OPCW

Scientific Advisory Board

Fourteenth Session
9 – 11 November 2009

SAB-14/1
11 November 2009
Original: ENGLISH

REPORT OF THE FOURTEENTH SESSION OF THE SCIENTIFIC ADVISORY BOARD

1. AGENDA ITEM ONE – Opening of the session

The Scientific Advisory Board (SAB) met for its Fourteenth Session from 9 to 11 November 2009 at the OPCW Headquarters in The Hague, the Netherlands. The session was opened by the Chairperson of the SAB, Philip Coleman of South Africa. 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

The SAB adopted the following agenda for its Fourteenth Session:

1. Opening of the session
2. Adoption of the agenda
3. *Tour de table* to introduce Scientific Advisory Board members
4. Welcome address by the Director-General
5. Overview of developments at the OPCW since the last session of the Scientific Advisory Board
6. Establishment of a drafting committee
7. Applications of nanotechnology to improve defensive countermeasures against chemical weapons
8. Consideration of the report of the fourth meeting of the temporary working group on sampling and analysis
9. Scheduled chemicals, including ricin and saxitoxin
10. Review of operational requirements and technical specifications for inspection equipment

11. Future work of the Scientific Advisory Board

12. Any other business

13. Adoption of the report

14. Closure of the session

3. AGENDA ITEM THREE – *Tour de table* to introduce Scientific Advisory Board members

The meeting was opened with the introduction of existing SAB members for the benefit of the five new members: Djafer Benachour from Algeria, Alejandra Graciela Suárez from Argentina, Michael Geist from Germany, Muhammad Zafar-Uz-Zaman from Pakistan, and Slavica Vučinić from Serbia.

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

4.1 The Director-General welcomed the members of the SAB, in particular the five new members.

4.2 The Director-General highlighted the importance of advances in science and technology with regard to the work of the OPCW. He welcomed the fact that there would be a presentation on the applications of nanotechnology for protection against chemical weapons and a presentation on optical and electrical detection of chemical weapons agents and implications of nanoscience. The Director-General also welcomed the ongoing cooperation between the OPCW Laboratory and the temporary working group on sampling and analysis. He thanked the SAB for the advice it had provided to the Technical Secretariat (hereinafter "the Secretariat") on the review of operational requirements and technical specifications for inspection equipment.

4.3 The Director-General expressed his appreciation to the two speakers mentioned below and thanked them for sharing their knowledge and for their support of the work of the SAB.

5. AGENDA ITEM FIVE – Overview of developments at the OPCW since the last session of the Scientific Advisory Board

5.1 The Secretary gave a presentation to the SAB on developments at the OPCW since the SAB's Thirteenth Session (which was held from 30 March to 1 April 2009). The members were informed about the status of destruction of Category 1 chemical weapons as at 30 September 2009.

5.2 The SAB was informed of the progress made on universality and of the fact that, as at 9 November 2009, there were 188 States Parties to the Chemical Weapons Convention (hereinafter "the Convention"). The Secretary also briefed the SAB on what the follow-up had been in relation 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"). In addition, the SAB was briefed on the financial status of its trust fund.

5.3 In the discussion following the presentation by the Secretary, the SAB members emphasised the importance of a meeting with the United States National Academy of Sciences/National Research Council Committee of Chemical Demilitarization (NAS/NRC CCD). The SAB proposed that the Secretary prepare a document containing the objectives and agenda of such a meeting. The document would support a possible request for voluntary contributions for the convening of the proposed meeting.

5.4 The SAB discussed the ongoing uncertainty regarding funding for two regular meetings per year of the SAB as well as of its temporary working group on sampling and analysis. The SAB is concerned that this uncertainty may have a negative impact on the continuity of the ongoing work and hopes that it can be resolved expeditiously.

6. AGENDA ITEM SIX – Establishment of a drafting committee

The SAB established a drafting committee, composed of four of its members, to prepare a draft report of its Fourteenth Session.

7. AGENDA ITEM SEVEN – Applications of nanotechnology to improve defensive countermeasures against chemical weapons

7.1 The joint IUPAC[1]/OPCW meeting on the impact of scientific developments on the Convention, held in 2007 in Zagreb, Croatia, recognised that nanotechnologies and particle engineering create opportunities for enhanced medical countermeasures (for example, drug delivery, the development of new sensors, and diagnostics) and for the development of more effective filter materials for respirators, protective clothing, and decontaminants[2]. At its Thirteenth Session, the SAB received presentations on the basics of nanotechnology and on the toxicology of nanomaterials. During that session, the SAB recommended that potential applications for protective purposes be addressed in greater detail in a future session. The SAB therefore invited Margaret Kosal (Assistant Professor at the Sam Nunn School of International Affairs, Atlanta, Georgia, United States of America) and Timothy M. Swager (Professor of Chemistry, Head of the Department of Chemistry and Associate Director of the Institute for Soldier Nanotechnologies at the Massachusetts Institute of Technology, Cambridge, Massachusetts, United States of America) to brief its members on applications of nanotechnology to improve defensive countermeasures against chemical weapons.

Subitem 7(a): Presentation 1

7.2 Margaret Kosal provided an overview of applications of nanotechnology for the protection against chemical warfare agents. Nanotechnology offers the opportunity of a ‘revolutionary’ approach to improved countermeasures, as opposed to the evolutionary development of the past 90 years. Ms Kosal described aspects of chemical and biological defence that may be influenced significantly by developments in nanotechnology in the next 20 years. These include physical protection, detection,

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1 IUPAC = International Union of Pure and Applied Chemistry.

2 Paragraph 7 of the 2008 IUPAC Technical Report: “Impact of Scientific Developments on the Chemical Weapons Convention”.

decontamination, diagnostics, and medical countermeasures. A survey of scientists from different disciplines indicated that research into the structure and function of nanomaterials and their interface with biological systems is regarded as having the greatest potential for scientific progress. Ms Kosal described qualities of an organisation that facilitate scientific breakthroughs in this interdisciplinary research area. These include a change in "risk tolerance". The fostering of international scientific cooperation is required in order to encourage and develop the beneficial application of nanoscience. Ms Kosal's presentation appears as Annex 2 to this report.

Subitem 7(b): Presentation 2

7.3 Timothy Swager discussed the design of novel chemical sensors based on conjugated polymers (CPs) and carbon nanotubes (CNTs). He described how a nano-sized CP can amplify the response of a fluorescence or resistance-based chemosensor, resulting from its ability to transport optical excitations or electrical charge over large delocalised distances. These transport properties provide the increased sensitivity and versatility of CPs and CNTs over small molecule chemosensors. The properties of the sensor can be modified by adding new functional diversity to the CPs and CNTs. For example, sensors are being explored for chemical-warfare agent detection that incorporate novel nucleophilic functionalities that react with the electrophilic chemical warfare agent to produce excited states. This research has high potential for enhancing the detection of chemical warfare agents. A vapour sensor that has high sensitivity to detect trinitrotoluene has been incorporated into a mobile robotic device for finding improvised explosive devices; this has been used successfully in field operations. Mr Swager's presentation appears as Annex 3 to this report.

Subitem 7(c): Discussion

7.4 In the discussion following the presentations, the SAB members emphasised that the two presentations were very useful, in terms of the interesting and relevant issues discussed, as well as the expertise, quality, and clarity of the two presenters. The SAB members reaffirmed the value of guest presenters being invited to SAB meetings to provide presentations on advances and developments in science and technology that are relevant to the Convention.

7.5 With respect to nanotechnology, the SAB members noted the considerable potential benefits of nanotechnology and nanomaterials in the improvement of protective measures against chemical weapons, including physical protection, detection, decontamination, and medical countermeasures. The SAB members also recognised the potential for misuse of certain developments in nanotechnology for purposes prohibited by the Convention.

Subitem 7(d): Recommendations of the Scientific Advisory Board

7.6 The SAB should continue to maintain a close watch on developments in nanotechnology and nanomaterials, for instance by inviting speakers to give presentations based on scientific advances relevant to the Convention at future sessions of the SAB.

8. AGENDA ITEM EIGHT – Consideration of the report of the fourth meeting of the temporary working group on sampling and analysis

8.1 The SAB received the report of the fourth meeting of the temporary working group on sampling and analysis, held on 5 and 6 November 2009, which appears as Annex 4 to this report. Robin Black, Chairperson of the group, presented the key findings, conclusions, and recommendations, which are summarised below.

Subitem 8(a): Conclusions and recommendations of the temporary working group on sampling and analysis

8.2 The temporary working group on sampling and analysis reaffirmed a previous recommendation that non-scheduled derivatives of scheduled chemicals, in particular derivatives of lewisites 1 and 2 that are necessary for gas chromatography-mass spectrometry (GC-MS) analysis, should be added to the OPCW Central Analytical Database (OCAD).

8.3 Shortening of analysis time for on-site analysis, particularly sample preparation time for aqueous samples, continues to be regarded as a high priority by the temporary working group on sampling and analysis and the OPCW Laboratory. The temporary working group noted the progress made by the OPCW Laboratory on shortening GC run times and the possible application of thermal desorption to aqueous samples. Hollow-fibre liquid-phase microextraction still appears to be the most promising technique for aqueous samples. The temporary working group recommended that laboratories pool their knowledge in the area of sample preparation of aqueous samples and collaborate on liquid-phase microextraction.

8.4 Desorption electrospray mass spectrometry and miniaturisation of mass spectrometry were identified as topics to be discussed at the next meeting.

8.5 The temporary working group noted that the first OPCW confidence-building exercise on biomedical samples had started, as recommended by the SAB at its Ninth Session (subparagraph 2.5(d) of SAB-9/1, dated 14 February 2007).

8.6 A correspondence group on trace analysis has been established within the temporary working group. Criteria must be defined if trace analysis is to be accepted for investigations of alleged use of chemical weapons. The use of an "identification points" system (in the manner of the system used by the European Commission for investigating the use of banned substances in animal products) was provisionally rejected because of its inconsistency with the system currently used in proficiency tests. As a first step, the results of the first confidence-building exercise on biomedical samples will be evaluated against criteria used by regulatory bodies such as the World Anti-Doping Agency and the United States Federal Drug Administration.

8.7 Methods for the identification of saxitoxin were proposed. Assuming that two independent techniques would be required, consistent with current practice in OPCW designated laboratories, liquid chromatography-tandem mass spectrometry (LC-MS/MS) (or capillary electrophoresis-tandem mass spectrometry (CE-MS/MS)) were proposed as the primary methods for an unequivocal identification, combined with a screening method such as an immunoassay or LC-fluorescence (GC is not applicable to saxitoxin).

8.8 Methods for the identification of ricin were proposed, although further consideration is required. LC-MS/MS sequencing of peptides formed on enzymatic digestion was considered necessary for an unequivocal identification, in combination with additional MS data, an immunoassay, functional assay or polymerase chain reaction. The results of a round-robin exercise recently held by the Global Health Security Action Group will be made available to the temporary working group.

Subitem 8(b): Recommendations of the SAB on the report of the fourth meeting of the temporary working group on sampling and analysis

8.9 The SAB reviewed the recommendations made by the temporary working group under subitem 8(a) and congratulated the group on the progress made on the important issues. The SAB endorsed the recommendations of the group and encouraged the temporary working group and its chairperson to continue the important work.

8.10 The SAB noted with concern that the addition of relevant non-scheduled chemicals to the OCAD, which is required for on-site analysis to determine the absence of Schedule 1 chemicals, is still awaiting the approval of the Executive Council.

9. AGENDA ITEM NINE – Scheduled chemicals, including ricin and saxitoxin

Subitem 9(a): Ricin

9.1 The SAB, during its Eighth Session (which was held from 8 to 10 February 2006), was asked to clarify what constitutes the Schedule 1 toxin ricin within the meaning of the Convention. Ricin is produced by the castor bean plant *Ricinus communis* and accounts for approximately one to two percent by weight of the castor bean. A large tonnage of deactivated ricin is discarded annually in the waste from castor-oil processing plants; the latter are exempt from the Convention's reporting procedures under Schedule 1 (C-V/DEC.17, dated 18 May 2000).

9.2 A precise definition of ricin poses a number of problems. Ricin is a glycoprotein (molecular mass ~65,000 Da), which is composed of two glycoprotein chains, referred to as the A and B chains, linked by a disulfide bond. The B chain facilitates penetration of ricin into cells; the A chain is responsible for the toxic action of ricin, which is a catalytic inhibition of protein synthesis. Neither the A nor B chains in isolation have high toxicity. A problem in defining ricin is that there are a number of natural variants, new ones may appear, and man-made variants may be produced by recombinant DNA technology. The following definition was proposed during the Eighth Session of the SAB (paragraph 3.2 of SAB-8/1, dated 10 February 2006 and Corr.1, dated 15 March 2006):

> "All forms of ricin originating from *Ricinus communis*, including any possible variations in the structure of the molecule arising from natural processes or man-made 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."

9.3 A representative of a National Authority pointed out to the SAB that it is not clear whether this definition encompasses ricin-like materials being developed as therapeutic agents. These materials have an additional linkage between recombinant A and B chains, in the form of a short peptide chain. This additional linkage has been designed in such a way that it is cleaved by proteases (enzymes that cleave peptide bonds in proteins) that are released or over-produced by tumour cells. These variants are several orders of magnitude less toxic than naturally produced ricin. It has also been pointed out that there are significant differences in the nucleotide sequences of the genes encoding these potential drugs, and consequently significant differences in the protein sequences.

9.4 The view of the SAB was that the inclusion of such materials within the definition of ricin does not serve the object and purpose of the Convention. These variants have been designed in such a way that they have greatly reduced toxicity, except towards tumour cells. Furthermore, the main reason for ricin having attracted interest as a chemical warfare agent is its natural production by the castor bean plant, which is harvested on a large industrial scale.

9.5 The SAB proposed the following modified definition of what constitutes ricin, which excludes materials with a second linkage between the A and B chains in addition to a disulfide bond:

> "All forms of ricin originating from *Ricinus communis*, including any variations in the structure of the molecule arising from natural processes, <u>or man-made modification designed to maintain or enhance toxicity</u>, are to be considered ricin as long as they conform to the basic 'native' bipartite molecular structure of ricin that is required for mammalian toxicity, <u>i.e. A and B chains linked only by a disulfide bond</u> (A-S-S-B). Once the inter-chain S-S bond is broken or the protein denatured, it is no longer ricin."

Subitem 9(b): Saxitoxin

9.6 The SAB, during its Thirteenth Session, agreed to prepare a fact sheet on saxitoxin (paragraph 6.5 of SAB-13/1, dated 1 April 2009). The fact sheet was prepared by Robert Mathews in the intersessional period, and was presented to the SAB for discussion. The fact sheet appears as Annex 5 to this report.

9.7 When the fact sheet was introduced, it was emphasised that this was still a draft, and other SAB members were invited to provide additional relevant information and drafting suggestions for the fact sheet, in particular for the section on usage of saxitoxin in civil society for peaceful purposes.

9.8 In the discussion that followed, it was emphasised that while the nomenclature of saxitoxin has changed since the toxin was first isolated in 1957, since the late 1980s, the doubly-charged cation has been referred to as saxitoxin, and that negotiators had wanted to include the agent TZ (saxitoxin dihydrochloride) and other forms of weaponisable saxitoxin in Schedule 1. The fact that the Convention lists saxitoxin with the Chemical Abstracts Service (CAS) registry number of the free base (saxitoxin dihydrate) is not inconsistent with this understanding, as the CAS registry numbers were intended as "identification aids" rather than "unique identifiers" for the various Schedules of chemicals.

9.9 A preliminary discussion ensued among SAB members on whether saxitoxin should continue to be listed in Schedule 1, or whether it might be more appropriate in Schedule 2. It was agreed that SAB members should consider this matter during the intersessional period with relevant stakeholders, including National Authorities, researchers working with saxitoxin, and traders in paralytic shellfish poisoning (PSP) diagnostic test kits. With respect to problems that had been highlighted on transfers of saxitoxin, including the implications of the prohibition of retransfers of Schedule 1 chemicals for the international trade in saxitoxin and the ready availability of PSP diagnostic test kits, another option raised was the possibility of a technical change to the Convention that would waive the retransfer prohibition on very small quantities of saxitoxin for medical/diagnostic purposes.

10. AGENDA ITEM TEN – Review of operational requirements and technical specifications for inspection equipment

10.1 The SAB was briefed by Irvine Swahn (Team Leader, OPCW Inspectorate Division) on the revision of the decision on the list of approved equipment with operational requirements and technical specifications (C-I/DEC.71 and Corr.1, both dated 23 May 1997). He informed the SAB that the document had been updated to include all relevant decisions taken since the First Special Session of the Conference of the States Parties to Review the Operation of the Chemical Weapons Convention, that an attempt had been made to make the specifications more generic in order to facilitate equipment procurement, and that operational instructions from the Preparatory Commission that are currently contained in the Secretariat's quality system documentation had been deleted. The aforementioned changes to the decision had allowed grouping of the inspection equipment into six appendices. The number of pages of the document had also been reduced.

Recommendations of the Scientific Advisory Board on the review of operational requirements and technical specifications for inspection equipment

10.2 Members of the SAB had been providing comments on the revised document during the intersessional period since the SAB convened for its Thirteenth Session. The SAB congratulated the Secretariat on its comprehensive revision of the decision and expressed its agreement with the content and structure of the proposed revision. The SAB conveyed its willingness to remain active in the matter of inspection equipment and requested that it be briefed regularly by the Secretariat on any substantial changes.

11. AGENDA ITEM ELEVEN – Future work of the Scientific Advisory Board

11.1 The SAB noted the importance of its maintaining a watching brief on the development of novel toxic chemicals relevant to the Convention, as highlighted at the Second Review Conference in subparagraph 2.49(b) of RC-2/S/1*, dated 31 March 2008, and paragraph 55 of RC-2/DG.2, dated 7 April 2008). The SAB recommended that this matter be discussed at a future session.

Agenda for the Fifteenth Session of the Scientific Advisory Board

11.2 The SAB discussed the agenda for its Fifteenth Session, currently planned to take place from Monday 12 to Wednesday 14 April 2010.

11.3 During its Fifteenth Session, the SAB will continue its discussion on the issue of saxitoxin, with the aim of preparing its final recommendation. In addition, the SAB will continue its discussion on ricin and will also consider the feedback from the confidence-building exercise on the analysis of biomedical samples. Furthermore, it will consider the outcome of the round-robin exercise on ricin recently held by the Global Health Security Action Group.

11.4 The following topics were proposed for discussion during the Fifteenth Session of the SAB or during future sessions:

(a) Applications of nanomaterials and nanotechnology in drug delivery;

(b) Molecularly imprinted polymers;

(c) Hazards associated with nanomaterials; and

(d) Methods of destruction for old chemical weapons.

12. AGENDA ITEM TWELVE – Any other business

Subitem 12(a): Possible OPCW involvement in the International Year of Chemistry 2011

12.1 Robert Mathews undertook to enquire about events being planned for the International Year of Chemistry 2011, and to report back to the SAB at its next session.

Subitem 12(b): Terms of office of Scientific Advisory Board members

12.2 With reference to the Note by the Director-General entitled "Terms of Office of Members of the Scientific Advisory Board" (EC-57/DG.14 C-14/DG.5, dated 1 July 2009), the Secretary updated the members of the SAB on the state of play regarding the staggered departure of those members whose mandate will finish in 2010. He drew their attention to the draft decision on that subject (C-14/DEC/CRP.1, dated 31 July 2009) that was to be considered by the Conference of the States Parties at its Fourteenth Session.

Subitem 12 (c): Possible ways of enhancing the interaction between the Scientific Advisory Board and States Parties, as well as the policy-making organs

12.3 The Secretary briefed the members of the SAB on the proposals contained in the Note by the Director-General entitled "Possible Ways of Enhancing the Interaction Between the Scientific Advisory Board and States Parties, as well as the Policy-making Organs, Making Best Use of Governmental Experts" (EC-58/DG.1, dated 22 July 2009).

13. AGENDA ITEM THIRTEEN – Adoption of the report

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

14. AGENDA ITEM FOURTEEN – Closure of the session

The Chairperson closed the session at 17:00 on 11 November 2009.

Annexes:

Annex 1: List of Participants in the Fourteenth Session of the Scientific Advisory Board

Annex 2 (English only, unedited): Presentation by Margaret Kosal: Nanotechnology: Applications for the Protection Against Chemical Warfare Agents

Annex 3 (English only, unedited): Presentation by Timothy M. Swager: Optical and Electrical Detection of CW Agents and Implications of Nanoscience

Annex 4 (English only, unedited): Report of the Fourth Meeting of the Temporary Working Group on Sampling and Analysis

Annex 5 (English only, unedited): Saxitoxin Fact Sheet

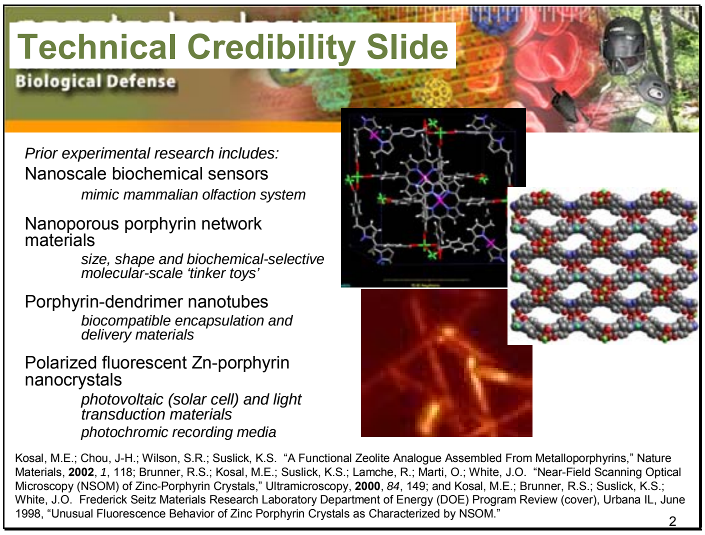
Annex 1

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

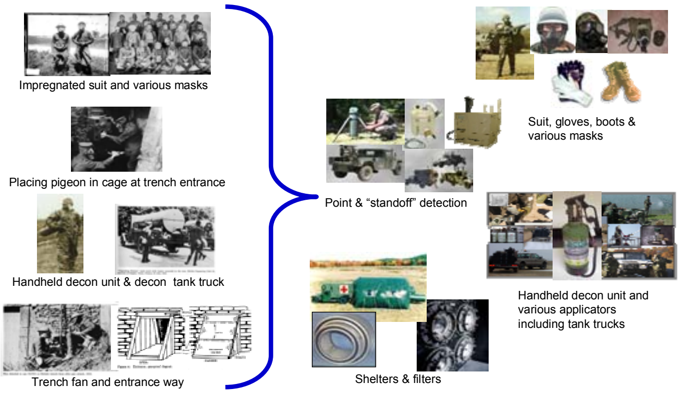
	Participant	State Party
1.	Djafer Benachour	Algeria
2.	Alejandra Graciela Suárez	Argentina
3.	Robert Mathews	Australia
4.	Zhiqiang Xia	China
5.	Danko Škare	Croatia
6.	Jean-Claude Tabet	France
7.	Michael Geist	Germany
8.	László Halász	Hungary
9.	R. Vijayaraghavan	India
10.	Mahdi Balali-Mood	Iran (Islamic Republic of)
11.	Alberto Breccia Fratadocchi	Italy
12.	Abdool Kader Jackaria	Mauritius
13.	José González Chávez	Mexico
14.	Godwin Ogbadu	Nigeria
15.	Muhammad Zafar-Uz-Zaman	Pakistan
16.	Titos Quibuyen	Philippines
17.	Igor V. Rybalchenko	Russian Federation
18.	Slavica Vučinić	Serbia
19.	Philip Coleman	South Africa
20.	Stefan Mogl	Switzerland
21.	Valery Kukhar	Ukraine
22.	Robin Black	United Kingdom of Great Britain and Northern Ireland

Annex 2

PRESENTATION BY MARGARET KOSAL: NANOTECHNOLOGY: APPLICATIONS FOR THE PROTECTION AGAINST CHEMICAL WARFARE AGENTS



Evolutionary Approach: 1918 vs 2009



A Operator's Perspective on Possible Nanotechnology Applications for Chemical Operations

COL Barry Lowe, Chief of Staff
20th Support Command (CBRNE)

Individual Protection

- Applications to make uniform material capable of providing protection against chemical agents, as well as other toxic materials
- Applications to make uniform material "react instantly" to become armor in the event of a bullet or fragment impact
- Applications for prophylaxis against inhalation or ingestion of chemical agents and toxic materials
- Applications for use as antidotes (this may be more feasible in the near term)

50 Years of Nanoscale Science

1959: Feynman: *"The principles of physics do not speak against the possibility of maneuvering things atom by atom."*

1974: *Notion* of molecular electronics

1981: Scanning Tunneling Microscope (STM)

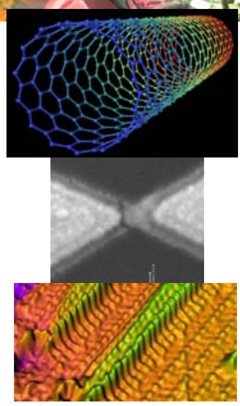
1985: Buckminsterfullerene (C_{60}) discovered

1986: Atomic Force Microscope (AFM) invented

1990s: Large scale "bottom-up" nanostructure synthesis: nanotubes, nanowires, clusters, molecular magnets, synthetic proteins, …

1998: Demonstration of Carbon Nanotube Transistor

2007: Graphene; Applications: Nanocomposites, Nanosensors, Molecular Electronics …



6

Two Scenario Types

Capability

Countermeasures Development

New or nano-enabled capabilities against current chem-bio capability gaps, i.e., problems that we currently lack solutions or have less than ideal passive defense capabilities.

Threat

Malfeasant Co-option of Nanotechnology

Potential misuse of nanotechnology by state or non-state actors, including proliferation challenges.

Organization: 9 Focus Areas

Countermeasure Capabilities

1. CW Detection & Diagnostics
2. BW Detection & Diagnostics
3. Physical Protection
4. Decontamination, Remediation, & Consequence Management
5. Medical Countermeasures

Threats (Malfeasant Co-option of Nanotechnology)

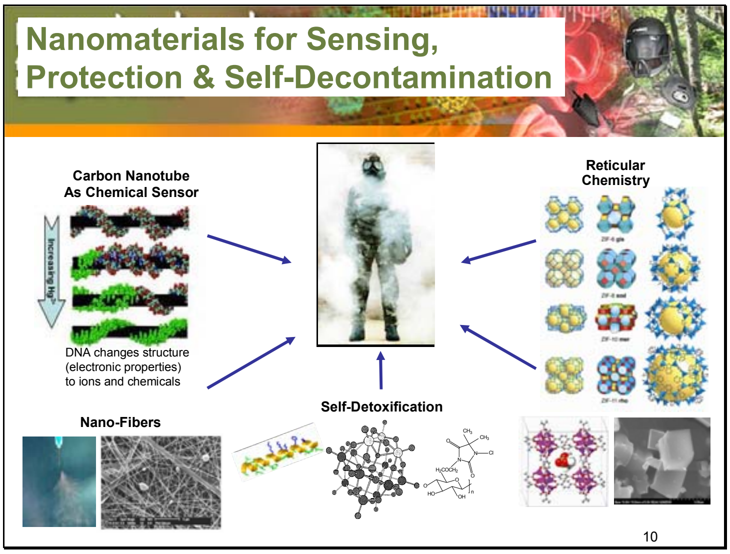
1. New and Nano-enabled Biochemical Agents
2. Malfeasant Exploitation of Toxicological or Other Deleterious Health Effects
3. Circumventing Vaccines and Evasion of Medical Countermeasures
4. Self-Assembled Materials & Devices to Molecular Assemblers

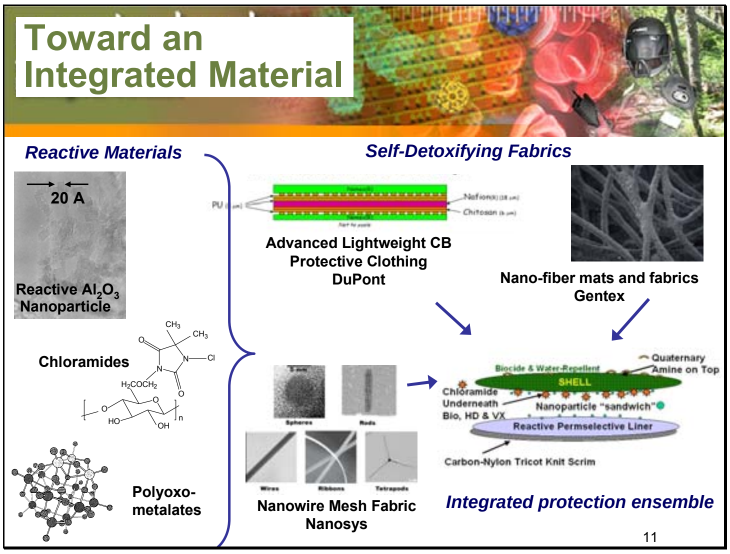
FOUO

Countermeasure Scenarios

- **Physical Protection**
  - Integrated Shielding System, i.e., a *"Borg Shield"*
- **Detection & Diagnostics**
  - Bio-mimetic/single-cell/tissue-based sensors
  - Tamper-resistant, self-powered, smart nano-tags
  - Synergistic "lab on a spec" hidden in a commercial device
  - Engineered seeds and genetically-adapted crops replicating detectors for global monitoring
- **Decontamination**
  - Post-exposure decontaminant
  - Decontaminating pre-coat
  - Large scale decontamination/demilitarization
- **Medical Countermeasures**
  - Nanoadjuvants, increase countermeasure efficacy (pre- & post-exposure)
  - Hyperimmunoglobulin
  - Nanoparticle for chemical absorption
- **BW Detection & Diagnostics**
  - Distributed networks
  - Massively multiplexed assays
  - Rapid identification & characterization
  - Anomaly detection

FOUO





S&T Capabilities

underlying the 2030 nano-enabled protection countermeasures

2020 – Enhanced Enabling Technologies and Manufacturing

Enhanced selectivity/specificity/discrimination in embedded sensors
- Less uncertainty/higher accuracy
- Rapid detection at minimal concentrations

Library of materials/properties
- Polymers and crystals
- Secondary, tertiary – specificity
- Deterministic control of functions/performance
- …neutralize, heal, shed

Hybrid properties: multifunctional materials, composites

Manufacturability of materials (industrial infrastructure)
- Integration of threat information, sensors with material response
- Testing and verification/validation of systems
- Verification and validation of manufacturing systems
- 2025 - Begin prototyping "Borg" shields

2010 – Enabling Technologies

Embedded sensors
- Piezoelectric sensors
- Quantum tunneling sensors
- Nanofiber arrays

Self decontaminating/self detoxifying materials

Control porosity
- Electroactive or other responsive textiles
- 0.5 nm to 10 microns
- Smart materials that open & close

Library of materials and properties
- Infrastructure for developing, maintaining the library
- Polymers and crystals are backbone structure (e.g. MOFs)
- Focus on design of primary structure: engineering the backbone
- At-will control of structures

12

2030 CW Detection Countermeasures

1. Tamper-resistant, self-powered, smart nano-tags:
   - Covert sensors
   - Air, Land, and Sea dispersion of smart nanodetectors, ability to disperse
   - Remotely interogatable nano-barcode material mixed into a chemical shipment of dispersed on a remote site
2. Bio-mimetic / cell / tissue-based sensors, instrumented cells (nano-canary) with pre-symptomatic sensitivity to biomarkers, e.g., neuronal-B-cell conjugate
   - Implantable
   - Chip-based bio-nanocircuit
   - Replicate major oxygen-carrying and neuromuscular signaling pathways
3. Engineered seeds and genetically-adapted crops replicating detectors for global monitoring of threats
4. Synergistic “lab on a spec”, e.g. no macroscopic analog

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S&T Capabilities

underlying the 2030 nano-enabled detection countermeasures

2020

- Scale energy sources to nanoscale
- Biotic/abiotic interface, e.g. robust transduction method with long-life scaled energetics
- Analytical tool / techniques for nanoscale
- Robust, controllable, reproducible manufacturing techniques for nanodevices
- Orthogonal transduction mechanisms based on mass, charge transfer, etc. that provide measurement capabilities, with preconcentration & preselection mechanisms

2010

- Targeted attachment of molecules &/or functional groups to nanostructures
- Advances in lithographic techniques, materials processing, nanoimprinting, nano-manufacturing processes
- Regio-selective structure of nanoparticles
- Demonstrated autonomous motion in nanoparticles
- Blood / tissue sensor interface
- Functional nanowires/tubes to sense/interrogate cells
- Robust, passive nanotag (RFID) that store information & interface or coupling /integration of RFID to nanodevice

14

2030 Scenarios: Decontamination & Hazard Mitigation

Decontaminating solutions to address the 2030 threat may fall into the following categories

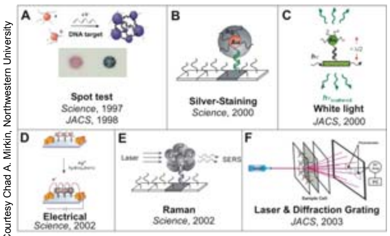
1. **Post-exposure Decontaminant**
2. **Decontaminating Pre-Coat**
3. **Large-scale decontamination/ demilitarization**

15

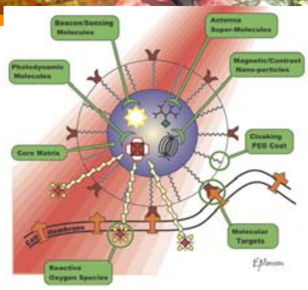
Nano-enabled Diagnostics & Therapeutics

DNA-based diagnostics without PCR ∴

- faster temporally
- diagnostically earlier
- more sensitive
- fewer false positives



Courtesy Chad A. Mirkin, Northwestern University



- Selective targeting
- Tissue or cell-specific
- Prolonged or delayed release

16

S&T Capabilities
underlying the 2030 nano-enabled medical countermeasures

2020

- Continued coupling with diagnostic, therapeutic, and detection efforts
- Demonstration of proof of concept for nanomaterials as countermeasure for multiple CWAs in a ruggidized environment.
- Protein/genomic library for threat agents for select threat agents.
- Manufacturing, disposal characterization of nanomaterials

2010

- Identification of selected targets (receptor:ligand interactions) for specific delivery to affected cells/tissues
- PK, toxicity, immune system interactions
- Nanomaterials stability, ease of administration, interactions with other compounds.

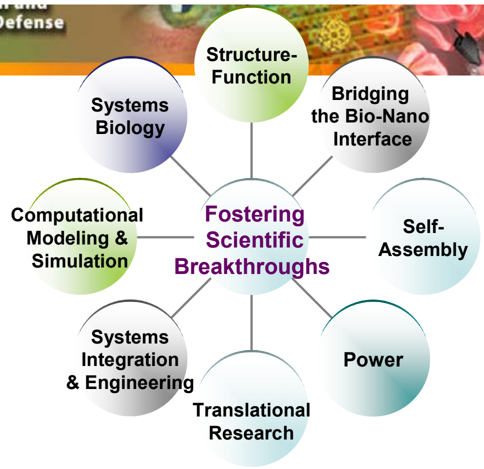
17

Prioritization of 2030 Countermeasures

		$	% of $
A3-1	Borg Shield: Develop backbone of ideal materials, secondary and tertiary structures (DOD = "System"), interface	943	16
A2-2	Bio-mimetic / single cell / tissue-based sensors, instrumented cells (nano-canary) e.g. B-cell sensors Implantable	659	11
A2-1	Tamper-resistant, self-powered, smart nano-tags	457	8
A5-3	Systems biology approach to exploitation of adversarial technology (develop of CM against threat)	453	8
A1-4	Massively Multiplexed Assays	373	6
A4-1	Post-exposure Decontaminant	353	6
A4-2	Decontaminating Pre-Coat	350	6
A1-3	Rapid ID/Characterization	339	6
A1-1	Distributed networks	320	5
A1-2	Anomaly detection	265	4
A2-4	Synergistic "lab on a spec" hidden in a commercial device, e.g. no macroscopic analog	223	4
A5-1	Hyperimmunoglobulin, convalescent sera rapid scale up of production	216	4
A5-2	Nanoadjuvants, increase countermeasure efficacy (pre- and post-)	202	3
A2-3	Engineered seeds and genetically-adapted crops replicating detectors for global monitoring of threats	166	3
A5-4	Nanoparticle for chemical absorption e.g. "nanoabsorbant"	161	3
A3-3	Large-scale manufacturing	160	3
A4-3	Large-scale decontamination/ demilitarization	132	2
A3-4	Nano chem/bio threats are neutralized and therefore pointless	85	1
A3-2	Outcome is a shielding system for individuals/groups, also equipment and materials	69	1
A4-4		0	0
	Total	5925	100

FOUO

Cross-Cutting Strategic Research Directions



nanotechnology
for Chemical and
Biological Defense

Structure/Function - Physiological

Understanding of nanomaterials structure/function at the systems biology level

- Studies of systems biology response of nanomaterials *in vitro* and *in vivo*
- Targeting (from tissue to subcellular)
- Toxicity

Security Implications

- Nanoparticles have significant potential for medical prophylactics and therapeutics for both chem. and biothreat agents
- Nanoparticles are clear, potential threat agents either by themselves or as agents of delivery; must understand and anticipate threat to design effective countermeasures

nanotechnology
for Chemical and
Biological Defense

Physiological Structure/Function of Nanomaterials
Potential Technical Approaches

- Study systems biology in various classes of mammalian cells exposed to various classes of nanoparticles (*in vitro*)
  - Gene expression/regulation
  - Proteomics
- Bioaccumulation in animal models vs. material and mode of delivery
  - Pathology
  - Histology
- Systems Biology
  - Test specific nanomaterials; understand exposure, accumulation, response
  - Design nanoparticles for known pathways associated with threat agents
- Use targeted delivery of nanoparticles to validate systems biology models
- Variability of structure/function vs. in vivo exposure to energy (EM, acoustic, thermal) and/or chemical stimuli
- Instrumentation/methods development for *in vivo* real-time, detection, imaging, and characterization of nanoparticles
- Methods for real-time, quantitative monitoring of biomolecules in a single cell
- Nanomaterials interactions with common biologics (proteins, nucleic acids)
  - Reverse panning
- Theory and Computation: Extension to interactions of complex, inorganic nanostructures with biomolecules (c.f. drug design)

Nanomaterials Structure/Functions – Control & Design

General principle for achieving each of the milestones listed below requires development of deeper knowledge of the physical chemistry of nanoparticle, including but not limited to:

- thermodynamics
- mechanical properties
- electrical properties
- optical & photonic properties
- magnetic properties
- chemical
- dynamics, kinetics, mechanisms
- toxicology of designed nanomaterials
- computation and modeling

Also requires confirmation that the reaction behaves as predicted

Power for Nanoscale Devices

Research Directions:

1. Scavenging energy from environment
   - Biological sources (Nano-parasitics) e.g. plant glucose
   - Non-biological sources, e.g. RF scavenging
2. Harvesting energy from human user
   - Nano-parasitics – e.g., derive energy from ATP
   - Mechanical energy from user activities/biological processes
3. Nano power generators for field use
   - Piezoelectric materials
4. Solar
5. Miniature Fuel Cells
6. Novel energy storage technologies

Self assembly, in vivo and in vitro

- ***Integration into organism***
- ***Replicating materials***
- ***How do things come together so we can defeat them***

Research Directions

1. Discovering the rules of molecular self assembly in aqueous environments
   - Essential for nano-bio interface
2. Establish reliable, robust, and transferable synthetic strategies for self-assembly of functional architectures
   - Essential for materials and devices with tailor-made, tunable functionality
3. Abiotic supramolecular self-replication
   - Enables low-cost, high-volume self-production of nanostructures
4. Self-repair
   - Enables damage-tolerant materials and systems with high purity
5. Understand how enzyme active sites work.
   - Required for creation of artificial enzymes (e.g. for decontamination, sensing)
6. Encapsulation of biological or synthetic payload
   - Required for robust delivery systems with low probability of detection (e.g. catalysts)
7. Stabilization of catalysts
   - Enable extended operations of artificial enzymes

Research Directions

		All	%	S&T Rsrch	S&T Prgm Mngt	Mil Ops	Fu-turist	Ana-lyst	Scrty Policy	DoD S&T	DoD Other	Other Gov	Non Govt
Potential for Nat Security Impact	Power	732	13	332	184	67	63	51	36	225	180	217	110
	System Biology	610	11	248	127	68	50	59	57	160	130	180	140
	Structure/Function of Nanomaterials	990	18	471	179	165	57	68	49	240	225	240	235
	Interface with biological systems	923	17	426	185	114	65	74	58	245	195	243	190
	Translational Research	441	8	164	96	94	23	34	30	115	95	151	80
	Systems integration and engineering	788	14	418	152	56	33	52	57	185	115	373	115
	Self assembly, in vivo and in vitro	751	14	230	120	88	50	104	78	175	195	306	75
	Modeling and Simulation	265	5	113	43	58	25	13	13	65	85	40	75
	Total	5500	100										
Potential for Scientific Progress	Power	627	11	265	168	62	56	41	34	200	172	160	95
	System Biology	717	13	284	176	78	66	55	58	210	142	240	125
	Structure/Function of Nanomaterials	1152	21	491	259	156	84	96	64	270	277	335	220
	Interface with biological systems	965	18	472	258	61	77	50	47	300	130	250	235
	Translational Research	526	10	171	154	75	38	41	45	190	127	134	75
	Systems integration and engineering	612	11	266	179	45	37	34	50	210	77	250	75
	Self assembly, in vivo and in vitro	480	9	167	129	65	46	35	37	170	102	118	90
	Modeling and Simulation	383	7	135	100	60	49	18	20	150	95	63	75
	Total	5460	100										

Fostering Scientific Breakthroughs

What qualities of an organization facilitate making major discoveries?

- Moderately high scientific diversity
- Capacity to recruit scientists who internalize scientific diversity
- Communication and social integration of scientists from different fields through *frequent* and *intense* interaction
- Leaders who integrate scientific diversity, have the capacity to understand the direction in which scientific research is moving, and provide rigorous criticism in a nurturing environment
- Flexibility and autonomy associated with loose coupling with the institutional environment

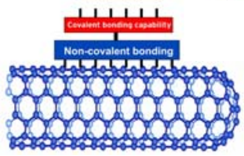
What qualities of an organization hamper the making of major discoveries?

- High differentiation – sharp boundaries among subunits such as departments, divisions, or colleges
- Hierarchical authority – centralized decision-making about research programs, number of personnel, work conditions, and/or budgetary matters
- Bureaucratic coordination – high standardization of rules and procedures
- Hyperdiversity – diversity to the degree that there cannot be effective communication among actors in different fields of science

Challenges of Export Controls

Case Study: Zyvex

- Richardson, Texas $10M revenues in 2005
- Nanoworks – nano tools, electron microscopes & photovoltaic applications
- Kentara™ – CNT dispersions in resins
- Current market
  - CNT-reinforced mountain bikes
  - Easton baseball bats
- Required to submit ITAR licenses for CNT-product exports to China
- In response, founder suggests may relocate to SE Asia



27

Recommendations & Conclusions

- Revolutionary, integrated and cross-cutting technologies are the key for future focus
  - Nanoscience offers the promise of future breakthroughs in "cutting edge" capabilities
- Need to foster revolutionary technology
  - Programmatic and institutional challenges paramount
  - Change in risk tolerance
- Fostering pro-active international scientific cooperation as means to encourage beneficial use of technology
  - Pro-active "Nano" Cooperative Threat Reduction
  - Track 2 diplomacy
- Strategic vision lacking

  We are currently attempting to limit the threat of "Biotechnology in an Age of Terrorism"
  - Serious threat anticipation with respect to nanotechnology should be initiated now
  - Balance "hype" & hope

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Sam Nunn School of International Affairs
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Georgia Institute of Technology

www.cistp.gatech.edu

Annex 3

PRESENTATION BY TIMOTHY M. SWAGER: OPTICAL AND ELECTRICAL DETECTION OF CW AGENTS AND IMPLICATIONS OF NANOSCIENCE

Optical and Electrical Detection of CWAgents and Implications of Nanoscience

OPCW-2009

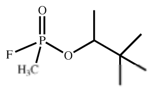
Timothy M. Swager
Massachusetts Institute of Technology

Key Points

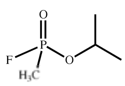
- Unique Chemical Reactivity is a Good Means for Selectivity.
- Synthesis of Unique Transduction Materials is a Productive Approach to Sensors
- Nanotechnology Principles are General and Transferable from on Sensng Scheme to Others
- There is Still a Need for Improved CW Detection

Chemical Warfare Agents

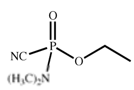
All are Strong Electrophiles



Soman

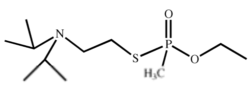


Sarin

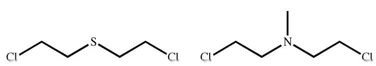


Tabun

Fluorine and Cyanide have Unique Reactivity



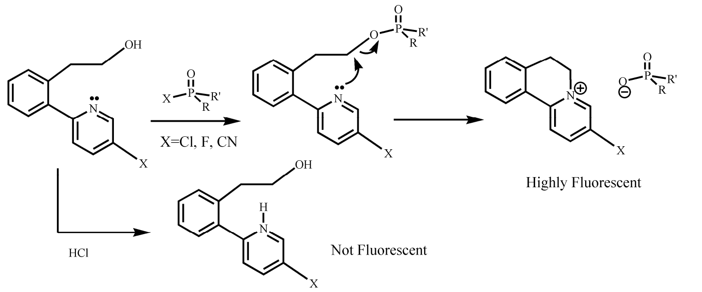
VX



Mustards

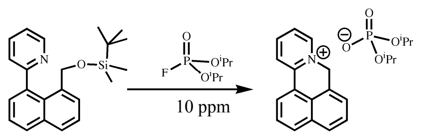
New Nerve Agent Indicators

Features: Near Instantaneous Response to Reactive Phosphates
High Specificity to Reactive Phosphates over Simple Brønsted Acids



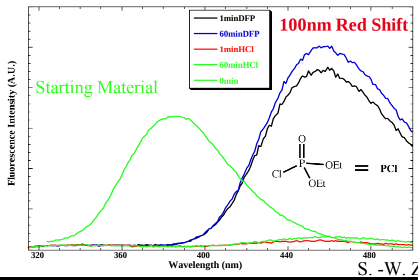
S. -W. Zhang

High Sensitivity Fluorescent Indicators



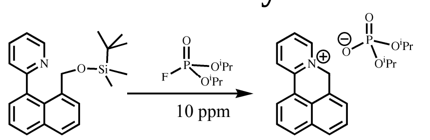
System is Highly Selective for DFP (Minimal Response to HCl)

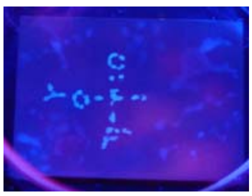
Greater than 20 times faster than the Ph-Pyr
$k_{obs} > 0.024\ s^{-1}$



S. -W. Zhang

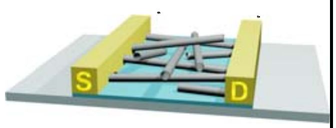
Applications of Reactivity Based Indicators

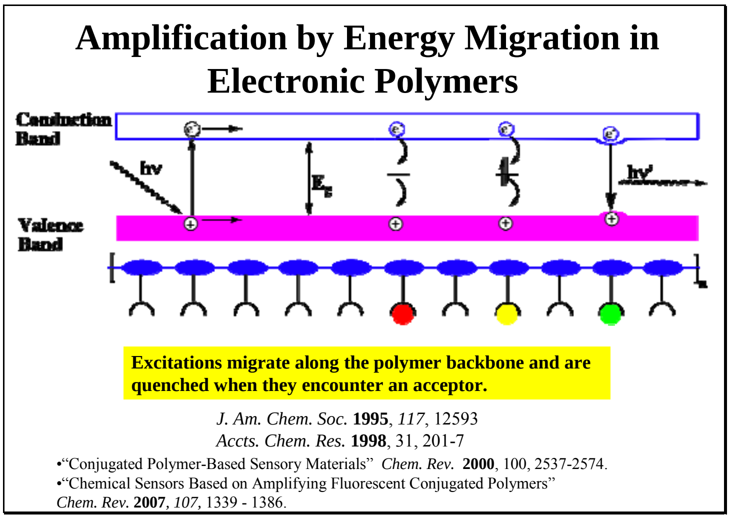


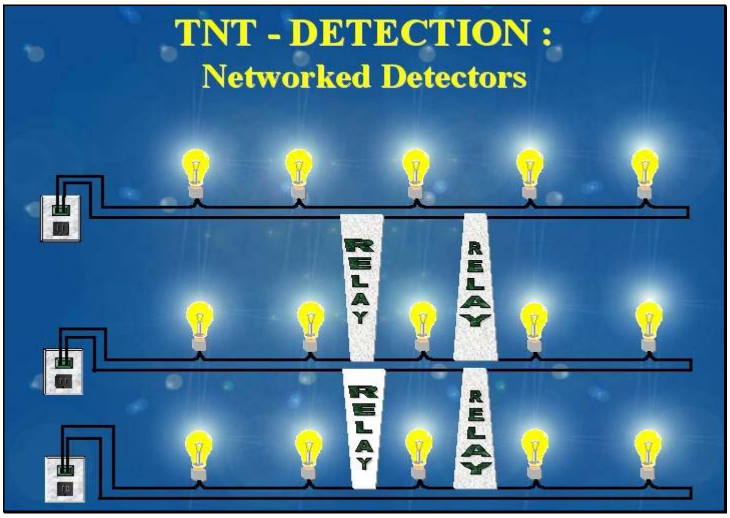


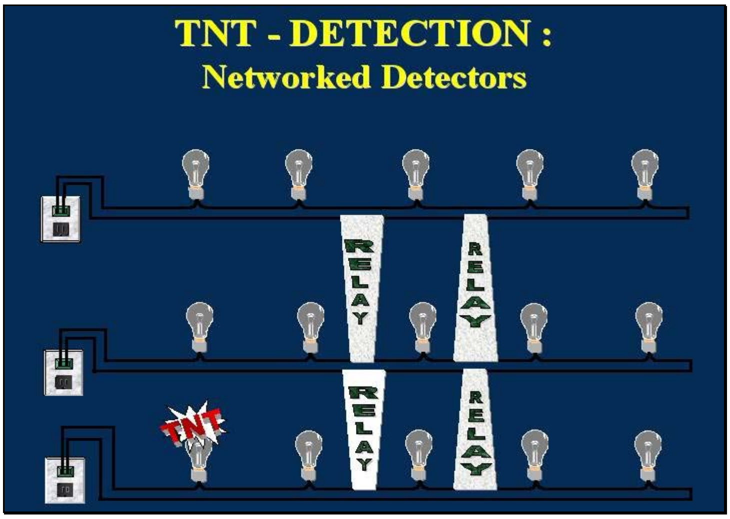
Surface Contamination Detection

Transduction Methods are Transferable to Nanotechnology Based Sensors

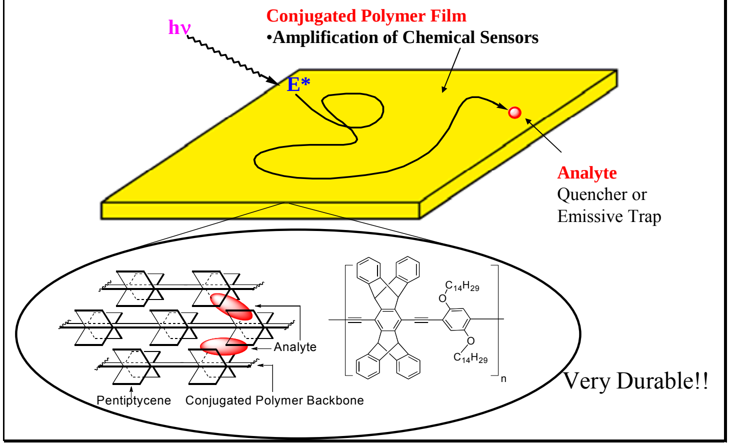



Amplification by Energy Migration in Electronic Polymers
Conduction Band
Valence Band
hv
E_g
hv'
Excitations migrate along the polymer backbone and are quenched when they encounter an acceptor.
J. Am. Chem. Soc. 1995, 117, 12593
Accts. Chem. Res. 1998, 31, 201-7
•"Conjugated Polymer-Based Sensory Materials" Chem. Rev. 2000, 100, 2537-2574.
•"Chemical Sensors Based on Amplifying Fluorescent Conjugated Polymers" Chem. Rev. 2007, 107, 1339 - 1386.


TNT - DETECTION :
Networked Detectors
RELAY
RELAY
RELAY
RELAY

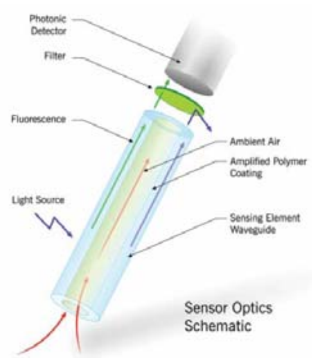

TNT - DETECTION :
Networked Detectors
RELAY
RELAY
RELAY
RELAY
TNT

Free Volume in Sensory Polymer Films



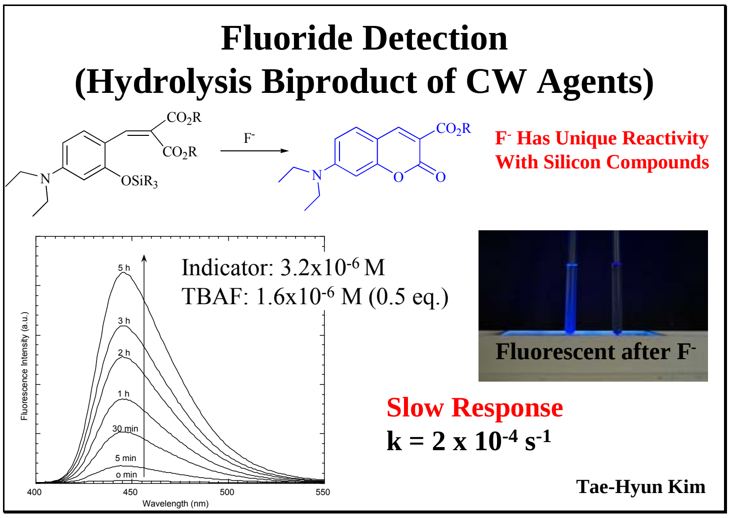
Single-Channel Fido Schematic

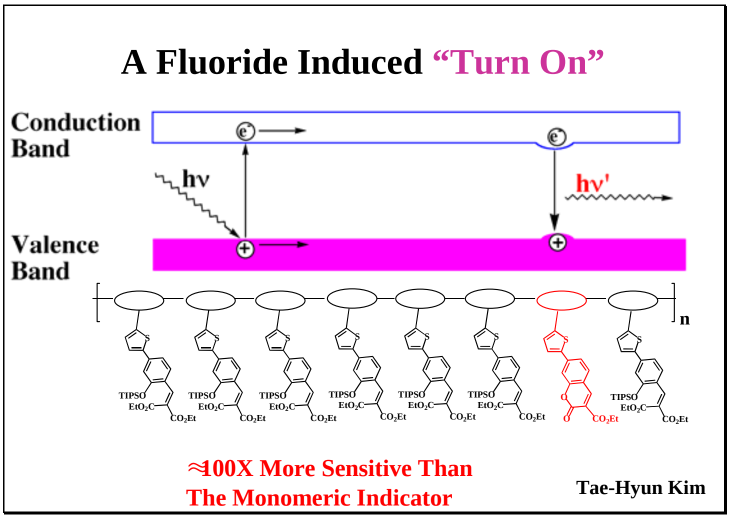
Design Provides Dramatically Better Performance over Fiber Optic Devices



Courtesy of ICx Technologies


icx nomadics
FIDO XT in Iraq + iRobot PackBot
Checkpoints-Robotic Platforms
Identifying Bombmakers
Fido Explosives Detector
iRobot
SAFETY FROM STANDOFF: PackBot carries the sensor to the vehicles, so Soldiers aren't exposed.
THE DETECTION OPPORTUNITY: Explosives leave invisible traces of vapors which FIDO can sniff and detect.
"The FIDO Sensor worked perfectly. With the physical setup forcing wind by the vehicles, FIDO was able to detect explosives 80 feet away. This allowed separation of the Soldiers and dog from the bomb, thus saving lives. . . ."


Fluoride Detection
(Hydrolysis Biproduct of CW Agents)
CO2R
CO2R
N
OSiR3
F-
CO2R
N
O
O
F- Has Unique Reactivity With Silicon Compounds
Indicator: 3.2x10-6 M
TBAF: 1.6x10-6 M (0.5 eq.)
5 h
3 h
2 h
1 h
30 min
5 min
0 min
Fluorescence Intensity (a.u.)
400
450
500
550
Wavelength (nm)
Fluorescent after F-
Slow Response
k = 2 x 10-4 s-1
Tae-Hyun Kim


A Fluoride Induced "Turn On"
Conduction Band
Valence Band
hν
hν'
TIPSO
EtO2C
CO2Et
n
≈100X More Sensitive Than The Monomeric Indicator
Tae-Hyun Kim

Moving Beyond Point Sensors: Prospects for Chemiresistors

Intrinsic Advantages of Chemiresistors
Very Low Power
Small Footprint
Wireless Network
Low Cost

Technical Needs
High Sensitivity
Selectivity
No Calibration

Enabling Technology for Creating Large Chemical Sensor Arrays

London Bombings: July 7, 2005

7:22 AM at Luton Station, Bombers Catch a Train to Kings Cross Prior to Being Filmed at 8:26am.

London Bombings: July 7, 2005

An Ordinary Photo, But is the First Point of Possible Detection:
Chemical Sensors at the Doorway Could Have Triggered Surveillance Systems and Provided Authorities More Than 1 hr to React

Trains and Other Closed Systems Provide Opportunities for Chemical Detection

Sensors in Air Recirculation Systems Could Trigger
Increased Surveillance

Large Areas for Coverage With Sensor Networks

Highly Distributed Chemical Sensor Arrays

Meeting the Technical Needs

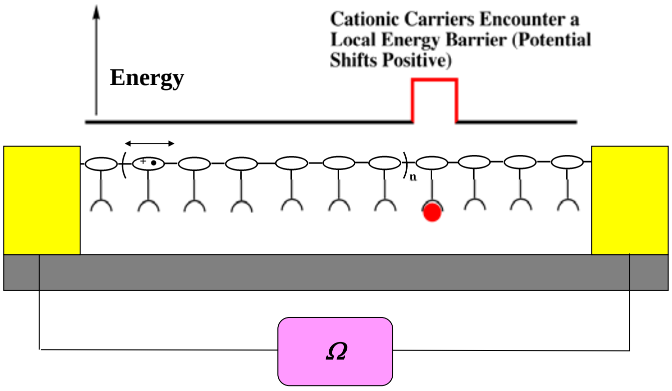
High Sensitivity- Molecular-/Nano-Wires

Selectivity- Molecular Recognition and Cross-Reactive Arrays

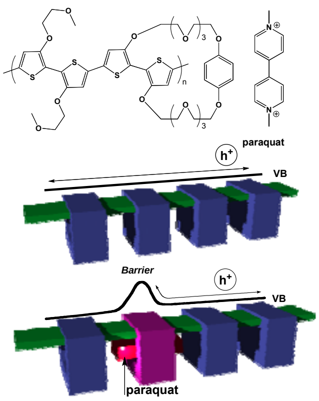
No Calibration- Robust Devices

Inspiration: Molecular/Nano Wire Sensors

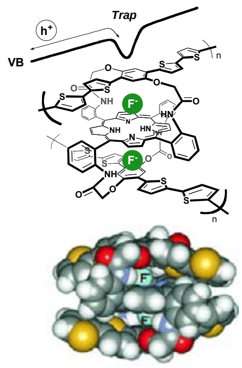
Fractional Binding Gives Large Conductivity Changes



Transduction Mechanisms



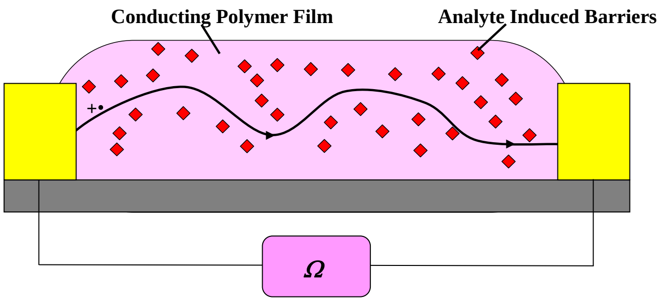
Marsella, M. J.; Carroll, P. J.; Swager, T. M. *J. Am. Chem. Soc.* **1994**, 116, 9347-8.



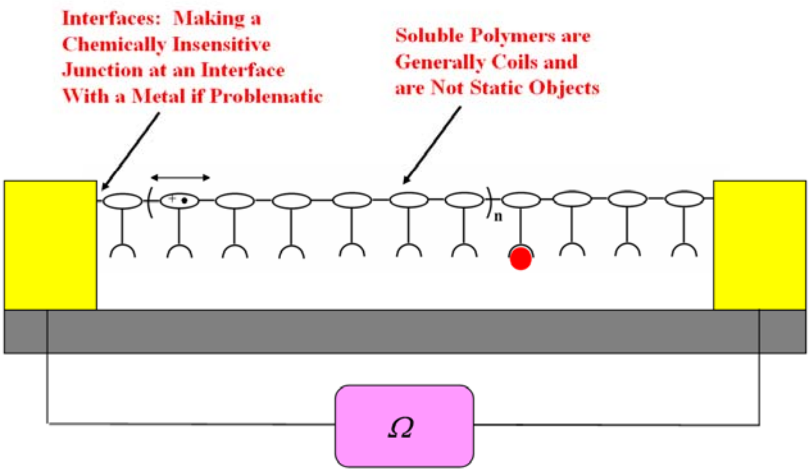
Takeuchi, M.; Shioya, T.; Swager, T. M. *Angew. Chem.* **2001**, 40, 3372-3376.

Current Situation in Resistive Conducting Polymer Sensors

But.. It Still Works
Binding of Analytes Creates Many Obstacles.
But There Are More Pathways!

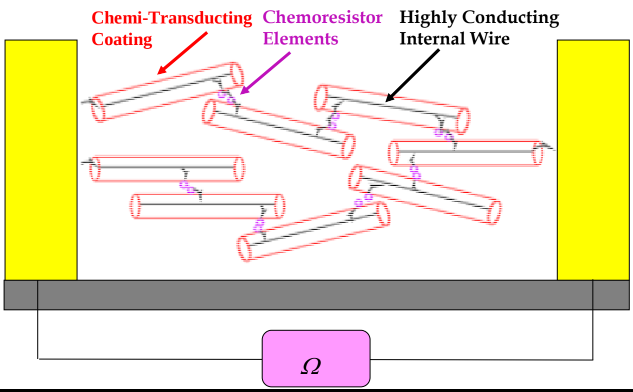


Difficulties



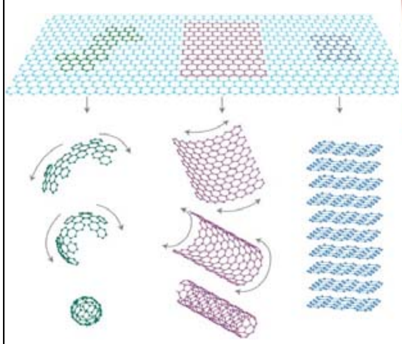
Random NanoWire Networks

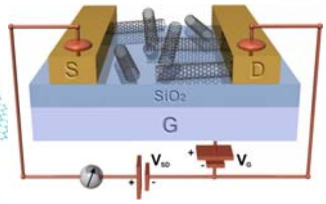
Defining a Finite Number of Conduction Pathways



Carbon and NanoElectronics

Carbon Structures





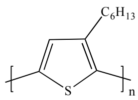
Mobilities
CNTs/Graphene> 10^5 $cm^2V^{-1}s^{-}$

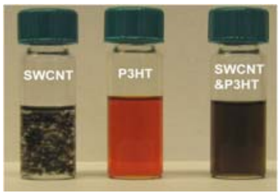
GaAs < 8,500 $cm^2V^{-1}s^{-1}$ (e^-)
<400 $cm^2V^{-1}s^{-1}$ (h^+)

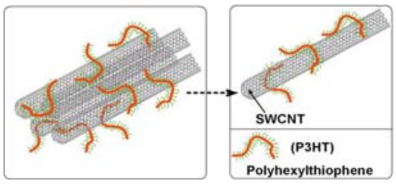
Si < 1,400 $cm^2V^{-1}s^{-1}$ (e^-)

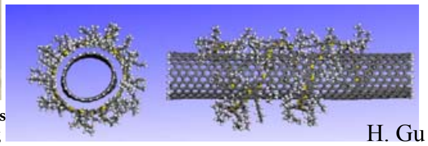
Dispersions of CNTs with Polythiophenes

Stable Dispersions are Formed with High M_n(>100,000) P3HT







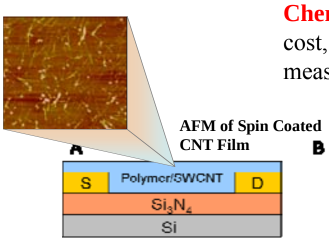


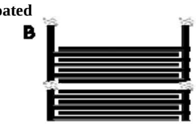
Stable 2 Years and Counting

H. Gu

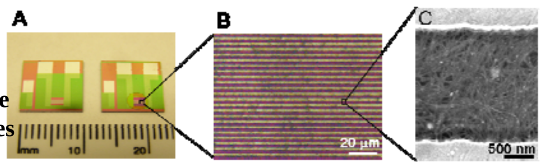
A Simple Chemoresistive Sensing Procedure

Chemiresistors: (1) Low power, low cost, portable sensing devices (2) Precise measurement with simple electronics

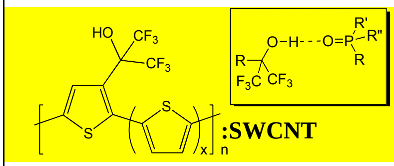


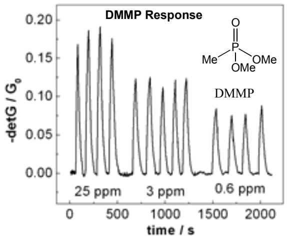


Device Images

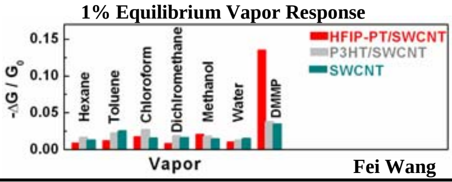


Detection of Chemical Warfare Simulants

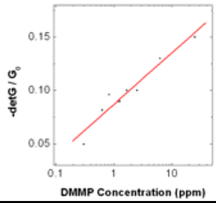




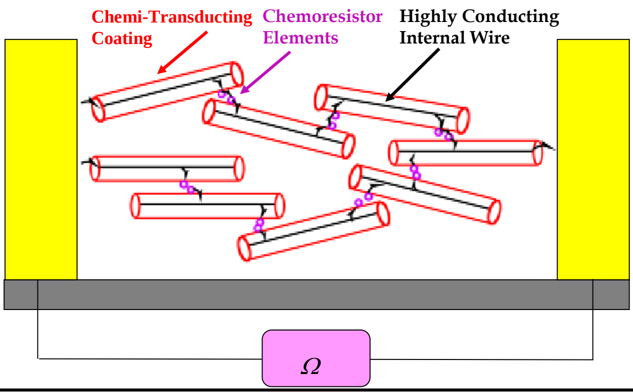
Mechanistic Studies Suggest DMMP Induced Charge Transfer, Carrier Pinning, and Polymer Conformational Changes



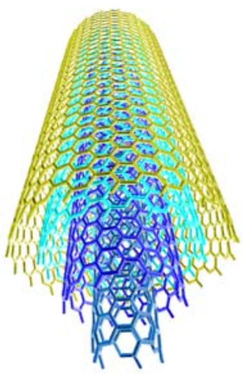
Fei Wang



Higher Stability → Crosslinked Coatings and Covalent Modification



Multiwalled CNTs (MWCNTs): High Conductance in Functionlized Nanotubes



Functionalization Only Destroys The Conductance of the Outer Layer

Key Points

- Unique Chemical Reactivity is a Good Means for Selectivity.
- Synthesis of Unique Transduction Materials is a Productive Approach to Sensors
- Nanotechnology Principles are General and Transferable from on Sensng Scheme to Others
- There is Still a Need for Improved CW Detection

Annex 4

**REPORT OF THE FOURTH MEETING OF THE TEMPORARY WORKING GROUP ON SAMPLING AND ANALYSIS
THE HAGUE, THE NETHERLANDS
5 – 6 NOVEMBER 2009**

1. INTRODUCTION

1.1 The Temporary Working Group (TWG) on Sampling and Analysis (S&A) of the Scientific Advisory Board (SAB) held its fourth meeting on 5 and 6 November 2009 at the OPCW in The Hague.

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 The following agenda was adopted:

(a) Opening of the meeting and adoption of the agenda
(b) Overview of on-site analysis procedures
    i) Presentation by the OPCW laboratory (Maciej Sliwakowski)
    ii) Discussion
(c) Sample preparation for aqueous solutions of degradation products
(d) Emerging techniques with possible applications to on-site analysis:
    i) Fast GC, SPME, LC-MS, miniaturization
(e) Toxin analysis (ricin and saxitoxin), off-site and on-site.
    i) Updates on correspondence group, literature, ricin round robin (Global Health Security Action Group) (Martin Schär, Sten-Åke Fredriksson)
    ii) Criteria for identification
(f) Criteria for trace analysis in investigations of alleged use (Paula Vanninen)
(g) Update on biomedical sample analysis (Robin Black)
(h) Any other business.
(i) Summary of conclusions and recommendations
(j) Closure of the meeting.

2. OVERVIEW OF ON-SITE ANALYSIS PROCEDURES

2.1 Maciej Sliwakowski provided the TWG with an overview of the logistics and preparation for missions involving on-site analysis, the time lines for on-site analysis, and efforts to reduce analysis time. His presentation is at Appendix 2. There is also a desire to reduce the logistic burden of equipment, particularly the additional pumps etc. required for concentrating aqueous samples using current operating procedures.

2.2 Faster GC has been investigated by the OPCW laboratory in collaboration with Verifin. GC run times have been shortened by approximately half without compromising retention indices or resolution. These revised conditions will be used in future missions where appropriate.

2.3 Adsorption of aqueous samples onto Tenax followed by on-Tenax derivatisation and thermal desorption GC-MS is currently being explored by the OPCW laboratory as an alternative to evaporation of aqueous samples to dryness and derivatisation. The sample is loaded onto Tenax solid adsorbent packed into a glass GC injector liner, dried using a gentle stream of nitrogen, derivatised in situ and analysed by thermal desorption GC-MS. Results to date look very promising, and the technique has been applied successfully to proficiency test samples. Additional work is needed to determine the robustness and scope of this procedure compared to the existing one.

2.4 A problem that has occurred in some inspections is the importation of a dangerous chemical, hexachlorobenzene[3], which is added to each analytical run for quality control. Several alternatives have been considered. Methyl stearate and dibenzothiophene, which are already used in the quality control system for other purposes, have now been accepted as viable alternatives.

2.5 The OPCW laboratory has acquired three portable FTIR instruments with the intended application of rapid analysis of certain types of bulk sample. TWG members were asked to encourage Member States to assist in the process of acquiring validated FTIR data for relevant analytes.

2.6 The TWG noted the significant advances being made by the OPCW laboratory in cooperation with partner laboratories. One aspect of on-site analysis that is a concern is the reluctance of some Member States to accept data for non-scheduled derivatives of scheduled chemicals and some degradation products into the OPCW Central Analytical Database (OCAD). Important examples are derivatives of Lewisites I and II that are required for GC-MS analysis. The TWG members were unanimous in their view that it is essential that these derivatives be included in the OCAD, in line with their recommendations of previous meetings of the TWG.

2.7 A problem that has arisen with some inspections of Schedule 2 production facilities is the presence of non-scheduled impurities in technical grade material that give mass spectra that resemble scheduled compounds in the OCAD. The use of the National

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[3] (HCB) – formerly used as a fungicide but is now a listed substance under the Stockholm Convention (2004) on persistent organic pollutants.

Institute of Standards and Technology database has been shown to resolve some of these ambiguities but it can only be used with the permission of the inspected party.

3. SAMPLE PREPARATION FOR AQUEOUS SOLUTIONS OF DEGRADATION PRODUCTS

3.1 The TWG discussed methods for the preparation of aqueous samples that avoid the need to evaporate aqueous solutions to dryness, a procedure that is time consuming, may be prone to error, and which requires additional heavy equipment if performed on-site. No advances over those presented to the November 2008 meeting[4] were reported other than the use of Tenax and thermal desorption described above. The general view of the TWG was that hollow fibre liquid phase microextraction showed the greatest potential in terms of applicability and cost, although there are some concerns on the manipulative skills required. The OPCW laboratory is investigating two phase (emulsion) extractive derivatisation. Other possible techniques include solid phase microextraction (SPME) and the use of ionic liquids. It was noted that there has been a huge increase in the number of scientific papers on liquid phase microextraction. Not only are these methods much faster than traditional ones, they use a minimal amount of organic solvent.

3.2 The TWG recommends Member States to pool their knowledge in this area. With the current squeeze on budgets, several laboratories have work that shows promising initial results, but which is not yet ready for publication. Methods also need direct comparison and particularly using realistic simulated samples. Current on-site procedures would be changed only if an alternative method was shown to have similar applicability and ruggedness. Consideration should be given to a discussion meeting with appropriate experts, and to holding a round robin exercise (in 2011) in which laboratories are asked to use a number of methods.

4. EMERGING TECHNIQUES WITH POSSIBLE APPLICATIONS TO ON-SITE ANALYSIS

4.1 Francesco Pilo described work being undertaken by the Provincial Firefighters Headquarters, Venice to evaluate solid phase microextraction (SPME) for sampling toxic atmospheres (see appendix 3). The TWG reconsidered its previous view of SPME (report of the third meeting held in November 2008[5]). The conclusions were the same, i.e. that SPME provides an easily automated, rapid and sensitive technique in selected applications, but could not be recommended for general application in on-site analysis because of the cost of the fibres, the need to use them only once in order to avoid cross contamination, time taken to condition the fibres, and their susceptibility to interference by high levels of contaminants. Hollow fibre-liquid phase microextraction had given superior results for degradation products in aqueous samples.

4.2 Desorption electrospray ionisation (DESI) was proposed as a technique that should be reviewed at the next meeting of the TWG. This technique allows rapid direct screening of samples such as wipes etc. Miniaturisation of MS will also be reviewed. Offers to

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4 Paragraphs 5.8 to 5.15, Annex 2 of SAB-12/1, dated 26 November 2008

5 Paragraph 5.7, Annex 2 of SAB-12/1, dated 26 November 2008

present were accepted from Armando Alcaraz and Jean Claude Tabet. Other techniques with increasing application are surface plasmon resonance in combination with molecular recognition, and molecularly imprinted polymers.

5. TOXIN ANALYSIS (RICIN AND SAXITOXIN), OFF-SITE AND ON-SITE

5.1 Based on the conclusions made in the third meeting of the TWG in November 2008, identification criteria for saxitoxin were proposed by Martin Schär and for ricin by Sten-Åke Fredriksson. (see their respective presentations in appendix 4 and appendix 5).

Saxitoxin

5.2 The following points were considered:

a. Should NMR be included as an identification method, in addition to LC-MS/MS (or CE-MS/MS), LC-fluorescence and immunoassays (lateral flow and ELISA)?
b. Should a points system be used for identification criteria to allow greater flexibility, and should a system adopted be open to additional methods?
c. Alternatively analytical methods could be differentiated as screening vs. identification methods.
d. If a point system is rejected are all screening methods to be considered as equal, e.g. lateral flow assay *vs.* ELISA?
e. What is the requirement for mass accuracy? An error of ±0.2 Da was considered to be too stringent. This degree of accuracy may not be achievable on ion trap instruments, which in other aspects are very useful (low cost, MS/MS capability).
f. Should a reference chemical be used to confirm identification?

<u>Conclusions on saxitoxin analysis:</u>

5.3 As a low molecular mass toxin, saxitoxin should not be treated differently from other low molecular mass scheduled chemicals. The main difference is that GC/GC-MS cannot be used for saxitoxin analysis and therefore an additional LC-based technique or immunoassay will need to be used as the second analytical method.

5.4 It was accepted that LC-MS/MS or CE-MS/MS (either product ion spectrum or ratio of MRM-transitions), in combination with LFA, ELISA or LC/Fluorescence as the second method, would be needed for unambiguous identification of saxitoxin. Specific criteria for the individual methods have yet to be defined. A point system for identification was not favoured by the TWG (see section 6).

5.5 NMR, if applicable, should be accepted as a screening/identification method for saxitoxin. Existing OPCW evaluation criteria (currently still under development) should be applied.

5.6 Identification should be in comparison to a reference chemical.

Next step

5.7 In order to elaborate identification criteria for the proposed methods, an exercise will be held on saxitoxin analysis among interested laboratories. Martin Schär will coordinate and evaluate the experimental data provided by laboratories. Draft criteria will be derived from the data for consideration by the TWG. Laboratories will be asked to prepare their own samples (saxitoxin is commercially available). Information on how to prepare and analyze the samples, and to report data, will be provided by the Spiez Laboratory, Switzerland.

Ricin

5.8 Screening methods proposed for ricin include immunoassays (LFA and ELISA), electrophoresis/chromatographic separation, molecular mass determination using MALDI mass spectrometry, PCR (for residual DNA in crude preparations) or a functional test (these require further consideration). These methods are not considered confirmatory either alone or in combination.

5.9 Methods suggested for confirmation of identification, in combination with one of the methods listed above, were based on comparisons of LC-MS/MS data obtained from enzymatic digests of the sample with those from a reference standard of ricin.

5.10 Ricin D was proposed as suitable reference material, since it is present in all known cultivars. Ricin E, the other main isoform, has the same amino acid sequence of chain A, while the carboxy terminal half of chain B has a sequence identical to the agglutinin (RCA_{120}).

5.11 It was proposed that data from a minimum of three peptides in an enzymatic digest, with one ricin-unique peptide from each of the A and B chains, should be required for confirmed identification.

5.12 A minimum of two product ions in the LC-MS/MS product ion spectra, or two transitions in LC-MS/MS MRM, was considered sufficient for confirmed identification of a peptide.

5.13 Existing OPCW evaluation criteria for chromatographic retention time, signal-to-noise ratio and mass spectral data should be used if practical.

5.14 A summary and assessment of the methods used in the recent interlaboratory comparison test on ricin (organised by the Global Health Security Action Group) will be compiled by Sten-Åke Fredriksson after the evaluation meeting on 30 November 2009 and made available to the TWG before the next meeting.

5.15 It is anticipated that firm proposals for ricin identification will be formulated at the next meeting of the TWG.

6. CRITERIA FOR TRACE ANALYSIS IN INVESTIGATIONS OF ALLEGED USE

6.1 Paula Vanninen reviewed the possible scenarios in which trace analysis of samples might be requested, and the criteria for trace analysis used by different regulatory bodies such as the World Anti-Doping Agency (WADA), Food and Drug Administration (FDA), and the European Commission (EC) (see appendix 6). The results of questionnaires distributed to a correspondence group were summarised. Investigations of alleged use were considered the most likely scenario where trace analysis would be required. The criteria for identification using trace analytical techniques (selected ion monitoring GC/LC-MS and multiple reaction monitoring GC/LC-MS/MS) differ in the fine detail amongst the various bodies but the general principals are similar in that they are based on the specificity of the method. The EC uses a system of identification points for detecting banned residues in animal products. This system assigns a certain number of points to each component of the analytical data and has the advantage of flexibility, but would not be consistent with criteria currently required for non-trace analysis. OPCW requirements would differ from those of WADA etc. in that two independent methods would almost certainly be required in line with current requirements at non-trace levels.

6.2 The acceptability of the results of trace analysis was discussed. Acceptability would depend on meeting pre-defined criteria deemed to give an unequivocal identification, and there would need to be consistency in the results from two designated laboratories. It was emphasised that results of trace analysis should not be considered in isolation but in combination with other evidence. Quantitation was considered not to be important in most scenarios. In addition to scheduled CW agents, trace analytical methods were required for riot control agents, specifically in the context of investigations of alleged use as a method of warfare.

6.3 It was noted that trace analysis would have to be targeted at certain analytes as directed by the OPCW in the context of the investigation. What information would need to be supplied to the laboratories requires further consideration by the Technical Secretariat.

6.4 As an initial step forward it was recommended that the results of the first confidence-building exercise on biomedical samples be assessed against criteria used by bodies such as WADA etc. This assessment will be provided to members of the TWG by the Chairperson.

7. UPDATE ON BIOMEDICAL SAMPLE ANALYSIS

7.1 The Chairperson provided the TWG with an update on preparations for the first OPCW Confidence-Building Exercise on biomedical samples. The main objectives of this exercise are:

(a) to broaden expertise in biomedical sample analysis across Member States;
(b) to compare different analytical methods;
(c) to commence a discussion on criteria for identification at trace levels.

7.2 Identification is the main requirement but laboratories are encouraged to report quantitative results if obtained. His presentation is at appendix 7.

7.3 Twenty three laboratories will participate in the exercise. Samples have been prepared by TNO Defence, Security and Safety, Rijswijk, the Netherlands, and will be dispatched by the OPCW laboratory on 6 November. To facilitate shipping and handling, commercial synthetic urine has been chosen as the matrix for this first exercise, spiked with urinary metabolites of vesicants and nerve agents. Laboratories have been supplied with references to appropriate analytical methods, and technical advice will be available during the exercise from Dstl, Porton Down, UK or TNO. Laboratories are asked to report results on or before 15 January 2010; a flexible template for reporting has been supplied. Reports will be evaluated by Dstl and TNO, and a meeting will be held to discuss the results in late February/early March 2010.

8. ANY OTHER BUSINESS

8.1 The Chairperson thanked Gary Mallard and Maciej Sliwakowski for attending and advising the TWG on the procedures of the OPCW Laboratory.

9. SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

9.1 The TWG reaffirmed its previous recommendation that non-scheduled derivatives of scheduled compounds, in particular analytical derivatives of lewisites, should be added to the OCAD.

9.2 The TWG noted that the OPCW laboratory has shortened the time for on-site GC-MS analysis by approximately 15 minutes per run cycle.

9.3 Shortening of the sample preparation time for on-site analysis of aqueous samples is still regarded as a high priority task. Progress on one possible solution (thermal desorption from Tenax) by the OPCW laboratory was noted. Previous work reported by the Verification Laboratory, DSO National Laboratories, Singapore, and Vertox Laboratory, India, indicated hollow fibre liquid phase microextraction to be a promising technique. The TWG recommended greater pooling of knowledge in this area by Member States, and proposed that a round robin exercise be considered in 2011 to compare different techniques.

9.4 The TWG noted the commencement of the first OPCW confidence-building exercise on biomedical samples.

9.5 Recommendations were made for the identification of saxitoxin. Assuming that two independent techniques would be required, LC-MS/MS (or CE-MS/MS) are proposed as the primary methods for an unequivocal identification, combined with a screening method such as an immunoassay or LC-fluorescence (GC is not applicable to saxitoxin).

9.6 A thorough discussion was held on criteria for the identification of ricin. It was recommended that further consideration should await the results of a round robin exercise on ricin currently being held by the Global Health Security Action Group. Firm recommendations will be discussed at the next meeting of the TWG.

9.7 The TWG considered various criteria for identification using trace analytical techniques. Criteria must be defined if trace analysis is to be accepted, e.g. in investigations of alleged use. The use of a system based on identification points (as used by the EC) was provisionally not recommended in order to maintain consistency with criteria in current use for verification analysis. It was agreed that results from the first confidence-building exercise on biomedical samples should be assessed with regard to meeting criteria in use in other regulatory bodies.

9.8 The TWG recommended that desorption electrospray ionisation and miniaturisation of mass spectrometry should be reviewed at the next meeting with regard to possible applications to verification analysis.

Appendices:

Appendix 1: List of Participants in the Fourth Meeting of the Temporary Working Group on Sampling and Analysis

Appendix 2: Sampling and Analysis Activities During an Inspection

Appendix 3: SPME Test Programme in Real Sample Conditions

Appendix 4: Saxitoxin: Identification Criteria Proposal

Appendix 5: Ricin (CAS 9009-86-3): Identification Criteria Proposal

Appendix 6: Examples of Minimum Criteria for Identification by Chromatography and Mass Spectrometry

Appendix 7: Update on Biomedical Sample Analysis

Appendix 1

LIST OF PARTICIPANTS IN THE FOURTH MEETING OF THE TEMPORARY WORKING GROUP ON SAMPLING AND ANALYSIS

	Participant	Member State
1.	Robert Mathews	Australia
2.	Jiří Matoušek	Czech Republic
3.	Paula Vaninnen	Finland
4.	Jean-Claude Tabet	France
5.	Anne Bossée	France
6.	Ralf Trapp	Germany
7.	R. Vijayaraghavan	India
8.	Francesco Pilo	Italy
9.	Jose Luz Gonzalez-Chavez	Mexico
10.	Mui Tiang Sng	Singapore
11.	Philip Charles Coleman	South Africa
12.	Roberto Martinez-Alvarez	Spain
13.	Sten Åke Fredriksson	Sweden
14.	Martin Schär	Switzerland
15.	Robin Black[6]	United Kingdom of Great Britain and Northern Ireland
16.	Armando Alcaraz	United States of America

6 Chairman of the TWG.

Appendix 2

SAMPLING AND ANALYSIS ACTIVITIES DURING AN INSPECTION

Sampling & Analysis (S&A) activities during inspection

TWG S&A meeting, 5 Nov 2009

Overview

- Logistic – What's involved
- Preparation for S&A mission
- On-site analysis
  - Activities
  - Analysis and timelines
- Shortening of certain activities
- Projects already conducted at the OPCW Lab. and Partner Laboratories
- Conclusions

Logistics – What's Involved

- Selection of Equipment
- Preparation of Analytical Equipment
- Notification to ISP of an Inspection with S&A
- Transportation of Equip & Chem to & from OPCW to the POE
- Transportation of Equip & Chem from the POE to the site; & setup at the site
- Turn-in and checking Equip & Chem back to the OPCW Stores

Equipment Transportation Issues

- Mission warning order comes out 8 weeks prior to the inspection
- 6-weeks prior to the departure - equipment meeting set-up to select equipment and organise transport
- 2-3 weeks prior to departure - start preparing equipment
- Equipment leaves OPCW Stores approx 1 week prior to departure for air transport, 2-4 days for road transport
- Equipment arrives at ISP's Point of Entry (POE) or site 1-2 days prior to inspection

S&A Equipment for e.g.: Industrial Inspection

Consist of normal industrial inspection equip plus:

- 1 - GC/MS plus laptop & printer;
- 1 - industrial sample preparation kit, (4-pelli cases);
- 1 - cooler with on-site transportation container;
- 1 - industrial sample collection kit, (1-pelli case);
- chemicals & gases (N_2 &He) & 1 portable fume hood.*

*depends on availability at inspected State Party

Preparation of Equipment for Analysis

- Unpacking the equipment
- Setting up the laboratory
- Setting-up the GC/MS (assembling the system, connecting power and signal lines, attaching carrier gas supply etc.)
- Cable and connections checking
- Pump-down of the GC/MS instrument
- Tuning and air/water checks
- Installation of libraries

Projects conducted at the OPCW Lab. and Partner Laboratories

- Fast GC/MS
- Thermodesorption
- HCB replacement
- New ODMS
- OCAD update

Analysis and timelines

- Blank run - 32 min
- Performance check run - 32 min
- BSTFA run - 32 min
- Sample run – 32 min
- Blank run – 32 min
- Sample run - 32 min
- Etc.

! Cooling and stabilisation time for the GC/MS oven is about 10 min.

Analysis and timelines cont.

- The current GC program used by OPCW inspectors:

  40ºC/2 min - 10ºC/min - 280ºC/6 min

Total run time: 32 min

Whole sequence including blank run, performance run and sample run takes around **2** hours.

FAST GC/MS

- Two projects conducted at the OPCW Lab. and one project conducted at VERIFIN
- All classes of Scheduled chemicals were examined
- AMDIS parameters evaluated: RT, RI, RiDiff, NET MF, Width, Tailing, S/N

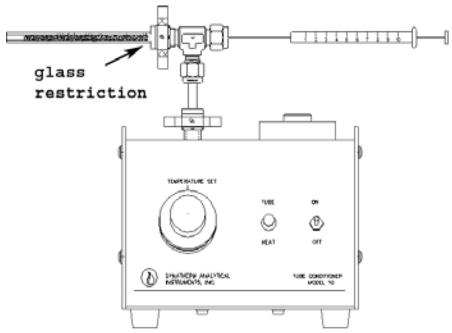
FAST GC/MS cont.

- 40°C/2 min - 16°C/min to 230°C,- 40°C/min to 280°C, hold 2 min.
- Runtime: 17.1 min

Thermodesorption

- Sample introduced directly onto the Tenax sorbent
- If necessary derivatization on the sorbent is performed
- Glass liner with sorbent is put directly into the injection port of GC/MS
- Applies to all types of liquid samples, limited sample preparation

Thermodesorption cont.



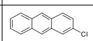
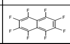
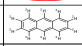
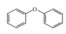
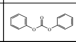
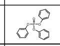
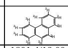
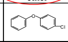
Thermodesorption cont.

- The time for sample preparation and sample introduction shortened to several minutes
- Some clean-up procedures for aqueous samples still necessary (e.g.: ion exchange)

HCB replacement

- Safety concerns: carcinogenic
- Need to be declared as DG every time when shipped
- ISP's restrictions on import of HCB

HCB replacement cont.

	2-chloro-Anthracene	Octafluoro-naphthalene	D10-Anthracene	1-Diphenyl ether	2-Diphenyl carbonate	Triphenyl phosphate	D10-Phenanthrene	4-chloro-Diphenyl ether
Chemical structure								
RI/RT	2034/21.22 Good	1119.5/10.16 Bad	1812.6/19.08 Good	1415.4/14.39 Bad	1710.1/17.88 Good	2423/24.66 Good	1801.4/19.00 Good	1608.2/16.86 Acceptable
Mass Spectra	212 Acceptable mass range A+2	272 Good mass range	188 Acceptable mass range	170 Short mass range	214 Acceptable mass range	326 Good mass range	178 Short mass range	204 Acceptable mass range A+2
Stability	Good	Good	Good	Acceptable	Acceptable Bad broken fragment	Acceptable	Good	Acceptable
Sensitivity	Acceptable	Good	Good	Acceptable	Acceptable	Bad	Good	Good
Column efficiency	Good	Good	Good	Good	Good	Bad	Good	Good
Column Affinity	Good	Good	Good	Good	Good	Bad	Good	Good
Solubility in DCM	Bad	Good	Good	Good	Good	Good	Good	Good

HCB replacement cont.

After some work it was decided that if an alternative was needed it would be either:

Methyl Stearate or Dibenzthiophene.

Reasons for choice of alternatives:

No new data need to be put into the OCAD

New ODMS

- Automatic evaluation of multiple GC/MS parameters (e.g.: Checker/Criteria check for the results of AMDIS analysis)
- Various options of the use of e-OCAD and NIST 05 libraries
- CHECKIT software for verification of integrity of the ODMS
- Pre-defined GC/MS methods for different applications

OCAD update

- Sch.2 S&A missions showed that some chemicals are missing from the OCAD – the OPCW Lab. conducted series of projects to fix that
- Update of FTIR (ATR) spectra

Conclusions

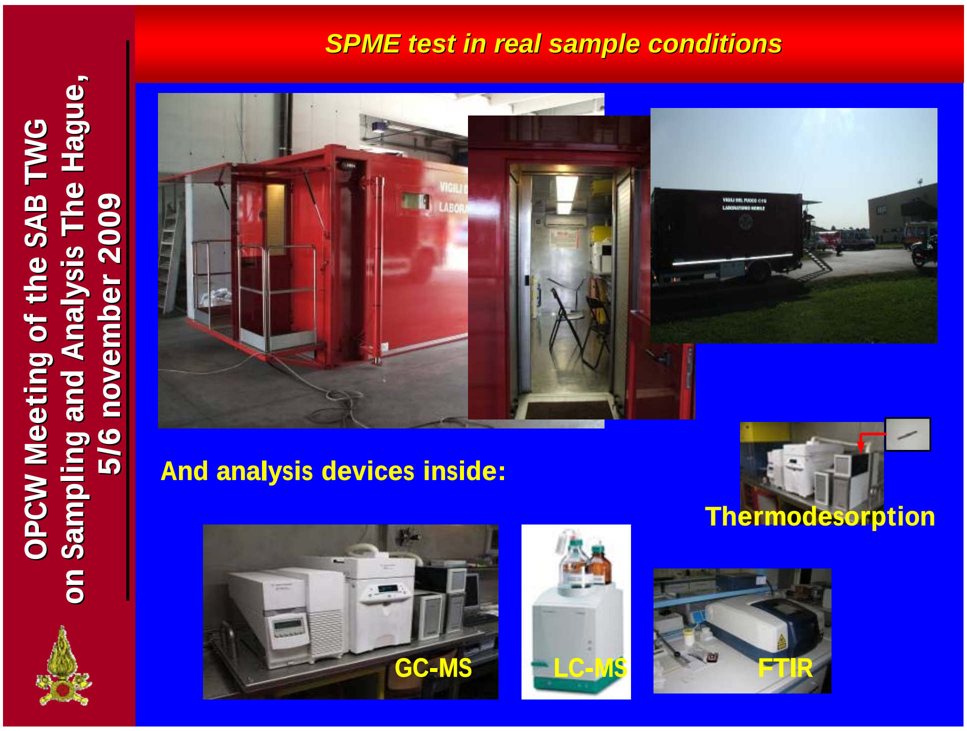
- The GC/MS run was shortened without loosing the performance of a system
- Thermodesorption seems to be promising alternative to the “classic” sample preparation
- HCB replacement still needs some investigation
- ODMS tools significantly enhanced the quality of analysis and shortened AC labour

Proposals for assistance

- Ricin and Saxitoxin issues in Proficiency Testing e.g.: what should be a control sample composition.
- New data for OCAD, especially FTIR
- Assistance in the process of accepting data for OCAD.

Appendix 3

SPME TEST PROGRAMME IN REAL SAMPLE CONDITIONS



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SPME test in real sample conditions

Test program objective

- Verify the use of SPME
- Develop and Test a SPME sampler
- Develop test sample room
- Define a database of fiber relate to target

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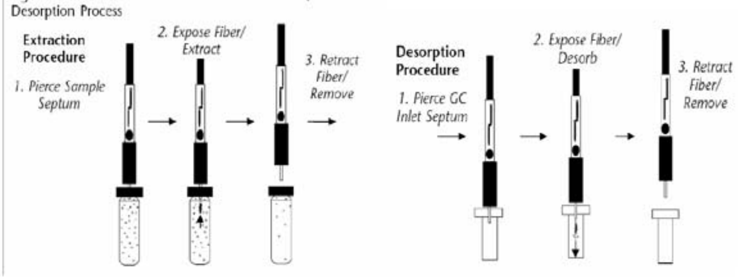
SPME test in real sample conditions

La microestrazione in fase solida è un metodo di trattamento del campione e contemporaneamente un sistema di introduzione del campione.

Ripartizione dell'analita tra il campione (soluzione acquosa o matrici solide) ed il polimero che riveste la fibra di silice fusa
(generalmente lunga 1 cm e con diametro di 100 µm).

La fibra è saldata alla parte terminale di un tubicino sottile di acciaio all'interno del sistema a siringa ed è protetta da un rivestimento anch'esso in acciaio. Lo stantuffo dell'apparecchiatura viene spinto per permettere l'esposizione della fibra al campione, quindi ritratto dopo l'opportuno tempo di campionamento e successivamente ri-spinto all'interno del sistema di iniezione del cromatografo (GC o HPLC).

Figure 1 – Solid Phase Microextraction – Extraction/
Desorption Process



http://www.sigmaaldrich.com/

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SPME test in real sample conditions

Develop a SPME sampler

This holder works only with exposed fiber with a protection system to prevent SPME damage. To expose the fiber we use the plunger and it is also the accessory that closes the holder when we have ended to sample. To plunger inside a spring mechanism is lodged in order to hold always sealed the fiber.

When we sample, the air pushed from the plunger is compressed in the holder without to use the external air; in this way we have not contamination of external air within the holder.

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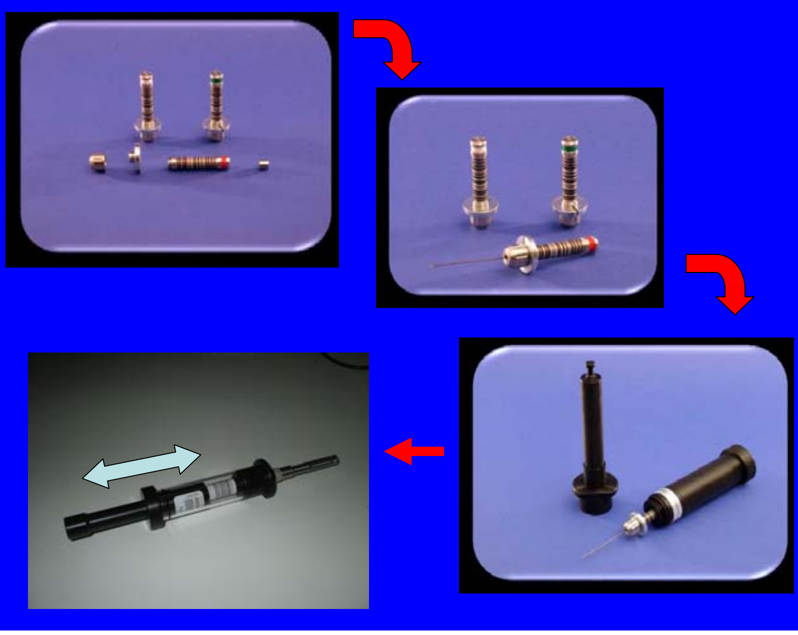
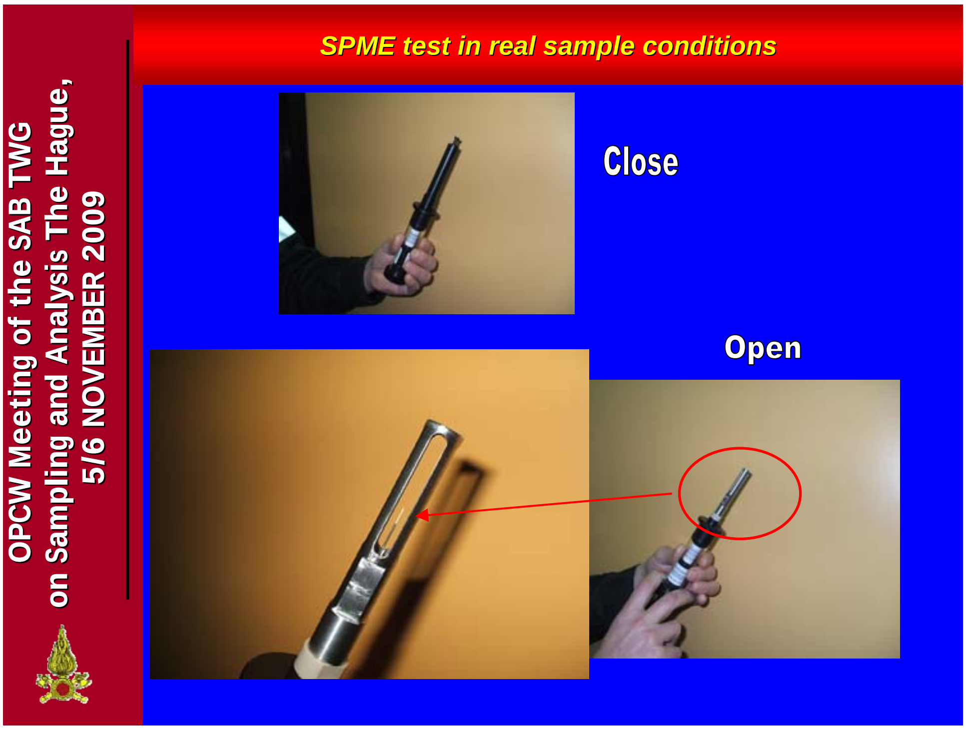
SPME test in real sample conditions





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SPME test in real sample conditions

Sample processing during tests

Commercial kit for manual sample

Kit 1

100 µm PDMS	red	T max 280°- T condiz. 250°C
7 µm PDMS	green	T max 340° - T condiz. 320°C
85 µm PA	white	T max 320° - T condiz. 280°C

Kit 2

75 µm Carboxen/PDMS	black	T max 320° - T condiz. 300°C
65 µm PDMS/DVB	blu	T max 270 - T condiz. 250°C
85 µm PA	white	T max 320° - T condiz. 280°C

Kit 3 "Stable flex" for volatile e semivolatile compound

65 µm PDMS/DVB	pink	T max 270° - T condiz. 250°C
50/30 µm DVB/CAR/PDMS	gray	T max 270° - T condiz. 250°C
85 µm Carboxen/PDMS	blu	T max 320° - T condiz. 300°C
85 µm PA	white	T max 320° - T condiz. 280°C

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SPME test in real sample conditions

Sample processing during tests

Fiber material	target	Target example
PDMS	Compound with no polarity or low polarity level	idrocarburi
PA	Compound with high polarity	fenoli, nitrobenzeni, alobenzeni
CAR/PDMS	gas, high volatily compound, trace analysis	alifatici alogenati leggeri
PDMS/DVB	Compound with high-medium polarity	ammine
CAR/PDMS/DVB	Compound with no polarity or low polarity level, C3 to C20	idrocarburi, aldeidi alifatiche

Suggest sampling verso with unknow target

CAR/PDMS
PDMS/DVB
CAR/PDMS/DVB

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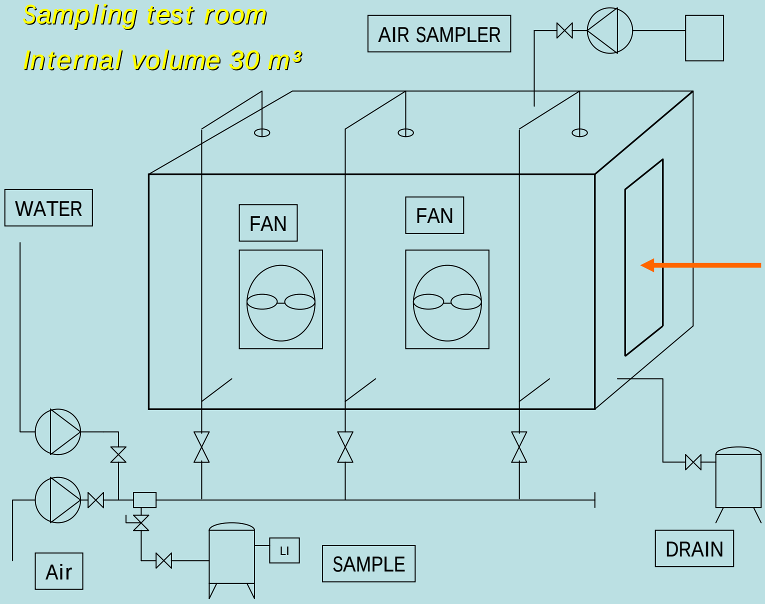
SPME test in real sample conditions

Sampling test room

Internal volume 30 m³



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SPME program test in real sample conditions

Conclusion

In order to define behavior of single fiber type with different chemical compound and in different concentration and the behaviour relative to other fiber will be done a study of overlay difference on GC-MS spectra. (gc-ms will be mantained in same condition for test).
From the overlay of previous databes in possible to see:

fiber	results	Sampling efficency level (score)
CAR/PDMS fiber	most sampling efficency but do not mantain a quantitative linearity	5
PDMS/DVB fiber	have same behaviour of car/pdms but for concentration below 10 ppm has a efficency level very low	4
CAR/PDMS/DVB fiber	good efficency but low level of linearity	2

From overlay of spectra of different type of fiber in same concentration we confirm:

- efficency level (same for all concentrations)

car/pdms fiber> pdms/dvb fiber > pdms/dvb fiber

- There are no significative difference on retention time

Appendix 4

SAXITOXIN: IDENTIFICATION CRITERIA PROPOSAL

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Saxitoxin (STX)

Identification Criteria Proposal

Meeting of the SAB TWG on Sampling and Analysis
The Hague, 5/6 November, 2009

Dr. Martin Schaer
Spiez Laboratory, Switzerland

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Questionnaire STX- Results 2008

		1) LC/ESI-MS M_w = 299.1±0.2 Da (Monoisotopic) Ret. time = R_t±0.2 min.			2) LC/ESI-MS/MS Positive signal vs. Blank (VERIFIN) Ret. time = R_t±0.2 min.			3) MRM-Transition(s), LC/ESI/MS/MS Parent ion m/z 300 Typical fragments: m/z 282, 204, 138 or others (VERIFIN) Ret. time = R_t±0.2 min.		
		A) LC/Fluorescence	B) Lateral Flow Assay (LFA)	C) Immunoassay (ELISA)	A) LC/Fluorescence	B) Lateral Flow Assay (LFA)	C) Immunoassay (ELISA)	A) LC/Fluorescence	B) Lateral Flow Assay (LFA)	C) Immunoassay (ELISA)
dstl	Ranking	-	-	3	-	-	2	-	-	4
	Comment	No experience of this assay	Screening Procedure?	Specificity of immunoassays needs discussion			Assumes precursor ion is MH+			Number of transitions required needs discussion
								Combination of 1+2+C (or A?)		
FOI	Ranking	3	3	4	1	2	1	2	3	2
	Comment	Insufficient			Good combination if chrom. systems are the same	Relies on the Ab specifity		Good combination if chrom. systems are the same		
LLNL	Ranking	2	3	4	2	3	4	2	3	4
	Comment	Acceptable good	Sufficient (pure samples)		Excellent, rapid, sensitive. Can be established in any lab.	Acceptable		Excellent, rapid, sensitive. Can be established in any lab.	Acceptable	
	Additional Method	LC / Nitrogen specific detector, Ranking for 1)-3): 1								
VERIFIN	Ranking	2	2	2	3	3	3	1	1	1
	Comment			Applied by VERIFIN						Applied by VERIFIN

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Questionnaire STX- Results 2008

		1) LC/ESI-MS M_w = 299.1±0.2 Da (Monoisotopic) Ret. time = R_t±0.2 min.			2) LC/ESI-MS/MS Positive signal vs. Blank (VERIFIN) Ret. time = R_t±0.2 min.			3) MRM-Transition(s), LC/ESI/MS/MS Parent ion m/z 300 Typical fragments: m/z 282, 204, 138 or others (VERIFIN) Ret. time = R_t±0.2 min.		
		A) LC/Fluorescence	B) Lateral Flow Assay (LFA)	C) Immunoassay (ELISA)	A) LC/Fluorescence	B) Lateral Flow Assay (LFA)	C) Immunoassay (ELISA)	A) LC/Fluorescence	B) Lateral Flow Assay (LFA)	C) Immunoassay (ELISA)
Spiez Lab	Ranking	2	4	3	2	4	3	1	3	2
	Comment							Know-how, LOD: 1ppb, DIN EN	Cross reaction, matrix influence	More robust than b)
	Additional Method	Intensity ratio of two MRM-Transitions, Ret. time, sample/reference vs. blank								
dso	Ranking	Combination of 1), 2) or 3) in addition with A, B or C shall constitute a confirmation								
	Comment							…and other ions. Deciphering of fragmentation routes is encouraged.		
CEB	Ranking	1			3	-	4			
	Comment	1) + 2) as 2 different identification techniques (no biochemical method suggested)			Derivatisation of STX needs ROP		Immunodetection on small molecules more critical than for large ones.			
	Additional Method	NMR 2D ^{1}H-^{1}H TOCSY: Sample/reference vs. blank Ranking 2: 2) + NMR (LOD: 10ppm with cryoprobe)								

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Questionnaire STX- Results 2008

"Ranking" of Methods listed in the Questionnaire

	1) LC/ESI-MS M_w = 299.1±0.2 Da (Monoisotopic) Ret. time = R_t±0.2 min.			2) LC/ESI-MS/MS Positive signal vs. Blank (VERIFIN) Ret. time = R_t±0.2 min.			3) MRM-Transition(s), LC/ESI/MS/MS Parent ion m/z 300 Typical fragments: m/z 282, 204, 138 or others (VERIFIN) Ret. time = R_t±0.2 min.		
	A) LC/Fluorescence	B) Lateral Flow Assay (LFA)	C) Immunoassay (ELISA)	A) LC/Fluorescence	B) Lateral Flow Assay (LFA)	C) Immunoassay (ELISA)	A) LC/Fluorescence	B) Lateral Flow Assay (LFA)	C) Immunoassay (ELISA)
Σpoints / Number of answers	9/4	12/4	16/5	11/5	12/4	17/6	6/4	10/4	13/5
Ø	2.3	3	3.2	2.2	3	2.7	1.5	2.5	2.6
Rank	3	7	8	2	7	6	1	4	5

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STX Identification Criteria Proposal

How to transform the results into criterias for the identification of STX?

- Ranking based on two methods A & B!
- Order according to the results of the questionnaire
- Use a point system
- Define number of points per combinatione of two methods
- Define necessary number of points for identification
- Include QDOC/LAB/WI/PT03 / NATO criteria

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STX Identification Criteria Proposal

Identification Points

Method A	**Requirements**	**Identification Points**	**Reference, Blank**	**Remarks**
Lateral Flow Assay	Positive	**3**	Against reference, blank	LOD approx. 200ppb
ELISA	Positive	**4**		LOD <10 ppb
LC/Fluorescence	Ret. time ±0.2 min S/N ≥ 5	**6**		LOD < 1ppb Pre/post column derivatization
Method B	**Requirements**	**Identification Points**	**Reference, Blank**	**Remarks**
LC/ESI-MS	Ret. time ±0.2 min MS full scan: $[M+H]^+$ plus 1 structure specific ion, Δm<0.2 Da, no intensity restriction	**3**	Against reference, blank	To add intensity variation restriction?
LC/ESI-MS/MS	Ret. time ±0.2 min Precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	**5**		Precursor: $[M+H]^+$
LC/MS/MS MRM-Transitions	Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	**4**		MRM-transition(s) can be chosen freely Average value of reference?
	Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value	**6**		

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STX- Identification Criteria Proposal

Confirmation level	Minimal Points Sum for combination of Methods A and B
Unambiguous identification	≥ 9
Confirmed	≥ 7
Provisional	≥ 3
No Confirmation	< 3

	No LC/MS method	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
No LC/Fl or immunoassay method	0	3	4	5	6
Lateral Flow Assay	3	6	7	8	9
ELISA	4	7	8	9	10
LC/Fluorescence	6	9	10	11	12

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STX- Identification Criteria Proposal

Method B / Method A	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
Lateral Flow Assay	**A**: Positive **B**: MS full scan: $[M+H]^+$ 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	**A**: Positive **B**: Ret. time ±0.2 min 1 MRM-Transition S/N ≥ 5	**A**: Positive **B**: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	**A**: Positive **B**: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
ELISA	**A**: Positive **B**: MS full scan: $[M+H]^+$ plus 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	**A**: Positive **B**: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	**A**: Positive **B**: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	**A**: Positive **B**: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
LC/Fluorescence	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: MS full scan: $[M+H]^+$ plus 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	**A**: Ret. time ±0.2 min, S/N ≥ 5 **B**: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value

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STX- Identification Criteria Proposal

General Remarks:

- **NMR:** Due to its limited spread among labs involved in CWC verification and the outcome of the questionnaire, NMR was not considered. However, NMR is a valuable analytical tool for STX identification.
- The **LC/Nitrogen specific detector** was not considered. The author is aware of only one lab which is currently using this type of detector.
- It has to be discussed which level, confirmed or unambiguous, is sufficient for STX to be correctly identified.

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STX- Identification Criteria Proposal

Pros:

- Necessary requirements for identification:
  Accomplishable with a relative broad range of combinations of at least two different analytical methods.
- Labs with existing LC/MS/MS equipment can fulfill the requirements for unambiguous identification by adding a simple LFA test to their analytical arsenal.
- Labs with an ELISA-test for STX at hand can fulfill the requirements for confirmed identification with the addition of a relative simple LC/MS system. A single quad LC/MS would be sufficient for confirmed identification.

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STX- Identification Criteria Proposal

Cons:

- No GC-technique
- LC/MS needed
- LFA / ELISA needed

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STX- Identification Criteria Proposal

Input from the Correspondence Group

- Not adopt NATO criteria “Confirmed” and “Unambiguous” (same meaning)?
- Point system: 3 or 4 points to LFA and ELISA, resp. vs. 6 points for LC/fluorescence?
  A consistent system across all areas of interest to the OPCW (toxins, biomedical, evironmental).
- Use intensity variation restrictions only in comparison with a reference.
- Add NMR (3 points) and LC/nitrogen detector (6 points)

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STX- Identification Criteria Proposal

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Confirmation level	Minimal Points Sum for combination of Methods A and B
Unambiguous identification	≥ 9
Confirmed	≥ 7
Provisional	≥ 3
No Confirmation	< 3

Confirmation level	Minimal Points Sum for combination of Methods A and B
Confirmed/Unambiguous	≥ 7
Provisional	≥ 3
No Confirmation	< 3

	No LC/MS method	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
No LC/FI or immunoassay method	0	3	4	5	6
Lateral Flow Assay	3	6	7	8	9
ELISA	4	7	8	9	10
LC/Fluorescence	6	9	10	11	12

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STX- Identification Criteria Proposal

Input from the Correspondence Group

- Not adopt NATO criteria "Confirmed" and "Unambiguous" (same meaning)?

Where to draw the line between "Identified" and "Not identified"?

	No LC/MS method	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
No LC/FI or immunoassay method	0	3	4	5	6
Lateral Flow Assay	3	6	7	8	9
ELISA	4	7	8	9	10
LC/Fluorescence	6	9	10	11	12

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STX- Identification Criteria Proposal

Input from the Correspondence Group

- Not adopt NATO criteria “Confirmed” and “Unambiguous” (same meaning)?
- Point system: 3 or 4 points to LFA and ELISA, resp. vs. 6 points for LC/fluorescence?
  A consistent system across all areas of interest to the OPCW (toxins, biomedical, evironmental).?

Method A	Requirements	Identification Points	Reference, Blank	Remarks
Lateral Flow Assay	Positive	3	Against reference, blank	LOD approx. 200ppb
ELISA	Positive	4		LOD <10 ppb
LC/Fluorescence	Ret. time ±0.2 min S/N ≥ 5	6		LOD < 1ppb Pre/post column derivatization
Method B	**Requirements**	**Identification Points**	**Reference, Blank**	**Remarks**
LC/ESI-MS	Ret. time ±0.2 min MS full scan: $[M+H]^+$ plus 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	3	Against reference, blank	To add intensity variation restriction?
LC/ESI-MS/MS	Ret. time ±0.2 min Precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	5		Precursor: $[M+H]^+$
LC/MS/MS MRM-Transitions	Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	4		MRM-transition(s) can be chosen freely
	Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value	6		Average value of reference?

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STX- Identification Criteria Proposal

Input from the Correspondence Group

- Not adopt NATO criteria “Confirmed” and “Unambiguous” (same meaning)?
- Point system: 3 or 4 points to LFA and ELISA, resp. vs. 6 points for LC/fluorescence?
  A consistent system across all areas of interest to the OPCW (toxins, biomedical, evironmental).
- Use intensity variation restrictions only in comparison with a reference.

Method B / Method A	LC/ESI-MS	LC/ESI/MS/MS 1 MRM-Transition	LC/ESI-MS/MS	LC/ESI/MS/MS 2 MRM-Transitions
Lateral Flow Assay	A: Positive B: MS full scan: $[M+H]^+$ 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-Transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
ELISA	A: Positive B: MS full scan: $[M+H]^+$ plus 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	A: Positive B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Positive B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Positive B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value
LC/Fluorescence	A: Ret. time ±0.2 min, S/N ≥ 5 B: MS full scan: $[M+H]^+$ plus 1 structure specific ion, $\Delta m<0.2$ Da, no intensity restriction	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min 1 MRM-transition S/N ≥ 5	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min, precursor: quasi-molecular ion, ≥ 4 daughters with ion intensities >10% of base peak	A: Ret. time ±0.2 min, S/N ≥ 5 B: Ret. time ±0.2 min Ratio of 2 MRM-transitions, must match within ±10% of the reference value

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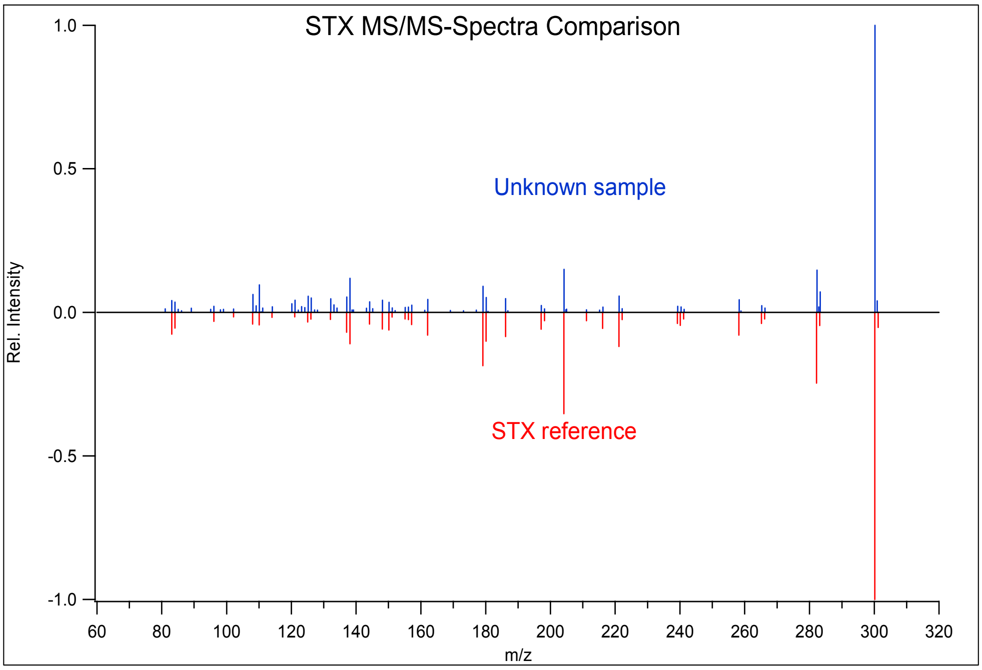
STX- Identification Criteria Proposal



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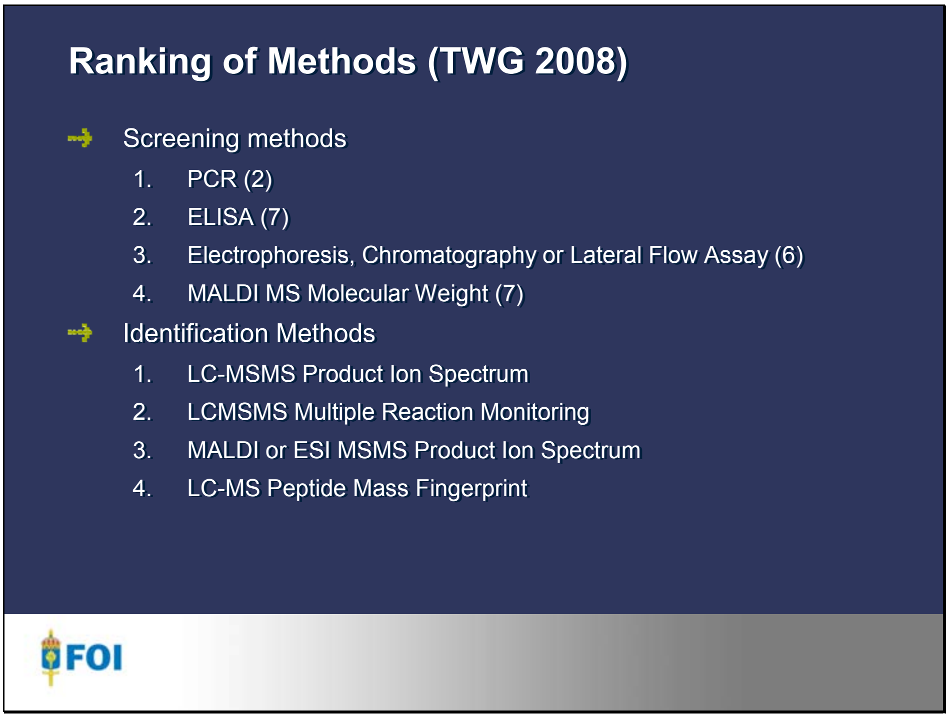
- Use intensity variation restrictions only in comparison with a reference.
- Add NMR (3 points) and LC/nitrogen detector (6 points)
  - **NMR:** Due to its limited spread among labs involved in CWC verification and the outcome of the questionnaire, NMR was not considered. However, NMR is a valuable analytical tool for STX identification.
  - The **LC/Nitrogen specific detector** was not considered. The author is aware of only one lab which is currently using this type of detector.

Question: Should there be more techniques added?

Appendix 5

RICIN (CAS 9009-86-3): IDENTIFICATION CRITERIA PROPOSAL



TWG 2008 Suggested discussion subjects

- Number of peptides necessary for identification
- Need for an S-S bridged peptide?
- Requirement for functional assays?
- LC-MS MRM criteria need discussion
- Real time PCR a reliable screening technique?
- LC-MSMS: ROP needed for ricin

FOI

Identification criteria for small organic molecules (molecular spectroscopic methods)

- **Existing criteria**
  - OPCW
  - NATO SIBCRA
    - Qualitative analysis
  - European Commission, FDA, WADA
    - Qualitative analysis
    - Quantitative & trace analysis

FOI

Identification criteria (molecular spectroscopic methods)

- **Proteins: NATO SIBCRA**
- Immunological, chromatographic & electrophoretic techniqes
- Full scan MS And MSMS data
- **LC MRM data (trace analysis)**

– **OPCW criteria applies**

FOI

European Community Decision 2002D0657-EN-10.01.2004

1. **Screening method (low false negative rate)**
2. **Confirmatory method (molecular spectroscopic technique)**

- **Requirements and performance criteria**
  - Reference material, blanks and positive control samples
- **Criteria for quantification**
  - Accuracy
  - Reproducibility
- **Criteria for qualitative analysis**
  - Retention time, peak width
  - Ion intensity ratio

FOI

European Community Decision 2002D0657-EN-10.01.2004

Identification points for confirmation by Mass Spectrometry

MS technique	Identification points per ion
Low resolution MS (LRMS)	1.0
$LRMS^n$ precursor ion	1.0
$LRMS^n$ product ion	1.5
High resolution MS (HRMS)	2.0
$HRMS^n$ precursor ion	2.0
$HRMS^n$ product ion	2.5

FOI

Ion intensity ratio

Tolerance for ion intensity ratios relative to the reference standard compound

EC 2002 D0657-EN-2004 (Small organic molecules)		WADA TD2003IDCR Thevis *et al.*, RCM 2007, 21, 297-304 (Peptides & proteins < 8kDa)	
Relative intensity	Tolerance (±%)	Relative intensity	Tolerance (±%)
> 50	20	>50	15 abs
20-50	25	25-50	25 rel
10-20	30	<25	10 abs
<10	50		

FOI

Proposal - Screening techniques

Technique	Requirements	Identification points	Comments
Lateral Flow Assay	Positive response	3	Positive and negative controls required
Electrophoresis (Gel/capillary electrophoresis) Chromatography (HPLC, HILIC, IEX)	Retention time ±0.2 min S/N ≥ 5	3	Electrophoresis migration parameter needs consideration
MALDI MS Molecular weight ESI MS Molecular weight	Mw 64,000 ±1000	3	Wide Mw distribution due to glycosylation heterogeneity
Immunoassay (ELISA)	Positive response	4	Quantification required
PCR	Positive response	4	Specific primers required

FOI

Proposal - Screening techniques for enzymatic digestion products

Technique	Requirements	Identification points	Comments
MALDI MS or ESI MS	Peptide mass ±0.2 Da	1 p /peptide (tot 3p)	Minimum 3 peptides S/N >10 Peptide mass accuracy < ±20 ppm: +1p/peptide
LC-MS	Retention time ±0.2 min Peptide mass ±0.2 Da	2 p /peptide (tot 6p)	

FOI

Proposal Confirmation of enzymatic digestion products

Technique	*Requirements*	*Identification points*	*Comments*
MALDI MS/MS or ESI MS/MS	Product ion mass accuracy ±0.2 Da Ions >10% relative intensity required	Parent ion: 1p + 1p /product ion (tot 15p)	Min. 3 peptides Uniqueness of peptides Min. 2 structure specific product ions. Major ions explained. Max. 4 points/spectrum
LC- MS/MS	Retention time ±0.2 min Product ion mass accuracy ±0.2 Da Ions >10% relative intensity required	Rt +parent ion: 2 p /peptide + 1 p /product ion (tot 18p)	Min. 3 peptides Uniqueness of peptides Min. 2 structure specific product ions Major ions explained. Max. 4 points/spectrum
LC- MS/MS MRM transitions	Retention time ±0.2 min Intensity ratio of 2 MRM transitions <10 % relative to reference value	Rt: 2 p /peptide + 1 p /transition (tot 9p)	Min. 3 peptides Uniqueness of peptides Min. 2 transitions/peptide

FOI

NATO SIBCRA Identification levels

Confirmation level	*Minimum points*
Unambiguous identification	≥ 15
Confirmed	≥ 6
Provisional	≥ 3
No confirmation	< 3

Example unambiguous id:

ELISA (3p)
Molecular mass (3p)
Mass of 3 peptides by MALDI or ESI MS (3p)
MALDI or ESI MSMS, 3 product ions/peptide (3x3p)
Total identification points: 18

FOI

Evaluation using NATO SIBCRA identification levels

	No MS enzym. digest.	MALDI MS ESI MS 3 peptides	LC-MS 3 peptides	MALDI or ESI MS/MS 3 peptides	LC-MS/MS MRM 3 peptides	LC-MS/MS 3 peptides
No screening technique	0	3	6	15	12	18[1]
LFA	3	6	9	18[2]	15[2]	21[2]
Electrophoresis Chromatography	3	6	9	18	15	21
MALDI MS Molecular weight	3	6	9	18	15	21
ELISA	4	7	10	19[2]	16[2]	22[2]
PCR	4	7	10	19[2]	16[2]	22[2]

FOI

Identification Criteria Proposal

Method B / Method A	MALDI MS ESI MS	LC-MS	MALDI MS/MS ESI MS/MS	LC-MS/MS MRM	LC-MS/MS
LFA	A: Positive B: Molecular weight of min. 3 peptides, Δm<0.2, S/N>10	A: Positive B: Ret.time ±0.2 min. Mol. weight, min. 3 peptides, Δm<0.2, S/N>5	A: Positive B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10	A: Positive B: : Ret.time ±0.2 min. Min. 3 peptides, min. 2 MRM transitions, S/N>5	A: Positive B: : Ret.time ±0.2 min. MSMS spectrum min. 2 product ions, Δm<0.2, S/N>5
Electrophoresis Chromatography	A: Ret.time ±0.2 min. B: Molecular weight of min. 3 peptides, Δm<0.2, S/N>10	A: Ret.time ±0.2 min. B: Ret.time ±0.2 min. Mol. weight, min. 3 peptides, Δm<0.2, S/N>5	A: Ret.time ±0.2 min. B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10	A: Ret.time ±0.2 min. B: Ret.time ±0.2 min. Min. 3 peptides, min. 2 MRM transitions, S/N>5	A: Ret.time ±0.2 min. B: : Ret.time ±0.2 min. MSMS spectrum min. 2 product ions, Δm<0.2, S/N>5
MALDI MS Molecular weight	A: 64000 ±1000 B: Molecular weight, min. 3 peptides, Δm<0.2, S/N>10	A: 64000 ±1000 B: Ret.time ±0.2 min. Mol. weight, min. 3 peptides, Δm<0.2, S/N>5	A: 64000 ±1000 B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10	A: 64000 ±1000 B: Ret.time ±0.2 min. Min. 3 peptides, min. 2 MRM transitions, S/N>5	A: 64000 ±1000 B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>5
ELISA	A: Positive B: Molecular weight. Min 3 peptides, Δm<0.2, S/N>10	A: Positive B: Ret.time ±0.2 min. Mol. weight, min. 3 peptides, Δm<0.2, S/N>5	A: Positive B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10	A: Positive B: : Ret.time ±0.2 min. Min. 3 peptides, min. 2 MRM transitions, S/N>5	A: Positive B: : Ret.time ±0.2 min. MSMS spectrum min. 2 product ions, Δm<0.2, S/N>5
PCR	A: Positive B: Molecular weight. Min 3 peptides, Δm<0.2, S/N>10	A: Positive B: Ret.time ±0.2 min. Mol. weight, min. 3 peptides, Δm<0.2, S/N>5	A: Positive B: MSMS spectrum min. 2 product ions, Δm<0.2, S/N>10	A: Positive B: : Ret.time ±0.2 min. Min. 3 peptides, min. 2 MRM transitions, S/N>5	A: Positive B: : Ret.time ±0.2 min. MSMS spectrum min. 2 product ions, Δm<0.2, S/N>5

FOI

Comments

- Confirmation by MSMS analysis of 3 peptides including 1 unique peptide from each of the chains
- Evidence of intact ricin (A and B chains connected) can be obtained by detection of the enzymatic digest product with the corresponding peptides connected by an intact disulfide bond (T24A-T1B)
- Selective sample clean-up in combination with MS or MS/MS earns an additional 3 p (chromatographic/electrophoretic or affinity clean-up)

- Suggested method combination for unambiguous identification of ricin:
  - LFA or standardised ELISA with commercially available antibodies
  - MALDI TOF MSMS or LC-MSMS equipment

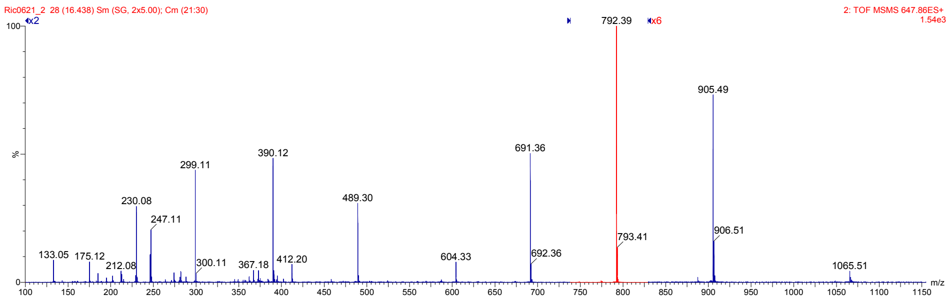
FOI

Comments

- Identification levels: screening and confirmation
- PCR: false positive risk
- MALDI & LC-MS PMF: peptide uniqueness
- Electrophoresis migration – comparison with Mw standards
- Additional IPs for cleanup before LC-MS and MSMS as for MALDI MS and MSMS
- Functionality test based on immunocapture – enzymatic reaction + LC-MSMS of released adenine (Anal. Chem. 2007)

FOI

Evaluation example

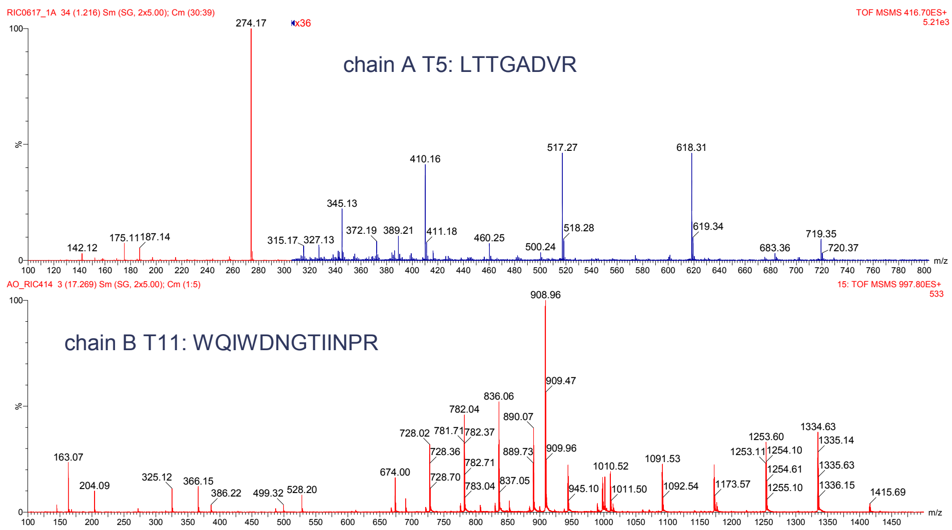


b	116.03	230.08	390.11	503.19	604.24	691.27	806.30	893.33	1007.37	1120.46	-
	1	2	3	4	5	6	7	8	9	10	11
	Asp	Asn	CAM	Leu	Thr	Ser	Asp	Ser	Asn	Ile	Arg
	11	10	9	8	7	6	5	4	3	2	1
y"	-	1179.54	1065.50	905.47	792.39	691.34	604.31	489.28	402.25	288.20	175.12

		RT (min)	ion 1	ion 2	ion 3	ion 4	ion 5	ion 6	ion 7	ion 8	ion 9	ion 10	ion 11
Product ion (*m/z*)	Analyte	16.30	792.40	691.36	390.12	299.11	489.30	905.50	230.08	247.11	604.33	175.13	1065.4
	Ref.	16.44	792.40	691.36	390.12	299.11	489.30	905.50	230.08	247.11	175.13	604.33	1065.5
Difference		0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	-0.01
Product ion Rel. intens.	Analyte		1.000	0.250	0.244	0.221	0.154	0.149	0.122	0.103	0.040	0.040	0.007
	Ref.		1.000	0.264	0.242	0.214	0.149	0.139	0.132	0.093	0.040	0.038	0.007
Identification points		1	1	1	1	1	1	1	1	1	1	1	1
Σ IP		5 (12)											

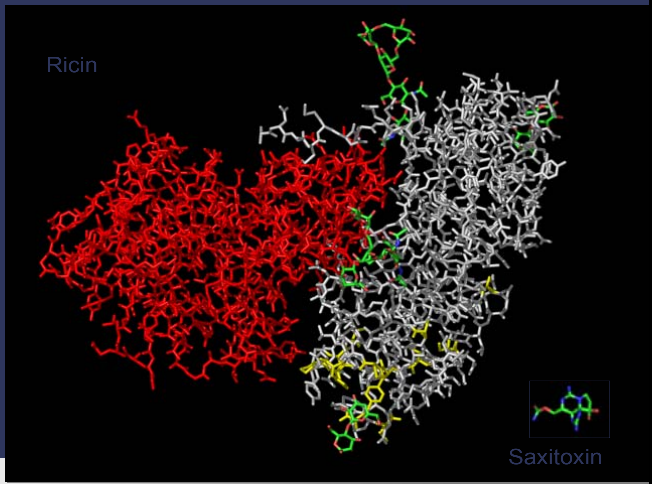
FOI

Example peptides



FOI

Unambiguous identification: Ricin & Saxitoxin



Structure of ricin determined by X-ray crystallography.

E. Rutenber *et al.* *Proteins* 10 (1991)

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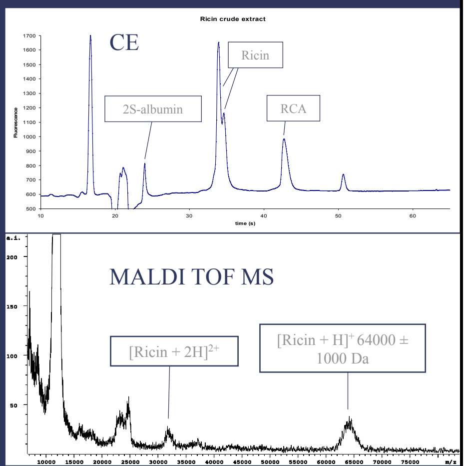
Screening for ricin - containing samples

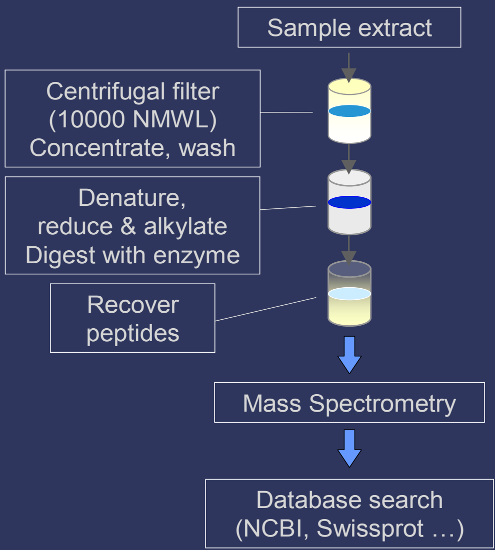
- Functional assays
- **Screening for protein content**
  - Extraction
  - Capillary gel electrophoresis
  - MALDI TOF Mass Spectrometry

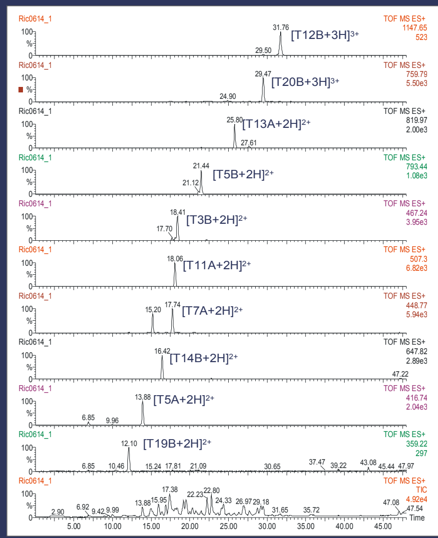


CGE and MALDI MS of a crude ricin extract

FOI

Ricin ROP – enzymatic digestion





LC-MS: trypsin digest of crude ricin
Anal Chem. 2005, 77, 1545-1555

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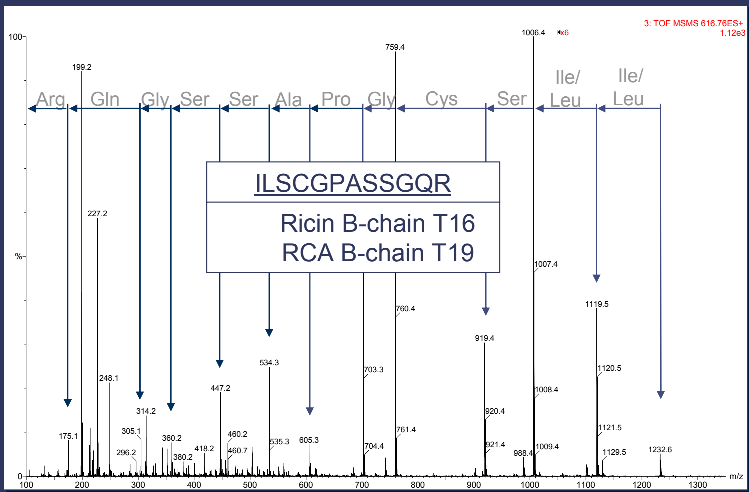
Ricin chain A trypsin digest peptides

T# [a]	Peptide sequence [b]	Mass	Observed sequence ions
T1	IFPK	504.32^{+}	y''_1-y''_3, b_2, a_2, FP
T2	QYPIINFTTAGATVQSYTNFIR	1225.90^{3+}	y''_1-y''_{11}, b_5, T_A2^{2+}, $[T_A2–HexNAc+2H]^{2+}$, HexNAc, $(HexNAcHex_{1-3})$
T3	AVR	[344.22]	
T4	GR	[231.13]	
T5	LTTGADVR	416.73^{2+}	y''_1-y''_2, b_2/DV, z_1-z_2, a_2/AD, a_4/TGAD, GAD
T6	HEIPVLPNR	537.81^{2+}	y''_1, y''_3-y''_8, b_1-b_3, a_1-a_3, z_3-z_7, PV
T7	VGLPINQR	448.77^{2+}	y''_1-y''_7, b_2, z_1-z_5, PL/LP, NQ, GLP, PIN
T8	FILVELSNHAELSVTLALDVTNAYVVGYR	1069.57^{3+}	y''_1-y''_{14}, b_{18}^{2+}-b_{21}^{2+}, b_{23}^{2+}-b_{28}^{2+}, $[M+3H-H_2O]^{3+}$,
T9	AGNSAYFFHPDNQEDAEAITHLFTDVQNR	827.63^{4+}	y''_1, y''_4- y''_{12}, y''^{2+}_9-y''^{2+}_{13}, y''^{2+}_{20}- y''^{2+}_{21}, y''^{3+}_{22}-y''^{3+}_{28}, b_3-b_5, NS, PD-H_20, FH, GNSA, GNSA-NH_3, QED-NH_3, FFH
T10	YTFAFGGNYDR	655.79^{2+}	y''_1, y''_4-y''_{10}, b_4, i_1, i_7/i_9
T11	LEQLAGNLR	507.29^{2+}	y''_1- y''_2, y''_3-y''_8, b_2, z_7-z_8, AGN-CO
T12	ENIELGNGPLEEAISALYYYSTGGTQLPTLAR	1147.58^{3+}	y''_1-y''_{14}, b_4-b_5, b_{14}, b_{17}, b_{17}^{2+}-b_{21}^{2+}, b_{24}^{2+}- b_{27}^{2+}, $[M+3H-H_2O]^{3+}$
T13	SFIICIQMISEAAR	791.41^{2+}	y''_1, y''_3-y''_{12}, b_2-b_6, a_2
T14	FQYIEGEMR	586.77^{2+}	y''_1-y''_2, y''_4-y''_8, z_1, z_4-z_8, c_1
T15	TR	[275.16]	
T16	IR	[287.20]	
T17	YNR	[452.23^{+}]	y''_1, a_1
T18	R	[174.11]	
T19	SAPDPSVITLENSWGR	864.93^{2+}	y''_2-y''_{14}, y''^{2+}_{12}, y''^{2+}_{14}, b_3-b_6, PD/ DP, PDPSVI
T20	LSTAIQESNQGAFASPIQLQR	1130.09^{2+}	y''_1-y''_9, y''_{11}-y''_{16}, b_4-b_7, b_{10}, b_{12}-b_{15}
T21	R	[174.11]	
T22	NGSK	[404.20]	
T23	FSVYDVSILIPIIALMVYR	738.09^{3+}	y''_1-y''_{11}, SI-H_20, IP/PI, YDVS-H_20/SVYD-H_20, VSILI-CO, VYDVS-H_20/SVYDV-H_20, VSILIPIIA-H_20
T24A-ss-T1B	ADVCMDPEPIVR [T_B1] CAPPPSSQF [T_A24]	759.01^{3+}	[$T_B1+T_A24b_2$], [$T_B1+T_A24b_3$-T_A24b_8], T_B1; y''_1, y''_4-y''_8, T_B24; y''_1-y''_3, y''_5-y''_7, SS, PP, SQ, PPPS, APPPSSQ

Ricin chain B trypsin digest peptides

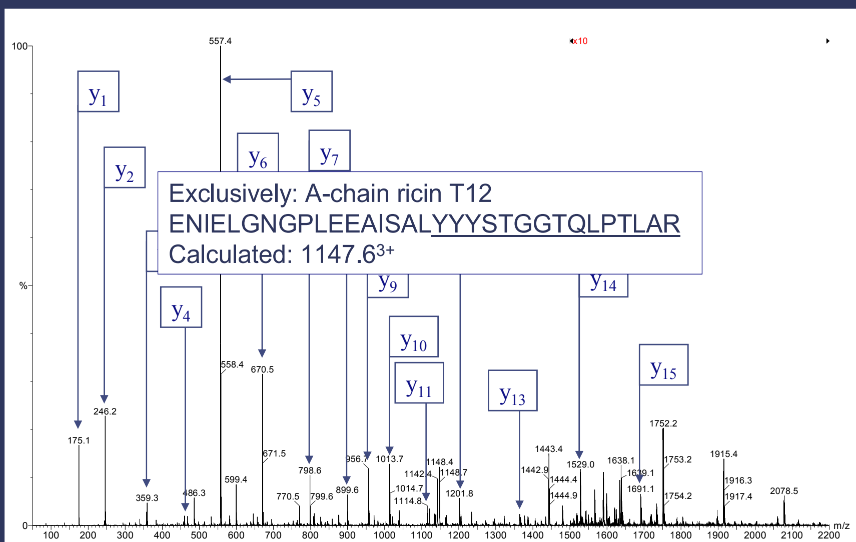
T# [a]	Ricin D peptide sequence [b]	Mass	Observed sequence ions
T2	IVGR	444.29^{+}	y''_1-y''_3, b_2-b_3, a_2
T3-ss-T5	NGLCVDVR FHNGNAIQLWPCK	800.68^{3+}	$[M+3H-H_2O]^{3+}$, $[T_B3\text{-ss-}T_B5y''_3\text{-}T_B5y''_4]^{1+}$, $[T_B3\text{-ss-}T_B5b_{12}]^{3+}$, $[T_B5\text{-ss-}T_B3y''_5]^{2+}$, T_B3; y''_1-y''_2, T_B5; b_2-b_3, b_6-b_{10}, i_{10}
T4	DGR	[346.16]	
T6	SNTDANQLWTLK	695.85^{2+}	y''_1-y''_{10}, b_2-b_3, z_5, $[M+2H-H_2O]^{2+}$
T7	R	[174.11]	
T8	DNTIR	618.32^{+}	y''_1- y''_2, y''_4, b_2-b_3, z_4, TI,
T9	SNGK	[404.20]	
T10 (-ss-)	CLTTYGYSPGVYVMIYDCNTAATDATR	983.10^{3+}	y''_1- y''_9, i_5/i_{16}, PG, PGV, PGV, PGVY, PGVYV, PGVYVM, PGVYVMI, PGVYVMIY, $[M+3H-H_2O]^{3+}$, $([y''\text{-}b]^{2+})$ [g]
T11	WQIWDNGTIINPR	997.77^{3+}	y''_2- y''_5, b_2-b_5, INP, T11, $T11^{2+}$, $[T_B11+HexNAc+2H]^{2+}$, $[T_B11+2HexNAc+2H]^{2+}$, $[T_B11+2HexNAc+Hex+2H]^{2+}$-$[T_B11+HexNAc+4Hex+2H]^{2+}$, HexNAc, (HexNAc-$Hex_1$)-(HexNAc-$Hex_3$)
T12	SSLVLAATSGNSGTTLTVQTNIYAVSQGW LPTNNTQPFVTTIVGLYGLCLQANSGQV WIEDCSSEK	[6936.75]	
T13	AEQQWALYADGSIRPQQNR	744.37^{3+}	b_2-b_6, y''_1, y''_5, y''^{2+}_9-y''^{2+}_{17}, DG, QQ, EQ, EQQ-NH_3, QQ-NH_3, QQWA-NH_3
T14-ss-T16	DNCLTSDSNIR ILSCGPASSGQR	648.3^{2+}	$[T_B14\text{-ss-}T_B16y''_9\text{-}y''_{11}]^{2+}$, $[T_B16\text{-ss-}T_B14y''_9]^{2+}$, T_B14; y''_1-y''_8, T_B16; y''_1-y''_8, b_2-b_3, a_2
T15	ETVVK	575.34^{+}	y''_1-y''_4, b_2-b_3
T17	WMFK	611.30^{+}	y''_1-y''_3, b_2, a_2
T18	NDGTILNLYSGLVLDVR	621.34^{3+}	y''_1-y''_5, y''_7-y''_9, b_2, b_4, b_7-b_8, i_9, LN/NL, SGL
T19	ASDPSLK	359.19^{2+}	y''_1-y''_3, b_3, z_1, PS/SD-H_2O, PS-CO, PS- H_2O, SL-CO, DP- H_2O, DP, PSL- H_2O, DPS-H_2O/SDP-H_2O, PSL,
T20	QIILYPLHGDPNQIWLPLF	1139.13^{2+}	y''_2-y''_5, y''_{14}, b_{14}-b_{15}, b_{14}^{2+}-b_{18}^{2+}

Example MS/MS peptide sequencing (M+2H)$^{2+}$ m/z 616.80



FOI

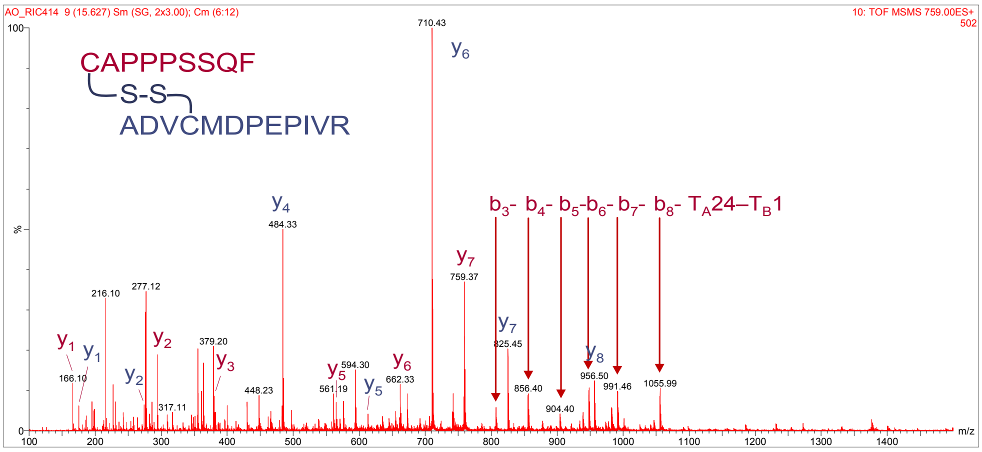
MS/MS spectrum of a peptide (M+3H)$^{3+}$, *m/z* 1147.6



FOI

Intact A-B disulfide bond indicates active ricin

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



Differentiating Ricin & RCA - A-chain

Ricin/RCA A-chain sequence homology: 94 %

ricin-specific trypsin peptides covers 67 % of the sequence

```
Ricin1   IFPKQYPIIN FTTAGATVQS YTNFIRAVRG RLTTGADVRH EIPVLPNRVG LPINQRFILV ELSNHAELSV   70
RCA2     IFPKQYPIIN FTTADATVES YTNFIRAVRS HLTTGADVRH EIPVLPNRVG LPISQRFILV ELSNHAELSV   70

Ricin1   TLALDVTNAY VVGYRAGNSA YFFHPDNQED AEAITHLFTD VQNRYTFAFG GNYDRLEQLA GNLRENIELG  140
RCA2     TLALDVTNAY VVGCRAGNSA YFFHPDNQED AEAITHLFTD VQNSFTFAFG GNYDRLEQLG G-LRENIELG  139

Ricin1   NGPLEEAISA LYYYSTGGTQ LPTLARSFII CIQMISEAAR FQYIEGEMRT RIRYNRRSAP DPSVITLENS  210
RCA2     TGPLEDAISA LYYYSTCGTQ IPTLARSFMV CIQMISEAAR FQYIEGEMRT RIRYNRRSAP DPSVITLENS  209

Ricin1   WGRLSTAIQE SNQGAFASPI QLQRRNGSKF SVYDVSILIP IIALMVYRCA PPPSSQF   267
RCA2     WGRLSTAIQE SNQGAFASPI QLQRRNGSKF NVYDVSILIP IIALMVYRCA PPPSSQF   266

XXX = underlined sequence determined by LC-ES MS/MS
XXX = peptide exclusive to ricin
X = ricin differentiating residue
```

1. GI:132567 Ricin precursor (Ricin D)
2. GI:113504 Agglutinin precursor (RCA)

Appendix 6

EXAMPLES OF MINIMUM CRITERIA FOR IDENTIFICATION BY CHROMATOGRAPHY AND MASS SPECTROMETRY

Table 1. Criteria for Mass Spectrometric Detection

Proposed priority order: (full scan (MS1) >) full scan (MS^n) > GC/LC-MSMS-SRM >GC/LC-MS-SIM

Please give your score on the following criteria

1 = best applicable, 2 = second 3= third, NO = should not be taken in use. If you cannot use the scoring give verbal explanation for your preferences.

#	Number of diagnostic ions	Relative abundances (RA)	Reference	Score & Comments
Full scan (MS1) Is this relevant at all in Trace Analysis?				
1.1	At least three structurally-specific ions, but no strict numerical criteria.	An acceptability range of ± 20 % on RA of major ions is a useful thumb rule, but not required.	FDA [1]	
1.2	Minimum of 3 ions (molecular ion, quasi-molecular ion or fragment ion)	If fragment ion's RA is greater than 5 %, must also include molecular ion or quasi-molecular ion. (See Table 2 for more information)	AORC [2]	
1.3	All measured diagnostic ions (molecular ion, characteristic adducts of the molecular ion, characteristic fragment ions and isotope ions)	When their relative intensity is more than 10 % in the reference spectrum of the calibration standard. (See table 3 for more information).	EC [3] EC [10]	
1.4	A minimum of three structurally significant ions.	± 20 %, when compared to the same relative abundances observed from standard solution	USDA [4]	
1.5	All diagnostic ions	When their relative intensity is more than 10 % in the reference spectrum of the calibration standard. (See table 4 for more information).	WADA [5] Rivier [11]	
1.6	The presence of all measured diagnostic ions with a relative intensity of more than 10% in the reference spectrum of the standard analyte is obligatory. If full-scan spectra are recorded in single MS, a minimum of four diagnostic ions must be present. The molecular ion should be included if it is present in the reference spectrum with a relative intensity of ≥10%.	A relative intensity ≥10% of the base peak.	Andre [8]	
1.7	At least three, preferably more diagnostic ions should be monitored (the molecular ion has to be included if relevant). All ions from the analyte should appear at the same retention time as the reference substance.	Relative intensities (%) of the base ion should be within ≤20% in the CI mode and ≤10% in the electron impact mode with respect to the standard analyte.	Rivier [11]	

#	Number of diagnostic ions	Relative abundances (RA)	Reference	Score & Comments
Selected Ion Monitoring (SIM, MS[1])				
2.1a	Three structurally-specific ions	Should match the comparison standard within ± 10 %	FDA [1]	
2.1b	Four or more unique structurally-specific ions	Should match the comparison standard within ± 15 %		
2.2	The molecular ion shall preferably be one of the selected diagnostic ions (the molecular ion, characteristic adducts of the molecular ion, characteristic fragment ions and all their isotope ions). The selected diagnostic ions should not originate from the same part of the molecule.	S/N-ratio ≥ 3:1 for each diagnostic ion	EC [3] EC [10]	
2.3	A minimum of four structurally significant ions.	Should match the comparison standard within ± 20 %	USDA [4]	
2.4	At least three diagnostic ions	The S/N -ratio of the least intense diagnostic ion must be greater than 3:1.	WADA [5] Rivier [11]	
2.5	At least one qualifying ion for each analyte and internal standard, in addition to a primary ion for each, is strongly encouraged where possible.	Ion ratios for LC/MS assays may be more concentration and time dependent than for GC/MS and therefore acceptable ion ratio ranges of up to 25% or 30% may be appropriate.	SOFT [6]	
2.6	The molecular ion should preferably be one of the selected diagnostic ions. The selected diagnostic ions should not exclusively originate from the same part of the molecule.	The S/N- ratio for each diagnostic ion must be 3:1. The relative intensities of the detected ions, expressed as a percentage of the intensity of the most intense ion or transition, must correspond to those of the standard analyte, either from calibration standards or from spiked samples	Andre [8]	
2.7	2-3 ions	The tolerance intervals for the ion ratios should be within the limits of ± 30 % of absolute ion abundance ratios.	FAO [9]	
2.8	A minimum of three diagnostic ions. In any case, a minimum of two diagnostic ions is mandatory in each mass spectrum.	The S/N-ratio of the diagnostic ions must be greater than 3:1. The relative abundance of any of the ions shall not differ by more than 5% (absolute) to 20% (relative), whichever is the greater, from that of the positive control urine; values of 0 and less are not valid, i.e. an expected ion must be present.	Rivier [11]	

#	Number of diagnostic ions	Relative abundances (RA)	Reference	Score & Comments
MS^n Full scan				
3.1	At least three structurally-specific ions, but no strict numerical criteria.	There should be a general correspondence between relative abundances or ranked abundances obtained for sample and standard.	FDA [1]	
3.2	A minimum of three diagnostic ions.	(See table 2 for more information)	AORC [2]	
3.3	Not defined	The S/N -ratio of the least intense diagnostic ion must be greater than 3:1.	WADA [5] Rivier [11]	
3.4	Three diagnostic ions that may include the precursor ion.	Precursor ion must have abundance equal to or greater than 5% of that of the most intense diagnostic ion of the MS–MS spectrum. Three ions must be considered with a S/N-ratio >3:1 and the relative abundance of any of the ions shall not differ by more than 10% (absolute) or 25% (relative), whichever is the greater, from that of the positive control urine; values of 0 and less are not valid, i.e. an expected ion must be present.	Rivier [11]	
MS^n Selected reaction monitoring (SRM)				
4.1a	If precursor ion is completely dissociated and only two structurally-specific product ions are monitored in MS^{n+1}	Should match the comparison standard within ± 10 %	FDA [1]	
4.1b	If three or more structurally-specific ions are monitored	Should match the comparison standard within ± 20 %		
4.2.	The target analyte confirmation shall be performed by either monitoring transition of one precursor ion to at least two product ions OR Monitoring at least two precursor-to-product ion transitions	± 20 %, when compared to the same relative abundances observed from standard solution. S/N-ratio 3:1.	USDA [4]	
4.3	Not defined	The S/N-ratio of the least intense diagnostic ion must be greater than 3:1.	WADA [5] Rivier [11]	
4.4	Three diagnostic ions that may this time include the precursor ion.	Precursor ion must have abundance equal to or greater than 5% of that of the most intense diagnostic ion of the MS–MS spectrum. Three ions must be considered with a S/N ratio >3:1 and the relative abundance of any of the ions shall not differ by more than 10% (absolute) or 25% (relative), whichever is the greater, from that of the positive control urine; values of 0 and less are not valid, i.e. an expected ion must be present.	Rivier [11]	

Table 2. The maximum permitted difference (tolerance) in RA in test spectrum and how to calculate it (AORC [2]).

RA of matched ion in reference spectrum	Full scan single-stage MS: Acceptable RA in test spectrum (10 % absolute or 30 % relative)	Full scan MS/MS & related techniques: Acceptable RA in test spectrum (20 % absolute or 40 % relative)
100 % (Base Peak)	70-100 %	60-100 %
90 %	63-100 %	54-100 %
80 %	56-100 %	48-100 %
70 %	49-91 %	42-98 %
60 %	42-78 %	36-84 %
50 %	35-65 %	30-70 %
40 %	28-52 %	20-60 %
30 %	20-40 %	10-50 %
20 %	10-30 %	0-40 %
10 %	0-20 %	0-30 %
5 %	0-15 %	0-25 %
1 %	0-11 %	0-21 %

Table 3. Maximum permitted tolerances for relative ion intensities using a range of mass spectrometric techniques (EC [3] & [10]).

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

Table 4. Maximum tolerance windows for relative ion intensities to ensure appropriate uncertainty in identification (WADA [5], Rivier [11]).

Relative abundance (% of base peak)	EI-GC-MS	CI-GC-MS, GC-MS^n LC-MS, LC-MS^n
> 50 %	± 10 % (absolute)	± 15 % (absolute)
25 % to 50 %	± 20 % (relative)	± 25 % (relative)
< 25 %	± 5 % (absolute)	± 10 % (absolute)

Table 5. Signal-to-Noise ratio (S/N) of the peaks

S/N	Reference
Greater than 3:1	FDA [1]
Above 3:1	AORC [2]
Greater than 3:1	WADA [5]
≥ 3:1	EC [3]
At least 3:1	USDA [4]

Proposal by VERIFIN: Signal-to-noise ratio at least 3:1

Table 6. Criteria for chromatographic separation (GC or LC)

Please give your score on the following criteria:
1 = best applicable, 2 = second 3= third, NO = should not be taken in use. If you cannot use the scoring give verbal explanation for your preferences.

#	Description	Reference	Score & Comments
Retention time (RT)			
5.1	Range should not exceed 2% for GC-MS or 5% for LC-MS	FDA [1]	
5.2	± 1 % or 6 seconds for GC (whichever is the greater) or ± 2% or 12 seconds for LC (whichever is the greater)	AORC [2]	
5.3	A tolerance of ± 0.5 % for GC and ± 2.5 % for LC.	EC [3]	
5.4	RT of the sample should be within 0.05 minutes for GC or 0.5 minutes for LC compared to the standard.	USDA [4]	
5.5	RT of the analyte should not differ more than 1 % or ± 0.2 minutes for GC or more than 2 % or ± 0.4 minutes for LC.	WADA [5] Rivier [11]	
5.6	For GC based assays, deviations of 1 - 2% from the calibrators or controls may be acceptable. Slightly larger deviations may be acceptable for HPLC based assays, particularly where the mobile phase is being programmed non-isocratically.	SOFT [6]	
5.7	Retention time correlation of the incurred analyte should fall within a +2% error factor (as currently generally accepted for both LC and GC as compared to a contemporary reference standard.	Baldwin [7]	
5.8	Tolerance intervals of 1.5 to 3% of the absolute retention time may be applied for capillary GC depending on the peak shape. The tolerance interval should be less than 1 sec for an RT less than 500 sec. For retention times between 500 and 5000 sec an interval of 0.2% RRT is recommended. For higher retention times 6 sec is a suitable interval.	FAO [9]	

5.9	With GC or LC, the peak width at half-maximum height shall be within the 90-110 % range of the original width, and the retention times shall be identical within a margin of 5 %.	EC [10]	
5.10	The retention time of the analyte should match that of standard with a tolerance of ± 5 s on capillary columns.	Rivier [11]	
5.11	the RT of the analyte shall not differ by more than 1% from that of the same substance in the positive control (urine) analyzed in the same batch	Rivier [11]	
Relative retention time (RRT) [when using ISTD]			
6.1	± 1 % or 6 seconds for GC or ± 2% for LC.	AORC [2]	
6.2	A tolerance of ± 0.5 % for GC and ± 2.5 % for LC	EC [3]	
6.3	Should be within 0.01 minutes for GC or 0.1 minutes for LC	USDA [4]	
6.4	The relative retention time of the analyte, shall correspond to that of the calibration solution at a tolerance of ± 0,5 % for GC and ± 2,5 % for LC.	EC [10]	
6.5	When using an internal standard, the RRT of the analyte must match that of the reference substance with a tolerance of 5/*A*, where *A* is the absolute retention time of the internal standard in seconds.	Rivier [11]	
6.6	Relative retention time (RRT) of the analyte shall not differ by more than 1% from that of the same substance in the positive control urine analyzed in the same batch	Rivier [11]	

References:

[1] Guidance for Industry: Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues, U.S. Department of Health and Human Services, Food and Drug Administration (FDA) Center for Veterinary Medicine 2003. Available: http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052658.pdf

[2] AORC Guidelines for the Minimum Criteria for Identification by Chromatography and Mass Spectrometry, Association of Official Racing Chemists USA 2003. Available: http://cobra.vdl.iastate.edu/aorc-2/AORC%20MS%20Criteria.pdf

[3] European Union Decision 2002/657/EC 17.8.2002, Off. J. Eur. Commun. 221 (2002) 8–36. Available: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:221:0008:0036:EN:PDF.

[4] United States Department of Agriculture, Agricultural Marketing Service, Science & Technology, Pesticide Data Program, SOP for Qualitative analysis. Available: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5074681

[5] World Anti-Doping Agency, Identification Criteria for Qualitative Assays; Incorporating Chromatography and Mass Spectrometry 1 Jan 2004. Available: http://www.wada-ama.org/rtecontent/document/criteria_1_2.pdf

[6] Society for Forensic Toxicologists / American Academy of Forensic Sciences, Forensic toxicology laboratory guidelines 2002.

[7] Report 1996 ASMS FALL WORKSHOP: Limits to Confirmation, Quantitation, and Detection. American Society for Mass Spectrometry (ASMS) 1997. Baldwin et al.

[8] Trends in the identification of organic residues and contaminants: EC regulations under revision. Andre et al. Trends in analytical chemistry, vol. 20, no. 8, 2001

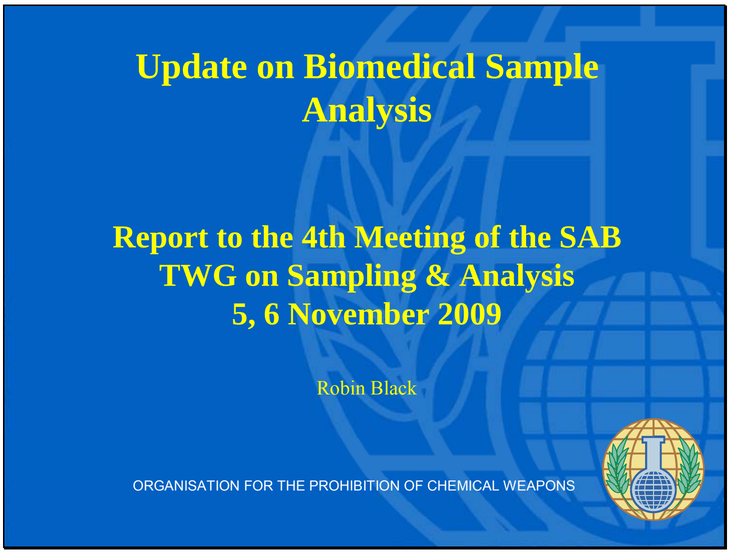
[9] FAO/WHO, Guidelines on The Use Of Mass Spectrometry (MS) For Identification, Confirmation And Quantitative Determination Of Residues CAC/GL 56-2005. Available: http://www.codexalimentarius.net/download/standards/10185/cxg_056e.pdf

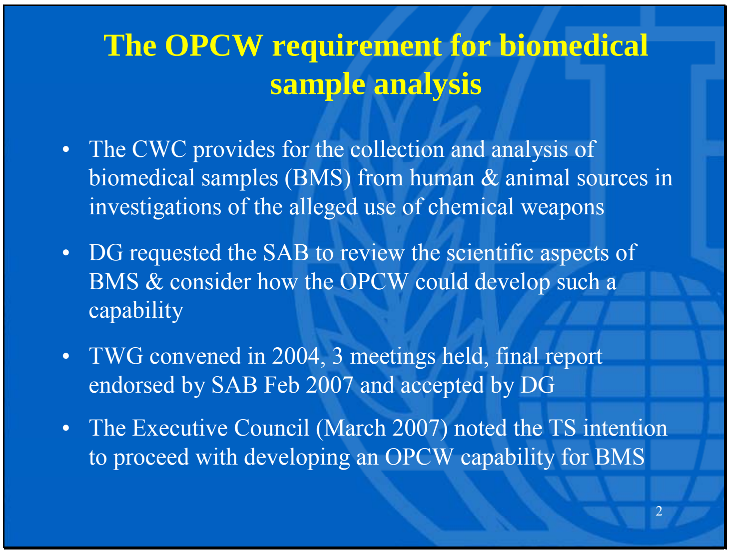
[10] EU COMMISSION DECISION implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. 2002D0657 - EN - 10.01.2004 - 002.001 - 1. Available: http://www.bsmi.gov.tw/wSite/record/file_act.jsp?ixCuAttach=3597

[11] Laurent Rivier, Analytica Chimica Acta 492 (2003) 69–82.

Appendix 7

UPDATE ON BIOMEDICAL SAMPLE ANALYSIS





Recommendation of SAB TWG on Biomedical Samples

- The next stage in building a capability should be coordinated by the OPCW laboratory with assistance from member states
- A progression recommended:

  collation/dissemination of knowledge
  ⇒ confidence building exercises
  ⇒ validated methods
  ⇒ proficiency tests
  ⇒ designation

3

Differences in requirements from OPCW analysis of chemical/environmental samples

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

4

1st First OPCW Confidence Building Exercise on Biomedical Sample Analysis

- To commence November 2009
- 25? participating laboratories
- Objectives:
  - To broaden the capability for biomedical sample analysis across Member States
  - To assess advantages & disadvantages of different methods
  - To commence a discussion on criteria for identification at trace levels
  - Identification is the main requirement but laboratories encouraged to report quantitative results if obtained

5

1st First OPCW Confidence Building Exercise on Biomedical Sample Analysis

- Matrix will be commercial synthetic urine
  - To facilitate handling in this first exercise
- Samples prepared by TNO Defence, Security and Safety, Rijswijk, The Netherlands
  - Stability tests completed
- Samples to be dispatched by OPCW laboratory (at 4°C)

6

1st First OPCW Confidence Building Exercise on Biomedical Sample Analysis

- Analytes will be urinary metabolites of sulfur mustard and nerve agents
  - Laboratories will be advised which types of analyte to target
  - List of references to different methods supplied
  - 5 spiked samples, 3 at 100 ng/ml, 2 at 10 ng/ml, plus blank urine
  - 4 standards also supplied
  - Technical advice & assistance available during the exercise from Dstl and TNO
  - Identification is the main requirement, preferably by two methods
    - but laboratories encouraged to report quantitative results if obtained

7

1st First OPCW Confidence Building Exercise on Biomedical Sample Analysis

- Laboratories asked to submit a report to OPCW lab on or before 15 January 2010
- Flexible reporting template supplied, with an example
- Reports will be evaluated by Dstl and TNO
- Target date for completion 15 February 2010
  - Meeting to discuss results end February
- Reports will not be ‘scored’ – it is not a test

8

Annex 5

SAXITOXIN FACT SHEET

Introduction

Saxitoxin (STX) is a neurotoxin which is naturally produced by certain species of marine dinoflagellates (including *Alexandrium sp.*, *Gymnodinium sp.*, *Pyrodinium sp.*) and cyanobacteria (including *Anabaena sp.*, some *Aphanizomenon spp.*, *Cylindrospermopsis sp.*, *Lyngbya sp.*, *Planktothrix sp.*). Ingestion of saxitoxin (usually through shellfish contaminated by toxic algal blooms) is responsible for the human illness known as paralytic shellfish poisoning (PSP).

The term Saxitoxin has also been used to refer to the entire suite of related neurotoxins produced by these microorganisms, which in addition to saxitoxin, includes neosaxitoxin (neoSTX), the gonyautoxins (GTX) and decarbamoylsaxitoxin (dcSTX). These molecules range in MW from 250 and 500Da, depending on the subsituent side groups.

Nomenclature

The term Saxitoxin originates from the species name of the butter clam (*Saxidona giganteus*) from which the toxin was first recognized.

The systematic IUPAC name for Saxitoxin is:
(3aS-(3a-α,4-α,10aR*))2,6-diamino-4-(((amino-carbonyl)oxy)methyl)-3a,4,8,9-tetrahydro-1H,10H-pyrrolo(1,2-c)purine-10,10-diol.

A survey of the literature demonstrates how the nomenclature of Saxitoxin has changed since the toxin was first isolated in 1957.[7] In particular, the term 'Saxitoxin' was originally used in reference to the dihydrochloride salt of the molecule.[8] In the early 1980s, one chemistry manual referred to the free base as Saxitoxin.[9] However, since the late 1980s, the doubly charged cation has been referred to as Saxitoxin.[10] More recently (and since the negotiations on the Chemical Weapons Convention were concluded in 1992), the nomenclature of Saxitoxin compounds has become more specific—distinctions are now made between Saxitoxin dihydrochloride and Saxitoxin dihydrate.[11]

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7 R J Mathews, 'Saxitoxin and the CWC: Personal Recollections and Reflections', Presentation to the Thirteenth Session of the Scientific Advisory Board, Annex 4 in Report of the Thirteenth Session of the Scientific Advisory Board, SAB-13/1 (1 April 2009).

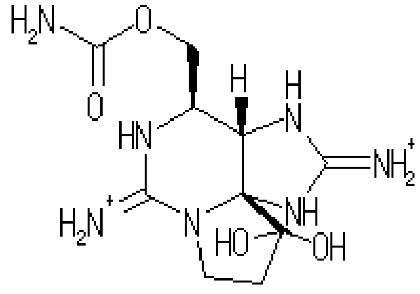
8 See, for example, Dictionary of Organic Compounds, 4th Edition (1965); SIPRI, The Problem of Chemical and Biological Warfare Vol. I, pp. 67-68, (1971), P.J. Scheuer, Chemistry of Marine Natural Products, (1973).

9 Dictionary of Organic Compounds, 5th Edition (1982).

10 See for example, The Concise Encyclopedia Biochemistry, 2nd Edition (1988), The Merck Index 11th Edition (1989); The Merck Index 14th Edition (2006).

11 Richard J Sax Sr, Sax's Dangerous Properties of Industrial Materials, 9th Edition (1995).

Structure of Saxitoxin[12]



Sources of Saxitoxin

Saxitoxin can be isolated from bivalve molluscs (such as the butterclam *Saxidona giganteus*) that have accumulated PSP-producing dinoflagellates (such as *Gonyaulax catanella*) during feeding. In one reported experiment, about 8 tonnes of clams were processed to produce a single gram of Saxitoxin.[13]

Saxitoxin has been synthesised in very small quantities and with considerable difficulty. Saxitoxin was first synthesised in 1977 in a 17-step synthesis with an overall yield of 0.2%.[14] More recently, (+) Saxitoxin has been synthesised in a 19-step synthesis with an overall yield of 1.6%.[15]

Main clinical features[16]

Saxitoxin is a powerful neurotoxin that binds with high affinity to sodium channels on cell membranes, inhibiting the influx of sodium ions into cells, with resulting suppression of cell action potentials, which results in paralysis.[17] Following ingestion of Saxitoxin, the onset of symptoms is typically within 10-60 minutes. Numbness or tingling of the lips and tongue (attributable to local absorption) spreads to the face and neck, followed by a prickling feeling in fingers and toes. With moderate to severe exposure, the paralysis spreads to the arms and legs. Motor activity is reduced, speech becomes incoherent and respiration laboured and subjects die from respiratory arrest. The terminal stages may occur within 2 – 12 hours. Fatalities in adults have been reported following ingestion of 0.5 – 12.4 mg.

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12 Source: ja.wikepedia.org.

13 WHO, Public health response to biological and chemical weapons, (World Health Organization, Geneva, 2004).

14 H. Tanino, T. Nakata, T Kanedo and Y. Kishi, A Stereospecific Total Synthesis of d,l-Saxitoxin, J. Amer. Chem. Soc., 1977, 2818.

15 J. Fleming and J. Du Bois, Total Synthesis of (+) Saxitoxin, J. Amer. Chem. Soc., 2006, 3926.

16 WHO, Public health response to biological and chemical weapons, (World Health Organization, Geneva, 2004).

17 It has been shown that the doubly-charged cation is the form of Saxitoxin that binds to the sodium channels on cell membranes.

Protective Measures[18]

Diagnosis of Saxitoxin poisoning is confirmed by detection of the toxin, using ELISA or mouse bioassay, in samples of, for example, stomach contents, water or food. No specific antidotes to Saxitoxin poisoning exist, and treatment is symptomatic. The toxin is normally cleared rapidly from the body via the urine, so that victims who survive for 12 – 24 hours usually recover. Diuretics may help. Specific antitoxin therapy has been successful in animals. No vaccine against Saxitoxin exposure has been developed for human use.

Saxitoxin: Peaceful applications

Saxitoxin is a component in diagnostic testing kits for PSP. It is also used in neurochemical research, including electrophysiological studies.

Saxitoxin as a CB weapon

Saxitoxin dihydrochloride was first isolated at the US Army Fort Detrick laboratory in the 1950s, designated as Agent TZ, and was investigated as a potential weapon.[19] Agent TZ was apparently weaponised in the M1 Biodart (E1) flechette system in the 1950s and 1960s.[20]

Saxitoxin is soluble in water and stable, and dispersal as an aerosol is feasible. No cases of inhalation exposure have been reported in the medical literature, but animal experiments suggest that the entire syndrome is compressed, and that death may occur within minutes.[21]

Saxitoxin and the CWC

Saxitoxin was proposed for inclusion in the CWC Schedules of Chemicals by the USA in 1984,[22] and was subsequently included in the CWC Rolling Texts within Schedule 1, with a footnote reflecting the view of some negotiators that Saxitoxin would be more appropriate in Schedule 2. From the record of negotiations it appears that what negotiators wanted to include in the Schedules was the form of Saxitoxin that had been weaponised in the past (that is, Agent TZ, the dihydrochloride salt), and other forms of weaponisable Saxitoxin.[23] When CAS Numbers were assigned to the chemicals in the CWC Rolling Text in the late 1980s, Saxitoxin was assigned that the CAS Number of Saxitoxin dihydrate on the understanding that the CAS Numbers were intended to be essentially 'identification aids' rather than

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18 WHO, Public health response to biological and chemical weapons, (World Health Organization, Geneva, 2004).

19 The military symbol TZ was derived after the name of its principal investigator, Dr Edward Shantz, who spent three decades working on toxins at the US Army Fort Detrick laboratory before joining the University of Wisconsin in 1972.

20 The M1 Biodart (E1) was a 7.62mm rifle cartridge flechette system filled with either Botulinum toxin A (XR), Saxitoxin (TZ), or possibly a combination of the two. There were reportedly 4,450 filled and 5,315 unfilled M1s in the US arsenal just prior to their destruction in the early 1970s. (Information from wikepedia).

21 WHO, Public health response to biological and chemical weapons, (World Health Organization, Geneva, 2004).

22 USA, CD/500, (1984)

23 R.J. Mathews, 'Saxitoxin and the CWC: Personal Recollections and Reflections', Annex 4 in Report of the Thirteenth Session of the Scientific Advisory Board, SAB-13/1 (1 April 2009).

'unique identifiers' for the various Scheduled chemicals.[24] In the CWC 'end-game' in 1992, it was agreed that Saxitoxin would be placed in Schedule 1.

Saxitoxin was the subject of the first simplified amendment procedure (technical change) to the CWC, based on concerns that delays in the transfers of diagnostic testing kits for PSP in shellfish (with each kit containing 5 micrograms of saxitoxin) by the 30 day advance notification requirement for Schedule 1 chemicals[25] could cause humanitarian problems. The technical change resulted in the 30 day advance notification requirement being waived for inter-states parties transfers of less than 5 milligrams of saxitoxin for medical/diagnostic purposes.[26]

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24 The issue of what constitutes Saxitoxin shows again that the CAS registry numbers given in the Convention cannot be considered to have regulatory power. They are essentially identification aids. See Paragraph 4.4 in Report of the Eighth Session of the Scientific Advisory Board, SAB-8/1 (19 February 2006).

25 CWC Verification Annex, Part VI, Paragraph 5.

26 CWC Verification Annex, Part VI, Paragraph 5bis, entered into force on 12 October 1999.