pressure producing a pressure differential of not less than 95 kPa (0.95 bar, 14 psi). Verify in advance that the manufacturer of either the blood tube or secondary packaging used in your facility is in compliance with the pressure differential requirement.

Outer Packaging for Blood Tubes

- Use polystyrene foam-insulated, corrugated fiberboard shipper (may be available from your transfusion service or send-outs department).
- For cushioning, place additional absorbent material in the bottom of the shipper.
- Add a single layer of refrigerator packs on top of absorbent material.
- Place the packaged specimens on top of the refrigerator packs.
- Use additional cushioning material to minimize shifting while the shipper is in transit.
- Place additional refrigerator packs on top of the secondary packaging to maintain a shipping temperature of 1° C-10° C for the duration of transit.
- Place blood shipping manifest in a sealable plastic bag and put on top of packs inside the shipper.
- Keep chain-of-custody documents for your files.
- Place lid on shipper and secure with filamentous shipping tape.
- Place your return address in the upper left-hand corner of the shipper top and put CDC's receiving address in center.
- Affix labels and markings adjacent to the shipper's/consignee's address that appears on the shipper.
- Place the UN 3373 label and the words "Biological Substance, Category B" adjacent to the label on the front of the shipper.

Secondary Packaging for Urine Cups

- Separate each urine cup from other urine cups, or wrap individual urine cups to prevent contact between urine cups. Regardless of the method used, the first layer of secondary packaging must be secured with one continuous strip of evidence tape and initialed half on the tape and half on the first layer of secondary packaging by the person making the seal. Examples of some ways to do this are to—
  - Pack urine cups in a gridded box lined with absorbent material. Seal the top half of the box to the bottom half with one continuous piece of evidence tape and write your initials half on the tape and half on the box.
  - Seal individually wrapped urine cups inside a clear, leak-proof biohazard polybag equivalent to Saf-T-Pak product STP-701, STP-711 or STP-731. Secure the closure of the bag with one continuous strip of evidence tape initialed half on the tape and half on the bag by the individual making the seal.
- Place urine cups, boxed or individually wrapped and secured properly with evidence tape, in the next layer of secondary packaging. An example of acceptable material is the Saf-T-Pak Disposable 2-Part Pressure Vessel system or its equivalent.
- Secondary packaging must have its closure secured with a single strip of evidence tape initialed half on the packaging and half on the evidence tape by the person making the seal.

Outer Packaging for Urine Cups

- Use polystyrene foam-insulated, corrugated fiberboard shipper (may be available from your transfusion service or send-outs department).
- For cushioning, place additional absorbent material in the bottom of the shipper.
- Place a layer of dry ice on top of the absorbent material. Do not use flakes or large chunks of dry ice for shipment because large chunks have the potential for shattering urine cups during transport.
- Ensure that specimens will remain frozen or will freeze during transport.
- Place packaged urine cups in the shipper.
- Use additional absorbent or cushioning material between wrapped urine cups to minimize shifting while shipper is in transit.
- Place an additional layer of dry ice on top of samples.
- Place the urine shipping manifest in a sealable plastic bag and put on top of dry ice inside the shipper.
- Keep chain-of-custody documents for your files.
- Place lid on shipper and secure with filamentous shipping tape.
- Place your return address in the upper left-hand corner of the shipper top and put CDC's receiving address in center.
- Place the UN 3373 label and the words "Biological Substance, Category B" adjacent to the label on the front of the shipper.
- Place a Class 9/UN 1845 hazard label on the same side of the shipper as the UN 3373 marking.
- If the proper shipping name, (either dry ice or carbon dioxide, solid) and Class 9/UN 1845 is not preprinted on the hazard label, add it in an area adjacent to the label.
- Note the weight of dry ice (in kg) on the preprinted area of the hazard label, or place that information adjacent to the Class 9/UN 1845 hazard label.
- Orientation arrows are not required on a shipper containing "Biological substance, category B." If you use arrows, be sure to orient the inner packaging so that closures are aligned with the arrows.
- If the shipper will be transported by a commercial air carrier, complete an airway bill. On the airway bill, note the proper shipping name and UN number for each hazardous material and identify a person responsible for the shipper per IATA packing instruction 650.

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SECTION THREE: SHIPPING SPECIMENS

Follow the guidance provided in your state's chemical-terrorism comprehensive response plan. If you are directed to ship the specimens to CDC, please ship the specimens to the following address:

Centers for Disease Control and Prevention
Attn: Charles Dodson
4770 Buford Hwy.
Building 110 Loading Dock
Atlanta, GA 30341
(770) 488-4305

Preparing Documentation

- Since blood tubes and urine cups cannot be shipped together in the same package, prepare a separate shipping manifest for each.
- Note on shipping manifest if urine sample is collected by means other than clean catch (e.g., catheterization).
- Place each shipping manifest (with specimen identification numbers) in a plastic zippered bag on top of the specimens before closing the lid of the polystyrene foam-insulated, corrugated fiberboard shipper.
- Do not transport chain-of-custody forms with specimens. Each entity or organization handling the specimens is responsible for the specimens only during the time that it has control of the specimens.
- Each entity or organization receiving the specimens must sign-off on the chain-of-custody form of the entity or organization relinquishing the specimens to close that chain. Electronic procedures such as electronic chain-of-custody and barcode readers will expedite this process.
- When receiving specimens, each new entity or organization must begin its own chain of custody. The entity or organization relinquishing the specimens must sign its chain of custody to close the chain and indicate that they have transferred the specimens.

Note: When the person relinquishing the specimens (relinquisher) and the person receiving the specimens (receiver) are not together at the time of specimen transfer, the relinquisher must document on its chain-of-custody form that the receiver is the express courier (e.g., FedEx, Delta Dash, DHL, UPS) and must document the shipment tracking number or have the person transporting the specimens sign the chain-of-custody to indicate that he or she has taken control of the specimens. Likewise, when receivers get the specimens, they will document on their chain-of-custody form that the relinquisher is the express courier (and provide the tracking number) or have the person transporting the specimens sign the chain-of-custody form.

Questions

If you have any questions or problems with specimen packaging or shipment, please send an e-mail to or call one of the following contacts:

- Jacob Wamsley, Incident Response Laboratory Coordinator, Division of Laboratory Sciences
  E-mail: JWamsley@cdc.gov Phone: 770-294-2491
- Jessica Mitchell, Chemical Terrorism Laboratory Program Liaison, Battelle Contractor
  E-mail: JCMitchell@cdc.gov Phone: 770-488-4166

Annex 3

REPORT OF THE TEMPORARY WORKING GROUP ON SAMPLING AND ANALYSIS THE HAGUE, 7 AND 8 FEBRUARY 2007

1. Introduction

1.1 The SAB Temporary Working Group on Sampling and Analysis held its first meeting on 7th and 8th February 2007 in The Hague.

1.2 The meeting was chaired by Miguel A. Sierra on behalf of the SAB.

1.3 The list of participants in the meeting is given in Appendix 1.

1.4 The following agenda was adopted.

i. Opening of the workshop and adoption of the Agenda (Chairman of the S&A TWG-Miguel A. Sierra).
ii. Welcome address by the Secretariat, Patrice Palanque.
iii. Tour de table for introduction of S&A-TWG members.
iv. VER/IVB briefing on current actions under Article VI S&A implementation plan, William Kane (OPCW-Head Industry Verification Branch).
v. General discussion on the provisional terms of reference.
vi. Current verification procedures:
vii. Review of the entire chain for sample collection and sample preparation. Identification of potential weakness and suggestions for improvements.
viii. On site analyses and routine inspections.
ix. Proposal and discussion of OPCW requirements for toxin analyses.
x. Expansion of the OCAD database to include non-scheduled degradation products.
xi. The current proficiency testing in a global context. Gary Mallard (Head OPCW Laboratory).
xii. Review of the success of the current proficiency testing system.
xiii. Presentation by Colin Pottage (UK) on proposed changes to the way Proficiency Tests are conducted and the trial run that was undertaken earlier last year.

xiv. Discussion of proposals for new objectives consistent with actual scenarios and inspection procedures.

xv. Study of criteria for a positive analysis in trace analyses.

xvi. New and/or additional techniques for on-site analyses, including toxin analyses.

xvii. Any other business.

xviii. Elaboration of the resume Report.

xix. Closure of the meeting.

1.5 Mr Patrice Palanque welcomed members of the TWG on behalf of the Director-General of OPCW. This is an important year for the OPCW in its preparation for the Second Review Conference to be held 7-18 April 2008. The work of the TWG was seen as an important contribution to the report and recommendations of the SAB to the Director General prior to the Review Conference.

TERMS OF REFERENCE

2.1 The proposed terms of reference for the TWG were discussed. These were broadly agreed upon, with minor modification as shown in Appendix 2.

STATUS OF VERIFICATION PROCEDURES

3.1 Dr. William Kane, Head of the Industry and Verification Branch, briefed the TWG on the current actions being undertaken under the implementation plan for Sampling and Analysis under Article VI. The presentation is included as Appendix 3. Dr Kane informed the TWG of the successful completion of two Article VI inspections in 2006 which included S&A.

3.2 The current OPCW procedures for on-site sampling and analysis were reviewed. The TWG concluded that the current procedures are still appropriate and effective for their intended purpose.

3.3 The TWG also accepted that GC-MS was still the most versatile technique, in terms of applicability, mobility and robustness. However, it recommends developments be reviewed in the preparation of aqueous samples or extracts, which avoid the need to concentrate aqueous solutions to dryness. Incorporation of other analytical techniques into the OPCW sampling and analysis procedures were discussed.

3.4 It was noted that off-site analysis had still not been employed during inspections. However, the TWG considered off-site analysis is still a key component of verification and the TWG reaffirmed the need for the system of designated laboratories. In a global context it is desirable that additional laboratories be designated.

CHEMICALS

4.1 The current status of the analysis of Schedule 1 toxins was discussed. Most designated laboratories have not been accredited to undertake toxin analysis and these types of samples have not been included in OPCW proficiency tests. This TWG considers this an unacceptable situation. The TWG recommends that the OPCW laboratory procure information on the capabilities of designated laboratories to perform toxin analyses. It may be necessary to seek one or more qualified laboratories that may or may not be part of the existing network of designated laboratories but which have demonstrated capability [ISO, QA/QC, etc] to analyze for toxins. The TWG recommends that the SAB consider the issue of the designation of such laboratories by the DG, in accordance with CWC VAII Par 56(b), including consideration as to whether this designation would require a modification of CSP Decision CSP/I/DEC.61.

4.2 The TWG discussed the addition of non-scheduled chemicals to the OCAD data base. Specifically: riot control agents in the context of allegations of use, non scheduled degradation products of scheduled chemicals considered relevant to verification, and chemicals related to OCW and ACW. A list of such chemicals as recommended in the report of the 4th meeting of the SAB is given in Appendix 4. The working group recommends the inclusion of these compounds in the OCAD, and the selective use of data from OCAD in accordance with the inspection aims as specified in the Convention.

PROFICIENCY TESTING

5.1 The TWG was briefed by Dr. Mallard and Mr. Pottage on the current proficiency testing system and successful work undertaken to incorporate procedures, which the OPCW would be using for the off-site analysis of authentic samples in an inspection. A new format for the conduct of and the reporting on the results of proficiency testing had been devised and tested in 2006 on a voluntary basis. The working group agreed that the new format more closely represented the situation that would exist with real samples and would also lessen the burden of reporting blanks in proficiency tests. The following recommendations were supported by the group:

- It is appropriate to move to Proficiency Tests based on this new format using the revised criteria proposed for this exercise (see Attachments) or Proficiency Tests using the new format there should be:
  2 sets of samples (authentic, control, blank)
  A similar total number of spiking chemicals to current tests

- For Proficiency Test and Authentic sample analysis there is a need to ensure that, as in this exercise, authentic, control & blank matrices are sufficiently different so that the two samples prepared at the OPCW laboratory are not readily identified. This can simply be achieved by varying the proportions of ‘contaminants used to prepare the blank and control samples.

- To help laboratories new to proficiency testing and to facilitate checking for report compilers, consideration should be given to preparing a reporting

example or simplified sheet to detail the minimum requirements needed for the report. Minimum reporting criteria need to be agreed for samples in which no reportable compounds are detected. We recommend inclusion of comprehensive preparation and/or flowchart pages for these samples together with a statement in the 'Comments' Section to say that no reportable compounds were found in these samples.

- When preparing the control sample the OPCW laboratory will make every effort, for example by consulting with the inspection team, to ensure that the spiking chemicals used to prepare the control sample are not those which might be expected to be present in the authentic samples.

- However, in the unlikely event that this does occur, consideration must be given to how the analysing laboratory proceeds, as it will not necessarily know if the results are real or due to cross-contamination. It is recommended that the laboratory repeats the analysis and, if the same chemical is still detected, reports as usual. The TS will be in a position to decide on the validity of these results based on comparison with those from other laboratories.

ANALYTICAL METHODOLOGY

6.1 Members of the TWG accepted that there may be a requirement for trace analysis in the context of allegations of use and expressed a range of views on its reporting. An identification points system that has been suggested for the trace analysis of biomedical samples was discussed. The view of the TWG was that this system should first be evaluated for biomedical sample analysis.

6.2 The TWG recommends the establishment of a new working group to evaluate the potential for LC-MS to be used in on-site analysis. This TWG should include in its scope issues such as standard sample preparation procedures and development of a spectral data base.

SUMMARY OF RECOMMENDATIONS

- The TWG recommends developments in the preparation of aqueous samples or extracts, which avoid the need to concentrate aqueous solutions to dryness, be reviewed.
- The TWG recommends that the OPCW laboratory procure information on the capabilities of designated laboratories to perform toxin analyses.
- The TWG recommends that the SAB consider the issue of the designation of laboratories for toxin analysis by the DG, in accordance with CWC VAII Par 56(b), including consideration as to whether this designation would require a modification of CSP Decision CSP/I/DEC.61.
- The working group recommends the inclusion of riot control agents in the context of allegations of use, non scheduled degradation products of scheduled chemicals considered relevant to verification, and chemicals related to OCW and ACW in the OCAD, and the selective use of data from OCAD in accordance with the inspection aims as specified in the Convention.

- The TWG recommends the establishment of a new working group to evaluate the potential for LC-MS to be used in on-site analysis

Appendix 1

List of Participants of the Temporary Working Group on Sampling and Analysis

	Participant	Member State
1.	Robert Mathews	Australia
2.	Paul A. D'Agostino	Canada
3.	Jiří Matoušek	Czech Republic
4.	Martin Söderström[1]	Finland
5.	Jean-Claude Tabet	France
6.	Anne Bossée	France
7.	Ralf Trapp	Germany
8.	R. Vijayaraghavan	India
9.	Jose Luz Gonzalez-Chavez	Mexico
10.	Marieke van Deursen	Netherlands
11.	Sng, Mui Tang	Singapore
12.	Philip Charles Coleman	South Africa
13.	Miguel Sierra[2]	Spain
14.	Roberto Martinez-Alvarez	Spain
15.	Stefan Mogl[3]	Switzerland
16.	Robin Black	United Kingdom of Great Britain and Northern Ireland
17.	Colin Pottage	United Kingdom of Great Britain and Northern Ireland
18.	James Gibson	United States of America
19.	Edward White	United States of America
20.	Armando Alcaraz	United States of America

[1] Replaced Dr Paula Vanninen.

[2] Chairman of the TWG.

[3] Replaced Dr Peter Siegenthaler.

Appendix 2

Terms of Reference for the Temporary Working Group on Sampling and Analysis

1) **Background:** This temporary working group on sampling and analysis will consist of experts in those sampling and analysis techniques, relevant to the current sampling and analysis network of the OPCW. The experts will cover the different interdisciplinary areas of knowledge and will have the state of the art scientific and technological knowledge necessary to undertake the tasks proposed by the SAB. The group is requested to report to the SAB on the following:

2) **Status of the Current Verification Procedures:**

i) Review and, if considered necessary, make recommendations for revision, of the following.
ii) The entire adopted chain for sample collection and sample preparation.
iii) Procedures for analysis in inspections using current methodology.

3) **Chemicals:**

i) Addition of non-scheduled compounds to the OPCW Central Analytical Database (e.g. riot control agents, non-scheduled degradation products), and selected use of data in accordance with inspection aims, as set out in the Convention.
ii) OPCW requirements for toxin analysis and how should OPCW address the issue of the analysis of toxins?

4) **Proficiency Testing:**

i) Review the purpose and design of the current scheme of proficiency testing, and the revised format currently under consideration.
ii) Consider modifying the proficiency testing to be consistent with actual inspection procedures and real scenarios.
iii) Review the success of proficiency testing in a global context.

5) **Analytical methodology**

i) Consider criteria for a positive analysis if full scan data are not obtainable, i.e. for trace analysis.
ii) Consider new/additional analytical techniques.

Appendix 3
Sampling and Analysis - Implementation for Article VI inspections

Sampling & Analysis – Implementation for Article VI Inspections

Bill Kane
Head of Industry Verification Branch
ORGANISATION FOR THE PROHIBITION OF CHEMICAL WEAPONS

Background – S&A

- CWC provides for S&A as routine tool for inspections
- TS not "ready" at EIF for S&A
- TS would have used S&A for Article VI -- had it been ready.
- 2002/03 - TS held bilateral consultations on S&A:
  - Views differed – outcome inconclusive
  - TS benefited from Member States suggestions

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Background - Continued

- 2005 – Verification/Inspectorate technically ready for S&A
- DG decided to implement S&A – taking all views into account
- DG instructed Verification Div. to take lead in planning with target to start in 3rd quarter, 2006

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DG Statement – Key Points

- Announcement made in Dec 2005 (EC-43)
- S&A is a routine tool expected to be used by inspectors under Article VI (used for CW)
- TS is prepared and ready to conduct S&A under Article VI (On-site laboratory analytical capabilities)

4

DG Statement – Continued

- Ensure protection of CBI
- Will start using on a limited basis for schedule 2 inspections
- TS to brief interested SP's for familiarization purposes with analytical procedures and equipment

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Sampling & Analysis - CWC

- CWC Article VI S&A provisions
  - General Provisions (VA, §52-58, Part II)
  - Schedule 2 (VA, §27, Part VII)
  - Schedule 3 (VA, § 22, Part VIII)
  - OCPF (VA, § 19, Part IX)

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S & A – Implementation

- "Start-up Period" for ~ 1-1/2 years (Start 3rd Qtr 06):
  - Schedule 2 subsequent inspections
  - OPCW analytical equipment brought on-site
  - Off-site analysis is not intended as part of the start up period
  - S&A in about 13 inspections (total S1,S2,S3 & OCPF inspections = 295 in 1-1/2 years)
  - Select one facility per State Party

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Sampling & Analysis

- Facilities to be selected during start up period:
  - Schedule 2 sites with potential technical capability to produce undeclared scheduled chemicals:
    - Batch-type facilities
    - Multipurpose facilities
    - Combination
- TS will consider further use of S&A after start-up period

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Sampling & Analysis

- Industry concerns taken into consideration:
  - Abiding by the "Least Intrusive Manner" principle
  - Ensure protection of Confidential Business Information (CBI)
  - Scope of access not increased unnecessarily

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TS Preparations for S&A

- Cross-Functional Task Force established in Sept. 2005:
  - Inspection Team Leaders
  - OPCW Laboratory
  - Operations and Planning Branch
  - Industry Verification Branch
  - Policy and Review Branch
  - Inspection Review Branch
- Purpose – develop implementation plan for S&A for the start-up period

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TS Preparations for S&A

- Cross-Functional Task Force – Activities
  - Logistics and resources
  - Sampling guidelines
  - Training
  - Inspection Planning, 2006 and 2007
  - Potential Problem Analysis
  - Information sharing with SP's
- Manage/Analyze Start-up Activities

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Article VI – Status of S&A

- Two missions completed successfully in 2006 (Sept. and Nov.)
- 8-10 S&A inspections planned in 2007
- S&A equipment and procedures worked
- Completed in required timeframe
- Analytical results - consistent with declared activities of the site.
- Some "learnings" from mission to be incorporated in future planning

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Thank you
Questions, Answers and Discussion
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Appendix 4

PRIORITY OF DATA TO BE OBTAINED FOR THE CENTRAL OPCW ANALYTICAL DATABASE

Non-scheduled degradation products of scheduled chemicals

1. Schedule 1.A.2 (Tabun family)

High priority:

O-ethyl N,N-dimethylphosphoramidate
O-isopropyl N,N-dimethylphosphoramidate

and their analytical derivatives (trimethylsilylesters).

Lower priority:

Data on other representatives of N,N-dialkyl O-alkyl phosphoramidates and their analytical derivatives (trimethylsilylesters).

2. Schedule 1.A.3 (VX-family)

High priority:

Bis(diethylaminoethyl)sulfide
Bis(diethylaminoethyl)disulfide
Bis(diisopropylaminoethyl)sulfide
Bis(diisopropylaminoethyl)disulfide

and their protonated salts.

Lower priority:

Bis(dimethylaminoethyl)sulfide
Bis(dimethylaminoethyl)disulfide
Bis(dipropylaminoethyl)sulfide
Bis(dipropylaminoethyl)disulfide

and their protonated salts.

3. Schedule 1.A.4 (sulfur mustards)

(a) Oxidised products of mustard gas:

High priority:

Bis(2-chloroethyl)sulfoxide
Bis(2-chloroethyl)sulfone

(b) Hydrolysis products:

High priority:

1,2-bis(2-hydroxyethylthio)ethane
bis(2-hydroxyethylthioethyl)ether

Low priority:

bis(2-hydroxyethylthio)methane
1,3-bis(2-hydroxyethylthio)propane
1,4-bis(2-hydroxyethylthio)butane
1,5-bis(2-hydroxyethylthio)pentane
bis(2-hydroxyethylthiomethyl)ether

(c) Oxidised hydrolysis products:

High priority:

bis(2-hydroxyethyl)sulfoxide
bis(2-hydroxyethyl)sulfone
1,2-bis(2-hydroxyethylsulfinyl)ethane
1,2-bis(2-hydroxyethylsulfonyl)ethane
bis(2-hydroxyethylsulfinylethyl)ether
bis(2-hydroxyethylsulfonylethyl)ether

Low priority:

bis(2-hydroxyethylsulfinyl)methane
bis(2-hydroxyethylsulfonyl)methane
1,3-bis(2-hydroxyethylsulfinyl)propane
1,3-bis(2-hydroxyethylsulfonyl)propane
1,4-bis(2-hydroxyethylsulfinyl)butane
1,4-bis(2-hydroxyethylsulfonyl)butane
1,5-bis(2-hydroxyethylsulfinyl)pentane
1,5-bis(2-hydroxyethylsulfonyl)pentane
bis(2-hydroxyethylsulfinylmethyl)ether
bis(2-hydroxyethylsulfonylmethyl)ether

In addition data on divinylsulfide and other vinyl analogues, formed by elimination, and of the analytical derivatives, if applicable, should be obtained.

4. Schedule 1.A.5 (lewisites)

High priority:

2-chlorovinylarsine oxide
2-chlorovinylarsonic acid
bis(2-chlorovinyl)arsinic acid
tris(2-chlorovinyl)arsine oxide

5. Schedule 2.A.1

Low priority:

diethylphosphate
diethylthiophosphate

Non-scheduled precursors and byproducts of the synthesis of scheduled chemicals

1. Non-scheduled precursors:

High priority:

Methyl benzilate
Ethyl benzilate
O-ethyl N,N-dimethylphosphoramidochloridates
O-isopropyl N,N-dimethylphosphoramidatochloridates

Low priority:

Other alkyl N,N-dimethylphosphoramidochloridates

2. Byproducts:

High priority:

Bis(2-chloroethyl)disulfide
1,4-Dithiane
1,4-Thioxane

Riot control agents and old/abandoned chemical weapons

High priority:

Name (code)	CAS number
Methyldichloroarsine (MD)	593-89-5
Ethyldichloroarsine (ED)	598-14-1
Phenyldichloroarsine (PD)	696-28-6
Diphenylchloroarsine (Clark I)	712-48-1
Diphenylcyanoarsine (Clark II)	23525-22-6
10-Chloro-5,10-dihydrophenarsazine (Adamsite)	578-94-9
Alpha-bromobenzyl cyanide (CA)	5798-79-8
Omega-chloroacetophenone (CN)	532-27-4
2-Chlorobenzylidenemalonitrile (CS)	2698-41-1
Dibenzoxazepine (CR)	257-07-8
Capsaicin	404-86-4
4-Nonanoylmorpholine	5299-64-9
Pelargonic acid vanillylamide	2444-46-4
Ethyl iodoacetate	623-48-3
Ethyl bromoacetate	105-36-2
Phosgene oxime (CX)	1794-86-1
Xylyl bromide	ortho: 89-92-9
	meta: 620-13-3
	para: 104-81-4
Benzyl bromide	100-39-0
Diphosgene	503-38-8
Triphosgene	32315-10-9

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