Science for Diplomats at EC-83
Chemical Weapons Sample Stability and Storage

12 October 13:30 – 14:45
Ooms Room
(light lunch available at 13:00)
SAB Response
to the Director-General’s request to the SAB to provide further advice on CW sample stability and storage

Dr Christophe Curty
SAB Member
SPIEZ LABORATORY
12.10.2016
Agenda

• Background
• DG’s request
• What is known?
• SAB response to the DG’s request
• Conclusion
Background Facts
Background
Investigation & Sampling
Background
Samples
Background Analysis Results

OPCW Designated Laboratories

[Map showing locations of designated laboratories worldwide, with indicators for various countries and laboratories.]
Background
Overview: from the facts to the analysis
DG’s request, 02.11.2015: Storage & Stability?
DG’s request, 02.11.2015:
Storage & Stability?

DIRECTOR-GENERAL'S REQUEST TO THE SCIENTIFIC ADVISORY BOARD TO PROVIDE ADVICE ON LONG-TERM STORAGE AND STABILITY OF SAMPLES COLLECTED IN RELATION TO POTENTIAL USE OF CHEMICAL WEAPONS

1. In order to be fully prepared to analyse any chemical potentially present in a wide range of types of samples in support of various operational missions, the OPCW must be able to store samples over several years and analyse those samples with high accuracy at any point in time.

2. In the context of the OPCW’s investigations and fact-finding missions the Technical Secretariat has since 2013 received samples in relation to potential use of chemical weapons. These samples are stored at the OPCW Laboratory at room temperature or refrigerated at 4°C.

3. Sample types (whether current or future) – containing chemicals of interest, such as various nerve and blister agents as well as their immediate precursors and degradation products – may include in particular:
   (a) Relatively pure samples;
   (b) Liquid (including extracts) and solid samples containing either relatively high levels or trace levels of the chemicals of interest;
   (c) Highly heterogeneous unprocessed samples – such as soil, metal fragments, paint chips, fragments of highly absorbent material, or wipes – containing either relatively high levels or trace levels of the chemicals of interest; and
   (d) Biomedical samples: blood, plasma, urine, tissue.

4. The Director-General requests the Scientific Advisory Board (SAB) to address the following questions:
   (a) Given the current storage conditions (set out in paragraph 2), how quickly and through what process could the types of samples mentioned in paragraph 3 degrade to a point where analysis of the samples would likely no longer return credible results?
   (b) What are the best-practice conditions for long-term storage of the types of sample mentioned in paragraph 3?
   (c) Given the best-practice storage conditions set out in the SAB’s answer to question (b), how quickly and through what process could the types of sample mentioned in paragraph 3 degrade to a point where analysis of the samples would likely no longer return credible results?
DG’s request, 02.11.2015: Storage & Stability?

Relatively pure samples

Liquid (including extracts) and solid samples containing either relatively high levels or trace levels of the chemicals of interest

Highly heterogeneous unprocessed samples – such as soil, metal fragments, paint chips, fragments of highly absorbent material, or wipes containing either relatively high levels or trace levels of the chemicals of interest

Biomedical samples: blood, plasma, urine, tissue

DIRECTOR-GENERAL’S REQUEST TO THE SCIENTIFIC ADVISORY BOARD TO PROVIDE ADVICE ON LONG-TERM STORAGE AND STABILITY OF SAMPLES COLLECTED IN RELATION TO POTENTIAL USE OF CHEMICAL WEAPONS

1. In order to be fully prepared to analyse any chemical potentially present in a wide range of types of samples in support of various operational missions, the OPCW must be able to store samples over several years and analyse those samples with high accuracy at any point in time.

Sample types
DG’s request, 02.11.2015: Storage & Stability?

- Given the current storage conditions (set out in paragraph 2), how quickly and through what process could the types of sample mentioned in paragraph 3 degrade to a point where analysis of the samples would likely no longer return credible results?

- What are the best-practice conditions for long-term storage of the types of sample mentioned in paragraph 3?

- Given the best-practice storage conditions set out in the SAB’s answer to question (b), how quickly and through what process could the types of sample mentioned in paragraph 3 degrade to a point where analysis of the samples would likely no longer return credible results?

Questions?
“Credible results”

- OPCW Proficiency Tests
- ISO 17025
- National Accreditation

«The SAB notes that the analytical findings of the Designated Laboratories (...) will always be scientifically accurate (...), the findings will always return ‘credible results’.”
Storage & Stability: what is known?

Degradation

Rotting

NATURAL PROCESS
Storage & Stability: what is known?
Influenced by?
Storage & Stability: what is known?
Influenced by?

- Composition
- Temperature
- Packaging
- Atmosphere
- Duration
- Light
- Moisture
- …
Storage & Stability: what is known?
Chemistry

- Chemical
- Composition of sample
- Temperature
- Atmosphere
- Packaging
- Duration
- Light
- Moisture
- …
Storage & Stability: what is known? Chemistry

Preparation

CWA

Degradation

Sarin

By-product

NATURAL PROCESS
Storage & Stability: what is known?
Chemistry

Degradation

Concentration

Time

A

LOD
Storage & Stability: what is known?

Chemistry

Chemical Composition of sample
Temperature Atmosphere Packaging Duration Light Moisture
Storage & Stability: what is known?
Chemistry

Concentration vs. Time

Degradation

A

LOD
Storage & Stability: what is known?
Chemistry

![Diagram showing concentration over time with A and B on the concentration axis and time on the horizontal axis. The diagram illustrates the degradation of A to B and the limit of detection (LOD).]
Storage & Stability: what is known?

Chemistry

Concentration

A

B

Degradation

A → B

Time

T1

T2

T3

LOD

A     B

95 A 5 B

60 A 40 B

5 A 95 B

Chemistry

Concentration

Degradation

A → B

Time

T1

T2

T3

LOD
Storage & Stability: what is known?
Chemistry

Degradation
A → B

Concentration

Time

T1

T2

T3

LOD
Storage & Stability: what is known?
Chemistry

Preparation

CWA

Sarin

CWC-related chemicals

Degradation

NATURAL PROCESS

By-product

CWC-related chemicals

Preparation

CWA

Sarin

CWC-related chemicals

Degradation

NATURAL PROCESS

By-product
Storage & Stability: what is known?

Chemistry

<table>
<thead>
<tr>
<th>Schedule number</th>
<th>(Estimated) number of chemicals</th>
<th>Schedule number</th>
<th>(Estimated) number of chemicals</th>
<th>Schedule number</th>
<th>Number of chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.A.1</td>
<td>&gt;20 000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.A.1</td>
<td>1</td>
<td>3.A.1</td>
<td>1</td>
</tr>
<tr>
<td>1.A.2</td>
<td>&gt;5 000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.A.2</td>
<td>1</td>
<td>3.A.2</td>
<td>1</td>
</tr>
<tr>
<td>1.A.3</td>
<td>&gt;200 000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.A.3</td>
<td>1</td>
<td>3.A.3</td>
<td>1</td>
</tr>
<tr>
<td>1.A.4</td>
<td></td>
<td>2.B.4</td>
<td></td>
<td>3.A.4</td>
<td>1</td>
</tr>
<tr>
<td>1.A.5</td>
<td>3</td>
<td>2.B.5</td>
<td></td>
<td>3.B.5</td>
<td>1</td>
</tr>
<tr>
<td>1.A.6</td>
<td>3</td>
<td>2.B.6</td>
<td></td>
<td>3.B.6</td>
<td>1</td>
</tr>
<tr>
<td>1.A.7</td>
<td>3</td>
<td>2.B.7</td>
<td></td>
<td>3.B.7</td>
<td>1</td>
</tr>
<tr>
<td>1.A.8</td>
<td>3</td>
<td>2.B.8</td>
<td></td>
<td>3.B.8</td>
<td>1</td>
</tr>
<tr>
<td>1.B.9</td>
<td>4</td>
<td>2.B.9</td>
<td></td>
<td>3.B.9</td>
<td>1</td>
</tr>
<tr>
<td>1.B.10</td>
<td>&gt;200 000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.B.10</td>
<td>10</td>
<td>3.B.10</td>
<td>1</td>
</tr>
<tr>
<td>1.B.11</td>
<td></td>
<td>2.B.11</td>
<td>8</td>
<td>3.B.11</td>
<td>1</td>
</tr>
<tr>
<td>1.B.12</td>
<td></td>
<td>2.B.12</td>
<td>10</td>
<td>3.B.12</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.B.13</td>
<td>1</td>
<td>3.B.13</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.B.14</td>
<td></td>
<td>3.B.14</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.B.15</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.B.16</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.B.17</td>
<td>1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Including branched chains and cyclo alkane chains, not including bicyclo alkane chains and stereoisomers and not including corresponding protonated and alkylated salts.

Storage & Stability: what is known? Chemistry

Response to the DG’s request
Response to the DG’s request

- Review of > 180 scientific papers

- Questionnaire to Designated Laboratories
Executive Summary

1. Given the current storage conditions in the OPCW Laboratory, how quickly and through what process could the aforementioned types of sample degrade to a point where analysis of the samples would no longer return credible results?

Storage conditions at the OPCW Laboratory
→ RT and 4°C

- Always credible analytical results
- Depends on many factors!
- Hydrolysis, oxidation and polymerisation
- Inevitable and natural (weeks to months!)
- Reduce time between collection and analysis
Executive Summary

1. Given the current storage conditions in the OPCW Laboratory, how quickly and through what process could the aforementioned types of sample degrade to a point where analysis of the samples would no longer return credible results?

Recommendation 1. Samples should be analysed as soon after collection as possible and the need for storage eliminated or, less favourably, the storage time minimised. **Prompt analysis** should be viewed as urgent, as the intact original chemicals will provide the strongest basis for confirming the use of chemicals prohibited by the Chemical Weapons Convention. (This is because the sample stability, and potential impacts of any matrix or environmental factors on the stability of any CWC-relevant chemicals in the sample, will not be known prior to analysis.)

Recommendation 2. **Further work** on the storage of samples just after sampling and during transport to the OPCW Laboratory, sample handling during splitting, handling and storage of samples at the OPCW Laboratory, should be pursued.
2. What are the best-practice conditions for long term-storage of the different types of sample?

**Recommendation 3. Commercial chemical** samples should be stored in glass containers with Teflon-lined caps in the dark: those in

(i) Schedules 1A01, 1A02, 1A03, 1A06, 1B09, 1B10, 1B11 and 1B12 at -18 °C under argon (to enable stability for 5-10 years).

(ii) Schedules 1A04 and 1A05 at room temperature (for stability > 10 years).

(iii) Schedule 1A08 (ricin) as a precipitate in 6 M ammonium sulfate at 4 °C (for stability > 10 years).
2. What are the best-practice conditions for long term-storage of the different types of sample?

Recommendation 3. Commercial chemical samples should be stored in glass containers with Teflon-lined caps in the dark; those in

Recommendation 4. Extracts of chemicals should be made in dichloromethane and stored in glass containers at 4 °C with Teflon-lined caps in the dark, to ensure stability of the intact original chemical for up to one year. (Swabs or wipes should be analysed within one month of collection or otherwise disposed of due to likely storage instability; wherever possible they should be extracted as soon as possible into dichloromethane and the extracts stored instead).

(iii) Schedule 1A08 (HFm) as a precipitate in 6 M ammonium sulphate at 4 °C (for stability > 10 years).
Executive Summary

2. What are the best-practice conditions for long term storage of the different types of sample?

Recommendation 3. Commercial chemical samples should be stored in glass containers with Teflon-lined caps in the dark; those in plastic containers in a refrigerator at 4 °C.

Recommendation 4. Extracts of chemicals should be made in dichloromethane and stored in glass containers at 4 °C with Teflon-lined caps.

Recommendation 5. Highly heterogeneous unprocessed samples – such as soil, metal fragments, paint chips, or fragments of highly absorbent material – containing relatively high levels of or trace levels of the chemicals of interest, should be stored in sealed glass or high-density polyethylene containers at -18 °C, to guarantee the stability of the samples for up to 6 months.

Recommendations 3 to 8 Type of containers, extracts, biomedical samples, splitting
Executive Summary

2. What are the best-practice conditions for long term-storage of the different types of sample?

**Recommendation 3.** Commercial chemical samples should be stored in glass containers with Teflon-lined caps in the dark; those in

**Recommendation 4.** Extracts of chemicals should be made in dichloromethane and stored in glass containers at 4 °C with Teflon-lined caps in the dark; tissue samples of the extracts should be split for

**Recommendation 5.** Highly heterogeneous unprocessed samples – such as soil, metal fragments, paint chips, or fragments of highly absorbent

**Recommendation 6.** Biomedical samples – for example, urine or plasma – should be stored in polypropylene or polyethylene terephthalate containers in a freezer at -80 °C (except for whole blood which should be refrigerated at 4 °C) to ensure the integrity of the samples for as long as possible (up to several years).
Executive Summary

2. What are the best-practice conditions for long term-storage of the different types of sample?

**Recommendation 3.** Commercial chemical samples should be stored in glass containers with Teflon-lined caps in the dark: those in dichloromethane can be stored at 4 °C.

**Recommendation 4.** Extracts of chemicals should be made in dichloromethane and stored in glass containers at 4 °C with Teflon-lined caps.

**Recommendation 5.** Highly heterogeneous unprocessed samples – such as soil, metal fragments, paint chips, or fragments of highly absorbent materials – should be stored in polypropylene or polyethylene terephthalate (PET) containers.

**Recommendation 6.** Biomedical samples – for example, urine or plasma – should be stored in polypropylene or polyethylene terephthalate containers.

**Recommendation 7.** Larger volumes of chemicals/samples should be split into subsamples and the subsamples used for repeated analytical manipulations. This will reduce the number of warming-cooling cycles the samples have to encounter. This is important especially for materials stored in a freezer or deep freeze (-80 °C). It will also help to minimise degradation of the chemical(s) in the unused portions of samples.

Recommendations 3 to 8 Type of containers, extracts, biomedical samples, splitting
Executive Summary

2. What are the best-practice conditions for long term-storage of the different types of sample?

Recommendation 3. Commercial chemical samples should be stored in glass containers with Teflon-lined caps in the dark; those in

Recommendation 4. Extracts of chemicals should be made in dichloromethane and stored in glass containers at 4 °C with Teflon-lined caps

Recommendation 5. Highly heterogeneous unprocessed samples – such as soil, metal fragments, paint chips, or fragments of highly absorbent materials – should be stored in polypropylene or polyethylene terephthalate

Recommendation 6. Biomedical samples – for example, urine or plasma – should be stored in polypropylene or polyethylene terephthalate

Recommendation 7. Larger volumes of chemicals/samples should be split into subsamples and the subsamples used for repeated analytical purposes

Recommendation 8. Samples of neat Scheduled chemicals required for long-term banking within the OPCW Laboratory should be flame-sealed in glass ampoules; the use of the flame-sealed ampoule technique appears to offer some storage and shipping advantages for which there is an evidence base.

Recommendations 3 to 8 Type of containers, extracts, biomedical samples, splitting
Executive Summary

3. Given these best-practice storage conditions, how quickly and through what type of process could the different types of samples degrade to a point where analysis of the samples would no longer return credible results?

- Always credible analytical results
- Inevitable and natural (weeks to months!)
- Extend “life” of original chemical
- Chemical forensics

Recommendation 9. The Technical Secretariat should monitor advances in sampling and analysis, and with the SAB, any new innovations relevant to chemical forensics.

Recommendation 10. A reference sample collection at the OPCW Laboratory should be kept to provide a range of chemical forensic options for current and future samples suspected of containing CWC-relevant chemicals.
Conclusion

Based on this review of processes by which CWC-relevant chemicals degrade, it is assessed that it is difficult, given the incomplete knowledge worldwide of the fate of CWAs in different matrices, to specify precisely when analysis of a sample ‘would likely no longer identify the intact original chemicals’. Analytical results, produced under stringent quality control in OPCW Designated Laboratories, are always ‘credible’. The main conundrum is how long after sample collection and storage will key markers of CWA use, or other CWC-prohibited activity, remain detectable? The passage of time will certainly lower the probability of identifying the original intact chemical(s), but the degradation products will remain detectable, proving CWA use.