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AN OVERVIEW OF THE OPCW'S PROGRAMME FOR BIOMEDICAL SAMPLES ANALYSIS

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Abstract: *Since 2009 the Organization for the Prohibition of Chemical Weapons (OPCW) has been organizing and conducting confidence-building exercises for biomedical samples analysis. The purpose of the exercises was to begin and continue building expertise in the analysis of biomedical samples relevant to the investigation of incidents of alleged use of chemical weapons. The exercise consists of a number of samples for which the laboratory will be request to identify and determine the approximate concentration of a specified set of metabolic products of Chemical Weapons Convention scheduled chemicals.*
The paper provides an overview of the current status of the confidence-building exercises and the implication of the Scientific Research Centre for CBRN Defence and Ecology to the OPCW's programme for biomedical samples analysis.

Keywords: biomedical samples, chemical weapons, Organization for the Prohibition of Chemical Weapons

1. Introduction In case of alleged use, the Chemical Weapons Convention (CWC) allows for the collection of environmental samples as well as biomedical samples. Samples of importance in the investigation of alleged use (IAU) include toxic chemicals, munitions and devices, remnants of munitions and devices, environmental samples, such as air, soil, vegetation, water, snow, etc. and biomedical samples from human or animal sources, such as blood, urine excreta, tissue etc. (CWC Verification Annex, part XI, §17). Biomedical sample analysis is different from the analytical strategies currently followed for the OPCW proficiency test samples.
In the environmental samples is requested to identify any chemical contained in the schedules of the CWC plus all the degradation products that can originate from them. Matrices most used are soil, water, and organic liquids and the spiking chemicals are present in low ppm levels. Biomedical samples offer a greater challenge because of the low concentrations of analytes likely to be encountered, the greater complexity of biological matrices, and the initial requirement to identify suitable biological indicators of poisoning. In addition to forensic applications, biomedical sample analysis may be used for diagnostic purposes to ensure that casualties receive appropriate therapy, and for monitoring exposure to chemical warfare (CW) agents, for example, in workers engaged in demilitarization activities.
Figure 1 shows the challenge of environmental samples vs. biomedical samples.

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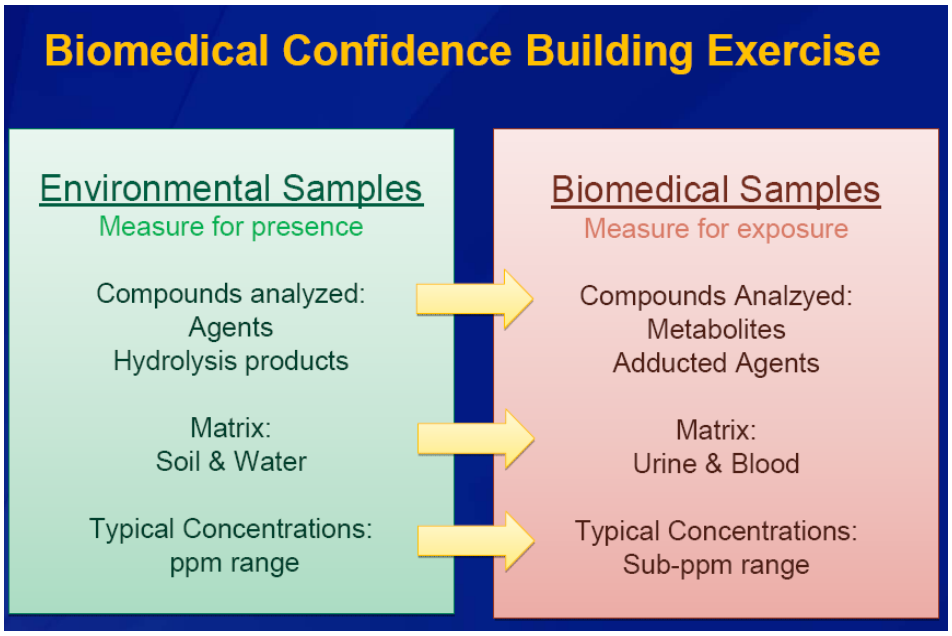

Figure 1: Environmental samples vs. biomedical samples

2. Goals of the OPCW biomedical sample analysis confidence building exercises

The purpose of the exercises is to begin and continue building expertise in the analysis of biomedical samples relevant to the investigation of alleged use.

The goals of biomedical sample analysis confidence building exercises are (a) to broaden the capability across Member States to analyze biomedical samples for biological markers of exposure to CWC Schedule 1 chemical warfare agents (currently this expertise resides in a small number of laboratories); (b) to assess the advantages and disadvantages of different analytical methods; (c) to commence a discussion on acceptable criteria for the identification of biomarkers, particularly where full scan mass spectra are not obtainable; (d) while the primary objective of the exercise was identification of the metabolite, laboratories were encouraged to report quantitative results if obtained; (e) to lead to a number of laboratories designated by the OPCW for the off-site analysis of biomedical samples in support of an IAU.

3. Characteristics of OPCW biomedical exercises

The biomedical exercises consist of a number of samples for which the laboratory will be requested to identify and determine the approximate concentration of a specified set of metabolic products of scheduled chemicals. Laboratories are encouraged to use their own methods of analysis as well as those suggested and, where possible, to compare the results in their reports.

Participants must analyse the samples using at least two different analytical techniques, one of these techniques must be a spectrometric technique, to identify the test chemicals (exception: in case of GC/MS or LC/MS together with High Resolution MS data only one technique will be sufficient). A system of identification points is followed for the evaluation of the data. A sum of at least 5 points should be obtained to achieve sufficient analytical data for the identification using a maximum of 3 techniques. The OPCW reporting templates should be used for reporting the identification results; a participant's report should be dispatched to the OPCW laboratory on or before the 28th calendar day after the confirmed date of arrival of the samples at the participant's laboratory (arrival day = day 1).

The OPCW laboratory in cooperation with evaluation laboratory publishes the report

of preliminary evaluation results and organizes meeting with participants to discuss preliminary evaluation results.

4. What is being measured in blood and urine samples?

Biomedical samples may include urine, blood, plasma, tissue, etc. As biomarkers of exposure are intact agents (biomarkers, metabolites), adducts with DNA and adducts with proteins.

Metabolites excreted in urine offer one of the simplest means of confirming that a casualty has been exposed to a CW agent. The major disadvantage of urinary metabolites as biological markers is that up to ~90% of the total amount excreted may be eliminated in the first 48–72 h following an exposure. Figure 2 shows a typical excretion profile for urinary metabolites and blood protein adducts. It is clear from Figure 2 that a much more sensitive analytical technique is required to detect metabolites in urine samples collected several days following an exposure, compared to samples collected within the first 48 hours. Depending on the scenario and application, it is clearly advantageous to have biological indicators that are more slowly eliminated, ideally allowing retrospective identification up to several months following an exposure. Covalent adducts with macromolecules, such as proteins and DNA, offer potentially much longer-lived biological indicators of exposure in comparison to free metabolites.

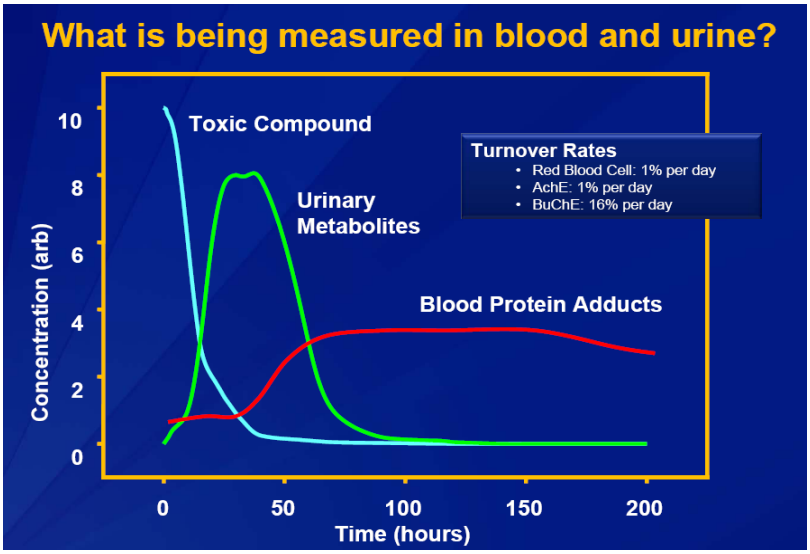

Figure 2: Typical excretion profile (Image from Thomas A. Blake presentation to the 3rd meeting of the participant laboratories)

5. History of the OPCW biomedical exercises

From 2009 to 2014, the OPCW laboratory conducted four biomedical exercises, to improve capabilities of the laboratories for the analysis of biomedical samples, to establish recommended sample preparation and analysis methods for the unambiguous identification of biomarkers of CW agents exposure and to establish a framework, including reporting, identification and evaluation criteria for a future system of proficiency tests.

The history of the OPCW biomedical exercises, matrix and spiked chemicals are presented in Table 1.

Table 1: History of the OPCW biomedical exercises

Biomedical test number	Sample dispatch	Matrix	Spiking chemicals	Concentration (ng/mL)
1st *	2009/2010	Synthetic urine	Mustard metabolites and phosphonic acids	100 - 10
2nd **	2012	Pooled human urine	Mustard metabolites and phosphonic acids	25 - 5
3rd ***	2013	Pooled human plasma; pooled human urine	Nerve agent producing adducts; nerve agent hydrolysis products	10 - 0.5
4th ****	2014	Pooled human plasma; pooled human urine	Nerve agent producing adducts; nerve agent hydrolysis products	10 - 0.5

* Samples prepared by TNO Defence, Security and Safety, Rijswijk, the Netherlands; Evaluation reports by TNO and Defence Science and Technology Laboratory (Dstl); 22 laboratories participated; 6 laboratories reported all spiking chemicals;
** Samples prepared by TNO; Evaluation by TNO and Dstl; 21 laboratories participated; 10 laboratories reported all spiking chemicals;
*** Samples prepared by The Verification Laboratory, Defence Medical and Environmental Research Institute, DSO National Laboratories, Singapore and OPCW Laboratory; 17 laboratories participated; 12 laboratories reported all spiking chemicals; first time many laboratories worked with human plasma;
**** Samples prepared by OPCW Laboratory with assistance from Lawrence Livermore National Laboratory, USA; 19 laboratories participated; 10 laboratories reported all spiking chemicals.

The first two exercises involved the analysis of metabolites of nerve agents and sulfur mustard agent in urine samples (both synthetic urine and pooled human urine).
In the 3rd exercise, it was desired to include the analysis of plasma samples as part of the exercise scenario: to assess whether 4 of the staff from the facility inspection have been exposed to sarin. 4 plasma and 2 urine samples plus 1 control were sent for biomedical sample analysis. Most participants used the fluoride regeneration method to analyze for the presence of nerve agents in plasma samples.
Regeneration of fluoridate nerve agents from butyrylcholinesterase produces the original agent; regeneration of tabun and V-agents produces corresponding fluoridate.
In the 4th exercise, it was desired to focus specifically on the analysis of plasma samples. The scenario was designed as an investigation of an alleged use of chemical weapons where plasma samples were collected. The target area for inspection was described as a small village, where the inspection team confirmed the presence of Ethyl S-2- diisopropylaminoethyl methylphosphonothiolate (VX) in munitions fragments and soil samples.
Tandem mass spectrometry was the most used MS technique for these exercises, with both selected reaction monitoring mode and product ion scan modes reported for analysis. High resolution mass spectrometry was reported using selected ion monitoring (SIM) and selected reaction monitoring (SRM). Single quad analysis was used with GC in SIM mode.
Participant laboratories to the OPCW biomedical exercises were from Sweden, UK, USA, Singapore, Finland, Romania, France, Germany, China, Hungary, India, Australia, Islamic Republic of Iran, Japan, Malaysia, Republic of Korea, Russian Federation, Spain, Turkey, and South Africa.

6. Benefits of involving the Scientific Research Center for CBRN Defense and Ecology to the OPCW's Programme for Biomedical Samples Analysis

The Scientific Research Centre for CBRN Defence and Ecology has been involved in 3 of the 4 OPCW biomedical sample analysis confidence building exercises. The Chemical Analysis and Testing Laboratory within the Centre developed procedures to prepare biomedical samples, especially for urine samples. Blood, a more difficult matrix to work with compared to urine, requires special equipments and personnel. In most scenarios associated with allegations of chemical weapons use, concentrations of free metabolites are likely to be higher in urine. Sample preparation included solid-phase extraction, liquid-liquid extraction, dilution, filtration, and derivatization. Analyses were performed by a single stage GC/MS (with selected ion monitoring). Chemicals *Ethyl methylphosphonic acid* and *Pinacolyl methylphosphonic acid* were found in a sample from the 2nd biomedical exercise, at 25 ng/mL spiking concentration, prepared by clean-up and derivatization with N-Methyl-N-(t-butyldimethylsilyl)trifluoroacetamide.

Figure 3 presents the mass spectrum of Ethyl methylphosphonic acid, confirmed by the reference chemical.

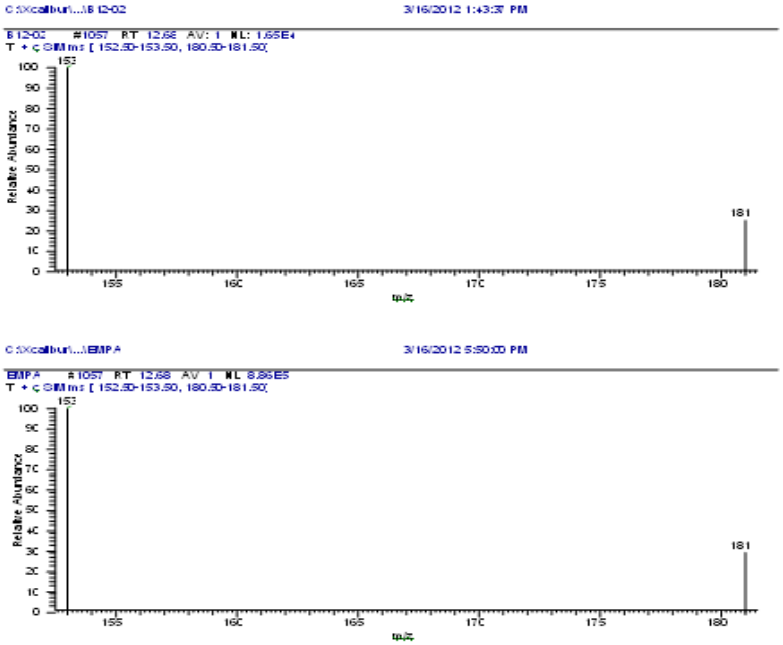

*Figure 3: EI SIM mass spectrum (m/z 153,181) supporting identification of **Ethyl methylphosphonate**: sample code B12-02 (top), reference chemical of TBDMS derivative of **Ethyl methylphosphonate**, sample code EMPA (bottom)*

The Chemical Analysis and Testing Laboratory developed 2 preparation methods of urine and plasma samples: the fluoride ion regeneration, followed by SPE with C18 sorbents, to liberate the nerve agent from the plasma, and conversion to perfluorinated derivatives, as derivatization method for detection of the phosphonic acids. Mass spectrometry was used for these exercises; single quad analysis was used with GC in SIM mode. The GC phase selected was 5% phenyl and 95% dimethylpolysiloxane column.

7. Conclusions

The OPCW has established a network of designated laboratories that are expert in the analysis of environmental samples.

The actual concern is building an international laboratories network with expertise in the domain of detection of chemical warfare agents from biomedical samples and introduction of analysis capabilities of biomedical samples in national strategies, as responses to chemical terrorism.

Biomedical sample analysis may provide key evidence in investigation of alleged use. Both free metabolites and covalent adducts provide unequivocal biological indicators of exposure to chemical warfare agents.

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