



**OPCW**

**Scientific Advisory Board**

Eighth Session  
8 – 10 February 2006

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## **REPORT OF THE EIGHTH SESSION OF THE SCIENTIFIC ADVISORY BOARD**

### **1. INTRODUCTION**

- 1.1 The Scientific Advisory Board (SAB) held its Eighth Session from 8 to 10 February 2006 in The Hague, the Netherlands. A list of participants is included as Annex 1 to this report.
- 1.2 The Session was opened by the Chairman of the SAB, Jiří Matoušek of the Czech Republic. The Director-General delivered a welcoming address in which he set out his views on the future work of the SAB, with particular emphasis on the contributions expected of it 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”).<sup>1</sup>
- 1.3 The SAB re-elected Jiří Matoušek of the Czech Republic as its Chair, and he will serve until its next annual Session. It also re-elected Mahdi Balali-Mood of the Islamic Republic of Iran as its Vice-Chair.
- 1.4 The SAB adopted the following agenda for its Eighth 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 Seventh Session
  4. Review of reports and adoption of recommendations on issues previously assigned to individual SAB members:
    - (a) Captive use of Schedule 1 chemicals
    - (b) Ricin
    - (c) Salts of scheduled chemicals

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<sup>1</sup> A Note by the Director-General containing his comments and recommendations to States Parties on the present report is being submitted to the Executive Council under separate cover (EC-44/DG.7, dated 8 March 2006).



5. Review of the second report of the temporary working group on biomedical samples, and adoption of recommendations on it
6. Discussion of the future work of the temporary working group on sampling and analysis
7. Education and outreach: review of the status of the joint project on codes of conduct and chemistry education with the International Union of Pure and Applied Chemistry
8. Initial discussion of the preparation of the report of the Scientific Advisory Board to the Second Review Conference
9. Future work
10. Election of the Chair and the Vice-Chair of the SAB
11. Any other business
12. Adoption of the report
13. Closure

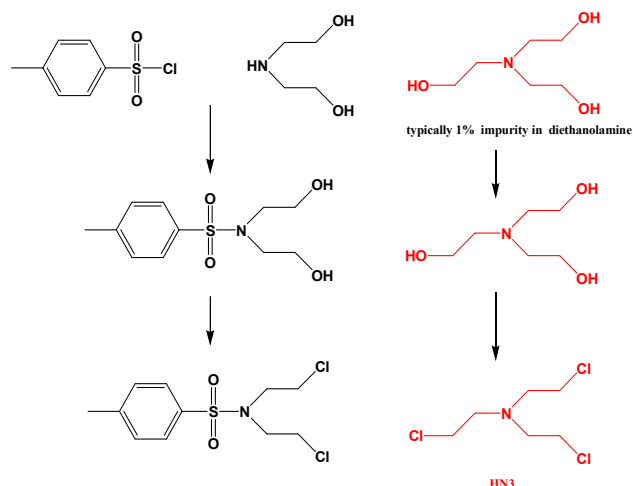
## **2. CAPTIVE USE OF SCHEDULE 1 CHEMICALS**

- 2.1 Herbert de Bisschop of Belgium, a member of the SAB, presented his findings regarding occurrences of the captive use of Schedule 1 chemicals. The Secretariat published these findings in 2005<sup>2</sup>, at the request of the Chair of the industry cluster of the Executive Council (hereinafter “the Council”), and they formed the basis for a decision by the Conference of the States Parties (hereinafter “the Conference”) on the matter.<sup>3</sup>
- 2.2 Mr de Bisschop indicated that there was a possibility that a Schedule 1 chemical (HN-3) would be formed as an impurity in the synthesis of pethidine-like compounds, for example, because of the presence (at approximately 1%) of triethanolamine in the diethanolamine used as a precursor (see the relevant part of the reaction scheme below). However, the nitrogen mustard would be present at such a low concentration that it would be difficult to isolate it from the reaction mixture. The SAB considered this to be an issue of perhaps some academic interest, but one that needed no practical adjustment of the regime for Schedule 1 chemicals.

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<sup>2</sup> S/528/2005, dated 1 November 2005

<sup>3</sup> C-10/DEC.12, dated 10 November 2005



### 3. RICIN

- 3.1 SAB member Miguel Sierra of Spain presented his findings with regard to the nomenclature of ricin and related analytical problems.
- 3.2 As for the question of what, within the meaning of the Convention, constitutes ricin, the SAB agreed to forward the following understanding to the Director-General. Such an understanding may be helpful to Member States, and could be incorporated into the OPCW Declarations Handbook:

“All forms of ricin originating from *ricinus communis*, including any possible variations in the structure of the molecule arising from natural processes or manmade modification, are to be considered ricin as long as they conform to the basic ‘native’ bipartite molecular structure of ricin (A-S-S-B) that is required for mammalian toxicity. Once the inter-chain S-S bond is broken or the protein denatured, it is no longer ricin.”

- 3.3 It should be noted that this understanding is consistent with a Conference decision that castor oil processing plants should not be subject to the Convention’s reporting procedures under Schedule 1 (C-V/DEC.17, dated 18 May 2000).
- 3.4 The SAB agreed that the analysis of ricin poses a number of problems. Because ricin is a protein, it exists in a considerable number of isoforms, and new mutations occur that will create additional isoforms in the future. As a consequence, there will be problems in conforming to the requirement established for OPCW proficiency testing, namely that the identification of test chemicals must be based on two independent analytical methods, at least one of which must be spectrometric. Applying that requirement to a molecule that exists in various isoforms may not be possible (or at least may not be easy), while using more-appropriate analytical methods based on biological principles, such as immunoassays, would not meet the criteria adopted for proficiency testing.

- 3.5 The SAB noted that this problem is not unique to ricin, and that it would also have to be addressed in the case of other toxins. The temporary working group on sampling and analysis has already placed the analysis of toxins on its agenda, and it was agreed that it would take up this issue in that context.

#### **4. SALTS OF SCHEDULED CHEMICALS**

- 4.1 SAB member Bob Mathews of Australia gave a detailed briefing on the evolution of the way salts of scheduled chemicals have been treated, beginning with the negotiations on this issue during the Geneva Conference on Disarmament in the 1980s. It is apparent from the record of the negotiations that the treatment of salts of scheduled chemicals was in fact considered by the negotiators, and that the inclusion of some (but not other) salts had remained controversial until the end. It was one of the issues that the then Chairman of the Ad Hoc Group on Chemical Weapons of the Conference on Disarmament eventually dropped as part of the “end game”. The final composition of the Schedules was influenced not only by scientific, but also by various political and economic considerations, and from a technical point of view the Schedules are not necessarily fully consistent. It is, however, clear from the record that the decision not to include certain salts was deliberate.
- 4.2 That is not to say that the rationale underlying the 1992 decision not to include certain salts of scheduled chemicals in the Schedules of Chemicals necessarily applies today. In the light of such factors as the possibility that terrorists might try to acquire and use toxic chemicals, the experience gathered in the actual implementation of the Convention, and the changing nature of chemical manufacturing, the inclusion of some of these salts in the Schedules of Chemicals might well be warranted. The SAB therefore agreed to include this issue in its review, for the Second Review Conference, of scientific and technical developments and their impact on the implementation of the Convention.
- 4.3 Related to this issue was the question of what constitutes saxitoxin, which is listed in Schedule 1 together with the Chemical Abstracts Service (CAS) registry number of the dihydrate (free base). This situation is of little help when it comes to considering which form or forms of the molecule are actually considered to be included in the Schedules of Chemicals.
- 4.4 A survey of the literature on the matter shows how the understanding of the molecular structure of saxitoxin has evolved over the past decades. Since the elucidation of the structure, the term “saxitoxin” has been used variously to describe the dihydrochloride of the molecule, or the free base, or its cation. More recently (and since the conclusion of the Convention), the nomenclature has become more specific, distinguishing between saxitoxin dihydrochloride and saxitoxin (di)hydrate. From the record of negotiations it appears that what negotiators wanted to include in the Schedules was the form of saxitoxin that had been weaponised in the past (the agent TZ, which is a salt), and other forms of weaponisable saxitoxin. However, there were also discussions about which Schedule, 1 or 2, saxitoxin should be included in. Problems related to this question became apparent after the entry into force of the Convention, when Part VI of the Verification Annex had to be adjusted to take account of practical realities: The notification regime for transfers of saxitoxin for medical and diagnostic purposes was changed so that it required notification at the

time of transfer instead of in advance.<sup>4</sup> It should be mentioned, as a side comment, that the issue of what constitutes saxitoxin shows again that the CAS registry numbers given in the Convention cannot be considered to have regulatory power. They are essentially identification aids.

- 4.5 The SAB concluded that the questions of what constitutes saxitoxin and of the placement of saxitoxin on Schedule 1 or 2 should also be taken up in the context of the aforementioned report it will be making to the Second Review Conference.

## **5. BIOMEDICAL SAMPLES**

- 5.1 The SAB received the second report of its temporary working group on biomedical samples. The report, which is included as Annex 2 to this report, was introduced by that group's Chair, Robin Black of the United Kingdom of Great Britain and Northern Ireland.
- 5.2 The SAB discussed in detail whether the OPCW should take steps to establish a system for analysing biomedical samples in investigations of alleged use, given the budgetary implications that such a process might have. It was noted, however, that the most of the resources required to develop such a capacity would be invested by the States Parties that would be seeking designation, and that only limited funds would be required of the OPCW to pay for additional staff, laboratory space, equipment, and supplies. It was also noted that the Convention requires the OPCW to undertake such analysis as part of investigations of alleged use. Furthermore, many States Parties are in any case developing the capability to analyse biomedical samples as part of their response to increasing threats of chemical and biological terrorism.
- 5.3 The SAB therefore considered it important that the OPCW establish such a capability, and that the Secretariat draw up a proposal on how to realise it, together with Member States that have developed, or are developing, capabilities in this area—in particular the laboratories thus involved. The SAB took the view that such a proposal should estimate how much it would cost to set up and maintain such a system, and that the work already done by the temporary working group would help in this regard.
- 5.4 The SAB also took the view that such a proposal should suggest:
- (a) that the system be separate from that for designating laboratories for the off-site analysis of chemical and environmental samples (though laboratories would be free to seek designation under either or both of the systems); and
  - (b) that there be a confidence-building phase during which interested laboratories would have an opportunity to:
    - (i) acquaint themselves more fully with the requirements for analysing biomedical samples;

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<sup>4</sup> EC-MII/DEC.1, dated 15 January 1999. A first change was notified by the Depositary in notification C.N.916.1999.TREATIES-7, issued on 8 October 1999; a second, in C.N.157.2000.TREATIES-1, issued on 13 March 2000.

- (ii) share experiences they have gained with analytical methods and standards; and
- (iii) work together to clarify the details of the evaluation criteria that should be applied in assessing inter-laboratory test results.

5.3 The SAB also felt that it might be useful to call for one more meeting of the temporary working group, at which it could make additional recommendations regarding this process.

## **6. SAMPLING AND ANALYSIS**

6.1 During a visit to the OPCW Laboratory in Rijswijk, the SAB was briefed by the Laboratory's Acting Head, Mieczyslaw Sokolowski, and his staff on the OPCW's current capabilities in on- and off-site analysis.

6.2 The SAB was concerned that the implementation of the OPCW policy on tenure has created certain difficulties for the OPCW Laboratory, including some that could endanger its accreditation status. The SAB also noted that it was very important that the knowledge and expertise acquired by departing Laboratory staff be transferred to new staff, and took the view that, to this end, there should be a sufficient period of overlap between new and departing Laboratory staff members.

6.3 The SAB reaffirmed the conclusion it had drawn at its Seventh Session that its temporary working group on sampling and analysis should review the whole issue of designated laboratories to determine to what degree proficiency testing furthers the goals behind the network of designated laboratories, whose focus is on challenge inspections and investigations of alleged use. The SAB took the view that among the items that proficiency testing should test candidate laboratories on are the following:

- (a) the analysis of complex matrices;
- (b) trace-level analysis;
- (c) the possibility of masking effects and of attempts to evade verification, and options to confound any such attempts; and
- (d) the detection of threat chemicals other than scheduled chemicals, including toxins other than ricin and saxitoxin, and riot-control agents.

6.4 The SAB confirmed that the temporary working group on sampling and analysis would also look at the analysis of toxins—an area where the SAB had in the past identified gaps in the OPCW's analytical capabilities.

## **7. EDUCATION AND OUTREACH**

7.1 The SAB received presentations from its Chair, Jiří Matoušek of the Czech Republic, and from one of its members, Professor Alberto Fratadochi of Italy, on the conceptual framework for addressing education and outreach under the Convention, as well as on the progress that had been made in a joint project of the OPCW and the International Union of Pure and Applied Chemistry (IUPAC) after an international workshop that

was held in July 2005 in Oxford, the United Kingdom of Great Britain and Northern Ireland.

- 7.2 The SAB was also briefed by Dr Alistair Hay of the University of Leeds, the United Kingdom of Great Britain and Northern Ireland, leader of the joint OPCW-IUPAC project that was agreed upon at the Oxford workshop, on the steps that had been taken since the project's inception. Dr Hay stated that four sets of written material had been prepared on the question of the multiple uses of chemicals and on the ethical issues arising from this, including codes of conduct and providing case studies to chemistry teachers and students. Dr Hay added that further papers are being prepared. He also reported that a successful pilot study had been conducted in conjunction with a conference on education in chemistry and on responsible stewardship that had been held on 30 October 2005 in Moscow, the Russian Federation. Dr Hay indicated that the study had confirmed the validity of the educational concept and the usefulness of the materials that had been prepared so far, and that it had led to improvements in the materials. A further pilot study is planned for August 2006, in conjunction with an international conference on education in chemistry, which will be convened in Seoul, the Republic of Korea, in February 2007.
- 7.3 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 among the entire scientific community (not only students, chemists and chemical engineers but, more broadly, scientists active in the life sciences) and the public. The SAB confirmed its support for the joint OPCW-IUPAC project, and encouraged IUPAC to make the materials Dr Hay had referred to more widely available as soon as possible, including over the Internet.
- 7.4 The SAB affirmed that it was important for National Authorities to be closely involved in education and outreach, and that they had a role to play in generating and maintaining support among 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 for the benefit of students and teachers. The sense of the SAB was that the OPCW should take steps to involve National Authorities more formally in creating an awareness and an understanding of the Convention, and that IUPAC should be encouraged to urge its constituent chemical societies and academies to become active in this area. The SAB also heard suggestions regarding the possible establishment of national task forces on scientific advice and action, and of laboratories that would provide technical support for the implementation of the Convention at the national level, and it considered these suggestions useful.
- 7.5 Professor Fratadochi of Italy informed the SAB that he had proposed to organise a second meeting of the SAB in 2006, to be convened in September in Bologna, with the support of the Italian National Authority, the Academy of Bologna, the University of Bologna, and the Italian chemical industry. Professor Fratadochi stated that such a meeting, for which the Director-General had already indicated his support, would be combined with an international conference to which IUPAC, and national chemical societies and industry representatives would also be invited, that it would address ethical issues in the context of the Convention and its implementation, and that it would further promote the joint IUPAC-OPCW project.

## 8. SECOND REVIEW CONFERENCE

- 8.1 The SAB began its discussion of how it would contribute to a review of developments in science and technology in the lead-up to the Second Review Conference. At the beginning of its deliberations, it was briefed on experiences from the preparations for the First Review Conference. Dr Alexander Kelle of Belfast University, United Kingdom of Great Britain and Northern Ireland, then gave an overview of the results of a workshop that had recently been held at his university under the theme “Preventing the Misuse of 21st Century Chemistry: State of the Art of Drug Development and Delivery, and Selected Enabling Technologies”. Dr Kelle reported that the workshop had identified and elaborated on a number of scientific developments that could affect the operation of the Convention.
- 8.1 The SAB had a preliminary discussion of the issues that will need to be reviewed in preparation for the Second Review Conference, including:
- (a) developments with regard to new chemicals, processes, and production equipment;
  - (b) micro-reactors;
  - (c) nanotechnology;
  - (d) verification technology and equipment;
  - (e) assistance and protection against chemical weapons;
  - (f) opportunities for the OPCW to further develop its international-cooperation portfolio to promote the peaceful application of chemistry; and
  - (g) awareness-raising, education, and outreach in order, *inter alia*, to enhance compliance with the Convention.
- 8.2 The SAB recommended that the OPCW again approach IUPAC with a proposal for an international symposium that would take place early in 2007 and that would involve participants from all regions and the full range of relevant disciplines, as well as experts from IUPAC, national chemical societies and science academies, the chemical industry, and governments and National Authorities. The sense of the SAB was that such a meeting would facilitate a thorough and comprehensive review of trends in science and technology and how they affect the implementation of the Convention.

## 9. FUTURE WORK OF THE SCIENTIFIC ADVISORY BOARD

The SAB decided that, during the remainder of 2006 and in 2007, it would continue working on the following issues (as already set out in the preceding paragraphs):

- (a) temporary working group on biomedical samples: another meeting to further discuss the analysis of biomedical samples in preparation for a proposed confidence-building exercise involving the Secretariat and laboratories interested in participating in the development of an OPCW capability in the field;



- (b) temporary working group on sampling and analysis: analysis of toxins, and discussion both of the objectives of the network of designated laboratories and of the design that shapes the further development of the network (including as regards how to confound the masking of target compounds);
- (c) temporary working group on education and outreach: continuation of the joint OPCW-IUPAC project; and
- (d) new temporary working group on advances in technology and their potential impact on the implementation of the Convention (such as micro-reactors, nanotechnology, and new methods of dispersion).

## 10. CONCLUSION OF THE SESSION

The SAB concluded its Eighth Session on 10 February 2006 at 15:58, with the adoption of this report.

### Annexes:

- Annex 1: List of Participants in the Eighth Session of the Scientific Advisory Board
- Annex 2: Report of the Second Meeting of the Temporary Working Group on Biomedical Samples, The Hague, 8 and 9 February 2006
  - Appendix 1: Participants in the Meeting of the Temporary Working Group
  - Appendix 2: Acceptability of Biomarkers
  - Appendix 3: Preparation of Control Samples and Matrix Blanks

**Annex 1**

**LIST OF PARTICIPANTS IN THE EIGHTH SESSION  
OF THE SCIENTIFIC ADVISORY BOARD**

	<b>Participant</b>	<b>Member State</b>
1.	Rolando A Spanevello	Argentina
2.	Bob 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 Männig	Germany
9.	László Halász	Hungary
10.	R Vijayaraghavan	India
11.	Mahdi Balali-Mood	Iran (Islamic Republic of)
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 Johnson	Norway
18.	Titos Quibuyen	Philippines
19.	Young-chul Lee	Republic of Korea
20.	Philip Coleman	South Africa
21.	Miguel A. Sierra	Spain
22.	Velery 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 SECOND MEETING OF THE TEMPORARY WORKING GROUP ON BIOMEDICAL SAMPLES THE HAGUE, 8 AND 9 FEBRUARY 2006**

#### **1. INTRODUCTION**

- 1.1 The SAB's temporary working group on biomedical samples held its second meeting from 6 to 7 February 2006 in The Hague.
- 1.2 Dr Robin Black of the United Kingdom of Great Britain and Northern Ireland chaired the meeting.
- 1.3 The list of participants of the meeting is included as Annex 1.
- 1.4 The group adopted the following agenda:
  1. Acceptable biomarkers
  2. Validation of analytical methods
  3. Recommendation of analytical methods and protocols
  4. Criteria for analytical results
  5. Acquisition of analytical standards
  6. Confidence-building measures
  7. Criteria for designation
  8. Safe handling and transport of biomedical samples
  9. Control samples
- 1.5 The purpose of the meeting was to review the scientific aspects of analysing biomedical samples, so as to facilitate decisions by the policy-making organs on the establishment of an OPCW capacity to analyse such samples in investigations of alleged use. The working group noted that the Council was still considering whether a proposal to this end should be developed. It would be developed by the Secretariat with the help and involvement of interested Member States.
- 1.6 The group reaffirmed the finding it recorded in the report on its first meeting (Annex 2 to SAB-7/1, dated 11 March 2005) that there are significant differences between the requirements associated with the sampling and analysis of, on the one hand, chemical and environmental samples, and, on the other, biomedical samples. The group took the view that a system for designating laboratories to analyse biomedical samples should therefore be developed separately by the OPCW, and that

this would allow laboratories to seek designation for environmental and chemical samples, or biomedical samples, or both.

## 2. ACCEPTABLE BIOMARKERS

- 2.1 The group reviewed which biomarkers would be acceptable for the analysis of biomedical samples, taking into account the following considerations. The choice of an appropriate biomarker is based on a number of factors. To achieve conclusive results, more than one biomarker should ideally be identified in samples from the same individual. In order for analytical results based on a single biomarker to be definitive, the biomarkers that have been chosen should be specific to the agent, and the biomarker should not be present at background levels in humans that have not been exposed to the agent.
- 2.2 The group discussed at length the acceptability or otherwise of biomarkers that are known to exist at trace concentrations in the population at large. Two analytes of particular concern are thiodiglycol (TDG) and its sulfoxide (TDGO), which is derived from the hydrolysis of sulfur mustard and subsequent oxidation. The consensus among the group was that TDG and TDGO in abnormally high concentrations (where “abnormally high” is to be defined at a later stage) should be accepted as supportive biomarkers, provided that laboratories present quantitative data.<sup>5</sup> Metabolites such as the  $\beta$ -lyase metabolites of sulfur mustard, for which no measurable background levels have been found, and that have been identified in samples from humans exposed to chemical-warfare agent, should be regarded as confirmative biomarkers. These are the preferred biomarkers for confirmation of an exposure. The group noted that human samples have been analysed only in cases of exposure to sulfur mustard, sarin, VX, and cyanide. Biomarkers that have been identified in animal studies, but whose presence has not been verified in cases of human exposure, should be regarded as presumptive if there are no human background levels and if no other sources for their presence are known—for example, chlorovinyl arseonous acid (CVAA), the hydrolysis product of Lewisite I. The group agreed that ethyl phosphoric acid, the secondary hydrolysis product of tabun, and triethanolamine, the primary hydrolysis product of HN-3, are unacceptable as biomarkers because of their ubiquitous occurrence, sometimes in high concentrations, in unexposed humans.
- 2.3 The group also repeated a remark it had made at its first meeting, namely that analytical evidence should not be regarded as standing on its own, but as supporting other evidence that may be available, such as eye-witness accounts, and clinical symptoms and signs reported in casualties.

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<sup>5</sup> This discussion uses a three-part typology for biomarkers (which is also used in the table in Appendix 2 below):

Supportive: Found in human samples from individuals known to have been exposed to the agent, but human background levels have been reported or there are alternative sources that could explain the presence of the biomarker.

Presumptive: Reported in animal studies (or in human studies for an analogous agent), but no studies in humans that have been exposed to the agents, no human background levels have been reported, and no alternative sources or pathways for the presence of the biomarker are known.

Confirmative: Found in human samples from individuals known to have been exposed to the agent, no human background levels have been reported and no alternative sources or pathways for the presence of the biomarker are known.

- 2.4 A list of biomarkers that includes Schedule 1 agents, 3-Quinuclidinyl benzilate (BZ) (Schedule 2), phosgene and hydrogen cyanide (Schedule 3), and that lists their current status, is given in Annex 2.

### 3. CRITERIA FOR THE EVALUATION OF ANALYTICAL RESULTS

- 3.1 The group agreed that criteria for the interpretation of mass-spectrometric results should be based on a flexible system of identification points rather than on a rigid set of criteria for each analyte. Mr Robert Read of the United Kingdom of Great Britain and Northern Ireland presented examples of methods in current use, where, for example, a rigid requirement to detect three selected ions in appropriate ratios could not be met at realistic levels. A recently published paper, he said, had demonstrated that a similar situation applied to several pesticides. A system using identification points could be modelled on the European Commission directive on the performance of analytical methods and the interpretation of results for the monitoring of substances in products of animal origin.<sup>6</sup> The scoring system would need to be modified to suit the requirements of the OPCW in the context of the alleged use of chemical weapons. The system would allow flexibility in terms of how much structural information was to be obtained in a single analysis, e.g. full-scan data if obtainable, the number of selected ions monitored by gas or liquid chromatography coupled with single-ion-monitoring mass spectrometry (GC/LC-MS-SIM), or the number of selected reactions monitored by gas or liquid chromatography coupled with single-reaction-monitoring mass spectrometry (GC/LC-MS-MS-SRM). A points system would favour those methods that are generally regarded as being highly specific (e.g., MS-MS as opposed to MS). It would also allow the flexibility to accept the use of different techniques or methods for the same biomarker, and the use of different biomarkers of the same agent to be detected, in order to achieve an acceptable number of identification points.
- 3.2 The group stressed the value of using internal standards, agreeing that they help minimise false negatives as well as false positives. The sense of the group was that this is important because biomedical matrices are “dirty” and quite variable, and there can be some variation in the levels of detection that can be achieved. The retention time for the analyte relative to an internal standard also increases confidence that the identification of a given biomarker is correct. Isotopically labelled standards for biomarkers would be most desirable.
- 3.3 The group did not try to develop criteria for such a system of identification points, agreeing that that ought to be done by the OPCW Laboratory in collaboration with laboratories from States Parties that have experience in the field. The development of such criteria would usefully form part of a phase of confidence-building among laboratories participating in the establishment of an OPCW capability to analyse biomedical samples. Other issues would also be addressed during this phase, including analytical methods and standards (see section 7 below).

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<sup>6</sup> See European Commission decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

#### **4. THE HANDLING OF SAMPLES**

- 4.1 The group took the view that many of the Secretariat's current procedures for the collection, transport, and distribution of samples could also be used for the handling of biomedical samples, and that the procedures that the Secretariat has developed for biomedical samples should now be tested so as to ensure their reliability.
- 4.2 The group discussed the need to have the OPCW Laboratory be the single point to which all authentic samples are moved, and from where they (and the matrix blanks and control samples) are distributed to designated laboratories. The group agreed that this arrangement should also apply to the off-site analysis of biomedical samples, and that distribution from a single point is the only feasible and anonymous way of distributing authentic samples (which may include control samples from an unexposed population living near the alleged incident site), matrix blanks, and control samples. The group agreed that the regime that the OPCW has adopted for environmental and chemical samples should therefore also be applied to the transfer of authentic biomedical samples and control samples.
- 4.3 The establishment of an OPCW capacity has implications for the allocation of resources. There will be a need for some additional staff, laboratory space, equipment, and supplies.
- 4.4 After a discussion of the technical aspects of using positive and negative controls as part of sampling and analysis, the group developed technical guidelines that it recommended the Secretariat use as it develops capabilities in this area. These guidelines are included as Appendix 3 to this report.
- 4.5 The group received and briefly discussed a paper by John Barr of the United States of America that discussed the collection, shipment, and storage of biomedical samples. The group recommended that the Secretariat take into consideration the technical comments in the paper as it develops further its procedures for the handling of biomedical samples, and that it prepare guidelines on such safety issues as checking samples for the absence of infectious contamination, for laboratories seeking designation for the analysis of biomedical samples.

#### **5. STANDARDS**

- 5.1 The group agreed that the unavailability of certain analytical standards is one of the obstacles to the broader adoption of some of the methods for the analysis of biomedical samples. This problem applies to a small number of metabolites, to most mass-labelled internal standards, and particularly to adducts with proteins and DNA.
- 5.2 The group recognised that some of the standards are difficult to synthesise, and that some are expensive, but it felt that it would be desirable for interested laboratories to procure the required standards commercially or synthesise them.
- 5.3 As a preliminary move towards making acquisition easier, the group agreed that details of synthetic methods and commercial sources should be compiled and made available to interested States Parties. The group requested all those who participated in its second meeting, and other laboratories active in the field, to submit full

experimental details of their synthetic methods (or methods for generation *in situ* in the case of blood-protein adducts) to the Chair of the temporary working group, so that they could be collated and made available to the OPCW Laboratory for wider distribution. The group also agreed that laboratories that wished to establish a method but that do not have the resources to synthesise a standard should be encouraged to approach the OPCW Laboratory, so that it could inquire into whether another laboratory could supply that standard.

## **6. ACCREDITATION**

- 6.1 One of the criteria that the Conference at its First Session adopted for the designation of laboratories is that they have been accredited by a national accreditation body for the types of analysis they are to conduct. The group agreed that accreditation, as a means of ensuring that quality-control systems are in place in laboratories being considered for designation, was in principle both desirable and feasible. However, it recognised that there were differences between the analysis of biomedical samples and the analysis of chemical and environmental samples.
- 6.2 The group also recognised that national accreditation systems differ—that some incorporate a more general accreditation system such as Good Laboratory Practice certification, while others require that each individual method be accredited. The group felt that, in view of the specialised nature of techniques for the analysis of biomedical samples (analyte-specific analysis, analysis at trace-concentration levels) and the small number of samples expected to be analysed, insisting on accreditation as a condition of designation might impose a major and financially prohibitive burden on those laboratories seeking designation, and be a disincentive for laboratories to seek designation.
- 6.3 For these reasons, the group considered that national accreditation need not be required in order to achieve a reliable system for the analysis of biomedical samples. The group did, however, recommend that, in deciding which laboratories to designate for the analysis of biomedical samples, the OPCW require suitable evidence of adequate quality-assurance measures and develop a system for assessing proficiency.

## **7. CONFIDENCE-BUILDING EXERCISES**

- 7.1 The group agreed that, before any designation system could be attempted, a series of confidence-building exercises, along the lines of the round-robin exercises undertaken before OPCW proficiency tests were introduced for environmental analysis, should be organised with the primary aim of encouraging more laboratories to become proficient in analysing biomedical samples.
- 7.2 Biomarkers can fall into three general categories, based on the complexity of the analysis: free metabolites, adducts from which target compounds can easily be released, and adducts that are essentially irreversible. The group agreed that confidence-building exercises should start with the simpler biomarkers, such as urinary metabolites, the release of TDG from aspartic/glutemic acid residues on blood proteins, and the fluoride-reactivation method for nerve-agent-inhibited cholinesterase.

- 7.3 The group discussed at length which spiking concentrations such exercises should start with. The final consensus was that they should begin with concentrations that would require techniques such as selected-ion and selected-reaction monitoring for identification, but at concentrations considered not too demanding to detect—for example, 50 ng/ml. The group felt that these exercises would also be of help in the development of a workable system for the interpretation of results, but stressed that it must be clear to all participating laboratories that later exercises would address trace concentrations of biomarkers.
- 7.4 The group was aware of the various OPCW capacity-building projects to help States Parties develop their capabilities with regard to chemical analysis in areas relevant to the Convention, and took the view that such approaches might also be used to distribute knowledge among laboratories about analytical methods and the preparation of standards that would be needed for the analysis of biomedical samples. It also felt that there might be some scope for the OPCW to solicit bids for commercial contracts for the synthesis of selected standards.

## **8. RESOURCE IMPLICATIONS**

- 8.1 The group was aware that, irrespective of which particular approach would eventually be decided on, the establishment of an OPCW capacity to analyse biomedical samples would undoubtedly require the hiring of some additional staff and create the need for some additional laboratory space, equipment, and supplies. It also recognised that States Parties would also need to address these resource implications and find solutions if they wished to have a reliable and effective system in place.
- 8.2 The group took the view that the Draft Programme and Budget for 2007 would have to include cost estimates for the initial confidence-building phase and for a modest extension of the capacity of the OPCW Laboratory so that it can support this phase.



## Appendix 1

### LIST OF PARTICIPANTS IN THE MEETING OF THE TEMPORARY WORKING GROUP ON BIOMEDICAL SAMPLES

	<b>Participant</b>	<b>Member State</b>
1.	Jiří Matoušek	Czech Republic
2.	Marja-Leena Kuitunen	Finland
3.	Bernard Brasme	France
4.	William Selvamurthy	India
5.	R Vijayaraghavan	India
6.	Daniel Noort	Netherlands
7.	Marcel van der Schans	Netherlands
8.	Phillip Coleman	South Africa
9.	Sten-Åke Frederikson	Sweden
10.	Robin Black	United Kingdom of Great Britain and Northern Ireland
11.	Robert Read	United Kingdom of Great Britain and Northern Ireland
12.	John R. Barr	United States of America
13.	James Gibson	United States of America
14.	John R. Smith	United States of America

## Appendix 2

### ACCEPTABILITY OF BIOMARKERS

Agent	Sample	Biomarker	Status	Comments
Sulfur mustard	Urine, blood	TDG Thiodiglycol sulfoxide	Supportive	TDG usually occurs at <1 ng/ml, and TDGO at <10 ng/ml in urine of unexposed individuals, but with occasional outliers. Significantly higher levels have been confirmed in human casualties.
	Urine	$\beta$ -lyase metabolites (2)	Confirmative	No background levels detected ( $>\sim 0.1$ ng/ml) in >120 samples. Detected in samples from human casualties. Two metabolites, although one may be formed from the other by oxidation after collection.
	Blood-albumin	Cysteine adduct	Confirmative	Detected in samples from human casualties. No background levels detected in >100 human samples.
	Blood-haemoglobin	N-terminal valine adduct	Confirmative	Detected in samples from human casualties. No background levels detected in a limited number of human samples.
	Blood-haemoglobin	Histidine adduct	Confirmative	As above
	Blood-albumin and -haemoglobin	Aspartic acid/glutamic acid adducts	Confirmative	Detected as TDG released by hydrolysis. Detected in a single human exposure. No background levels detected in a limited number of human samples.
HN-1	Urine	N-ethyldiethanolamine	Supportive	Confirmed as an excretion product in animals. No samples from human casualties. Not detected ( $>1$ ng/ml) in >100 human samples, but has industrial uses.
HN-2	Urine	N-methyldiethanolamine	Supportive	As above. HN-2 is still used as an anti-cancer agent.
HN-3	Urine	Triethanolamine	Not acceptable	Present in most unexposed subjects, sometimes at high levels. Widespread use in domestic products.
Lewisite 1	Urine	Chlorovinyl arsonous acid (CVAA)	Presumptive	Confirmed as an excretion product in animals; no samples from human casualties. No background levels detected in >100 human samples.
	Blood	CVAA (free, and bound to haemoglobin)	Presumptive	Presence confirmed in animal studies.

<b>Agent</b>	<b>Sample</b>	<b>Biomarker</b>	<b>Status</b>	<b>Comments</b>
Sarin	Urine, blood	Isopropyl methylphosphonic acid	Confirmative	Detected in samples from human casualties. No background levels in >100 human samples.
	Urine, blood	MPA	Supportive	Secondary hydrolysis product that may result from hydrolysis of iPrMPA in the sample. Could also arise from other nerve agents and from fire retardants.
	Blood: BuChE/AChE <sup>7</sup>	iPrMP-serine adduct	Confirmative	Detected in samples from human casualties. No background levels in a limited number of human samples. May be detected by F <sup>-</sup> displacement as sarin, by hydrolytic displacement as iPrMPA, or as a phosphorylated nonapeptide.
	Blood-albumin	iPrMP-tyrosine adduct	Presumptive	Confirmed adduct in animals; no human samples.
Soman	Urine, blood	Pinacolyl MPA	Presumptive	Confirmed as an excretion product in animals; no human samples.
	Urine, blood	MPA	Supportive	See above.
	Blood— BuChE/AChE	MP-serine adduct	Presumptive of a nerve agent	Indicative of an aged nerve-agent residue, but does not identify the agent.
	Blood-albumin	PinacolylMP-tyrosine adduct	Presumptive	Confirmed as an adduct in animals; no human samples.
GF (cyclosarin)	Urine, blood	Cyclohexyl MPA	Presumptive	Confirmed as an excretion product in animals; no human samples.
	Urine, blood	MPA	Supportive	See above.
	Blood: BuChE/AChE	CyclohexylMP-serine adduct	Presumptive	Not confirmed in animals or human samples, but analogous to sarin adduct.
	Blood: BuChE/AChE	MP-serine adduct	Presumptive of a nerve agent	Indicative of an aged nerve-agent residue, but does not identify the agent.
	Blood-albumin	c-HexMP-tyrosine adduct	Presumptive	Confirmed in animals; no human samples.

Agent	Sample	Biomarker	Status	Comments
VX	Urine, blood	Ethyl MPA	Presumptive of a nerve agent or confirmatory	Confirmed in animals and one human casualty; further studies required in unexposed subjects. Could arise from VX or the ethylated analogue of sarin. Confirmative of VX when detected with $\text{MeSCH}_2\text{CH}_2\text{N}(\text{iPr})_2$ .
	Blood	$\text{MeSCH}_2\text{CH}_2\text{N}(\text{iPr})_2$	Supportive or confirmatory	Confirmed in animals and one human casualty. Confirmative when detected with ethyl MPA.
	Blood: BuChE/AChE	EtMP-serine adduct	Presumptive of a nerve agent	Confirmed in animal studies. Could arise from VX or an ethylated analogue of sarin. Confirmative when detected with $\text{MeSCH}_2\text{CH}_2\text{N}(\text{iPr})_2$ . May be detected by $\text{F}^-$ displacement, by hydrolytic displacement as ethyl methylphosphonic acid, or as a phosphorylated nonapeptide.
Tabun	Urine	$\text{Me}_2\text{N}-\text{P}(\text{O})(\text{OEt})\text{OH}$ $\text{HO}-\text{P}(\text{O})(\text{OEt})\text{CN}$	Probably unacceptable	Appear to be too unstable to be useful biomarkers.
	Urine	$\text{EtO}-\text{P}(\text{O})(\text{OH})_2$	Not acceptable	Ubiquitous occurrence in unexposed human subjects.
	Blood: BuChE/AChE	$\text{Me}_2\text{N}(\text{HO})\text{P}(\text{O})$ -serine adduct	Presumptive of a nerve agent	Formed with tabun in human blood <i>in vitro</i> , but not confirmed in animal or human samples. Could arise from tabun, GV, or O-alkyl analogues. Full adduct from tabun appears to age or hydrolyse on digestion.
	Blood: BuChE/AChE	$\text{EtO}(\text{HO})\text{P}(\text{O})$ -serine adduct	Supportive	As above. Could arise from several pesticides.
	Blood-albumin	$\text{Me}_2\text{N}(\text{EtO})\text{P}(\text{O})$ - tyrosine adduct	Presumptive	Confirmed adduct in animals; no human samples.
BZ	Urine	Benzilic acid 3-quinuclidinol	Presumptive	No confirmation in animal or human samples.
Phosgene	Urine			No biomarkers identified.
	Blood			Forms adducts with haemoglobin and albumin, but background levels are present.
Hydrogen cyanide	Urine	2-aminothiazoline-4-carboxylic acid	Not acceptable	Background levels from smoke and some food constituents in unexposed subjects.

### Appendix 3

#### TEMPORARY WORKING GROUP ON BIOMEDICAL SAMPLES

#### PREPARATION OF CONTROL SAMPLES AND MATRIX BLANKS<sup>8</sup>

##### CONTROL SAMPLES

###### Urine

1. Control samples of urine should be prepared or purchased by the OPCW prior to the shipment of any samples for proficiency testing, or of samples taken during an investigation of alleged use of chemical weapons. Urine should be spiked with urinary metabolites in order to allow for the analysis of any chemical-warfare agent present. These positive control samples should be method-specific and can be prepared with any of the metabolites involved in a method, or can be closely related compounds (to decrease the possibility of cross-contamination and carry-over effects).

###### Blood

2. Control samples for adducts should also be prepared so that they can be included with shipments of samples. Preparation involves spiking the target chemical-warfare agent into blood or serum (whole blood for mustards, and serum or plasma for nerve agents). These are general positive control samples, not control samples from a matched population of individuals that were near the site of the alleged use of the chemical-warfare agent but are not believed to have been exposed to it. The preparation of spiked control samples from this population is not recommended, because live agents are required for the preparation of such samples, and the live agents would therefore have to be transported. These same samples can be used as positive controls for the measurement of blood metabolites.

###### Sulfur-mustard adducts

3. Sulfur mustard HD (in acetonitrile) is spiked at the level of 10µM into whole blood at 37°C for 2 hours. The blood is then separated into red blood cells and plasma. These can be diluted to any desired level by the addition of control plasma for use as positive control or proficiency-testing samples.

###### Nerve agent adducts

4. Plasma samples are spiked with 1 nerve agent (GA, GB, GD, GF, or VX). The cholinesterase activity of a control pool of serum should first be measured. This BuChE activity should be between 50 and 80 nM. A quantity of nerve agent sufficient to inhibit between 50% and 75% of the BuChE activity should be added to

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<sup>8</sup> This paper was prepared by John Barr, United States of America, a member of the temporary working group.

this control plasma. The samples can be kept at room temperature for at least 1 hour. A laboratory should check the plasma samples to ensure that there is no free agent.

#### **MATRIX BLANKS**

5. Matrix blanks (negative control samples) should also be included with each shipment. These can be negative-control urine for urinary-metabolite methods, and negative-control plasma for serum-protein-adduct and serum-metabolite methods. The blanks should be collected and pooled from individuals with no known or suspected exposure to any chemical-warfare agent. In addition to the pooled matrix blanks that are prepared in advance, it is useful to obtain control samples from a matched population of individuals that were near the site of the alleged use of chemical-warfare agent but are not believed to have been exposed to it.

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