OPCW

Scientific Advisory Board

Eleventh Session
11 – 13 February 2008

SAB-11/1
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REPORT OF THE ELEVENTH SESSION OF THE SCIENTIFIC ADVISORY BOARD

1. AGENDA ITEM ONE – Opening of the Session

The Scientific Advisory Board (SAB) met for its Eleventh Session from 11 to 13 February 2008 at the OPCW headquarters in The Hague, the Netherlands. The Session was opened by the Vice-Chairperson of the SAB, Mahdi Balali-Mood. The meeting was chaired by Philip Coleman of South Africa, and Mahdi Balali-Mood of the Islamic Republic of Iran served as Vice-Chairperson. A list of participants appears as Annex 1 to this report.

2. AGENDA ITEM TWO – Adoption of the agenda

2.1 The SAB adopted the following agenda for its Eleventh Session:

1. Opening of the Session
2. Adoption of the agenda
3. Tour de table to introduce new SAB Members
4. Election of the Chairperson and the Vice-Chairperson of the SAB[1]
5. Welcome address by the Director-General
6. Overview on developments at the OPCW since the last session of the SAB
7. Establishment of a drafting committee
8. Work of the temporary working groups:
   (a) Consideration of the report of the second meeting of the sampling-and-analysis temporary working group;

[1] In accordance with paragraph 1.1 of the rules of procedure for the SAB and the temporary working groups of scientific experts (EC-XIII/DG.2, dated 20 October 1998)

(b) Status report by the Industry Verification Branch on the implementation of sampling and analysis for Article VI inspections;

(c) Presentation by the OPCW Laboratory;

(d) Update on education and outreach; and

(e) Update on the formation of the temporary working group on advances in science and technology and their potential impact on the implementation of the Convention:

(i) composition of the group; and

(ii) its terms of reference

9. Presentations on old and abandoned chemical weapons (OACWs): Destruction techniques:

(a) briefing on the status of OACWs; and

(b) briefing on methods and technologies for the destruction of OACWs

10. Presentations on the identification of chemicals:

(a) presentation on the identification by the Technical Secretariat of scheduled chemicals; and

(b) presentation on the identification of chemicals for customs purposes

11. Preparation of the report of the Eleventh Session of the SAB and finalisation of the report of the SAB to the Second Special Session of the Conference of the States Parties to Review the Operation of the Chemical Weapons Convention on developments in science and technology

12. Future work of the SAB

13. Adoption of the report

14. Closure of the meeting

3. AGENDA ITEM THREE – Tour de table to introduce new SAB Members

The meeting was opened with introductions of SAB members for the benefit of three new members: Igor V. Rybalchenko from the Russian Federation, Shuzo Fujiwara from Japan, and Stefan Mogl from Switzerland.

4. AGENDA ITEM FOUR – Election of the Chairperson and the Vice-Chairperson of the SAB

By acclamation, the SAB members elected Philip Coleman of South Africa as the Chairperson of the SAB for a term of one year. Mahdi Balali-Mood of the Islamic Republic of Iran was re-elected as Vice-Chairperson for a term of one year.

5. AGENDA ITEM FOUR – Welcome address by the Director-General

5.1 The Director-General congratulated both Philip Coleman from South Africa for his election as Chairperson and Mahdi Balali-Mood from the Islamic Republic of Iran for his re-election as the Vice-Chairperson. He emphasised that both the Chairperson and the Vice-Chairperson bring with them vast experience and unique expertise on issues related to chemical engineering and disarmament.

5.2 The Director-General also welcomed three new members of the Board, namely Shuzo Fujiwara from Japan, Igor V. Rybalchenko from the Russian Federation, and Stefan Mogl from Switzerland. The Director-General expressed his view that the Technical Secretariat (hereinafter "the Secretariat") will certainly benefit from their considerable experience and expertise.

5.3 The Director-General emphasised that the SAB report to 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") will underline the important impact that recent developments in science and technology will have on the implementation of the Chemical Weapons Convention (hereinafter "the Convention"); he also expressed the view that the Second Review Conference will undoubtedly find the SAB's recommendations useful. He emphasised that further advice from the Board regarding sampling and analysis would be of great importance, and that the Board could also contribute to efforts to eliminate shortcomings in the OPCW Central Analytical Database (OCAD).

5.4 The Director-General reminded the SAB of the importance of establishing a temporary working group (TWG) on science and technology, and emphasised the need to have as large a geographical representation as possible, so that all geographical regions would be fully involved in these deliberations.

5.5 As for education and outreach in relation to the Convention, the Director-General encouraged the Board to continue to reach out to a broad global audience with a view to exploring innovative and practical approaches for promoting awareness of the Convention and its benefits.

5.6 The Director-General welcomed the voluntary contributions received from eight Member States since the establishment of the SAB Trust Fund. He emphasised that future voluntary contributions could help the Board and its TWGs to maintain the frequency with which they met, thus sustaining their important work.

6. AGENDA ITEM SIX – Overview on developments at the OPCW since the last session of the SAB

6.1 A presentation was heard from the Secretary to the SAB on developments at the OPCW since the SAB's Tenth Session (from 21 to 23 May 2007). As at 10 January 2008, there were 183 States Parties to the Convention and efforts to achieve universality are ongoing.

6.2 Since July 2006, there has been considerable activity in terms of preparations for the Second Review Conference. An open-ended working group has been formed, and a Chairperson and four Vice-Chairpersons have been appointed. The open-ended working group considered the draft report of the SAB during September and October of 2007, met with chemical industry representatives on 11 June 2007, and also met with non-governmental organisations on 19 November 2007.

6.3 The SAB was updated on the status of its Trust Fund. Currently the balance stands at EUR 53,000, an amount that is not sufficient to fund planned SAB activities in 2008 as described in paragraph 6.4 below. SAB members were asked whether they could promote the activities of the SAB with their National Authorities and thus encourage their respective countries to contribute to the fund.

6.4 Assuming that funding will become available, the Twelfth Session of the SAB is tentatively scheduled to take place from 10 to 12 November 2008. There is also a need to have meetings of the sampling-and-analysis and the science-and-technology TWGs. These meetings could also be held in November in conjunction with the SAB's Twelfth Session. The Thirteenth and the Fourteenth Sessions of the SAB are tentatively scheduled for May and November 2009, respectively.

7. AGENDA ITEM SEVEN – Establishment of a drafting committee

Drafting committees were established to prepare the report of the Eleventh Session of the SAB and the report of the SAB on developments in science and technology to the Second Review Conference.

8. AGENDA ITEM EIGHT – Work of the temporary working groups

Subitem 8(a): Consideration of the report of the second meeting of the sampling-and-analysis temporary working group

8.1 The SAB received the second report of th**e** TWG on sampling and analysis (Annex 2) presented by Robin Black, Chairperson of this TWG. The key findings and conclusions of the report were that:

(a) current verification procedures for on-site and off-site analysis can still be considered appropriate and effective, but a number of practical problems need to be resolved;

(b) gas chromatography-mass spectrometry (GC-MS) remains the most versatile technique for on-site analysis in terms of its applicability, mobility, and robustness. As a possible means of reducing analysis time, greater collaboration between Member States and the OPCW Laboratory on fast gas

chromatography (GC) is recommended. Although it is desirable to reduce the logistical burden in regard to GC-MS equipment, the current generation of commercialised man-portable instruments are not considered suitable for OPCW requirements for on-site inspection; they are essentially used for screening;

(c) the preparation of aqueous samples is an important issue. The current procedure requires the concentration of aqueous samples to dryness before the derivatisation of polar degradation products. This is time-consuming and the additional pumping equipment required adds to the logistical burden. Possible solutions to this issue were outlined and will be addressed in detail at the next meeting of this TWG. Several possible solutions involve extractive derivatisation;

(d) liquid chromatography-mass spectrometry (LC-MS) was discussed as an alternative approach to the on-site analysis of aqueous samples. LC-MS allows direct analysis of aqueous solutions and could also provide on-site screening for toxins. The TWG considered that current LC-MS instrumentation is not practical for on-site analysis during inspections, but that it has an important role to play in off-site analysis. The TWG recommended that developments in this field should be closely monitored;

(e) the TWG has started to address the issues of toxin analysis, and focussed particularly on saxitoxin and ricin (both Schedule 1 chemicals). An informal correspondence group within the TWG has been established to exchange information; and

(f) topics that will be addressed in detail at the next meeting are aqueous samples, trace analysis, and toxin analysis.

Subitem 8(b): Status report by the Industry Verification Branch on the implementation of sampling and analysis for Article VI inspections

8.2 The SAB heard an update from the Industry Verification Branch on the status of sampling-and-analysis efforts with respect to Article VI inspections. Since the start-up period in the third quarter of 2006, a total of 11 Schedule 2 inspections have been completed—nine of which were completed within the past 12 months. During 2008, 8 to 10 additional inspections are planned. All inspections have gone well. There have been some "lessons learned" that will be invaluable for future inspections.

Subitem 8(c): Presentation by the OPCW Laboratory

8.3 Gary Mallard, the Head of the OPCW Laboratory, reported on three topics: LC-MS instruments for on-site use, additions to the OCAD, and how to achieve better on-site analysis.

8.4 With respect to LC-MS for on-site use, he indicated that the OPCW Laboratory concurred with the sampling-and-analysis TWG's conclusion that there are no suitable instruments currently available to conduct such analyses. He asked the SAB whether approval needs to be sought from the Conference of the States Parties (hereinafter

"the Conference") for the addition of LC-MS to the list of approved equipment. The consensus of the SAB was that it would be premature to pursue approval of LC-MS at this time. The sampling-and-analysis TWG will monitor developments in this area of analytical technology and will advise the SAB when portable LC-MS has developed to a point when the Secretariat could seek approval for its use.

8.5 With respect to additions to the OCAD, the Validation Group (hereinafter "the Group") agreed to continue work on riot-control agents and non-scheduled degradation products. A substantial body of data has been approved by the Group, and more data are in the process of being added. A list of the data is provided in Annex 3.

8.6 There are two issues that are having an impact on on-site analysis: the time required for GC-MS analysis and the use of the internal standard hexachlorobenzene. This year, the OPCW Laboratory will initiate efforts to reduce the time required for GC-MS analysis through faster GC analysis and will actively pursue a replacement agent for hexachlorobenzene, which falls under import restrictions in some countries.

Preparations by the Secretariat in regard to investigations of alleged use

8.7 In response to a question from the SAB on various aspects of investigations of alleged use (IAUs), including the size of the teams, on-site analysis, and other related matters, the Board received a briefing from Alex Lampalzer (from the Verification Branch (VER)) on the Secretariat's readiness to conduct IAUs. The Board expressed its appreciation for the comprehensive briefing and considered various aspects related to the conduct of IAUs, including detection, sample-taking, and especially the relative merits of on-site versus off-site analysis.

Subitem 8(d): Update on education and outreach

8.8 Alberto Fratadocchi gave an update on the education-and-outreach project, which had been carried out jointly by the Secretariat with the International Union of Pure and Applied Chemistry (IUPAC). He outlined the various meetings involving representatives of both the OPCW and IUPAC that had been held to raise awareness of the Convention among the scientific communities, students, and the public, and that had also stressed the peaceful uses of chemistry. It is expected that a number of publications at an affordable price will soon be available from the IUPAC and other publishers—in particular, a final version of a proposed Code of Conduct.

8.9 An international workshop on the impact of advances in science and technology on the Convention was held in Zagreb, Croatia, from 22 to 25 April 2007. The workshop addressed the following subjects: the General Purpose Criterion; developments with regard to new chemicals; advanced technologies, with particular attention to micro- and nano-apparatus and products; research on drug development, synthetic biology, proteomics, or genomics bio-engineering production that could be relevant to the Convention; trends in protection against chemical weapons; further developments in regard to the promotion of peaceful applications of chemistry; and raising awareness in the scientific community and among the public in order to enhance compliance with the Convention.

8.10 At the 41st IUPAC World Chemistry Congress, held in Turin, Italy, from 5 to 11 August 2007, the following topics, inter alia, were discussed: the OPCW and the universality of the Convention in the world of chemistry, the role of science and education in raising awareness of the Convention, and ethics in science and education. A workshop also took place on the following topics: the dangers posed by chemical weapons, the multiple uses of chemicals, and how to raise awareness of the Convention among the public and the chemistry community.

8.11 The SAB acknowledged the excellent work undertaken by members of the Board on chemical education and outreach in collaboration with the IUPAC and other organisations. The SAB at its Sixth Meeting proposed the convening of a TWG on this subject area, but, for various reasons, this TWG was never established. Nevertheless, in conjunction with other organisations (for example, the IUPAC) SAB members and other individuals have made significant progress in these areas. As a result, the predominant view of the Board is that education and outreach has now progressed beyond the stage where a TWG would accelerate the progress being made. As an alternative to a TWG, it was proposed that the International and Cooperation and Assistance Division, in cooperation with the IUPAC and with the continued support from expertise within the SAB, should be asked to take the lead for future activities in this area. The establishment of a new TWG on science and technology is now the highest priority for the SAB.

Subitem 8(e): Update on the formation of the temporary working group on advances in science and technology and their potential impact on the implementation of the Convention

8.12 The SAB heard an update on the establishment of the TWG on advances in science and technology and their potential impact on the implementation of the Convention. In response to a note verbale (NV/ODDG/129780/07, dated 9 October 2007), the Secretariat received a number of applications from individuals wishing to participate in the work of this TWG, but this group could not be established because very few of the nominees had the requisite expertise. Some fields of expertise, such as advanced methods of dissemination of agricultural aerosols and micro-reactors were not available among the proposed candidates, nor were there suitable candidates from all geographical regions.

9. AGENDA ITEM NINE – Presentations on old and abandoned chemical weapons (OACWs): Destruction techniques

Subitem 9(a): Briefing on the status of OACWs

9.1 Jeff Osborne of the Chemical Demilitarisation Branch of the VER briefed the SAB on the status of OACWs. By definition, old chemical weapons (OCWs) are those manufactured before 1925 and those manufactured between 1925 and 1946 that are no longer in usable condition. Abandoned chemical weapons (ACWs) are chemical weapons left without consent on the territory of another State Party after 1 January 1925. The process for dealing with OACWs involves recovery and identification, declarations to the OPCW, verification by the OPCW, and ultimately, destruction by the States Parties. Mr Osborne reviewed the locations of OACWs by

country and weapon type. He also briefly described the methods that are used to destroy OACWs.

Subitem 9(b): Briefing on methods and technologies for the destruction of OACWs

9.2 Claude Eon, an expert on destruction techniques for OACWs, gave the SAB a detailed presentation of the destruction methods employed for OACWs. He also highlighted the safety, health, and environmental issues associated with OACWs and their destruction. At the end of his presentation, Mr Eon recommended that the SAB review relevant destruction technologies.

9.3 The SAB discussed this matter and considered that, for technical reasons, undertaking such a review would be inappropriate at this stage.

10. AGENDA ITEM TEN – Presentations on the identification of chemicals

Subitem 10(a): Presentation on the identification by the Technical Secretariat of scheduled chemicals

10.1 Daniel Cardozo of the VER informed the SAB about activities to improve the identification nomenclature for scheduled chemicals and explained different identification systems (for example, the IUPAC Name, the Chemical Abstracts Service Registry Number (CASRN), the International Non-Proprietary Name (INN), the IUPAC International Chemical Identifier (InChI), and the Harmonized Commodity Description and Coding System (HS) of the World Customs Organisation), all of which lead to different results. In particular, the HS, which is used on a global basis by customs organisations, generally does not allow the identification of chemicals that are listed in the Convention's schedules of chemicals. On a national level, identification of chemicals might be possible, but might conflict with other control regimes. Fortunately, only 35 chemicals on the schedules are traded in quantities exceeding one tonne per year.

10.2 The Secretariat is working together with the European Chemical Industry Council (CEFIC) and the European Union on creating an updated Handbook on Chemicals, which will be made available to Member States and individual companies via the internet to help identify and declare scheduled chemicals.

Subitem 10(b): Presentation on the identification of chemicals for customs purposes

10.3 Hervé Schepers of the European Commission outlined the difficulties associated with moving chemicals through customs on a global basis. He pointed out the problems of different classification systems, naming systems, databases, languages, and so on. The overall system should be improved on a global basis, and Mr Schepers recommended that, in conjunction with the European Committee for Interoperable Systems (ECIS), the CASRN/Customs Union and Statistic Number (CUS) be used in the future.

11. AGENDA ITEM ELEVEN – Preparation of the report of the Eleventh Session of the SAB and finalisation of the report of the SAB to the Second Special Session of the Conference of the States Parties to Review the Operation of the Chemical Weapons Convention on developments in science and technology

Robin Black presented the final SAB Report to the Second Review Conference, which emphasised modifications and additions that had been made since the approval of the preliminary report during the SAB's Tenth Session. The SAB approved and accepted the final report, which has been distributed as an Annex to a Note by the Director-General (RC-2/DG.1, dated 28 February 2008).

12. AGENDA ITEM TWELVE – Future work of the SAB

12.1 Subject to the availability of funding in its Trust Fund, the SAB decided to hold an additional session in November 2008, ideally, close to the Thirteenth Session of the Conference. Possible dates suggested for this meeting were from 24 to 26 November 2008.

12.2 The SAB also decided that, subject to the availability of funding in its Trust Fund, the TWG on sampling and analysis and the TWG on science and technology would meet in the week prior to the SAB session in November 2008.

12.3 It also decided that terms of reference for the TWG on science and technology would need to be prepared, preferably before June 2008.

13. AGENDA ITEM THIRTEEN – Adoption of the report

The SAB considered and adopted the report of its Eleventh Session.

14. AGENDA ITEM FOURTEEN – Closure

The Chairperson closed the Session at 17:10 on 13 February 2008.

Annexes:

Annex 1: List of Participants in the Eleventh Session of the Scientific Advisory Board
Annex 2 (English only):
Report of the Second Temporary Working Group on Sampling and Analysis, Madrid, 10 – 11 December 2007

- Appendix 1: List of participants to the second meeting of the temporary working group on sampling and analysis
- Appendix 2: Presentation on Industry and Protection Forum
- Appendix 3: Proposed additions to the OCAD
- Appendix 4: Presentation on Italian Fire Brigade CBRN Laboratory
- Appendix 5: Fast-GC for field laboratories – Possibilities and limitations
- Appendix 6: Presentations on toxins

Annex 3 (English only):
Chemicals with Data for On-Site Use Accepted by the Validation Group but Not Approved by the Executive Council

Annex 1

LIST OF PARTICIPANTS IN THE ELEVENTH SESSION OF THE SCIENTIFIC ADVISORY BOARD

	Participant	State Party
1.	Rolando A. Spanevello	Argentina
2.	Robert Mathews	Australia
3.	Herbert de Bisschop	Belgium
4.	Zhiqiang Xia	China
5.	Danko Škare	Croatia
6.	Jean-Claude Tabet	France
7.	Detlef Maennig	Germany
8.	László Halász	Hungary
9.	R. Vijayaraghavan	India
10.	Mahdi Balali-Mood	Iran (Islamic Republic of)
11.	Alberto Breccia Fratadocchi	Italy
12.	Shuzo Fujiwara	Japan
13.	Abdool Kader Jackaria	Mauritius
14.	José González Chávez	Mexico
15.	Godwin Ogbadu	Nigeria
16	Young-chul Lee	Republic of Korea
17.	Igor V. Rybalchenko	Russian Federation
18.	Philip Coleman	South Africa
19.	Miguel A. Sierra	Spain
20.	Stefan Mogl	Switzerland
21.	Valery Kukhar	Ukraine
22.	Robin Black	United Kingdom of Great Britain and Northern Ireland
23.	James Robert Gibson	United States of America

Annex 2

REPORT OF THE SECOND TEMPORARY WORKING GROUP ON SAMPLING AND ANALYSIS

MADRID, 10 – 11 DECEMBER 2007

1. INTRODUCTION

1.1 The Scientific Advisory Board Temporary Working Group on Sampling and Analysis (S&A TWG) held its second meeting on 10th and 11th December 2007 in Madrid.

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

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

1.4 Fernando Herencia from the Spanish National Authority addressed the working group and outlined that his country has been supportive of the OPCW activities such as training courses for OPCW inspectors, basic and advanced courses for National Authorities, legislative seminars, working groups on transfers of scheduled chemicals as well as assistance and protection courses. He also emphasized that his country has a strong belief in the work related to the activities of the Scientific Advisory Board and for the first time was providing funding to support it.

1.5 Patrice Palanque expressed the warm thanks of the Technical Secretariat of the OPCW to the Spanish National Authority for its generous financial contribution and its strong support and cooperation, which allowed for the convening of this meeting in Madrid. He welcomed members of the temporary working group on behalf of the Director General of the OPCW and outlined the importance of the work of the group, particularly its advice that relates to the sampling and analysis inspections.

1.6 The following agenda was adopted:

(i) Welcome by Fernando Herencia of the Spanish National Authority

(ii) Opening of the workshop and adoption of the Agenda (Chairman of the S&A TWG)

(iii) Welcome address by the Secretariat, Patrice Palanque

(iv) Tour de table for introduction of S&A-TWG members

(v) Matters on Sampling and Analysis (S&A) arising from the Industry and Protection Forum

(vi) Non-scheduled degradation products, Riot Control Agents and old/abandoned CW agents as possible additions to OPCW Central Analytical Database (OCAD)

(vii) New/additional techniques for on-site analysis:

(i) Developments in GC-MS instrumentation

(ii) Developments in LC-MS instrumentation

(iii) Sample preparation of aqueous solutions of degradation products

(viii) Off-site analysis

(ix) Criteria for a positive identification in trace analysis

(x) Toxin analysis (ricin and saxitoxin), off-site and on-site, and proposals for the way forward

(ix) Any other business

(x) Date of next meeting

(xi) Summary of recommendations

(xii) Closure of the meeting

2. MATTERS ARISING FROM INDUSTRY FORUM

2.1 Ralf Trapp summarised matters relevant to S&A arising from the Industry and Protection Forum. The OPCW has now undertaken 11 inspections of Schedule 2 industrial facilities, with on-site sampling and analysis to verify the absence of undeclared scheduled chemicals. The most salient points were:

(i) Confidentiality was less of an issue than was anticipated; inspections were performed with the instrument operated in open mode.

(ii) On average, only two samples were analysed in each inspection. The logistics of setting up the equipment and sample analysis time were limiting factors.

(iii) Inspections would be facilitated if the inspected facility were better informed and prepared with regard to logistics and support.

(iv) There was caution against over protection of data because operating the system in blinded mode and using only OCAD as the database may produce false positives.

(v) There was a need for OCAD to be as comprehensive as possible.

2.2 A presentation prepared by Colin Pottage, who was unable to attend the meeting, is given in Appendix 2.

3. ADDITIONS TO OCAD

3.1 The temporary working group discussed the additions of non-scheduled chemicals to the OCAD. In addition to the chemicals that were proposed at the first meeting of the group, it proposed the following:

(vi) disulphides arising from mustard and its higher homologues, which are common impurities, plus their degradation products;

(vii) additional arsenicals that are components of the so-called arsine oil (specifically triphenylarsine), plus degradation products of arsenicals phenyldichloroarsine, diphenylchloroarsine and triphenyl arsine;

(viii) xylylene bromide, an irritant from the First World War period;

(ix) Capsaicin analogues as riot control agents.

3.2 There was some discussion on the previous proposal to include diethyl phosphate and diethyl thiophosphate, because of their ubiquitous occurrence as degradation products of pesticides and/or plasticizers. They may however be relevant in the context of amiton.

3.3 A revised list of recommended additions to OCAD, which incorporates the chemicals proposed above, is given in Appendix 3. The temporary working group reaffirmed its recommendation that these chemicals should be added to the OCAD in order to facilitate the verification provisions of the Convention, and the selective use of sub-sets data from OCAD in accordance with the aims of the inspection (routine, challenge, allegations of use, Old and Abandoned Chemical Weapons (OACWs).

4. NEW/ADDITIONAL TECHNIQUES FOR ON-SITE ANALYSIS

Developments in GC instrumentation.

4.1 Francesco Pilo described methods and procedures used by the Italian Fire Brigade CBRN Laboratory for identifying toxic/industrial chemicals produced during fires and industrial accidents involving bulk chemicals. In these cases benchtop GC-MS instrumentation is transported to the scene of the incident in a portable laboratory by road transport. Typical analyses involve air sampling and conventional GC-MS with a requirement for rapid identification for first responders. The presentation is given in Appendix 4.

4.2 Developments in fast GC were discussed as a means of shortening on-site analysis time. Paula Vanninen described investigations undertaken at VERIFIN that had demonstrated GC analysis time for the OPCW QC test mixture could be shortened approx five fold (from ~25 to ~5 min), using similar equipment to that

used by the OPCW on-site. Retention indices (RIs) for the OPCW test mix were still within acceptable tolerances. Robin Black reported that work at Dstl on fast GC with a range of GC columns could not reproduce RIs within the criteria currently required for on-site identification, but one column could meet the tolerances allowed for proficiency tests. The VERIFIN presentation is given in Appendix 5.

4.3 Work undertaken in the OPCW Laboratory had suggested that a more modest reduction in sample time (50%) may provide a more robust procedure with regard to retention index and peak identification using AMDIS software. The question was asked if a 50% reduction in a GC run would significantly shorten analysis time, particularly when the time for recycling of the column temperature was taken into account. The point was also made that any change to fast GC could be counterproductive unless it was robust and universally applicable with regard to reproducibility of RIs and spectra.

4.4 The temporary working group recommended that greater collaboration in this area should be encouraged between laboratories and with the OPCW laboratory.

4.5 The temporary working group was of the view that commercialised small portable GC-MS instruments used in other fields, e.g. the Hapsite instrument, are not suitable for the requirements of on-site inspection; they are essentially screening systems.

Developments in LC-MS instrumentation

4.6 One of the major limitations of on-site GC-MS analysis as currently used by inspectors is the additional time and equipment required for the identification of polar degradation products of CW agents in environmental samples. These require concentration of aqueous samples to dryness and derivatisation. This process is lengthy, requires an additional heavy pump, and can be a source of error. One solution to this problem would be on-site LC-MS, but not at the expense of additional heavy equipment for transportation.

4.7 The members of the temporary working group were not aware of any LC-MS instruments currently available or likely to be commercialised in the near future that would meet requirements for reduced size and robustness. Several laboratories in the US are working on small portable systems but these are years from full development. The main commercial drivers at present were for new ionisation methods and more powerful instrumentation rather than miniaturisation. Additional barriers to the use of LC-MS on-site would be the lack of a database and the limited amount of structural information inherent in LC-MS spectra using atmospheric pressure ionisation methods. VERIFIN and Spiez Laboratory are collaborating in compiling a LC-MS database. A subcommittee within the OPCW Validation Group will be comparing LC-MS spectra produced in different laboratories.

4.8 There could be a role for on-site LC-MS in challenge inspections and investigations of alleged use. This would have to be weighed against the

additional costs and expertise required for on-site LC-MS analysis, and the alternative of using off-site LC-MS analysis.

4.9 It was acknowledged that LC-MS could significantly improve on-site analysis, and could include saxitoxin and ricin for which GC-MS cannot be used. Experts should maintain a watching brief on developments in LC-MS instrumentation, and methods for direct analysis of samples such as using DESI and DART.

Sample preparation of aqueous solutions of degradation products.

4.10 An alternative solution to the time and pumping equipment required for GC-MS of polar analytes in aqueous samples may be to adopt newer extraction/derivatisation techniques. A number of approaches have been explored in other fields of analysis. These include:

(x) on-SPE derivatisation

(xi) on-SPME derivatisation

(xii) hollow-fibre extraction and derivatisation

(xiii) stir-bar sorptive extraction/derivatisation

(xiv) microemulsion extractive derivatisation

(xv) two-phase extractive derivatisation

(xvi) aqueous phase alkylation

(xvii) aqueous phase derivatisation with chloroformates

(xviii) molecularly imprinted polymers

4.11 Relatively little work in this area appears to have been undertaken on analytes relevant to the CWC. One of the problems is the range of reactivities and polarities of the various degradation products such as phosphonic acids, thiodiglycol, ethanolamines etc.. The most promising technique so far reported (DSO National Laboratories, Singapore) appears to be hollow fibre-protected liquid phase microextraction with in-situ derivatisation to tert.-butyldimethylsilyl (TBDMS) derivatives. Good levels of detection (LODs) in water were reported for a range of CW agent hydrolysis products. The additional advantage of this procedure is that some TBDMS derivatives are in OCAD. Alkylation of phosphonic acids has been demonstrated in aqueous solution (Vertox Laboratory, India) with subsequent hollow fibre liquid phase microextraction, although these derivatives (propyl and pentyl esters) are not in the OCAD library. The same laboratory has reported aqueous phase alkylation using microemulsion derivatisation. There have been many literature reports on aqueous phase derivatisation with chloroformates. Exploratory studies at Dstl, UK had shown that such derivatisation was not easily applied to CW related analytes.

4.12 The OPCW Laboratory has identified this as an important area and the temporary working group encourages collaboration between laboratories. One solution to the applicability of different techniques to different classes of degradation products might be to have 2-3 different procedures but it would be preferable to have one that was universally applicable.

4.13 The temporary working group proposed a more detailed discussion of aqueous phase derivatisation at the next meeting, with invited presentations from the laboratories that have undertaken work in this area.

5. OFF-SITE ANALYSIS

Criteria for a positive identification in trace analysis

5.1 The OPCW Laboratory in cooperation with Member States has established rigid criteria for the unequivocal identification of chemicals relevant to the Convention at levels where full scan spectral data can be acquired. These criteria are at least up to the standards required by other regulatory bodies, e.g. (European Commission (EC), the United States Food and Drug Administration (FDA)) for residues in animal products and the World Anti-Doping Agency (WADA) and International Olympic Committee (IOC) for drug testing in sport.

5.2 The OPCW Laboratory has not yet addressed trace environmental analysis, where identification requires compound targeted analysis under non-scanning conditions. Other regulatory bodies, including the EC, FDA and WADA have developed criteria for unequivocal identification at trace levels based on the selectivity of the various techniques.

5.3 The OPCW is planning to address trace analysis in the context of confidence building exercises for biomedical sample analysis but this will take time.

5.4 A number of different views were expressed on what approach should be taken by the OPCW Laboratory with regard to trace analysis in environmental samples.

5.5 It was proposed by some members of the temporary working group that the OPCW should at least be prepared with regard to the criteria that should be required for an acceptable identification using targeted techniques such as selected ion monitoring and multiple reaction monitoring. These should be able to withstand scientific scrutiny and should therefore be at least up to the standard required by other regulatory bodies such as the EC, FDA and WADA. The International Laboratory Accreditation Co-operation Guide (ILAC-G7) states that the identification of a prohibited substance must result from a direct comparison with a reference material analysed in parallel or series with the test sample using a mass spectrometric technique and there must be written laboratory criteria as to what constitutes a match.

5.6 It was emphasised that trace analysis should be considered with other evidence and not in isolation.

5.7 A suggestion previously made by the Head of the OPCW laboratory, which has yet to be discussed by laboratories engaged in Proficiency Tests, is to distribute an optional sample with a future proficiency test that would require compound targeted techniques (distribution with test samples would reduce costs, and reporting would presumably be separate and within a different time scale.) Laboratories would be invited to report data so that the OPCW Laboratory could assess the quality of this data (the sample would not be part of the test).

Toxin analysis (ricin and saxitoxin), off-site and on-site

5.8 Detailed presentations on toxin analysis were given by Martin Schaer of the Spiez Laboratory and Crister Åstot of FOI. The presentations, are at Appendix 6, focussed specifically on saxitoxin and ricin, the only toxins identified in Schedule 1.

5.9 The most appropriate method for the identification of saxitoxin is LC (or CE)-MS/LC-MS-MS. GC-MS is not applicable to saxitoxin analysis. With little prospect of on-site LC-MS in the near future, the only practical solution to on-site analysis of saxitoxin is immunoassay. Immunoassays have varying degrees of selectivity and specificity and should therefore be regarded only as screening assays for possible additional analysis off-site.

5.10 LC-MS/LC-MS-MS (or CE-MS/CE-MS/MS) are the main techniques for the identification of saxitoxin; NMR may be applicable dependent on quantity and purity. It was noted that current OPCW Laboratory criteria for identification regard LC-MS and LC-MS-MS as a single technique.

5.11 The temporary working group recommends that the OPCW Laboratory review the relevant criteria for LC-MS/LC-MS-MS, not only in the context of saxitoxin but for the broader application to relevant chemicals. Provided that a minimum number of product ions are required for LC-MS-MS there appears to be no reason why LC-MS-MS cannot be used to provide a 'fingerprint' cf GC-EI-MS, and single stage LC-MS confirmation of the molecular mass, cf GC-CI-MS. This would require careful assessment with regard to some classes of chemicals, particularly where isomer differentiation is required. It should also be noted that for some classes of compounds, e.g. alkylphosphonic acids and dialkyl alkylphosphonates, low energy collisions in ion trap and triple quadrupole instruments may give only one or two fragment ions.

5.12 Criteria for the unequivocal identification of proteinaceous toxins, particularly a heterogeneous one such as ricin, are much more difficult to develop. By definition, proteins consist of a large number of amino acids linked by amide bonds, in some cases (e.g. ricin) also through disulphide linkages between cysteine residues, and folded into a specific conformation. An added complication is that ricin is glycosylated. There are many isoforms of ricin with different glycosylation patterns. There are also ricin variants with modified amino acid sequences. Crister Åstot recommended that ricin D should be the

preferred target molecule as it appears to occur in castor beans from all strains of *Ricinus communis* so far studied.

5.13 As is the case for saxitoxin, immunoassay appears to be the only current practical option for on-site analysis, and only as a screening assay. PCR (to identify ricin coding DNA) was suggested as an alternative, although it was acknowledged that it would be more expensive than an immunoassay.

5.14 There is now a fairly standard approach to the mass spectrometric identification of proteins, but the information obtained is very much instrument dependent. A simple mass spectrum of a protein provides either an approximate or accurate measure of the molecular mass, depending on the instrument used. For example, a typical MALDI-TOF instrument gives a broad peak covering a mass range of ~300 Da; a deconvoluted ESI-MS spectrum will show various isoforms with mass accuracy within a few Da or with greater mass accuracy depending on the instrument.

5.15 Other possible techniques include SDS-page (as a screening procedure) and NMR.

5.16 The first issue with regard to toxin analysis is what information is required, and this will be context dependent.

5.17 Much debate centred on a possible requirement to demonstrate functional ricin (i.e. A-S-S-B linkage intact and the active protein conformation maintained). There are a number of assays now available, e.g. measurement of protein synthesis inhibition, demonstration of specific binding of sugars by NMR.

5.18 The temporary working group reiterated its recommendation made in the first meeting that the OPCW Laboratory circulate a questionnaire to Member States asking for current capabilities for toxin analysis. It also asked the OPCW Laboratory to consider the most likely scenarios that may require toxin analysis.

5.19 The temporary working group recommended the establishment of a correspondence group within its members to discuss criteria for identification. Members are encouraged to consult other experts within their country where appropriate. Crister Åstot offered FOI to coordinate the group and keep other members of the TWG informed of progress. Analysis of two types of sample will initially be considered, a solid sample and an aqueous solution at medium to high concentration.

6 ANY OTHER BUSINESS

6.1 Paula Vanninen noted that the inclusion of hexachlorobenzene in the OPCW QC mix for GC-MS analysis had caused problems in a Member State because of its status as a restricted substance (under the Stockholm Convention). The chair of the temporary working group will ask the Head of the OPCW Laboratory if a substitute compound has been identified, or if he would like the temporary working group to address this issue.

6.2 It was noted that the OPCW Laboratory do not have criteria as to what constitutes an acceptable match with regard to EI mass spectra. The acceptability of spectra is therefore to a degree subjective. The Head of the OPCW laboratory has previously indicated that he would like to address this issue.

7 DATE OF NEXT MEETING

Should funding be available in the Scientific Advisory Board Trust Fund, the temporary working group could convene in The Hague in spring or fall 2008.

8 SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

8.1 The temporary working group reaffirmed its recommendation that additional chemicals, as given in Appendix 3, should be added to OCAD, plus the selective use of sub-sets data from OCAD in accordance with the aims of the inspection.

8.2 The temporary working group recommended that greater collaboration should be encouraged between laboratories and the OPCW laboratory on fast GC.

8.3 Current generation of commercialised portable GC-MS instruments used in other fields are not suitable for the OPCW requirements for on-site inspection; they are essentially screening systems.

8.4 The temporary working group assessed that LC-MS would not be practical for on-site analysis in the near future. It does have an important role in off-site analysis. There may be a role for on-site LC-MS in challenge inspections and investigations of alleged use but the benefits would have to be weighed against the additional costs, the expertise required, and the alternative use of off-site analysis.

8.5 The temporary working group agreed with the OPCW Laboratory that alternative aqueous derivatisation methods for polar degradation products should be investigated for on-site analysis. A more detailed evaluation should be undertaken as part of the next meeting of the temporary working group.

8.6 The temporary working group recommended that the OPCW Laboratory addresses techniques used in trace analysis such selected ion monitoring and multiple reaction monitoring.

8.7 The temporary working group requests that the OPCW Laboratory consider LC-MS/LC-MS-MS as independent techniques for identification.

8.8 The temporary working group reiterated its recommendation that the OPCW Laboratory circulate a questionnaire to Member States asking for current capabilities for toxin analysis, in particular saxitoxin and ricin.

8.9 The temporary working group has established a correspondence group within its members to discuss the analysis of saxitoxin and ricin.

9. CLOSURE OF THE MEETING

The meeting was closed at 5.15pm.

Appendix 1. List of participants to the second meeting of the temporary working group on sampling and analysis

	Participants	**Member States**	**e-mail addresses**
1	Robert Mathews	Australia	robert.mathews@dsto.defence.gov.au
2	Jiří Matoušek	Czech Republic	matousek@recetox.muni.cz
3	Jiří Cermak	Czech Republic	jiri.cermak@vuos.com
4	Paula Vaninnen	Finland	paula.vanninen@helsinki.fi
5	Jean-Claude Tabet	France	tabet@ccr.jussieu.fr
6	Anne Bossée	France	anne.bossee@dga.defense.gouv.fr
7	Ralf Trapp	Germany	Ralf.trapp@gmail.com
8	R. Vijayaraghavan	India	Jai_vijay@hotmail.com
9	Shigeyuki Hanaoka	Japan	hanaoka-shigeyuki@ceri.jp
10	Francesco Pilo	Italy	francpil@libero.it
11	José Luz González-Chávez	Mexico	joseluz@servidor.unam.mx
12	Philip Charles Coleman	South Africa	philipc@protechnik.co.za
13	Miguel Sierra[1]	Spain	sierraor@quim.ucm.es
14	Roberto Martinez-Alvarez	Spain	rma@quim.ucm.es
15	Crister Lundmark Åstot[2]	Sweden	crister.astot@foi.se
16	Martin Schär	Switzerland	martin.schaer@babs.admin.ch
17	Robin Black[3]	United Kingdom of Great Britain and Northern Ireland	rmblack@dstl.gov.uk
18	Armando Alcaraz	United States of America	alcaraz1@llnl.gov

[1] Vice-Chairman of the TWG
[2] Replaced Sten-Åke Fredriksson
[3] Chairman of the TWG

Appendix 2. Presentation on Industry and Protection Forum

OPCW Industry & Protection Forum - 1st & 2nd November 2007

Personal Observations – focussing on S & A

Colin Pottage, Dstl Porton Down

Background

- One of events to celebrate 10th anniversary
- Financial support EU & supported the International Council of Chemical Associations (ICCA) & CEFIC
- To cover key issues concerning OPCW verification procedures
- Discuss possible establishment of a platform for consultation and co-operation among governments, the chemical industry and the OPCW
- Forum could also be used to launch and co-ordinate future events and activities

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Format

- ~ 200 participants
  - National Delegations in the Hague, National Authorities, Industry & TS
- Plenary Session followed by 3 Workshops:
  - *Issues surrounding verification and implementation that are of relevance to the chemical industry, including sampling and analysis*
  - *Assistance and protection*
  - *Safety and security at chemical plants*
- Equipment exhibition, including a demonstration of the OPCW mobile laboratory

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Issues surrounding verification and implementation that are of relevance to the chemical industry, including sampling and analysis - Facilitator Ralf Trapp

- Presentations on technical aspects
  - OPCW S&A Concept Present & Future – Per Runn & Bill Kane
  - Protection of Confidential business information in S&A – Gary Mallard
    - OPCW Dual Mode Software (ODMS)
  - Logistics of Sampling and analysis – Andrew Othieno
- NA perspectives on S&A in chemical industry inspections
  - 10 S&A inspections since autumn 2006
  - Series of case studies from recent experiences

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Summary

- Most speakers were supportive of S&A which was seen as being successfully carried out
- Currently analysis is for absence of intact Schedule1 chemicals, but in future TS would also wish to look for degradation products
- 8/10 inspections analysis carried out in open mode
  - Therefore concerns of commercial confidentiality not as great as envisaged
- ~ 2 samples analyzed per inspection
- Logistical issues – size and portability of equipment
- Need for more comprehensive OCAD
  - Data on scheduled and non-scheduled chemicals

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Conclusions – 1

S&A points noted from Ralf Trapp's summary to the plenary session

- Need to educate & prepare sites on logistic requirements
- SP's need to check legal considerations regarding transport of OPCW equipment
- Beware of over protection of data as the degree of blinding may lead to false positives
  - can be resolved
- Need for OCAD to be as comprehensive as possible and to include data on non-scheduled chemicals

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Conclusions – 2

S&A points noted from Ralf Trapp's summary to the plenary session

- The IT needs maintain a flexible approach to S&A which may be site dependent
- S&A is expensive but:
  - It can provide the only definitive proof of absence of undeclared chemicals
  - It is a fundamental requirement of the Convention
  - The OPCW has proved that it can be done effectively
- Presentations findings etc will be available on website:

www.opcwipf.org

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Appendix 3. Proposed additions to the OCAD

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 by-products of the synthesis of scheduled chemicals

1. Non-scheduled precursors:

High priority:

Methyl benzilate
Ethyl benzilate
O-ethyl N,N-dimethylphosphoramidochloridate
O-isopropyl N,N-dimethylphosphoramidochloridate

Low priority:

Other alkyl N,N-dimethylphosphoramidochloridates

2. By-products:

High priority:

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

Lower priority:

disulfides of higher mustard homologues
hydrolysis products of disulfides

Riot control agents and old/abandoned chemical weapons

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
Triphenylarsine	603-32-7
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
Dihydrocapsaicin	
Nordihydrocapsaicin	
Pelargonic acid vanillylamide (nonivamide)	2444-46-4
4-Nonanoylmorpholine	5299-64-9
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
Xylylene bromide	
Benzyl bromide	100-39-0
Diphosgene	503-38-8
Triphosgene	32315-10-9

Degradation products of arsenicals

Name (code)	CAS number
Phenylarsine oxide	
Phenylarsonic acid	
bis(Diphenylarsine)oxide	
Diphenylarsinic acid	
bis(Diphenylaminearsine)oxide	
Diphenylamine arsonic acid	
Triphenylarsinie oxide	

Appendix 4. Presentation on Italian Fire Brigade CBRN Laboratory

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10-11 DECEMBER 2007

Fire-fighters, Public Rescue and Civil Defence Department - Italy

Sampling and analysis in emergency condition of Volatile Organic Compound:

TWO CASES OF INDUSTRIAL PLANTS RELEASES

Francesco Pilo

Provincial Headquarters of Venezia

Ministry of the Interior

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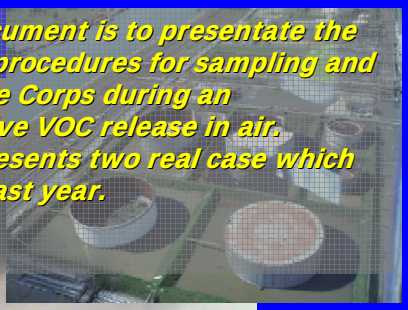
AIR SAMPLING AND ANALYSIS DURING EMERGENCY WITH VOCs RELEASES IN AIR

The purpose of this document is to presentate the devices and operating procedures for sampling and analysis of National Fire Corps during an emergency which involve VOC release in air. Moreover document presents two real case which were occurred during last year.



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AIR SAMPLING AND ANALYSIS DURING EMERGENCY: SPECIAL FEATURES

- *24/24 hour sampling and analysis disponibility*
- *Response time confrontable with emergency time (max 1 hour from the release)*
- *Define chemical substances and relative adbundance*

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AIR SAMPLING AND ANALYSIS DURING EMERGENCY: TARGETS

- *Identify chemical elements relevant for human safety (stocastic effects in short time)*
- *Define release limits in air.*
- *Define level limits for toxic elements in air*
- *Define population's risk level*
- *Define action to avoid risk for population (application of emergency plan)*
- *Identify source and contribute to reduce release*

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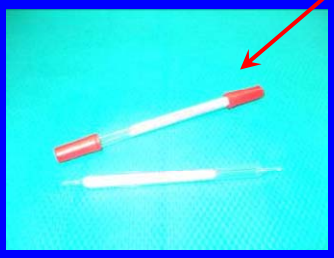
VOC AIR PORTABLE SAMPLING DEVICES

SAMPLE PUMP

TUBE

GLASS TUBE



CARBON TUBE

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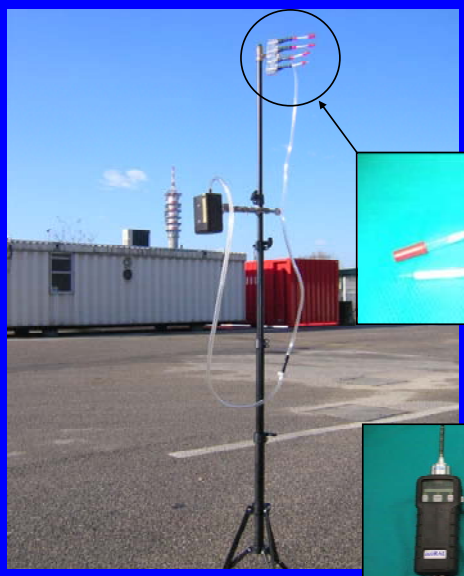
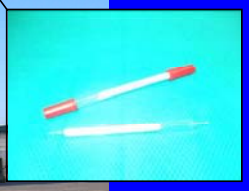
AIR SAMPLING STATION



10 AIR SAMPLING STATION



PID DEVICE
presampling operation

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VOC AIR PORTABLE SAMPLING DEVICE

GLASS TUBE/CARBON TUBE

Tube internal material:
Active Charcoal: organic polar elements
Alluminia, silica gel: polar organic, inorganic elements

Targets:

Use for air sampling with inorganic or polar organic substances

Use procedures:

Max air flow: 1 l/min (100-500 cc/min)

Sampling time:

5 minutes, for low concentration 10 minutes

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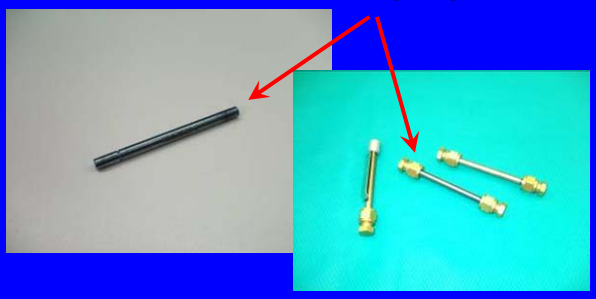
VOC AIR PORTABLE SAMPLING DEVICES

AIR PUMP

IRON TUBE



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AIR SAMPLING INSTRUMENTS

IRON TUBE

Tube internal material:
- tenax-glass fiber for organic compound C7-C30
- tenax for organic compound C7-C30
- tenax-unicarb for organic compound C3-C8 and C7-C30

Target:
Use for air sampling organic elements (VOC)

Use procedures:
Max air flow: 2 l/min

Sampling time:
5 minutes, for low concentration 10 minutes

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MOBILE CHEMICAL LABORATORY

And analysis devices inside:



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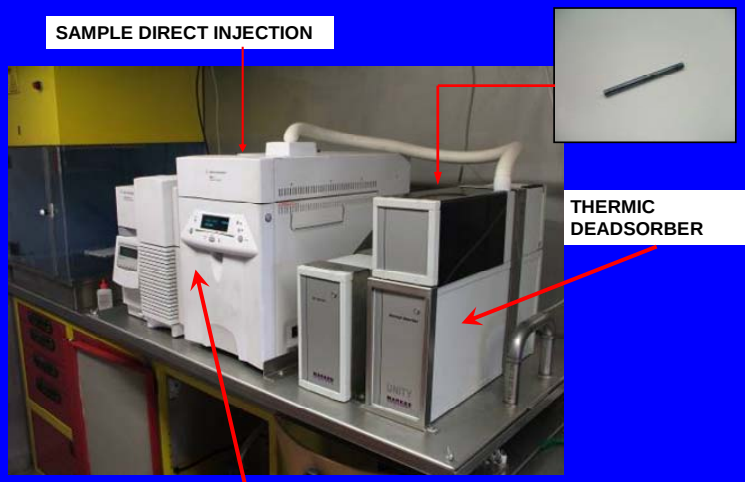
ANALYSIS INSTRUMENTS



(Volatile Organic Compounds)

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ANALYSIS INSTRUMENTS (Inorganic Compound)

(Volatile and liquid Inorganic Compounds)

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ANALYSIS INSTRUMENTS (Inorganic Compound)

FT-IR PORTABLE INSTRUMENT

(Solid Compound)

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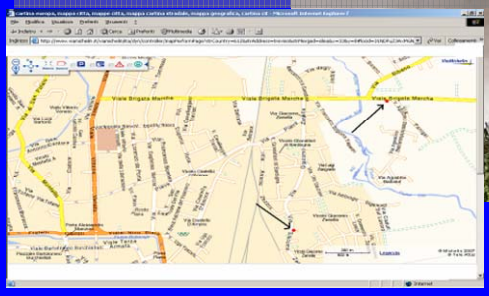
FIRST CASE: FIRE OF PLASTIC PLANT

Fire of important plastic plants determine an important smoke release rate which contain a large quantities of VOCs and possible presence of inorganic compounds.



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AIR SAMPLING SPOTS

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SAMPLING AND ANALYSIS TIME

Sampling procedures:

Active sampling on thermal deabsorbal iron tube, sampling time between 8 and 12 minutes, air volume of 2 or 3 litres.

Activities:

Arrival time of mobile laboratory on emergency scenario: about 15.40.
Arrival place : via Da Milano, 100 m far from plant, upwind . Position of chemical laboratory is useful in order to reduce arrival time of sample.
Dowwind sample spots with a distance from plant between 100 m and 2 km.

Sample spot	Arrival time of Sample Time in mobile laboratory	Time of results
Via Da Milano	15.40	16.27
Via Zanella	16.38	17.18
Viale Brigata Marche	17.35	18.45
Plant proximity	19.00	19.35
Vicolo Corti	19.00	20.18
Via Don Sturzo	21.05	22.14
Via Don Sturzo	22.15	23.48

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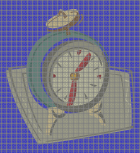
SAMPLING AND ANALYSIS RESPONSE TIME

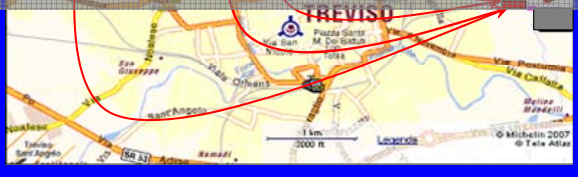
Sampling and analysis response time:

Response time: 1 hour ; max 1 hour and half for complete screening divided by:

- 15' sampling
- 10' first analysis step (thermal desorber)
- 25-50' second analysis step (GC-MS)

60-90': time of first chemical response about chemical subtances and relative abundance





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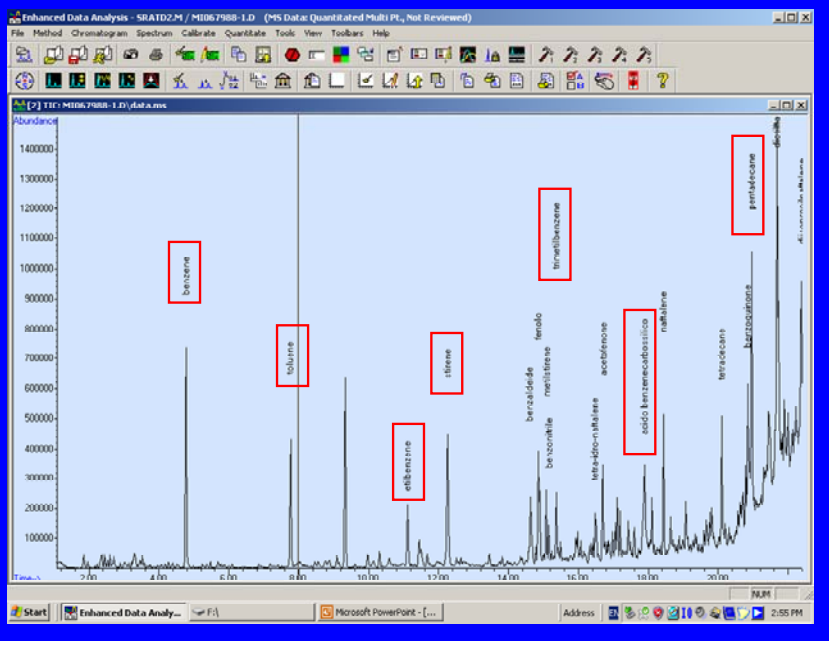
RESULTS



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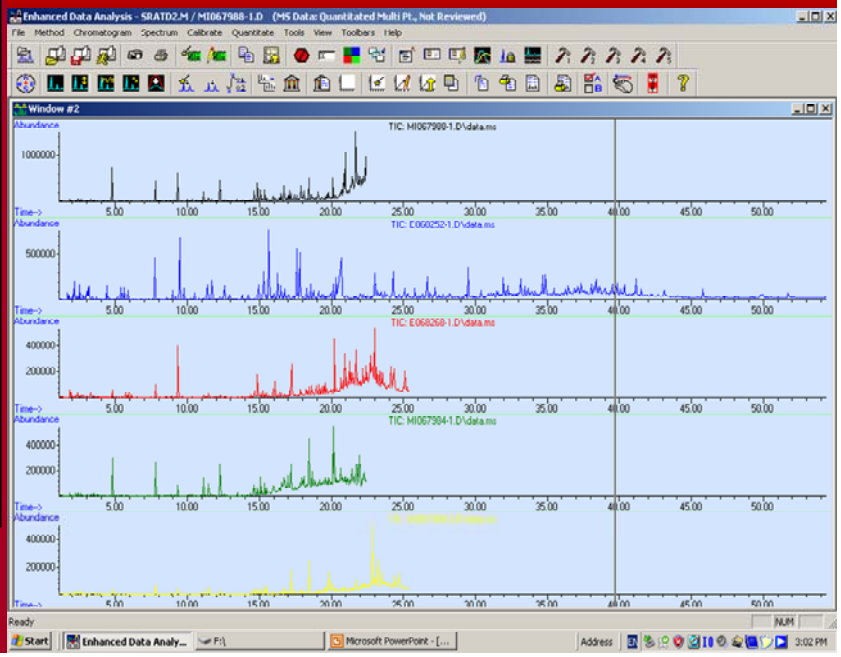
RESULTS



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RESULTS

ANALYSIS REPORT

benzene
Toluene
etilbenzene
xilenE
benzaldeide
otta-nona-decanale
stirene
metil-stirene
tetraidronaftalene
tri, tetra, penta decani.
trimetil-benzene
naphthalene
acetofenone

Sampling and analysis procedure permit to identify elements and define a semiquantitative results.
Concentration of toxic elements like benzene is quite low. Also concentration of chloride composts is low concentration and they cannot give problems in air. This analysis does not permit to evaluate toxic elements ground disposal.

OPWC Sample and Analysis TWG
Madrid, Ministry of Industry
10-11 DECEMBER 2007

SECOND CASE: VOC RELEASE FROM INDUSTRIAL PLANT

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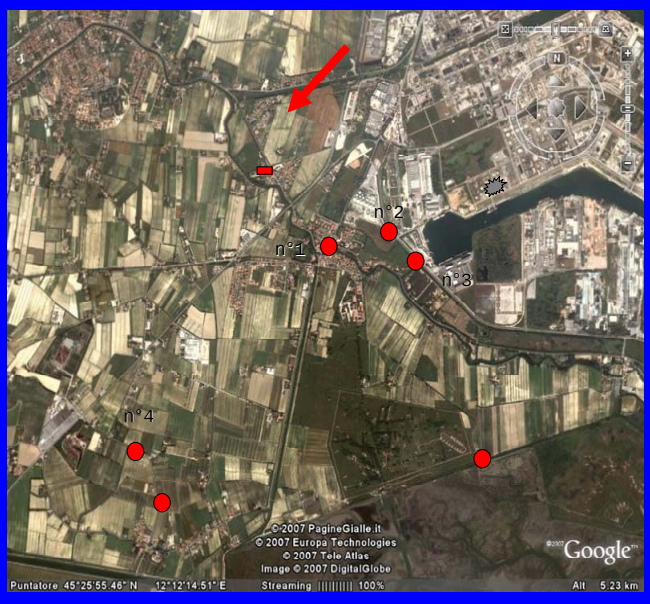
AIR SAMPLING SPOTS



- Plastic industry
- Mobile Chemical Laboratory
- Sampling spot
- Wind direction

OPWC Sample and Analysis TWG
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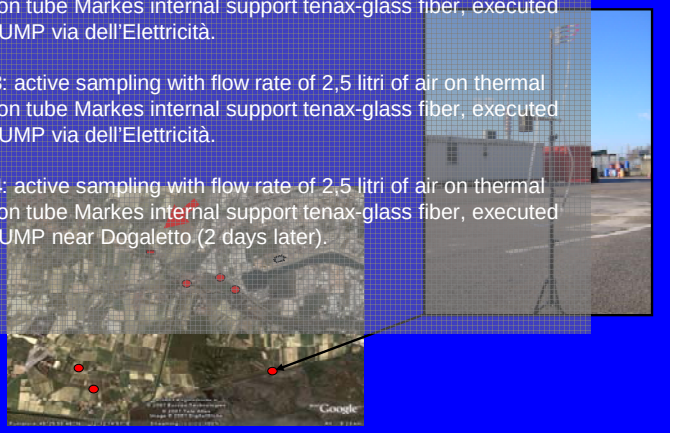
10-11 DECEMBER 2007

SAMPLING PROCEDURES

- **Sample n°1**: active sampling with flow rate of 1,25 liter of aria on thermal deabsorbal iron tube Markes internal support tenax-glass fiber, executed by Gil Air 5 air pump in Malcontenta.
- **Sample n°2**: active sampling with flow rate of 5 litri of air on thermal deabsorbal iron tube Markes internal support tenax-glass fiber, executed by Gil Air 5 PUMP via dell'Elettricità.
- **Sample n°3**: active sampling with flow rate of 2,5 litri of air on thermal deabsorbal iron tube Markes internal support tenax-glass fiber, executed by Gil Air 5 PUMP via dell'Elettricità.
- **Sample n°4**: active sampling with flow rate of 2,5 litri of air on thermal deabsorbal iron tube Markes internal support tenax-glass fiber, executed by Gil Air 5 PUMP near Dogaletto (2 days later).



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10-11 DECEMBER 2007

ANALISYS PROCEDURES

ANALYSIS INSTRUMENTS

Analysis with GC/MS Agilent model 6850/5973, column HP5MS, head thermal desorber Markes Unity.

ANALYSIS METHODOLOGY

Analysis with thermal desorber, double split e splitless relative to quantities of sample.

REFERENCE METHODOLOGY

EPA TO-17

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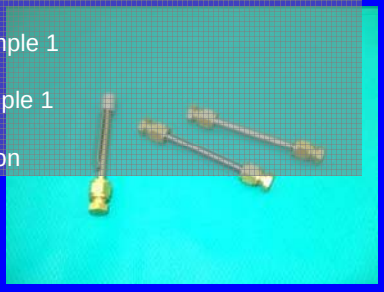
RESULTS

Sample n°1 : Sample with presence of light oli fraction pentane, eptane, cyclopentane, ecc., more heavy fraction like decano, metil-undecano and aromatic elements toluene, trimetil-benzene, ecc..

Sample n°2 :Quite similar to sample 1

Sample n°3 : Quite similar to sample 1

Sample n°4: Ground concentration



OPWC Sample and Analysis TWG
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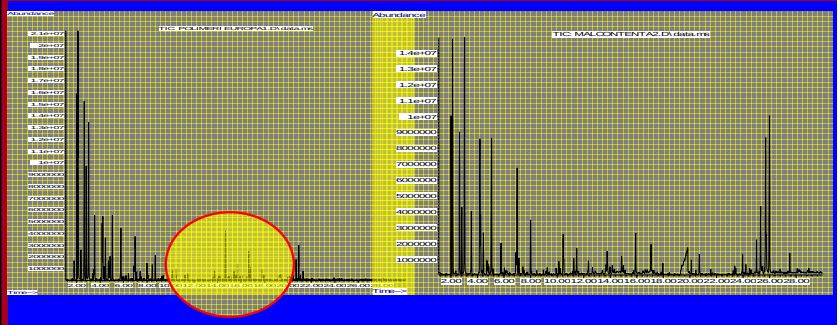
RESULTS



Sample n°1

Sample n°4

Analysis results permit to:

• Define source of release and contribute to reduce air aereosol by use of foam directy on oil surface. Definition of a procedure to reduce oil level inside all tanks.

• Monitor VOC level in air in order to avoid risk for population


THANK YOU
FOR
YOUR ATTENTION!
francesco.pilo@vigilfuoco.it

Appendix 5. Fast-GC for field laboratories – Possibilities and limitations

GC-MS

- GC-MS is the most commonly used technique for the analysis of CWC-related chemicals
- In some cases shorter analysis time is attractive
  - if a large number of samples have to be analysed in a limited time (on-site inspections of the OPCW)
  - situations where the results of the analysis are needed as soon as possible (field laboratory, on-site analysis)

VERIFIN

2

Fast-GC

- Methods to increase speed of GC analysis
  - by reducing
    - column length
    - column inner diameter
    - thickness of stationary phase
  - by increasing
    - heating rate of the oven
    - carrier gas flow-rate
  - by using hydrogen as carrier gas

VERIFIN

1

Fast-GC

- In the fast GC typically used column is
  10 m x 0.10 mm x 0.10 µm
- The efficiency of the column is equivalent to
  25 m x 0.25 mm x 0.25 µm
- Excellent efficiency of the Fast-GC column allows the use of higher carrier gas linear velocity and higher temperature program rate, typically 50 – 100 ºC/min

VERIFIN

4

Fast-GC limitations

- Reduction of the column inner diameter means low sample capacity of a column
- With very narrow bore columns, split injection is often used to avoid peak broadening and to produce a narrow injection band
- Poor sensitivity of split injection compared to splittless injection is partly compensated by narrower peaks and improved signal-to-noise ratio
- Thin film increases the column activity thereby increasing peak broadening of polar chemicals

VERIFIN

5

Fast-GC

- Typically analysis time is only few minutes and the peak width at half height is below 1 sec
- Time-of-flight, TOF instrument is the most suitable mass spectrometer for detection of narrow peaks
- Scanning rate of a mass spectrometer is a limiting factor in some instruments
- However, modern scanning mass spectrometers like ion trap and quadrupole instruments can be operated in full scan mode with scanning rate from 10 to 20 spectra per second allowing the collection of peaks with a peak width of 0.2 s

VERIFIN

6

Method translation

- Simplest way to start the modification of conventional GC method to Fast-GC method is modification of the GC parameters by using method translation software freely available from internet (www.agilent.com)
- Agilent 6890N gas chromatograph and 5975B mass selective detector have been applied

VERIFIN

7

Method translation

Criterion: Fast Analysis — **Speed gain: 6,34805**

	Original Method	Translated Method
Column		
Length, m	*30,0*	*10,0*
Internal Diameter, µm	*250,0*	*100,0*
Film		
Thickness, µm	*0,250*	*0,100*
Phase Ratio	250,0	250,0
Carrier Gas	*Helium*	*Helium*
Head Pressure, bar	0,8080	3,9749
Flow Rate, mLn/min	*1,000*	*0,8000*
Outlet Velocity, cm/sec	35,80	179,02
Average Velocity, cm/sec	24,92	52,74
Hold-up Time, min	2,00615	0,316027
Outlet Pressure (absolute), bar	*1,0133*	*1,0133*
Ambient Pressure (absolute), bar	*1,0133*	*1,0133*

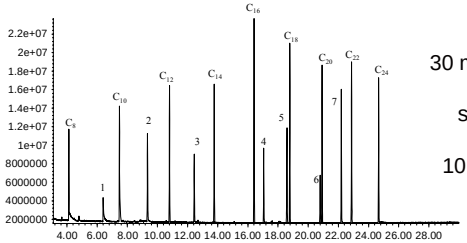
Oven Temperature Program

	Ramp Rate	Final Temp.	Final Time	Ramp Rate	Final Temp.	Final Time
	°C/min	°C	min	°C/min	°C	min
Initial		*40*	*1,000*		*40*	0,158
Ramp 1	*10,000*	*250,00*	*5,000*	63,481	250,00	0,788

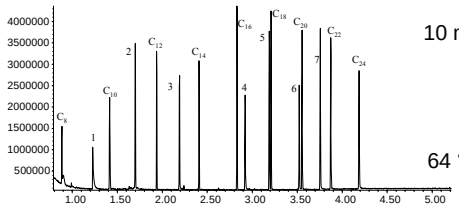
VERIFIN

8

Analysis of the OPCW QC-test solution (5ppm)



Conventional GC
30 m x 0.25 mm I.D. x 0.25 µm
gas flow 33 cm/sec
splitless injection (1 µl, 1 min)
40 °C (1 min) –
10 °C/min – 280 °C (5 min),



Fast-GC
10 m x 0.10 mm ID x 0.10 µm
gas flow 56 cm/sec
split injection (1 µl)
split ratio 1:25
40 °C (0.16 min) –
64 °C/min – 280 °C (1.3 min)

VERIFIN

(1) trimethylphosphate, (2) 2,6-dimethylphenol, (3) 5-chloro-2-methylphenol, (4) tri-n-butylphosphate, (5) dibenzothiophene, (6) malathion and (7) methyl stearate

Quality of Retention Indices and Mass Spectra using AMDIS software

Test compound	Library values	Conventional method (reference)		Fast-GC method	
	RI	RI	fit	RI	fit
Trimethylphosphate	938	935.5	98	929.3	97
2,6-Dimethylphenol	1112	1112.3	98	1108.9	98
5-Chloro-2-methylaniline	1308	1310.9	97	1307.5	97
Tri-n-butylphosphate	1655	1653.8	98	1647.4	97
Dibenzothiophene	1774	1783.7	95	1789.0	93
Malathion	1986	1987.9	96	1981.5	95
Methyl stearate	2130	2130.0	98	2126.8	96

VERIFIN

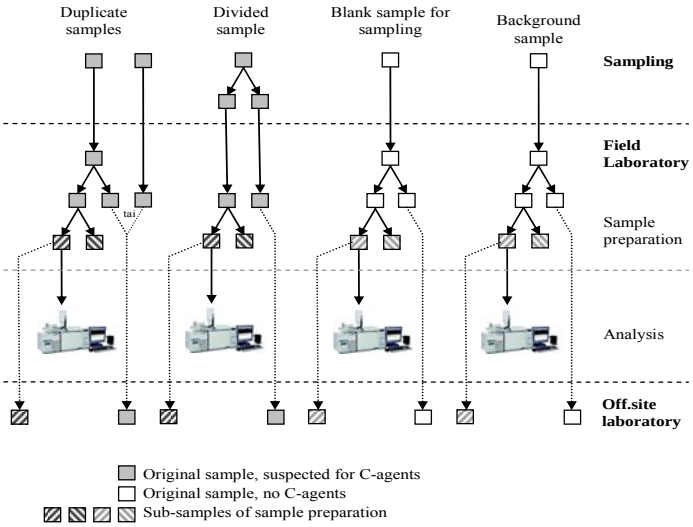
Preliminary results of Fast-GC

- When maximum scanning rate of the quadrupole instrument is used, peaks are splitted and MS fit values are between 60-70
- Therefore, we operate in full scan mode with scanning rate of 7 spectra per second; maximum scanning rate is 17
- In qualitative analysis, 3-4 sampling points over the peak are sufficient for identification
- In quantitative analysis, ca. minimum of 10 sampling points over the peak are needed for accurate results

VERIFIN

11

Total analytical procedure



VERIFIN

12

Total analysis time in laboratory

- The total analysis time is the sum of the time needed for
  - Sample preparation
  - GC analysis
  - Cool down and reequilibration of GC
  - Reporting and documentation
- In most cases, sample preparation limits sample throughput, not the GC-MS analysis itself

VERIFIN

13

Conclusions

- To totally utilize Fast-GC, also faster sample preparation method has to be developed
  - solid phase micro extraction fibers (SPME)
- For higher analyte concentrations, split ratio can be easily increased to obtain good separation efficiency
- When chemical backgound is high, conventional bore columns are more efficient for separation
- Fast-GC compared to conventional GC is slightly less sensitive
- When neat toxic chemicals need to be identified, fast-GC is potential technique to give fast response

VERIFIN

14

Summary

- Use of fast GC is very attractive for on-site analysis in field laboratories of the inspection team of the OPCW and the SIBCRA sampling and analysis teams
- During year 2007, in our laboratory the fast GC-methods will be optimised to find the most suitable GC and MS conditions for reliable, fast and sensitive analysis
- Different sample matrices will be tested including diesel background
- The method has to fulfill the requirements established by the OPCW for the use of retention indices as part of identification process together with EI-mass spectra

VERIFIN

Appendix 6. Presentations on toxins
Screening and verification of ricin by chemical analytical methods; Crister Åstot, FOI.

Screening and Verification of Ricin by Chemical Analytical Methods

Dr. Crister Astot
FOI, Swedish Defence Research Agency
CBRN Defence and Security

FOI

TWG on Sampling and Analysis
Madrid 10-11 Dec 2007

Outline

- Screening
- Verification
- Remarks with respect to CWC

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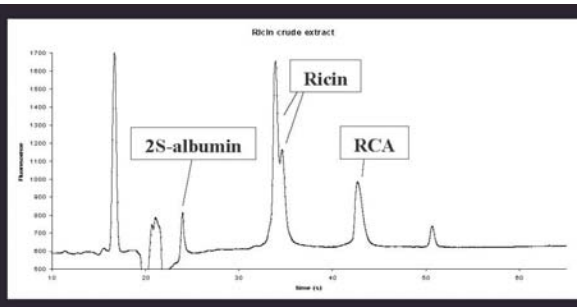
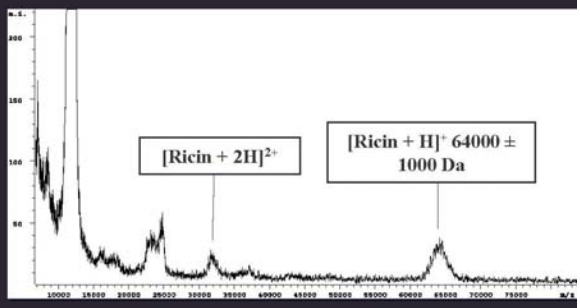
Screening

- For protein content
  - Gel electrophoresis
- Mass Spectrometry
  - MALDI TOF Mass Spectrometry
  - MALDI TOF peptide mapping



Gel electrophoresis of crude ricin extract



MALDI MS of crude ricin extract

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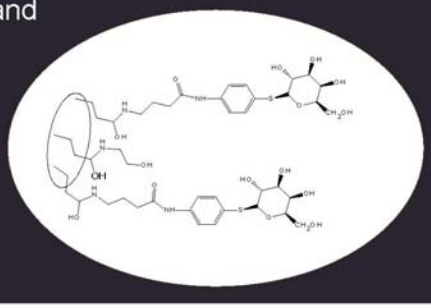
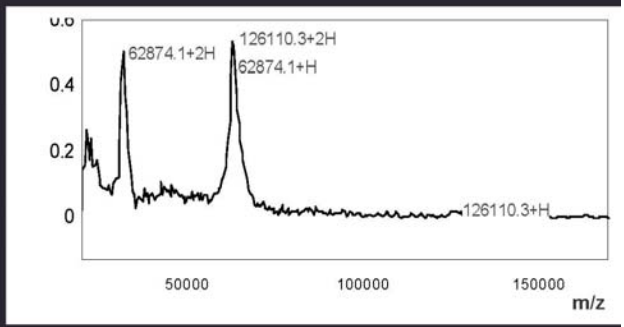
Screening, cont.

- Surface Enhanced Laser Desorption Ionisation on activated target chip (SELDI MS)
  - Affinity capture of ricin
    - Antibody
    - Galactosyl Ligand-modified surface
      - » Enhanced specificity
      - » Enhanced sensitivity

Target surface modified with Galactosyl Ligand





SELDI MS of a crude ricin extract

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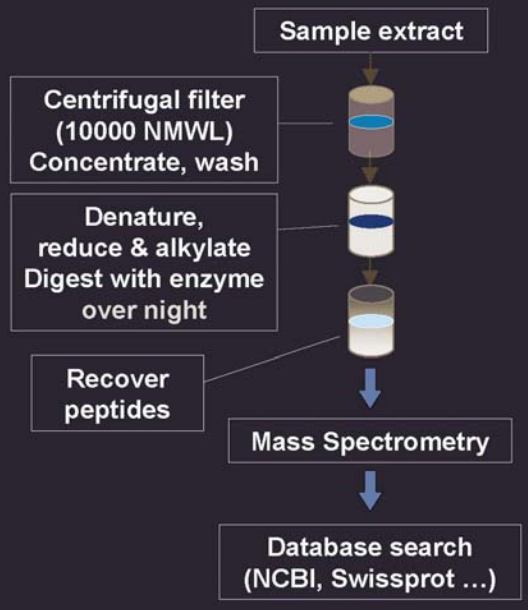
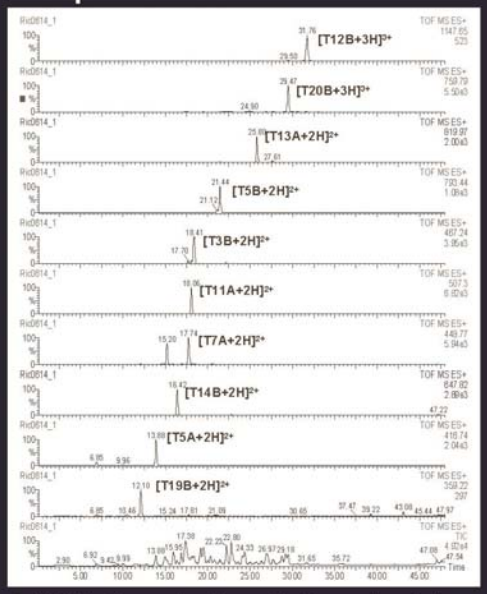
Enzymatic digestion – ”standard procedure”





LC-MS: trypsin digest of crude ricin

Fredriksson *et al*, Anal. Chem. 2005, 77, 1545-1555.

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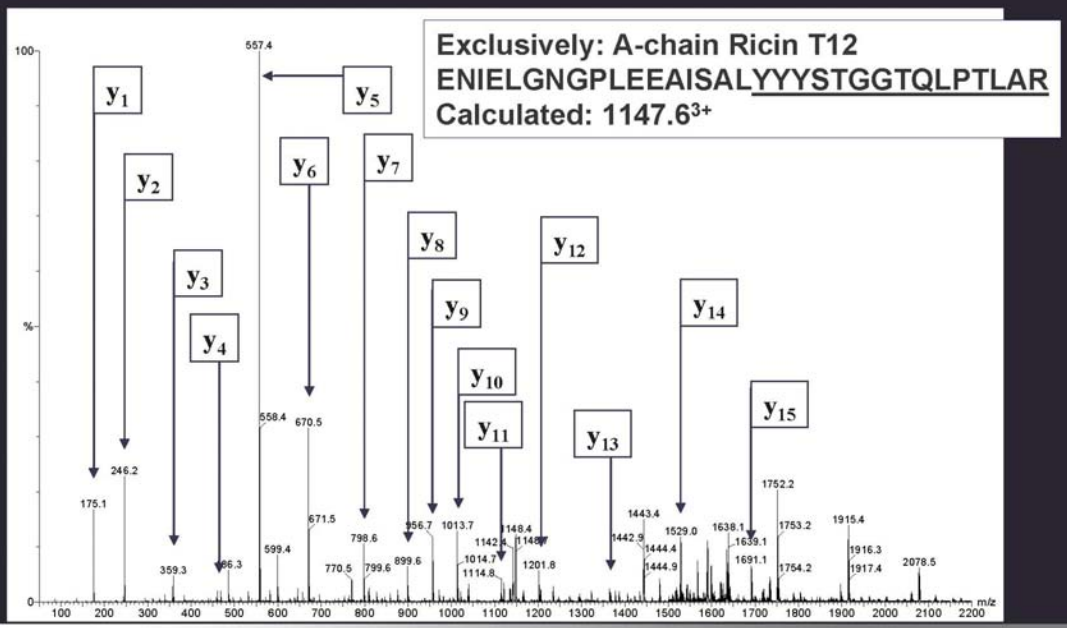
MS/MS spectrum of a peptide $(M+3H)^{3+}$, *m/z* 1147.6



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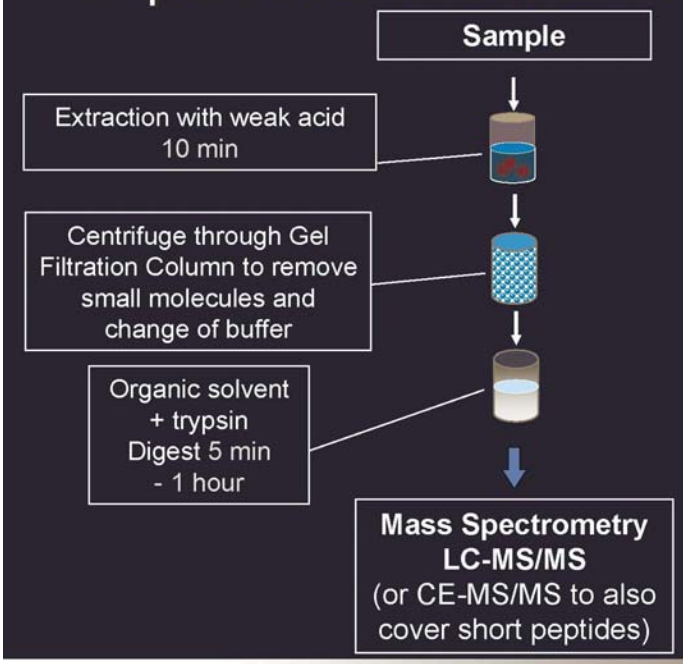
Rapid solvent assisted enzymatic digestion



Östin *et al.* Anal. Chem. 2007, 79, 6271-6278

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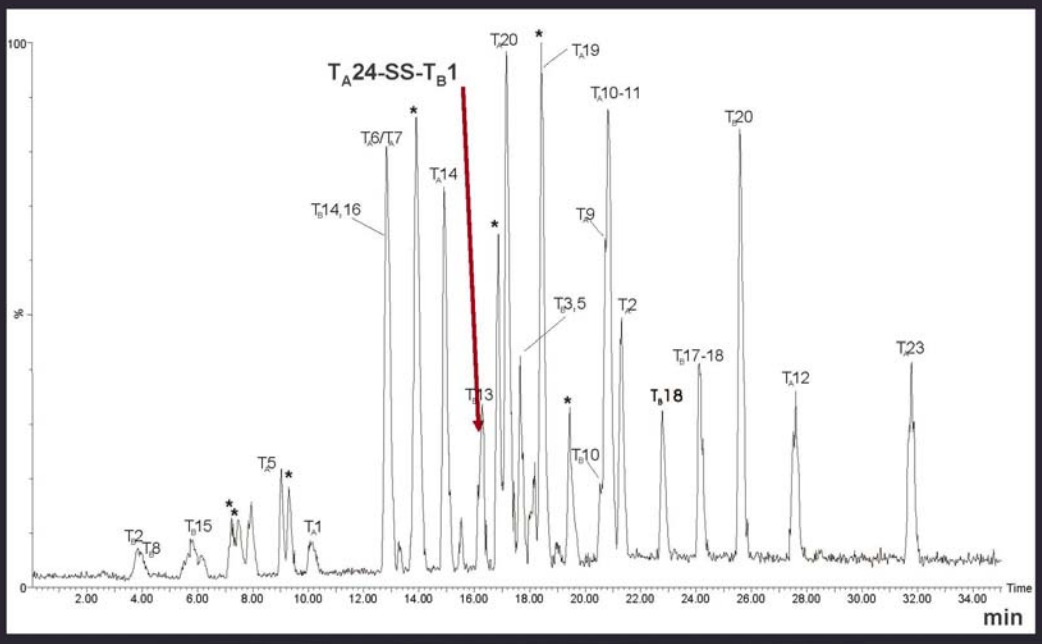
Peptide map from solvent assisted trypsin digestion



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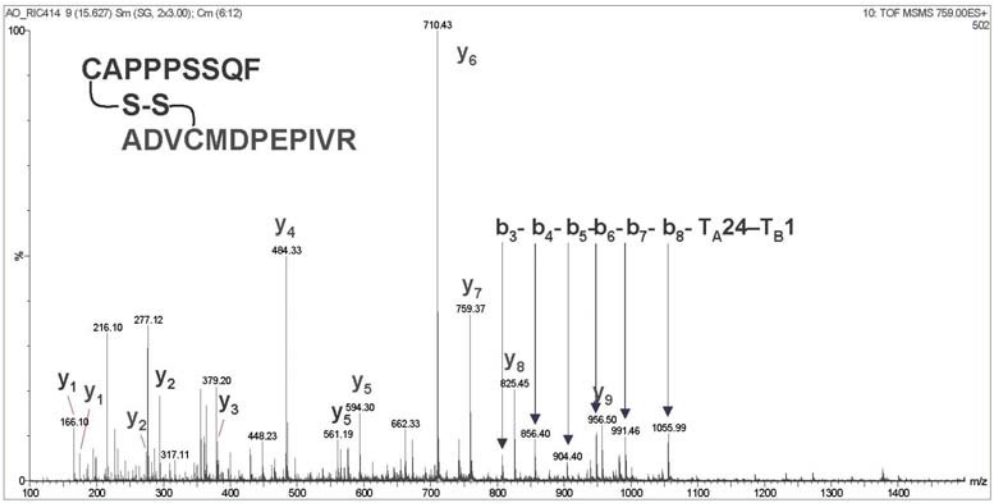
Intact A-B disulfide bond indicates active ricin

Chain A-B disulfide linked peptide: $(T_A24{-}SS{-}T_B1)^{2+}$



Ricin Specific Peptides and Sequence Coverage

Method	% A- chain sequence coverage	Ricin specific A-chain peptides	% B-chain sequence coverage	Ricin specific B-chain peptides
Reduction, carboxymethylation and trypsin digestion	**78**	**8**	**72**	**10**
Organic solvent assisted trypsin digestion, 1 h (5 min)	**96**	**10**	**71**	**2* + 8**
Formic acid cleavage , 2 h	**20**	**2**	**20**	**5**
Reduction and Formic acid, 2h	**20**	**2**	**49**	**7**

*** Double peptides with intact – SS – bonds**

Verification

- Sample preparation for LC-MS/MS
  - Microgram sample amount sufficient
    - LC-MS/MS detection limit for identification < 10 ng of ricin (100 fmol)
- Impure and dilute samples samples may require clean-up and concentration
  - Gel filtration column
  - Molecular weight cutoff filter
  - Affinity capture of ricin (column format) - Enhanced specificity and sensitivity

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Verification, cont

- Enzymatic digestion
  - Reduction and carboxymethylation – trypsin digestion
  - Solvent assisted trypsin digestion
- LC-MS and LC-MS/MS
  - Peptide map
  - MS/MS of peptides for sequence information
- Unambigous verification?

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Unambiguous identification - OPCW / CWC
Where to set the requirements?

- Comparison with authentic reference data (reference compound)
  - Amino acid sequence of two ricin-specific peptides from each of the A- and B-chains?
  - Ricin D?
  - Database concideration? Reference material?
- … "Ricin remains accountable as long as the A-SS-B bond is not broken, irrespective of the isoforms present." … [1]
  - Functional assay or MALDI-MS?
  - Intact A-SS-B bond as shown by double peptide in solvent assisted trypsin digestion – MS/MS?
- Two separate techniques? As for "traditional" CWA´s?

[1] OPCW. SAB-II/DG.1. 3 June 1999.

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The "Ricin-group" at FOI,
CBRN Defence and Security

Sten-Åke Fredriksson
Anders Östin
Tomas Bergström
Elisabeth Artursson
Margaretha Lundqvist
Calle Nilsson

Co-operation with Ben van Baar and co-workers at TNO is gratefully acknowledged

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Analytical methods for the detection and identification of the CWC schedule 1 compounds saxitoxin and ricin; Martin Schär, Spiez Laboratory.

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Analytical Methods for the Detection and Identification of the CWC Schedule 1 Compounds Saxitoxin and Ricin

Meeting of the SAB TWG on Sampling and Analysis
Madrid, 10/11 December, 2007

Dr. Martin Schaer
Spiez Laboratory, Switzerland

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

LABOR SPIEZ - Analytical Chemistry

Peter Siegenthaler

Urs Meier

Jean-Claude Dutoit

Thomas Clare

Martin Schär

Schweizerische Eidgenossenschaft
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CWC Schedule 1 – Toxin analysis

- Which question to answer? - Possible scenarios
- Which analytical methods?
- Which set of methods could be reasonable for a designated lab?
- Pros and Cons of related identification criteria

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Toxins - Defining the Analytical Problem

Preliminary Report of the Scientific Advisory Board:

SAB-10/1
Annex 2
page 8

Ricin

3.9 The SAB was also asked by the Director-General to consider what, within the meaning of the Convention, constitutes ricin. Such an understanding may be helpful to States Parties, and could be incorporated into the OPCW Declaration Handbook. The SAB recommends the following definition of ricin:

> "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."

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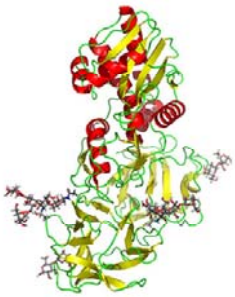
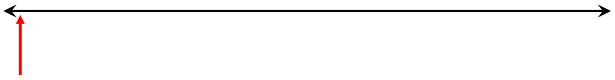
Toxins - Defining the Analytical Problem

The appropriate analytical method depends on the particular problem



C, H, N,
O, S,..



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Ricin - Possible Scenarios

Attack against a catering company with Ricin	
Activity:	(Civilian) Protection
Samples:	Sweet leftovers (food), maybe clinical samples
Compounds:	Intact Ricin
Detection:	Immunoassays (ELISA) Peptide map (MALDI-TOF MS, enrichment step prior to MS analysis) Bioassay: Cell culture

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Ricin - Possible Scenarios

Attack against a catering company with Ricin

Activity:	(Civilian) Protection
Samples:	Sweet leftovers (food), maybe clinical samples
Compounds:	Intact Ricin
Detection:	Immunoassays (ELISA) Peptide map (MALDI-TOF MS, enrichment step prior to MS analysis) Bioassay: Cell culture

Poisoning of domestic animals with biological fertilizer

Activity:	(Civilian) Protection
Samples:	fertilizer leftovers or fertilizer samples (from seller, user etc.)
Compounds:	Intact Ricin
Detection:	Immunoassays (ELISA) Peptide map (MALDI-TOF MS, enrichment step prior to MS analysis) Bioassay: Cell culture

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Ricin - Possible Scenarios

Military area or industry

Activity:	Forensic investigation (e.g. OPCW: aleged use, UN-Mission)
Samples:	Castor oil production residues wipe test samples (production equipment) protein solutions
Compounds:	Intact Ricin Denatured Ricin A-Chain glycosylated B-Chain glycosylated (Any fragments of Ricin)
Detection:	Immunoassays (ELISA) Peptide map (MALDI-TOF MS) GC-MS: Ricinin

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Ricin - Possible Scenarios

Military area or industry

Activity:	Forensic investigation (e.g. OPCW: aleged use, UN-Mission)
Samples:	Castor oil production residues
	wipe test samples (production equipment)
	protein solutions
Compounds:	Intact Ricin
	Denatured Ricin
	A-Chain glycosylated
	B-Chain glycosylated
	(Any fragments of Ricin)
Detection:	Immunoassays (ELISA)
	Peptide map (MALDI-TOF MS)
	GC-MS: Ricinin

Terrorist activities

Activity:	Forensic investigation (Governmental)
Samples:	Plant residues (seeds R.c.)
	wipe test samples (production equipment)
	Soil samples
Compounds:	Intact Ricin
	Denatured Ricin
	A-Chain, B-chain glycosylated
	(Any fragments of Ricin)
Detection:	Immunoassays (ELISA)
	Peptide map (MALDI-TOF MS)
	GC-MS: Ricinin

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NATO Criteria for Toxin Identification

Level of confidence	Analytical methods	
	Proteinaceous toxins	Nonprotein-aceuos toxins
Provisional identification	The chromatographic retention data acquired for the mid-spectrum agent under two different experimental conditions matches that of known mid-spectrum agent data or A specific immunological assay registers a positive response or The molecular mass of the mid-spectrum agent, determined by MS, matches that of known mid-spectrum agent data	

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NATO Criteria for Toxin Identification

Level of confidence	Analytical methods	
	Proteinaceous toxins	Nonprotein-aceuos toxins
Provisional identification	The chromatographic retention data acquired for the mid-spectrum agent under two different experimental conditions matches that of known mid-spectrum agent data or A specific immunological assay registers a positive response or The molecular mass of the mid-spectrum agent, determined by MS, matches that of known mid-spectrum agent data	
Confirmed identification	Any two of the three criteria for provisional identification are met	
	or The molecular mass and corresponding mass map of the enzymatic digestion products (with a minimum of three products) matches that of known mid-spectrum agent data	

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NATO Criteria for Toxin Identification

Level of confidence	Analytical methods	
	Proteinaceous toxins	Nonprotein-aceuos toxins
Provisional identification	The chromatographic retention data acquired for the mid-spectrum agent under two different experimental conditions matches that of known mid-spectrum agent data or A specific immunological assay registers a positive response or The molecular mass of the mid-spectrum agent, determined by MS, matches that of known mid-spectrum agent data	
Confirmed identification	Any two of the three criteria for provisional identification are met	
	or The molecular mass and corresponding mass map of the enzymatic digestion products (with a minimum of three products) matches that of known mid-spectrum agent data	
Unambiguous identification	The chromatographic retention data acquired for the mid-spectrum agent under two different experimental conditions matches that of an authentic reference standard acquired under identical experimental conditions or a specific immunological assay registers a positive response plus The molecular mass and corresponding mass map of the enzymatic digestion products (with a minimum of three products) matches that for an authentic reference standard acquired under identical experimental conditions plus Sequence data for the mid-spectrum agent matches that for an authentic reference standard acquired under identical experimental conditions	Similar to OPCW criteria for chemicals

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Ricin – Current Situation

1. LC/MS analysis
   - Molecular mass range
   - Peak broadening of the convoluted electrospray ion signals due to the glycan heterogeneity
   - Retention time
   - Comparison with reference

2. Immunoassay

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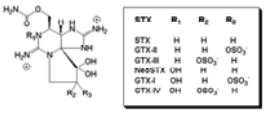
Saxitoxin (STX)

Identification Problem	Analysis	Method	Criteria for Unambiguous Identification	
			Criteria	Pros and Cons
Intact STX	Molecular weight M_w Retention time R_t	LC/ESI-MS	M_w = 299.1±0.2 Da (Monoisotopic) Ret. time = R_t±0.2 min.	+ Well defined values - LC/MS/(MS) equipment needed
	Fragments of STX	LC/ESI-MS/MS	Parent ion m/z 300 Typical fragments: m/z 282, 204, 138 Ret. time = R_t±0.2 min.	
	Retention time, selective fluorescence detection	LC/Fluorescence	Ret. time = R_t±0.2 min.	- Fluorescence exitation/detection needed
	Epitope recognition	Lateral Flow Assay	Detection yes / no	+ Speed / Sensitivity - Cross reactivity
		Immunoassays[1)]	Detection yes / no	+ Sensitivity /Selectivity + Quantitative analysis - Cross reactivity
	Biological activity	Bioassay: Cell Culture	Concentration for 50% mortality (EC_{50}-value)	- Time needed
STX in difficult matrices	Chromatographic separation, specific detection MS: MRM-Method	LC/Fluorescence	Ret. time = R_t±0.2 min.	+ Well defined values
		LC/ESI-MS/MS	M_w = 299.1±0.2 Da (Monoisotopic) Ret. time = R_t±0.2 min.	
Variants[2)]	Same analysis methods as above for STX. Isobaric molecules may only be distinguished by LC/ESI-MS/MS if retention times or fragment mass spectra are different. Variants not yet measured, however CWC-Schedule 1 "saxitoxin" refers to STX (free base) exclusively[3)].			

[1)] Immunoassays:
- ELISA (Enzyme Linked Immunosorbent Assay)
- ECL-I (Electrochemiluminescence Immunoassay; e.g. BioVeris M1M)
- Suspension Array System (e.g. BioPlex-200)

[2)] Variants of saxitoxin
Source: FDA



[3)] OPCW-Document: "THE TREATMENT OF SALTS OF SCHEDULED CHEMICALS WITHIN THE SCHEDULES TO THE CHEMICAL WEAPONS CONVENTION", RC-1/NAT.15, p. 4, 29. April 2003.

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Intact Ricin

Problem	Analysis	Method	Criteria for Unambiguous Identification	
			Criteria	Pros and Cons
Identification of Intact ricin	Molecular weight[1]	ESI-MS, MALDI-MS	M_w(Intact Ricin) ± Δ Da	- ESI: M_w with deconvolution processing not well defined - MALDI: Resolution too low
		SDS-PAGE	M_w(Intact Ricin) ± Δ Da	- Mass accuracy too low
	Molecular weight, Retention time, Heterogeneity[1]	LC/ESI-MS	M_w(Intact Ricin) ± Δ Da Ret. time = R_t±0.2 min. (Pseudo molecular ion region shows heterogeneity)	- ESI: M_w with deconvolution not well defined - heterogeneity dependent on compound - LC: Well defined value
	Epitope recognition	Lateral Flow assay	Detection yes / no	+ Speed / Sensitivity - Cross reactivity
		Immunoassays[2]	Detection yes / no	+ Sensitivity /Selectivity + Quantitative analysis - Cross reactivity
	Biological activity	Bioassay: Cell culture	Concentration for 50% mortality (EC_{50}-value)	+ Sensitivity - Time needed
	Intact S-bridges (Biological activity)	M_w by MS	M_w(Intact Ricin) ± Δ Da No A- or B-chain detected	+ No A- and B-chain present - ESI: M_w with deconvolution not well defined - Oxidation/reduction can be reversible
		Bioassay: Cell culture	Concentration for 50% mortality (EC_{50}-value)	+ Sensitivity - Time needed
		Reducing and non-reducing SDS/PAGE	M_w of intact ricin vs. M_w of A- and B-chain	+ Qualitative result - Quantitation?
	Peptide map Sequence tag MS/MS fragmentation ion search	Tryptic digest, LC/ESI-MS/MS, MALDI-MS, Static Nanospray-MS	Tryptic fragments m/z	+ Fragments well defined + Peptide masses with ESI and MALDI easily measurable - MALDI and/or nanospray needed
Glycan / Glycan structure	Total molecular mass of all glycans	Deglycosylation of ricin. Comparison of molecular weights by LC/ESI-MS, MALDI-MS	Δm=M_w($Ricin_{Glycosylated}$)-M_w($Ricin_{Deglycosylated}$) M_w($Ricin_{Deglycosylated}$)	+ ESI: Heterogeneity vs. homogeneity detectable and M_w($Ricin_{Deglycosylated}$) - Δm not well defined (peak distr. of the glyc. species)
Isoforms / Variants	Same analysis methods as above for ricin. Reference material needed. In principle, all methods established for the ricin samples should also be applicable to other isoforms and variants. However masses and retention times may be different.			

[1] Heterogeneity/homogeneity of the pseudo molecular ion.

[2] Immunoassays:
- ELISA (Enzyme Linked Immunosorbent Assay)
- ECL-I (Electrochemiluminescence - Immunoassay; i.e. BioVeris M1M)
- Suspension Array System (i.e. BioPlex-200)

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A- and B-chain of Ricin

Problem	Analysis	Method	Criteria for Unambiguous Identification	
			Criteria	Criteria
Glycosylated A-chain / Glycosylated B-chain	Molecular weight, Retention time, Heterogeneity[1]	LC/ESI-MS MALDI-MS	LC/ESI-MS: M_w ± Δ Da Ret. time = R_t±0.2 min. MALDI: M_w ± Δ Da	- ESI: M_w with deconvolution not well defined - heterogeneity dependent on compound - LC: Well defined value
	Molecular weight	SDS-PAGE	Mass ± 10%	- Mass accuracy too low + Detection of impurities
	Peptide map Sequence tag MS/MS fragmentation ion search	Tryptic digest, LC/ESI-MS/MS, MALDI-MS, Static Nanospray-MS	Tryptic fragments A-Chain, B-Chain: specific m/z-values	+ Fragments well defined + Peptide masses with ESI or MALDI measurable - Tryptic digest eventually impaired by glycans + Separation of A- and B-chain not necessary - MALDI, nanospray needed
	Epitope recognition	Immunoassays[2]	Detection yes / no	+ Sensitivity /Selectivity + Quantitative analysis - Cross reactivity
	Sequence analysis	LC/ESI-MS/MS	CAD-Fragments: specific m/z-values	+ Fragments well defined - Sample prep (digest)
Deglycosylated A-chain / Deglycosylated B-chain	Molecular mass, Retention time, Homogeneity[1]	ESI-MS, MALDI-MS	LC/ESI-MS: M_w ± Δ Da Ret. time = R_t±0.2 min. MALDI: M_w ± Δ Da	+ ESI: M_w with deconvolution well defined + MALDI: Mass accuracy and resolution eventually too low for identification - Deglycosylation step
	Peptide map Sequence tag MS/MS fragmentation ion search	Tryptic digest, LC/ESI-MS, MALDI-MS, Static Nanospray-MS	Tryptic fragments A-Chain, B-Chain: specific m/z-values	+ Fragments well defined + Tryptic digest not impaired by glycans + Peptide masses with ESI and MALDI easily measurable + Separation of A- and B-chain not necessary - MALDI, nanospray needed
	Sequence analysis complex matrices	LC/ESI-MS/MS	CAD-Fragments: specific m/z-values	+ Fragments well defined - Sample prep (digest)

[1] Heterogeneity/homogeneity of the pseudo molecular ion.

[2] Immunoassays:
- ELISA (Enzyme Linked Immunosorbent Assay)
- ECL-I (Electrochemiluminescence - Immunoassay; i.e. BioVeris M1M)
- Suspension Array System (i.e. BioPlex-200)

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The End

Thank you for you attention!

Annex 3

**CHEMICALS WITH DATA FOR ON-SITE USE
ACCEPTED BY THE VALIDATION GROUP
BUT NOT APPROVED BY THE EXECUTIVE COUNCIL**

1,2-Bis(2-trimethylsilyloxyethylsulfonyl)ethane
1,2-Bis(2-trimethylsilyloxyethylthio)ethane
1,3-Bis(2-trimethylsilyloxyethylsulfonyl)propane
1,4-Dithiane
1,4-Oxathiane
1,5-Bis(2-trimethylsilyloxyethylsulfonyl)pentane
10-Chloro-5,10-dihydrophenarsazine
2-Chlorobenzylidene malononitrile
2-Chloroethyl 2-hydroxyethyl sulfide
2-Chloroethyl vinyl sulfone
2-Chloroethyl vinyl sulfone
2-Chloroethyl vinyl sulfoxide
2-Chloroethyl vinyl sulfide
2-Hydroxyethyl vinyl sulfide
Benzyl bromide
Bis(2-chloroethyl)disulfide
Bis(2-chloroethyl)disulfide
Bis(2-chloroethyl)sulfone
Bis(2-chloroethyl)sulfone
Bis(2-chloroethyl)sulfoxide
Bis(2-N,N-diisopropylaminoethyl)sulfide
Bis(2-N,N-dimethylaminoethyl)sulfide
Bis(2-N,N-dipropylaminoethyl)disulfide
Bis(2-trimethylsilyloxyethyl)sulfone
Bis(2-trimethylsilyloxyethylsulfonyl)methane
Bis(2-trimethylsilyloxyethylthioethyl)ether
Bis(2-trimethylsilyloxyethylthiomethyl)ether
Capsaicin
Dibenz[b,f][1,4]oxazepine
Diethyl methyl phosphate
Diethyl methyl phosphate
Diethyl trimethylsilyl phosphate
Diethyl trimethylsilyl phosphate
Diphenylchloroarsine
Diphenylchloroarsine
Diphenylcyanoarsine
Diphenylcyanoarsine
Divinylsulfone
Divinylsulfone
Divinylsulfoxide
Divinylsulfoxide
2-Ethoxyethyl N-ethyl-N-isopropylphosphoramidocyanidate

2-Ethoxyethyl N-ethyl-N-propylphosphoramidocyanidate
2-Ethoxyethyl N-isopropyl-N-methylphosphoramidocyanidate
2-Ethoxyethyl N-methyl-N-propylphosphoramidocyanidate
Ethyl bromoacetate
Ethyl iodoacetate
Ethyl methyl phosphorocyanidate
Ethyldichloroarsine
2-Methoxyethyl N-ethyl-N-isopropylphosphoramidocyanidate
2-Methoxyethyl N-ethyl-N-propylphosphoramidocyanidate
2-Methoxyethyl N-isopropyl-N-methylphosphoramidocyanidate
2-Methoxyethyl N-isopropyl-N-propylphosphoramidocyanidate
Methyldichloroarsine
m-Xylyl bromide
N-(2-Hydroxyethyl)thiomorpholine
N-(2-Trimethylsilyloxyethyl)thiomorpholine
O,O-Diethyl S-methyl phosphorothiolate
O,O-Diethyl O-trimethylsilyl phosphorothionate
O,O-Diethyl O-trimethylsilyl phosphorothionate
o-Xylyl bromide
Pelargonic acid vanillylamide
Phosgene oxime
p-Xylyl bromide
w-Chloroacetophenone
Triphenylarsine
Propyl diphenylarsinothioite
Ethyl diphenylarsinothioite
Methyl (diphenylarsino)thioacetate
Tetraphenyldiarsoxane
S,S-Diethyl N,N-dipropylphosphoramidodithiolate
S-Ethyl S-isobutyl N,N-dipropylphosphoramidodithiolate
S,S-Diisobutyl N,N-dipropylphosphoramidodithiolate
S,S-Dipropyl N,N-dipropylphosphoramidodithiolate
Ethyl 2-methoxyethyl N,N-dimethylphosphoramidate
2-Chloroethylthioethyl 2-chloroethyldithioethyl ether
Bis(2-chloroethyl)trisulfide
2-(2-Chlorovinyl)-1,3,6,2-dioxathiarsocane
Trimethyl arsenite
Dimethyl 2-chlorovinylarsonite
Methyl bis(2-chlorovinyl)arsinite
Tetrakis(2-chlorovinyl)diarsoxane
O,O-Diethyl O-methyl phosphorothionate
Diethyl tert-butyldimethylsilyl phosphate
tert-Butyldimethylsilyl N,N-dimethylaminoethyl-2-sulfonate
Trimethylsilyl N,N-dimethylaminoethyl-2-sulfonate
Methyl trimethylsilyl N,N-dipropylphosphoramidate
Methyl N,N-dimethylaminoethyl-2-sulfonate
Methyl N,N-dimethylaminoethyl-2-sulfonate
Methyl N,N-diethylaminoethyl-2-sulfonate
Methyl N,N-diethylaminoethyl-2-sulfonate

Methyl N,N-diisopropylaminoethyl-2-sulfonate
Methyl N,N-dipropylaminoethyl-2-sulfonate
Methyl trimethylsilyl N,N-diethylphosphoramidate
Isopropyl trimethylsilyl N,N-diethylphosphoramidate
Methyl tert-butyldimethylsilyl N,N-diethylphosphoramidate
Methyl tert-butyldimethylsilyl N,N-dipropylphosphoramidate
Ethyl trimethylsilyl N,N-diethylphosphoramidate
Isopropyl trimethylsilyl N,N-dipropylphosphoramidate
Ethyl tert-butyldimethylsilyl N,N-diethylphosphoramidate
Methyl N-ethyl-N-methylaminoethyl-2-sulfonate
Methyl N-methyl-N-propylaminoethyl-2-sulfonate
Methyl N-isopropyl-N-methylaminoethyl-2-sulfonate
Methyl N-ethyl-N-isopropylaminoethyl-2-sulfonate
Methyl N-isopropyl-N-propylaminoethyl-2-sulfonate

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