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RESPONSE TO THE DIRECTOR-GENERAL'S REQUEST TO THE SCIENTIFIC ADVISORY BOARD TO PROVIDE FURTHER ADVICE ON CHEMICAL WEAPONS SAMPLE STABILITY AND STORAGE

1. EXECUTIVE SUMMARY

1.1 The Scientific Advisory Board (SAB) has considered the long-term storage and stability of samples collected in the context of the OPCW's investigations, including fact-finding missions and the Declaration Assessment Team, according to the Director-General's questions of 2 November 2015 (see Annex 1).

1.2 In the context of the OPCW's investigations, the Technical Secretariat has since 2013 received numerous samples, which are stored in the OPCW Laboratory at room temperature or refrigerated at 4 °C.

1.3 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 for example:

(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.

1.4 The Director-General requested the SAB to address three overarching questions:

(a) Given the current storage conditions in the OPCW Laboratory, how quickly and through what process could the aforementioned types of samples 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 aforementioned types of samples?

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

1.5 Before answering these questions, it is necessary to comment on the OPCW off-site verification mechanism in light of 'credible results' referred to in questions (a) and (c) from paragraph 1.4:

1.6 The OPCW has a system of laboratories designated by the Director-General for off-site analysis of samples. These samples would primarily be collected from challenge inspections, investigations of alleged use (IAUs) of chemical weapons, or other OPCW investigations. Laboratories are designated for the analysis of environmental and biomedical samples on the basis of their performance in OPCW Proficiency Tests (PTs).[1] This system has created a robust network of laboratories, which act as a deterrent factor to non-compliance to the Chemical Weapons Convention (CWC) by adding a high level of confidence to the verification regime. The scientific rigour of these PTs, following the criteria laid down by the International Organisation for Standardization (ISO) 17025 quality system and OPCW quality management system,[2,3,4,5] ensures this high level of confidence.

1.7 The SAB notes that the analytical findings of the Designated Laboratories from analysis of samples collected in OPCW investigations will always be scientifically accurate because of the stringent forensic checks and balances[6] in place: the findings will always return 'credible results' ('credible' is defined in the Oxford English Dictionary as 'able to be believed; convincing'). The results of the analyses will always be convincing and withstand scrutiny both scientifically and legally, especially if presented as evidence in court. The integrity of the procedures established in OPCW Designated Laboratories provides all necessary safeguards and thus protects the off-site analysis process from any suggestion of tampering.

1.8 It is with these important points in mind that the Director-General's questions can now be answered in turn.

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

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1 Note by the Director-General. Evaluation of the Results of the Thirty-Eight OPCW Proficiency Test. S/1368/2016, dated 30 March 2016.

2 "Standard Operating Procedure for the Organisation of OPCW Proficiency Tests" (QDOC/LAB/SOP/PT01 (Issue 2, Revision 4, dated 10 April 2015)).

3 "Work Instruction for the Preparation of Samples for OPCW Proficiency Tests" (QDOC/LAB/WI/PT02 (Issue 2, Revision 4, dated 10 April 2015)).

4 "Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests" (QDOC/LAB/WI/PT03 (Issue 3, Revision 2, dated 10 April 2015)).

5 "Work Instruction for the Reporting of the Results of the OPCW Proficiency Tests" (QDOC/LAB/WI/PT04 (Issue 2, Revision 1, dated 10 April 2015)).

6 United Nations Mission to Investigate Allegations of the Use of Chemical Weapons in the Syrian Arab Republic. Final Report, Appendix 2. Methodology used during the United Nations Mission, pp. 23-26.

(a) Any chemical stored for a sufficiently long time, no matter what the storage conditions, can degrade to one or more products. If the chemical degrades entirely and is no longer observable in the sample, scientists can often reconstruct the identity of the original chemical from analysis of its breakdown products. These products in a sense constitute a 'memory' of the original chemical. The situation for chemical warfare agents (CWAs) and related chemicals, such as precursors, is no different: their concentration may reduce upon storage, although their breakdown products will increase in concentration. This change, allowing the identity of the disappearing chemical to be pieced together from the molecules constituting the breakdown products, makes chemical analysis a powerful tool for retrieving evidence of chemical weapons use. Samples may also contain by-products of their synthetic route and unreacted starting materials, which will further enhance their analytical value. (To visualise how molecular breakdown products can be used to reconstruct the identity of the original CWA, the non-scientist may wish to think about reconstructing a broken object, such as a vase, from its fragments. Similarly, in chemical forensics, the identity of a CWA or precursor can be reconstructed from the types of breakdown products observed through sample analysis by Designated Laboratories). It must be noted however, if the agent or precursor is initially present only at trace level, prolonged storage may result in adsorption of the original chemical and/or its degradation product(s) to the container walls, for example. In such cases re-analysis could result in a non-finding of the original chemical and/or its degradation product(s) due to their presence in extremely low concentration, at levels below the instrument detection levels.

(b) The storage conditions used by the OPCW Laboratory will inevitably and naturally lead to loss of intact original chemicals by degradation in most cases (this phenomenon occurs in every laboratory in the world). It is impossible to put a precise time on how long any chemical will take to degrade, as shelf-life or degradation rate depends on the chemical structure, matrix, the presence of stabilisers and storage conditions, as well as the initial concentration of the chemical. It is only possible to estimate, with considerable uncertainty, a likely storage time, and impossible to state accurately when the various sample types will degrade to a point where analysis would not identify the intact original chemical(s).

(c) However, it is possible to state that the intact original chemical(s) in the sample types stored in the OPCW Laboratory might degrade naturally in, at worst, weeks to months, and at best, months to several years. (In some cases, degradation is so slow that the intact agent is present for many decades.) The analysis of these samples will return credible analytical results, but with less specific information. The characteristic degradation compounds will still contain the molecular evidence for proving CWA use, or in the case of other investigations, the presence of a CWC-related chemical.

(d) The main degradation of CWAs, and other CWC-related chemicals, in environmental samples occurs through reaction with water (hydrolysis) or oxygen in air (oxidation).

(e) To reduce the potential for degradation in the samples, as little time as possible should elapse from the time of collection of any sample to the time of analysis; lengthy delays of weeks to years may diminish the concentration of the intact original chemicals in the samples, but not diminish their usefulness as evidence in IAUs or other CWC-related investigations.

(f) **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.)

(g) **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.

1.10 What are the best-practice conditions for long-term storage of the different types of samples?

(a) The SAB has reviewed the scientific literature, and the answers to a SAB questionnaire returned by nine OPCW Designated Laboratories, on the best-practice conditions for the sample types described. Based on the findings, to optimise the conditions for reduced degradation of the CWC-relevant chemicals in the samples, the SAB makes the following recommendations:

(b) **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).

(c) **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).

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

material – containing relatively high levels 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.

(e) **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).

(f) **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.

(g) **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.

1.11 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 likely no longer return credible results?

(a) The previous comments in this Executive Summary on the uncertainties of prediction of shelf-lives of chemicals should be noted. Based on the review herein of processes by which CWC-relevant chemicals degrade, the SAB assesses that it is difficult, given the incomplete knowledge worldwide of the fate of CWAs and other CWC-relevant chemicals in different matrices, to specify precisely when analysis of a sample would likely no longer identify the intact original chemicals. The best-practice storage conditions provided in answer to the previous question will extend the time the original chemical in the sample will persist. Although some loss of this chemical may occur even under these conditions, the analysis of the samples will return credible analytical results, but with less specific information. The characteristic degradation products and other chemical residues (such as synthesis by-products and unreacted starting materials) will still provide the molecular evidence necessary for proving CWA production, chemical weapons use or other CWC-related compliance judgement.

(b) Further information on the provenance of the chemicals in a sample might be accessible by using chemical forensics. In this respect, the SAB recognises that attribution of CWA use will become easier as the science of chemical forensics advances. These observations led the SAB to propose two additional recommendations relevant to addressing the Director-General's questions:

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

(d) **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.[7]

2. OBJECTIVE

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

2.2 In the context of the OPCW's investigations, including fact-finding missions (FFMs)[8,9,10,11] and the Declarations Assessment Team, the Technical Secretariat has since 2013 received numerous samples. These samples are stored currently at the OPCW Laboratory at room temperature or refrigerated at 4 °C.[12,13]

2.3 The diversity of sample types containing chemicals of interest - such as nerve and blister agents, and immediate precursors and degradation products - is potentially vast, and could include:

(a) Relatively pure samples;

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

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7 Retention of a sample reference collection would support two result areas of the OPCW Medium-Term Plan: 'verification for continued confidence in compliance' and 'capacity development to prevent and respond to the hostile use of toxic chemicals and to foster international cooperation' (Note by the Technical Secretariat, "Medium-Term Plan of the Organisation for the Prohibition of Chemical Weapons 2017-2021", at the Eighty-Third Session of the Executive Council, from 11to 14 October 2016, (EC-83/S/1, C-21/S/1, dated 8 April 2016)).

8 Dr R. Trapp. Lessons Learned from the OPCW Mission in Syria, 16 December 2015: http://www.the-trench.org/wp-content/uploads/2016/01/Trapp-20151216-OPCW-Syria-lessons-learned.pdf

9 1st FFM in Syria report: http://www.the-trench.org/wp-content/uploads/2016/01/OPCW-FFM-20140616-1st-Chlorine-investigation-report.pdf

10 2nd FFM in Syria report: http://www.the-trench.org/wp-content/uploads/2016/01/OPCW-FFM-20140910-2nd-Chlorine-investigation-report.pdf

11 3rd FFM in Syria report: http://www.the-trench.org/wp-content/uploads/2016/01/OPCW-FFM-20141218-3rd-Chlorine-investigation-report.pdf

12 OPCW Executive Council, Seventy-Seventh Session, 7-10 October 2014. Note by the Technical Secretariat. Retention of samples of Syrian chemical weapons, EC-77/S/3, dated 12 September 2014.

13 OPCW Executive Council, Seventy-Seventh Session, 7-10 October 2014. Draft decision. Retention of samples of Syrian chemical weapons. EC-77/DEC/CRP.2, dated 12 September 2014 and EC-77 DEC/CRP.2/Rev.1, dated 23 March 2016.

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

(d) Biomedical samples, such as blood, plasma, urine, tissue.

2.4 In November 2015, the OPCW Director-General requested the SAB to estimate how quickly and through what processes these types of samples, when stored under the conditions used in the OPCW Laboratory, could degrade to a point where their analysis would no longer result in the identification of the intact original chemicals. Additionally, best-practice conditions for long-term storage of the sample types were sought. The next question to address was how quickly and through what processes could samples stored under these conditions degrade to a point where their analysis would likely no longer identify the intact original chemicals?

2.5 This report answers these questions: the answers have been formulated after consideration of the scientific literature and the collected views of the experts in nine OPCW Designated Laboratories.

3. FINDINGS

Processes by which relevant chemicals degrade:

(a) The scientific literature describes processes by which CWAs and other CWC-relevant chemicals degrade. The information is incomplete and scattered across publications. However, compilations of data exist [1-10][14]. These suggest that many CWAs and other CWC-relevant chemicals, if pure, and stored in the absence of water, can last for years without appreciable deterioration. This high stability has enabled them to be stockpiled historically. In some cases, 'stabilisers' (chemical additives) have been added to preserve them from degradation. Processes that affect chemical storage are physical (evaporation and absorption) and/or chemical (mainly hydrolysis, oxidation, and polymerisation). Degradation is a complex phenomenon that is still not fully understood.

(b) CWAs are generally reactive; they react with water and often other chemicals. Nerve agents (tabun, sarin, soman, and VX) and vesicants (sulfur mustard, nitrogen mustards, and Lewisites) react with water (they hydrolyse) at a rate dependent on their aqueous solubility and susceptibility to be attacked by water [11-13].

(c) Sulfur and nitrogen mustards have low solubility in water and this protects them to an extent from hydrolytic degradation. This is demonstrated by the current hazards posed by sea-dumped chemical munitions containing sulfur mustard, despite the fact that disposal occurred decades ago [14,15].

(d) It is the hydrolysis of nerve and blister agents during storage, in the environment or human body, which usually results in their degradation (by one or multiple pathways depending on their chemical structure) and

[14] Numbers in square brackets refer to literature cited in the Reference section (pages 21-36).

detoxification [7,8]. The rate of hydrolysis depends on temperature and pH for environmental samples and for biomedical samples (37 °C, pH 7.4) on the presence of additional substances that may catalyse hydrolysis (e.g. enzymes such as carboxylesterase present in plasma [16]). Some chemicals of interest can further undergo oxidation by oxygen in the air, or during metabolism [3-10], as shown in Figure 1 for the nerve agent *O*-ethyl.

(e) *S*-(2-diisopropylaminoethyl) methylphosphonothiolate (VX) and *bis*(2-chloroethyl)sulfide (sulfur mustard or HD).

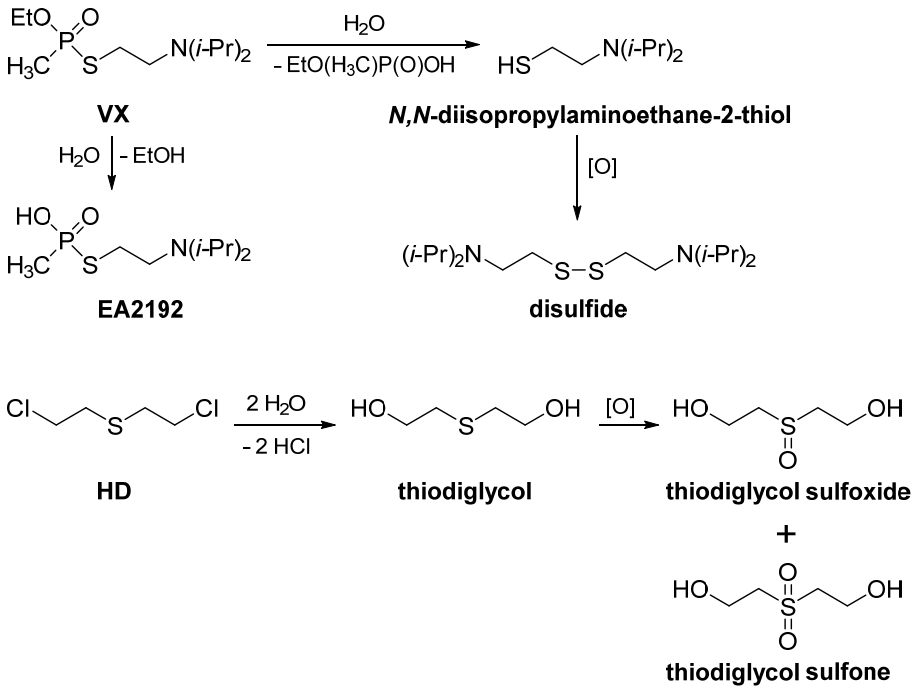

FIGURE 1: DEGRADATION OF VX (*TOP*) OR HD (*BOTTOM*) OCCURS THROUGH HYDROLYSIS THEN OXIDATION. HYDROLYSIS OF VX IS COMPLEX AND PH DEPENDENT AND CAN PRODUCE EA2192. THE SULFUR ATOM OF THE THIOL PRODUCED BY ONE OF THE VX HYDROLYSIS PATHWAYS IS OXIDISED BY OXYGEN IN THE AIR TO A DISULFIDE. THIODIGLYCOL FROM HD HYDROLYSIS OXIDISES TO THIODIGLYCOL SULFOXIDE AND THIODIGLYCOL SULFONE.

(f) Oxidation has also been employed for the destruction [17-20] and decontamination [21,22] of CWC-relevant chemicals. Oxidation is only possible when the affected atom is not in its maximum oxidation state. A carbon atom (C) bonded to a phosphorus atom (P) and three hydrogen atoms (H), to give a P-methyl group (P-CH_3), cannot be hydrolysed or oxidised easily (it is in its maximum oxidation state, +4). Other atoms in low oxidation states, such as sulfur in thiols RSH or sulfides RSR can oxidise to

give disulfides RSSR, sulfoxides RS(O)R or sulfones RSO_2R, respectively (the sulfur atom is in a higher oxidation state in sulfones). The resilient P-CH_3 group remains intact throughout environmental hydrolysis of nerve agents such as *O*-isopropyl methylphosphonofluoridate (sarin) [4,13]. In this case the P-F bond, then more slowly the P-O*i*-Pr bond, is cleaved by water. The P-CH_3 motif in the hydrolysis products isopropyl methylphosphonic acid (iPMPA) and methylphosphonic acid (MPA) indicates the prior presence of sarin [23,24], especially when found together with the production impurity diisopropyl methylphosphonate (DIMP) (Figure 2).

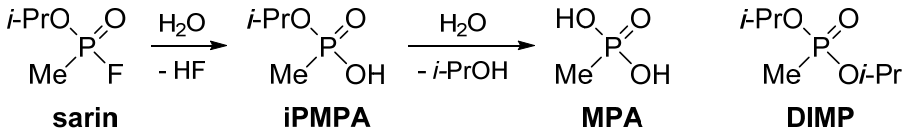

FIGURE 2: SARIN REACTS WITH ENVIRONMENTAL MOISTURE TO PROVIDE IPMPA. THIS REACTS SLOWLY WITH WATER WITH LOSS OF ISOPROPANOL TO PROVIDE MPA. THE DISCOVERY OF SARIN, IPMPA, MPA AND DIMP BY THE UNITED NATIONS INVESTIGATING TEAM, FROM ANALYSIS OF SOIL SAMPLES COLLECTED AFTER THE 21 AUGUST 2013 ATTACK ON CIVILIANS IN GHOUTA IN THE SYRIAN ARAB REPUBLIC, CONFIRMED THAT SARIN-FILLED ROCKETS HAD BEEN DEPLOYED [25,26].

(g) Sulfur and nitrogen mustards degrade along several pathways: (a) to give vinyl species, e.g. $HOCH_2CH_2SCH{=}CH_2$ from loss of hydrogen chloride from the initial product of hydrolysis of HD; (b) through cyclisation, for example of HD, during hydrolysis, e.g. to give 1,4-thioxane $O(CH_2CH_2)_2S$; (c) sulfonium or quaternary nitrogen salt formation from intermolecular reaction of hydrolysis products of sulfur and nitrogen mustards, respectively, and (d) the oxidation of HD to form the sulfoxide and sulfone. This complexity of degradation is typical for mustard-type vesicants bearing at least one 2-chloroethyl group ($ClCH_2CH_2$-). The arsenical agent Lewisite 1 contains a trivalent arsenic atom (+3 oxidation state) and hydrolyses rapidly with loss of hydrogen chloride to 2-chlorovinylarsenous oxide (Lewisite oxide, CVAO). This then oxidises to 2-chlorovinylarsonic acid (CVAA) where the arsenic atom has achieved pentacovalent status (+5 oxidation state) (Figure 3) [11,12]. This is another example of a hydrolysis and oxidation process of CWA degradation. Note that this process can occur in environmental samples containing vesicant agents on storage, or decontamination, and will be time dependent (it is more likely to occur over time). Fortunately, the hydrolysis and oxidation products contain much of the structural information of the CWA itself and are indirect proof of the prior presence of the CWA. [4].

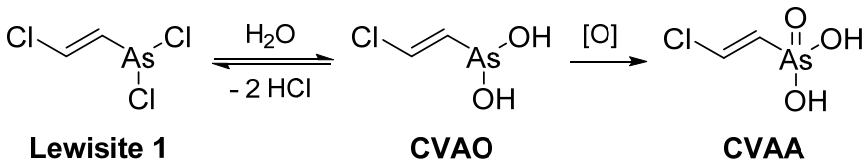

FIGURE 3: LEWISITE 1 HYDROLYSIS TO CVAO, WHICH RETAINS THE VESICANT AND TOXIC PROPERTIES OF LEWISITE 1 [27]. CVAO OXIDISES TO CVAA WHICH IS NOT APPRECIABLY VESICANT OR TOXIC.

(h) CWAs and other CWC-relevant chemicals also react with certain solvents and care should be taken to choose an appropriate solvent for short-term storage. Sulfur mustard and some nerve agents react readily with low molecular-weight alcohols (for example, methanol, and ethanol). Reactive solvents, such as these, should be regarded as unsuitable. In one Designated Laboratory, organophosphorus nerve agents are regarded as stable in isopropanol, and sulfur mustard is stored in hexane. Care is required in the choice of other solvents for CWAs and other CWC-relevant chemicals. Care must also be taken to select appropriate grades of solvent for extraction and storage of CWAs and other CWC-relevant chemicals. Drying solvents wherever possible is likely to minimise unwanted degradation via hydrolysis. Some commercial solvents contain more water than others: acetonitrile is an example of one having a relatively high water content; all commercial solvents, including those sold for chromatographic applications, contain significant quantities of water (up to 500 parts per million). Some sample solutions containing reactive chemicals, such as sulfur mustard sulfone, prepared in dimethyl sulfoxide (DMSO) should be stored in a vacuum desiccator rather than a refrigerator due to the strong hygroscopic nature of this solvent. Chromatographic performance during sample analysis can also be a storage consideration; the preferred solvents for chromatography include hexane, dichloromethane, and ethyl acetate.

(i) Technical difficulties associated with assessing CWA purity are considerable and to some extent have confounded definitive data on the subject. No single analytical technique is suited to measuring all components of mixtures simultaneously. For example, the acidic hydrolysis products of nerve agents cannot directly be analysed by gas chromatography and data based solely on this technique could give an artificially high impression of purity. Likewise, low thermal stability of some impurities such as pyrophosphonates can lead to a false impression of agent purity. The most suitable technique for assessing CWA purity is nuclear magnetic resonance (NMR) spectroscopy. Even using this technique, more than one measurement is required to account for the possibility of the presence of non-phosphorus containing degradation products or stabilising chemicals; and detailed work is required to characterise the range of possible degradation products in mixtures [1-10]. Sensitivity limitations can also prevent the characterisation of lower concentration impurities by NMR spectroscopy. However, it remains the technique of choice for routine purity assessments of CWAs and CWC-related chemicals, noting

that trace impurities in samples - such as unreacted starting materials, synthetic by-products, and stabilisers - can provide valuable information on the history and perhaps provenance of the chemicals.

(j) Stability studies on neat chemical agents are normally based on the storage of material for use as reference or surety material to support research for protective purposes, as permitted by the Convention. In general, high purity (>95%) chemical agents are required for such work. In some cases the storage of neat chemical agents so that they remain at this purity specification requires careful control of storage and use conditions. The storage of small quantities of CWA is likely to result in large headspaces above the agent. These smaller quantities of CWAs may be less stable than larger quantities of CWA stored in vessels with a reduced headspace. Frequency of use is also important: one Designated Laboratory has shown that solutions (~1 $mg \cdot mL^{-1}$) of CWAs degrade faster when opened weekly compared to those that remain unopened for a longer time period [28,29].

(k) The stability of low concentration solutions of CWAs has been studied in one Designated Laboratory by gas chromatography with flame photometric detection (GC-FPD) and with mass spectrometry (GC-MS) [28,29]. It was difficult to control instrument response over sufficient time (months) to be certain that changes resulted from degradation rather than changes in instrument response. This resulted in low concentration solutions of CWAs for quantitative work being stored for no more than one month (because of absence of reliable evidence to support longer-term storage). Note, however, the difference in requirements for a CWA needed for quantitative analysis and that required for a CWC-related investigation where “presence” or “absence” is a key criterion.

(l) The US Environmental Protection Agency compared the stability of dilute solutions of CWAs in dichloromethane and hexane in screw-capped vials and flame-sealed ampoules [30]. The analytical measurements contained some uncertainty, but in general flame-sealed ampoules resulted in greater stability than screw-capped vials. In most cases, degradation, where observed, was confirmed by complementary techniques (observation of products by GC-MS). Of the CWAs studied, sarin and VX were most susceptible to degradation during storage; up to a maximum of 80% degradation of VX occurred over one year. Solutions of CWAs in dichloromethane were more stable than those in hexane. The study highlighted that CWA impurities can affect storage stability; VX degraded faster in the presence of sarin.

(m) Insufficient information is available to ascertain if CWA degradation in solution depends on the concentration of the CWA in the solvent. It is often assumed that low concentration solutions will be less stable because of a higher water-to-analyte ratio or because of the possible adsorption to the container walls, but this requires further study.

(n) The rate of degradation of CWAs in the environment depends on many factors: the precise nature of the chemical agent and contaminated surface, and the temperature. For example snow contaminated with sarin, soman, tabun,

VX and sulfur mustard was analysed after exposure for one, four, seven, 15 and 30 days under Norwegian winter conditions [31]. After 15 days, the nerve agents tested were still present in concentrations sufficient to allow positive verification and quantitative analysis by GC-MS. After 30 days, the concentration of sarin, tabun and sulfur mustard had fallen below the limits of detection; analysis could only identify soman and VX. Sulfur mustard freezes on contact with snow and does not penetrate it as easily as the other agents. This illustrates the difficulty of predicting CWA fate as physical and chemical factors affect the persistence of each agent differently, making their stability in environmental matrices difficult to forecast accurately.

(o) The examples provided illustrate the general principle that most CWAs are unstable in the presence of moisture and hydrolyse at different rates to give characteristic products. These products can transform further - in the environment or body - via oxidation to provide non-toxic, often stable products [32]. Detection of the starting agent, hydrolysis and/or oxidation products, may be possible from analysis of samples collected days to weeks after a chemical attack [4,32].

(p) Identification of CWAs in biomedical samples – e.g. blood, plasma, urine, or tissue – is also possible, but CWAs do not remain intact in the body for long. The products of hydrolysis/oxidation are much more likely to be detected. Sometimes a CWA enters the body and reacts with a specific amino acid of a protein present in the bloodstream (e.g. albumin, haemoglobin, or butyrylcholinesterase) to give an addition product (adduct). DNA-adducts are also reported, mainly with sulfur mustard. The adduct retains some of the structural information of the CWA and provides information on the CWA used. Unambiguous identification of adducts and/or urinary metabolites may be possible, using mass spectrometry, and provide evidence of CWA exposure. Such an approach has been applied successfully to retrospective identification of poisoning by sulfur mustard [34-68], nitrogen mustards [69-72], Lewisite [73-78], organophosphorus nerve agents [33,79-133], the incapacitant BZ [134], phosgene [135] and hydrogen cyanide [136].

(q) Approaches to the analysis of biomedical samples to assess exposure to nerve agents [137] and chemical forensics relevant to the Convention [138] have been reviewed by the OPCW Laboratory. An edition of the peer-reviewed journal “Analytical Bioanalytical Chemistry” on *Analysis of Chemical Relevant to the Chemical Weapons Convention*, guest-edited by scientists from the OPCW Laboratory, also contains pertinent information [139].

(r) How long after human exposure to CWAs can biomarkers be detected is not easily predicted, especially given the paucity of confirmed human exposures, and difficulty of extrapolating data from animal experiments. The problem extends to estimating how long samples containing such biomarkers can be stored without loss of information critical as evidence to an IAU or other CWC-related investigation. In environmental samples, hydrolysis/oxidation products are likely to remain retrievable by solvent extraction sometime after the CWA was disseminated. In this case, there may be an opportunity to visit

the samples at a later date, maintaining the possibility of finding evidence of CWA use.

(s) Biodegradation of CWAs by microorganisms might affect the storage of CWA samples. Biodegradation of organophosphorus compounds has been studied as a possible means of nerve agent destruction [140]. Only a few microorganisms screened to date and isolated have the capacity to degrade CWAs, and this capacity is limited at present. Data on the microbial degradation of CWAs in samples, and CWA-containing sample matrices, are largely lacking.

(t) Characteristic degradation products for chemicals in Schedules 1, 2 and 3 in the Annex on Chemicals to the Convention [141] are summarised in Annex 2 [142-183]. These products are not exhaustive but cover many of those of anticipated relevance to IAUs or other CWC-related investigations. Mass spectra of many of the products are already in the OPCW Central Analytical Database (OCAD).

(u) 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.

(v) Only estimates can be provided to answer the question of the time window to ensure the integrity of the intact original chemicals in stored samples. To provide these estimates the SAB composed a questionnaire (Annex 3) for the OPCW Designated Laboratories. Nine responded and their views on sample storage are collated and analysed in the next section.

4. RESPONSES FROM THE OPCW DESIGNATED LABORATORIES

Best-practice sample storage conditions provided by OPCW Designated Laboratories in response to the SAB's questionnaire are discussed hereafter in order of sample type in the Director-General's question, namely:

(a) Relatively pure samples (commercial, own-made, and solutions of chemicals)

(b) Samples containing chemicals of interest; including heterogeneous samples

(c) Biomedical samples: including blood, plasma, urine, tissue

5. RELATIVELY PURE SAMPLES

5.1 Relatively pure samples have been subdivided into commercial chemicals, own-made chemicals, and solutions of chemicals, because of their different storage requirements, and are now discussed in turn:

5.2 *Commercial chemicals*: These should be stored in their original packaging in the dark at a temperature recommended by the manufacturer (room temperature, 4 °C, or -18 °C). Maximum storage times are chemical-dependent; the supplier expiration date should be used as an indication of shelf-life. Commercial chemicals can be used for the manufacturer's recommended storage time or stored until no longer required; purity checks before use are advised.

5.3 One Designated Laboratory noted that the International Organization for Standardization (ISO) 17025 quality system[15] required commercial chemicals to be purity checked at least every two years. Another replied that to prolong storage life pure chemicals can be diluted in an inert solvent, such as acetonitrile or dichloromethane. A third Designated Laboratory estimated maximum storage times of one, three and five year(s) for pure chemicals kept at -20 °C, 2-10 °C and 25 °C respectively (Table 1). These comments refer to analytical standards and not analytical samples.

TABLE 1: DESIGNATED LABORATORY RESPONSES: COMMERCIAL CHEMICALS

Type	Chemical	Storage Condition	until assignment completed	manufacturer's recommendation	until signs of degradation	up to 2 weeks	2 weeks	up to 1 month	several months	up to 3 months	up to 6 months	at least 6 months	up to 1 year	several years	up to 3 years	up to 5 years	more than 10 years
Commercial, pure	General	room temperature														■	
		refrigerator													■		
		freezer (-18°C)											■				
		original packaging		■	■										■	■	
		manufacturer's recommendation		■	■												

room temperature: usually 25°C

5.4 *Own-made chemicals*: These should be stored in glass, high-density polyethylene (HDPE) or Teflon containers, and resealed between uses. Those not accessed regularly, especially chemicals in Schedule 1 (S1), can be stored in sealed glass ampoules under an inert gas atmosphere (e.g. argon). Recommended storage temperature varies according to the chemical but -18 °C is preferred by most Designated Laboratories (Table 2) that replied to the questionnaire. The glass or plastic containers should be housed in containers made of metal (e.g. stainless steel) and these should contain active charcoal to absorb any accidental spillage of the chemicals. A Designated Laboratory commented that rare or difficult-to-obtain chemicals should be stored indefinitely, noting that even impure samples could assist analysis at some point in future.

5.5 One Designated Laboratory with long-term experience provided guidelines for the best-practice storage of S1 chemicals, grouping them into classes according to storage requirements (Tables 2 and 3). In general, the purer the CWAs, the longer they stayed pure (although >95% purity at the start of the storage period was advised).

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15 ISO 17025 is the main ISO standard used by testing and calibration laboratories. In many countries, it is the accreditation standard most laboratories must hold to be deemed technically competent. OPCW Designated Laboratories require this to be permitted to analyse environmental samples.

TABLE 2: DESIGNATED LABORATORY RESPONSES: OWN-MADE CHEMICALS

Chemical	Type	Storage Condition	until assignment completed	manufacturer's recommendation	until signs of degradation	up to 2 weeks	2 weeks	up to 1 month	several months	up to 3 months	up to 6 months	at least 6 months	up to 1 year	several years	up to 3 years	up to 5 years	more than 10 years
Synthesized, pure	General	refrigerator													■		
		freezer (-18°C)	■		■										■		■
		glass container	■		■										■		■
		HDPE container													■		
		Teflon lined caps															■
		Ar atmosphere													■		
		sealed ampoules													■		
	S1 (General)	freezer (-18°C)															■
		glass container															■
	Nerve Agents	freezer (-18°C)										■					■
		glass container															■
		Teflon lined caps															■
		Ar atmosphere															■
	Vesicants	freezer (-18°C)										■					
	Sulfur Mustard	room temperature										■					■
		glass container															■
		glass stopper															■
		Teflon lined caps															■
		sealed, wax															■
		Ar atmosphere															■
	Lewisite	room temperature															■
		glass container															■
		Teflon lined caps															■
		Ar atmosphere															■

room temperature: usually 25°C
Ar: Argon
HDPE: High-density polyethylene

TABLE 3: DESIGNATED LABORATORY RESPONSES: SCHEDULE 1 CHEMICALS AND PRECURSORS

Chemical	Type	Storage Condition	until assignment completed	manufacturer's recommendation	until signs of degradation	up to 2 weeks	2 weeks	up to 1 month	several months	up to 3 months	up to 6 months	at least 6 months	up to 1 year	several years	up to 3 years	up to 5 years	more than 10 years
Synthesized, pure	Nitrogen Mustard	freezer (-18°C)															■
		glass container															■
		Teflon lined caps															■
		Ar atmosphere															■
	Ricin	as precipitate in 6M NH4SO4															■
		glass container															■
		Teflon lined caps															■
	S1 Precursors	freezer (-18°C)															■
		glass container															■
		Teflon lined caps															■
		Ar atmosphere															■
	DF	freezer (-18°C)															■
		Teflon container															■
		Ar atmosphere															■
	P-Cl Precursors	freezer (-18°C)															■
		glass container															■
		Teflon lined caps															■
		Ar atmosphere															■

room temperature: usually 25°C
Ar: Argon
HDPE: High-density polyethylene

5.6 Additional information on best-practice storage conditions for S1 chemicals from this same Designated Laboratory are provided here, with additional data in square brackets from another Designated Laboratory:

(a) Schedule 1A01: *O*-Alkyl alkylphosphonofluoridates (e.g. sarin, soman)

(b) Schedule 1A02: *O*-Alkyl *N*,*N*-dialkylphosphoramidocyanidates (e.g. tabun)

(c) Schedule 1A03: *O*-Alkyl *S*-2-dialkylaminoethyl alkylphosphonothiolates (e.g. VX)

(d) Schedule 1A06: Nitrogen mustards (i.e. HN-1, HN-2, HN-3)

(e) Schedule 1B10: *O*-Alkyl *O*-2-dialkylaminoethyl alkylphosphonites (e.g. QL)

(f) Schedule 1B11: *O*-Isopropyl methylphosphonochloridate (i.e. chlorosarin)

(g) Schedule 1B12: *O*-Pinacolyl methylphosphonochloridate (i.e. chlorosoman)

Stored at -18 °C in glass containers with Teflon-lined caps in the dark under argon, degradation is slow: 5-10% over 5-10 years (except Schedule 1B10 chemicals which show 20-40% degradation over 5 years, as they are generally highly reactive).

[Pure samples (>95%) of sarin or soman store at room temperature - and cyclosarin, tabun, and V-agents store at -18 °C - for over a year. The storage stability of pure samples of V-agents is structure dependent and seems to decline, in the absence of any added stabilisers, apparently in the order: *O*-isobutyl *S*-2-diethylaminoethyl methylphosphonothiolate > *O*-ethyl *S*-2-diethylaminoethyl methylphosphonothiolate (VM) > *n*-butyl *S*-2-diethylaminoethyl methylphosphonothiolate > *O*-ethyl *S*-2-diisopropylaminoethyl methylphosphonothiolate (VX).]

[*Tris*(2-chloroethyl)amine (HN-3) stores for a month at -18 °C before visible degradation (discolouration and precipitation), but analysis shows the supernatant to be pure despite this time-related change.]

(h) Schedule 1A04: Sulfur mustards

(i) Schedule 1A05: Lewisite 1, Lewisite 2, Lewisite 3

Stored at room temperature in the dark under argon in glass containers with Teflon-lined caps, they are stable for over 10 years.

[Sulfur mustard stores at room temperature for decades. Lewisites 1, 2 and 3 store unchanged for over a year at room temperature.]

(j) Schedule 1A08: Ricin

Stored as a precipitate in 6 M ammonium sulfate at 4 °C in the dark in glass containers with Teflon-lined caps (insoluble if freeze-dried), the ricin is stable for over 10 years.

(k) Schedule 1B09: Alkylphosphonic difluorides

These moisture-sensitive liquids are stored at -18 °C in the dark under argon in Teflon containers. Under these conditions they degrade slowly (5 – 10% over 5 – 10 years).

[Perfluoroalkoxyalkane plastic is suitable for storing methylphosphonic difluoride (DF) or an equimolar mixture of methylphosphonic dichloride and DF (the so-called "di-di mixture"). Both DF and the "di-di mixture" will degrade slowly over a year when stored at room temperature in these containers and are easily purified without appreciable loss.]

(l) Degradation products and others

These are stored at -18 °C in the dark under argon in Teflon containers. They are generally more stable than CWAs and their precursor chemicals.

[Solutions of V-agent precursors $RO(Me)P(O)S^-Na^+$ in water (from their syntheses) store without appreciable decomposition; an aqueous solution (R = Et) declined in concentration from 1.4 to 1.2 mol/l over 13 years.]

5.7 One Designated Laboratory checks purities of stored S1 nerve and vesicant agents on a monthly basis because of uncertainties related to the stabilities of solutions upon storage. Reference standards are checked for purity before use. The ISO 17025 quality system requires purity checking of nominally stable chemicals, such as CWA degradation products, at least every two years.

6. SOLUTION OF CHEMICALS

6.1 Solutions of commercial chemicals used for laboratory standards are stored in clean glass containers (e.g. vials or volumetric flasks) under conditions specified in safety data sheets or the scientific literature. Dilute solutions of Scheduled chemicals in dichloromethane (10 ppm) are stable at ~4 °C in sealed ampoules/vials for 6 months (qualitative standards) or 12 months (qualitative standards) (Table 4). If the chemical or its container show signs of degradation, they should be disposed of safely.

TABLE 4: DESIGNATED LABORATORY RESPONSES: SOLUTIONS OF CHEMICALS

Type	Chemical	Storage Condition	until assignment completed	manufacturer's recommendation	until signs of degradation	up to 2 weeks	2 weeks	up to 1 month	several months	up to 3 months	up to 6 months	at least 6 months	up to 1 year	several years	up to 3 years	up to 5 years	more than 10 years
Commercial or Synthesized, solution	General	refrigerator						■		■			■				
		freezer (-18°C)											■				
		glass container			■		■			■			■		■		
		Teflon lined caps											■				
Commercial or Synthesized, solution	Scheduled	in dichloromethane									■						
		refrigerator									■						
		sealed									■						

6.2 Solutions of CWAs and degradation products used as standards are generally not stored for longer than one month in screw-capped vials in cases where sub-sampling of these solutions is a regular event. Nerve agents can be stored short-term in isopropanol and sulfur mustard in hexane. CWAs are stable in dichloromethane for a month but their stability in this solvent has not been studied in detail.

6.3 The results of analysis suggest that CWAs absorbed onto painted surfaces [33] could be extracted and detected many years after dissemination, and studies have shown stability of CWAs on sorbent tubes for up to one month sufficient to permit identification [142].

7. LIQUID (INCLUDING EXTRACTS) AND SOLID SAMPLES CONTAINING RELATIVELY HIGH LEVELS OR TRACE LEVELS OF THE CHEMICALS OF INTEREST AND HIGHLY HETEROGENOUS 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

7.1 Responses from the Designated Laboratories are summarised in Table 5. There is no evidence for long-term storage of solutions of CWAs used as standards beyond six months. Anecdotal information suggests these types of samples can be stored for longer (years) at -18 °C. There is limited evidence that supports storage of dilute (10 ng ml^{-1}) and concentrated (1 mg ml^{-1}) solutions of standards for at least six months [28,29].

TABLE 5: DESIGNATED LABORATORY RESPONSES: SAMPLES IN ORGANIC AND AQUEOUS SOLUTIONS, SOLID/HETEROGENEOUS SAMPLES, AND AIR SAMPLES

Chemical	Type	Storage Condition	until assignment completed	manufacturer's recommendation	until signs of degradation	up to 2 weeks	2 weeks	up to 1 month	several months	up to 3 months	up to 6 months	at least 6 months	up to 1 year	several years	up to 3 years	up to 5 years	more than 10 years
Sample, organic solutions	General	refrigerator	■			■											
		freezer (-18°C)	■					■			■						
		glass container	■			■					■						
		original container				■											
		sealed									■						
Sample, aqueous solutions	General	refrigerator	■			■					■						
		glass container	■			■					■						
		original container				■											
		PP container									■						
		sealed									■						
Sample, solid/hetero-geneous	General	freezer (-18°C)	■														
		refrigerator	■			■					■						
		glass container				■					■						
		HDPE container									■						
		original container				■											
		sealed									■						
Sample, air	General	refrigerator	■														
		freezer (-18°C)	■														

room temperature: usually 25°C
HDPE: High-density polyethylene
PP: Polypropylene

7.2 GC-based techniques may lack long-term stability for repeat quantitative measurements for CWA solutions of concentration of 1 ng ml^{-1} and lower [28,29]. Solvent choice (quality, including water content) is likely to be of great importance as well as specific storage conditions, including how often solutions are used.

8. BIOMEDICAL SAMPLES: BLOOD, PLASMA, URINE, TISSUE

8.1 Designated Laboratories generally store these sample types at +4, -18 or -80 °C in glass, polypropylene or polyterephthalate containers (Table 6). Estimates of maximum storage times are matrix, analyte and concentration dependent; they range from months to years depending on storage conditions.

8.2 Little is known about the ageing of nerve agent blood adducts following long term storage. Sulfur mustard blood adducts, and urinary metabolites of sulfur mustard and nerve agents, have been re-analysed following several years of storage.

TABLE 6: DESIGNATED LABORATORY RESPONSES: BIOMEDICAL SAMPLES

Chemical	Type	Storage Condition	until assignment completed	manufacturer's recommendation	until signs of degradation	up to 2 weeks	2 weeks	up to 1 month	several months	up to 3 months	up to 6 months	at least 6 months	up to 1 year	several years	up to 3 years	up to 5 years	more than 10 years
Sample, biomedical	General	refrigerator				■					■						
		freezer (-18°C)	■														
		freezer (-80°C)												■			
		glass container				■					■						
		original container															
		sealed									■						
	Hair exp. to Sarin	room temperature							■								
	Protein, frozen	freezer (-18°C)												■			
		freezer (-80°C)												■			
	Protein, lyophilized	freezer (-18°C)												■			
		refrigerator												■			
	Protein, in solution	refrigerator				■											
	Protein, in solution with 50% glycerol	freezer (-18°C)											■				
	Blood	freezer (-18°C)									■						
		PET container									■						
		PP container									■						

room temperature: usually 25°C
PP: Polypropylene
PET: Polyethylene terephthalate
exp. to: exposed to

8.3 Detailed guidelines for storing and handling of protein-containing samples from one Designated Laboratory are provided in Annex 4 [143].

9. OTHER TECHNOLOGIES THAT COULD BE USED TO STORE/PACKAGE SAMPLES

9.1 Flame-sealed ampoules can be used to extend the storage stability of in-house reference chemicals. Certan® capillary bottles (LGC Group), available from Sigma-Aldrich Ltd., can be used to store highly-volatile samples.

9.2 Solid phase microextraction fibres might be used to store nerve agent urinary metabolites for extended periods of time. Blood spot papers [184] and related technologies seem promising for long-term storage of blood and other biological matrices. Much of the perceived benefit is in the sampling aspects (less invasive, low volume) but there is also a range of direct mass spectrometric analysis technologies appearing on the market (for example, paper spray analysis [185]) that might prove valuable.

10. CONCLUSIONS

10.1 In the context of the OPCW's investigations, including fact-finding missions and the declarations assessment team, the Technical Secretariat has since 2013 received numerous samples, which are stored in the OPCW Laboratory at room temperature or refrigerated at 4 °C.

10.2 The SAB notes that these conditions may naturally lead to loss of the intact original chemicals by degradation, in, at worst, weeks to months, and at best, months to several years. The analysis of these samples thereafter may give results with less

specific information, but still containing the molecular evidence, in the form of characteristic degradation compounds and other residues, for proving CWA use or making other compliance-related judgements. The main degradation of CWAs or other CWC-relevant chemicals in environmental samples occurs through hydrolysis and/or oxidation.

10.3 To minimise degradation of chemicals in the samples, as little time as possible should elapse from the time of collection of any sample to the time of analysis; lengthy delays of weeks to years will diminish the concentration of the intact original chemicals in the samples. Identification of the presence of the intact original chemical(s) is desirable, but not essential, for providing evidence of use of chemicals relevant to the Chemical Weapons Convention.

10.4 Best-practice conditions for various samples summarised in Tables 1-6 have been used to make the recommendations in the Executive Summary.

10.5 The Technical Secretariat should monitor advances in sampling and analysis, and with the SAB, innovations relevant to chemical forensics. Knowledge of storage conditions for CWA and other CWC-relevant samples remains vital to the work of the OPCW in non-proliferation and the prevention of re-emergence of chemical weapons [186,187].

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Annexes:

Annex 1: 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

Annex 2: Scheduled Chemicals and Major Degradation Products Identified From the Scientific Literature

Annex 3: Questionnaire on the Storage of Samples in Relation to the Potential Use of Chemical Weapons

Annex 4: Storing and Handling of Protein Containing Samples

Annex 1

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:

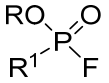
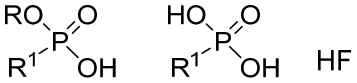
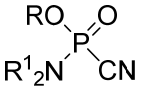
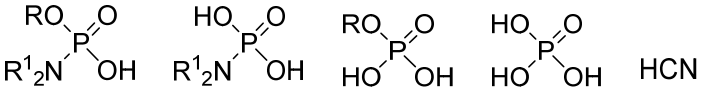
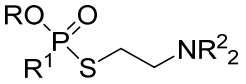
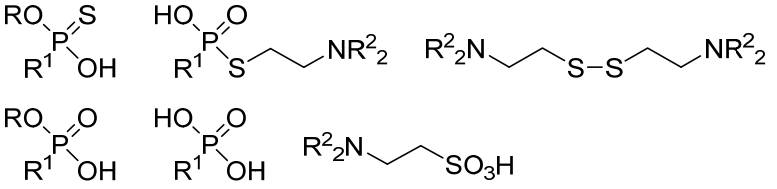
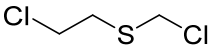
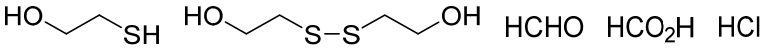
   (a) Given the current storage conditions (set out in paragraph 2), how quickly and through what process could the types of samples mentioned in paragraph 3 degrade to a point where analysis of the samples would likely no longer return credible results?

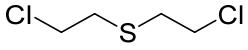
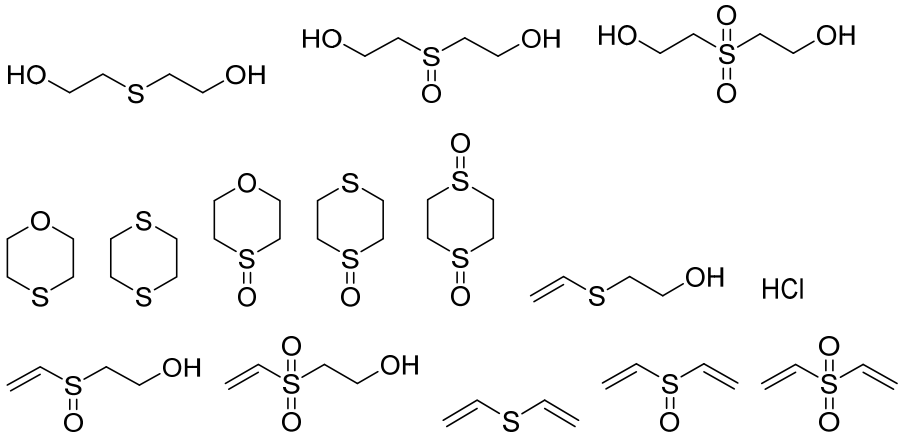
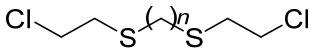
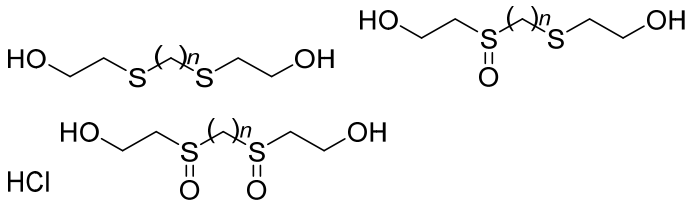
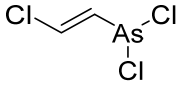
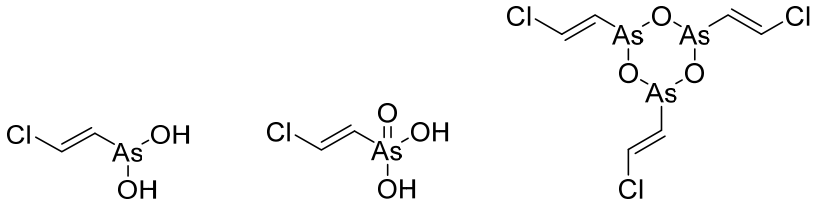
   (b) What are the best-practice conditions for long-term storage of the types of sample mentioned in paragraph 3?

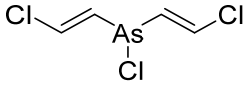
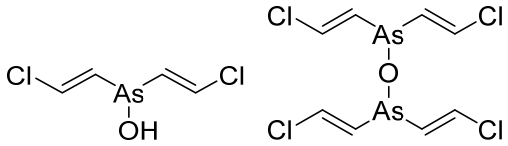
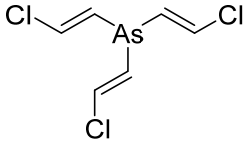
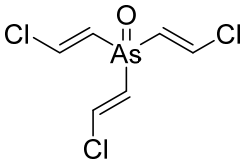
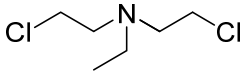
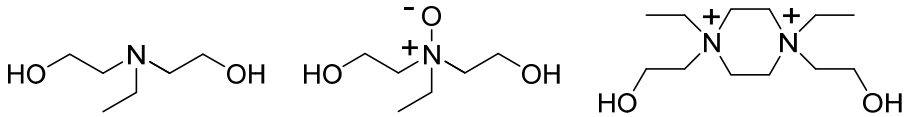
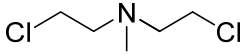
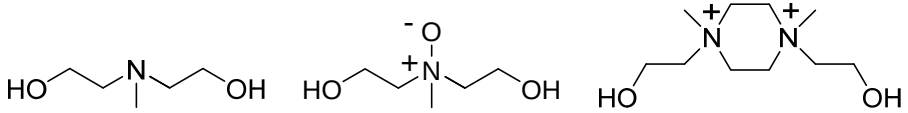
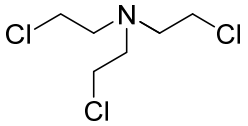
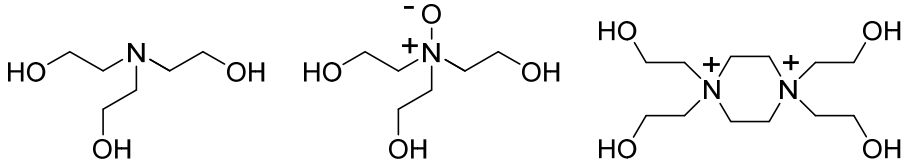
   (c) Given the best-practice storage conditions set out in the SAB's answer to question (b), how quickly and through what process could the types of sample mentioned in paragraph 3 degrade to a point where analysis of the samples would likely no longer return credible results?

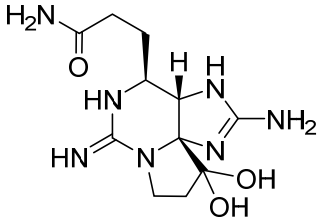
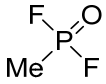
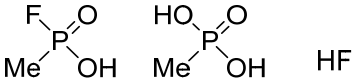
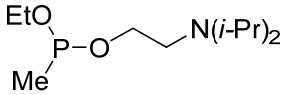
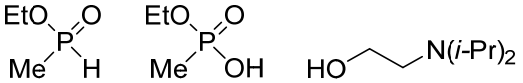
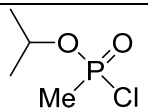
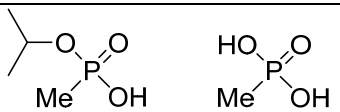
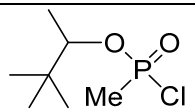
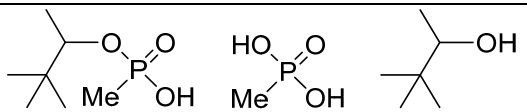
Annex 2

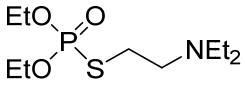
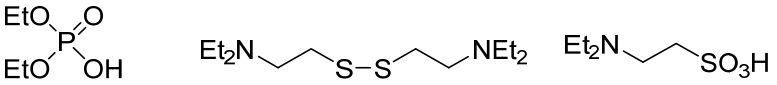
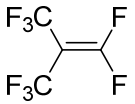
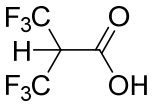
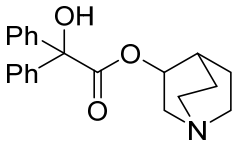
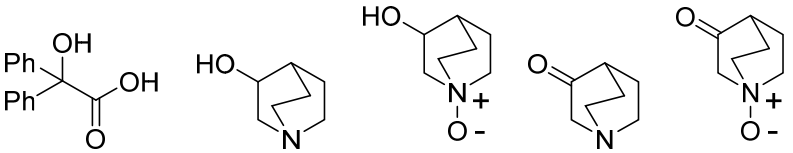
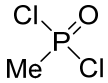
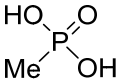
SCHEDULED CHEMICALS AND MAJOR DEGRADATION PRODUCTS IDENTIFIED FROM THE SCIENTIFIC LITERATURE

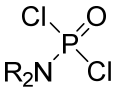
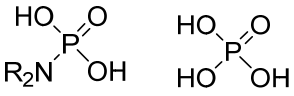
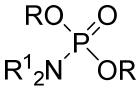
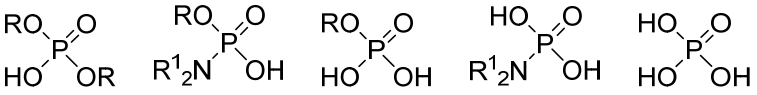
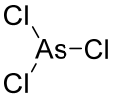
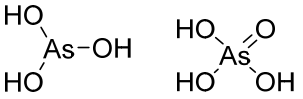
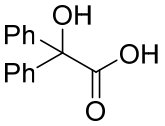
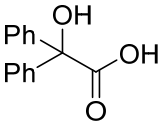
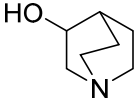
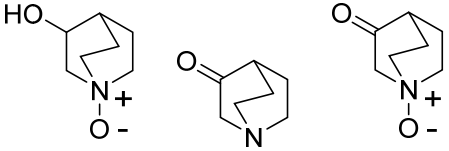
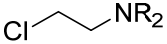
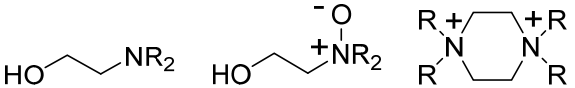
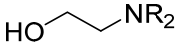
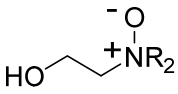
SCHEDULE	STRUCTURE	MAIN DEGRADATION PRODUCTS	REF/S
Schedule 1A01 *O*-Alkyl alkylphosphonofluoridates (e.g. sarin, soman)			4,33, 143,145
Schedule 1A02 *O*-Alkyl *N*,*N*-dialkyl phosphoramidocyanidates (e.g. tabun)			4,13,145
Schedule 1A03 *O*-Alkyl *S*-2-dialkylaminoethyl alkylphosphonothiolates (e.g. VX)			4,13, 145-153
Schedule 1A04 **Sulfur mustards**			
2-Chloroethylchloromethyl-sulfide			145

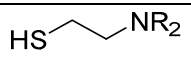
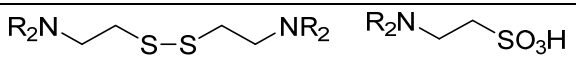
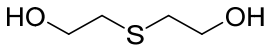
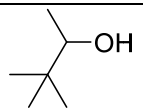
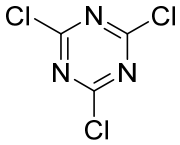
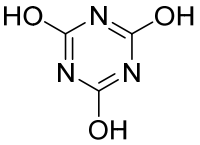
SCHEDULE	STRUCTURE	MAIN DEGRADATION PRODUCTS	REF/S
Bis(2-chloroethyl)sulfide (mustard gas)			154-157
Bis(2-chloroethyl)thioalkanes (*n* = 1 to 5)			155
Schedule 1A05 **Lewisites**			
2-Chlorovinyldichloroarsine (Lewisite 1)			158-165

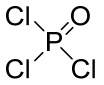
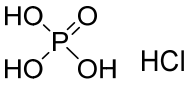
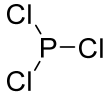
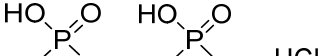
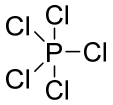
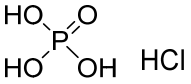
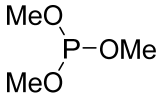
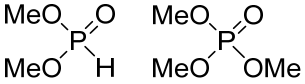
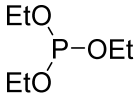
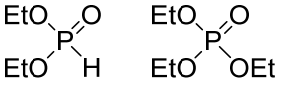
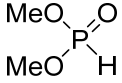
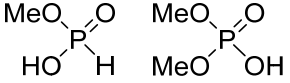
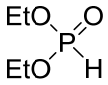
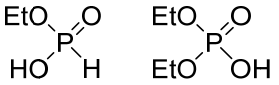
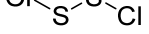
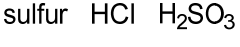
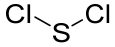
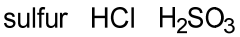
SCHEDULE	STRUCTURE	MAIN DEGRADATION PRODUCTS	REF/S
Bis(2-chlorovinyl)chloroarsine (Lewisite 2)			158-165
Tris(2-chlorovinyl)arsine (Lewisite 3)		usually does not degrade, but can oxidise to this 	158-165
Schedule 1A06 **Nitrogen mustards**			
Bis(2-chloroethyl)ethylamine (HN-1)			11,12,166
Bis(2-chloroethyl)methylamine (HN-2)			11,12
Tris(2-chloroethyl)amine (HN-3)			11,12,166

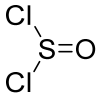
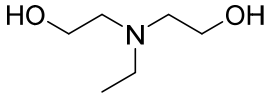
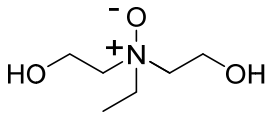
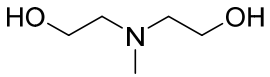
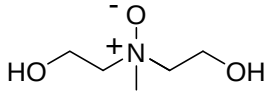
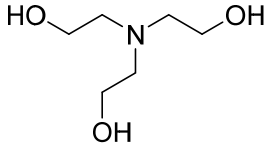
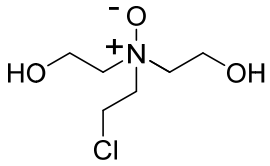
SCHEDULE	STRUCTURE	MAIN DEGRADATION PRODUCTS	REF/S
Schedule 1A7 Saxitoxin		likely to remain intact - the possible degradation products are unknown	167
Schedule 1A8 Ricin	complex protein	likely to denature - the possible degradation products are unknown	168-170
<u>Schedule 1B</u> **<u>Precursors</u>**			
Schedule 1B09 Alkylphosphonic difluorides (e.g. DF)			13
Schedule 1B10 *O*-Alkyl *O*-2-dialkylaminoethyl alkylphosphonites (e.g. QL)			13
Schedule 1B11 *O*-Isopropyl methylphosphono-chloridate (chlorosarin)			13
Schedule 1B11 *O*-Pinacolyl methylphosphono-chloridate (chlorosoman)			13

SCHEDULE	STRUCTURE	MAIN DEGRADATION PRODUCTS	REF/S
Schedule 2A **Toxic chemicals**			
Schedule 2A01 *O*,*O*-Diethyl *S*-[2-(diethylamino)ethyl] phosphorothiolate (Amiton)			13
Schedule 2A02 1,1,3,3,3-Pentafluoro-2-(trifluoromethyl)-1-propene (PFIB)		 HF	145, 171-173
Schedule 2A03 3-Quinuclidinyl benzilate (BZ)			174
Schedule 2B **Precursors**			
Schedule 2B04 Chemicals, except those listed in Schedule 1 containing a phosphorus atom to which is bonded one methyl, ethyl or propyl (*n* or *iso*) group but no	For example, methyl-phosphonic dichloride (DC) 	 HCl	13,171,175

SCHEDULE	STRUCTURE	MAIN DEGRADATION PRODUCTS	REF/S
further carbon atoms			
Schedule 2B05 *N*,*N*-Dialkylphosphoramidic dihalides		 HCl	13
Schedule 2B06 Dialkyl *N*,*N*-dialkylphosphoramidates			13
Schedule 2B07 Arsenic trichloride		 HCl	11,12
Schedule 2B08 2,2-Diphenyl-2-hydroxyacetic acid		 generally will not degrade any further	145,174
Schedule 2B09 Quinuclidin-3-ol			145,174
Schedule 2B10 *N*,*N*-Dialkylaminoethyl-2-chlorides			176
Schedule 2B11 *N*,*N*-Dialkylaminoethane-2-ols		generally will not degrade further 	176

SCHEDULE	STRUCTURE	MAIN DEGRADATION PRODUCTS	REF/S
Schedule 2B12 *N*,*N*-Dialkylaminoethane-2-thiols			147
Schedule 2B13 Bis(2-hydroxyethyl)sulfide (thiodiglycol)		stable in the environment can oxidise to the sulfoxide and sulfone (degradation products of sulfur mustard)	33,145, 154-157
Schedule 2B14 3,3-Dimethylbutan-2-ol (pinacolyl alcohol)		stable in the environment (degradation product of soman)	177
<u>Schedule 3A</u> **<u>Toxic chemicals</u>**			
Schedule 3A01 Carbonyl dichloride (phosgene)		non-persistent, hydrolysing with ease to HCl and CO_2	172,178
Schedule 3A02 Cyanogen chloride	ClCN	only slightly soluble in H_2O; polymerises to  which hydrolyses to give this triol 	11,12
Schedule 3A03 Hydrogen cyanide	HCN	persists in the open for only a few minutes after release - acid hydrolyses it to $HCONH_2$	179,180
Schedule 3A04 Trichloronitromethane (chloropicrin)	CCl_3NO_2	stable in the environment	11,12

SCHEDULE	STRUCTURE	MAIN DEGRADATION PRODUCTS	REF/S
Schedule 3B **Precursors**			
Schedule 3B05 Phosphorus oxychloride			13
Schedule 3B06 Phosphorus trichloride			13
Schedule 3B07 Phosphorus pentachloride			13
Schedule 3B08 Trimethyl phosphite			13
Schedule 3B09 Triethyl phosphite			13
Schedule 3B10 铈imethyl phosphite			13
Schedule 3B11 Diethyl phosphite			13
Schedule 3B12 Sulfur monochloride			181
Schedule 3B13 Sulfur dichloride			-

SCHEDULE	STRUCTURE	MAIN DEGRADATION PRODUCTS	REF/S
Schedule 3B14 Thionyl chloride		HCl SO_2 H_2O	-
Schedule 3B15 Ethyldiethanolamine			182
Schedule 3B16 Methyldiethanolamine			182
Schedule 3B17 Triethanolamine			182

Annex 3

QUESTIONNAIRE ON THE STORAGE OF SAMPLES IN RELATION TO THE POTENTIAL USE OF CHEMICAL WEAPONS

1. Please provide details of storage conditions used at your organisation for pure reference chemicals (chemical warfare agents, precursors, synthesis by-products, and degradation products) including container type, temperature, use of any stabilising materials or other special conditions associated with storage and/or packaging. Please provide an estimate of maximum storage times after which the reference materials degrade and are no longer fit for purpose. In the comments section, please provide details of any evidence for these estimates.

Sample type	Best practice storage conditions (incl. container type, temperature, use of stabilisers or any special conditions associated with storage and/or packaging)	Estimates of maximum storage times after which analysis of the samples is unlikely to show the intact original chemical(s) of interest	Comments (including details of evidence base for the estimates of maximum storage times)
Commercially-available pure chemicals			
Synthesized/own-made pure chemicals, including Schedule and non-Schedule chemicals			
Solutions of commercially-available and synthesized/ own-made pure chemicals			

2. Please provide details of storage conditions that you regard as best practice for the following sample types, in relation to potential use of chemical weapons. Please provide an estimate of maximum storage times after which analysis is unlikely to show the intact original chemical(s).

Sample type	Best practice storage conditions (incl. container type, temperature, use of stabilisers or any special conditions associated with storage and/or packaging)	Estimates of maximum storage times after which analysis of the samples is unlikely to show the intact original chemical(s) of interest	Comments (including details of evidence base for the estimates of maximum storage times)
Organic liquid samples containing chemicals of interest, including extracts			
Aqueous liquid samples containing chemicals of interest			

Solid and/or highly heterogeneous/unprocessed samples containing the chemicals of interest, incl. soil, vegetation, wood, paint/plastic fragments, metal fragments, clothing, wipes, solid absorbents (e.g. Orbo, Tenax)			
Air samples			
Biomedical samples (for the analysis of bio-adducts and metabolites), for example whole blood, red blood cells, plasma, serum, sputum, urine, hair, human remains			

3. Is the management of the storage of the samples part of the accreditation/quality assurance at your organisation? If yes, which accreditation system is in use and what are the recommendations regarding sample management?

4. Are you aware of any emerging technologies that could be used to store/package samples more effectively (e.g. blood spot papers, vacuum packing, or flame-sealed ampoules for liquid samples)?

5. Which further approaches/strategies would help reduce the effect of sample storage on the stability of the chemicals of interest? Please consider aspects of the storage of the samples before and after the analytical work.

Annex 4

STORING AND HANDLING OF PROTEIN CONTAINING SAMPLES [143]

1. Overview of structure, stability and sample handling of proteins

Proteins are highly complex biomolecules with specific functions. Their biological function and molecular stability in terms of an intact primary structure depends on a variety of factors.

Proteins have a primary, secondary, tertiary, and quaternary structure. The primary structure is the basic, intact chain of amino acids which form the protein. A lot of applications rely on this primary structure (SDS-PAGE, 2D-electrophoresis etc.). However proteins are not usually active in this form. Activity requires an intact secondary, tertiary, and sometimes quaternary structure.[16] Active protein is required when the assay relies on a specific protein function (antibodies, enzymes etc.). Primary (and usually secondary) structure of proteins is quite rugged but tertiary structure can be disrupted quite easily. The probability of denaturing the protein increases with the number of processing steps. These steps include protein sample preparation, purification, and storage.

Proteins lose easily their biological function and/or overall stability (due to multiple freeze/thaw cycles, changes in pH etc.). Over time, changes will take place in protein structure that could potentially alter experimental results. There are also many problems that can arise from improper handling of proteins. Contaminants in samples can affect the results and may even damage equipment. However, there are many steps that can be brought into play with handling and storage processes of proteins to minimise any damage and in turn, maximise accuracy of results.

2. Storage of proteins

The maximum acceptable storage time in solution at +4 °C depends highly on the type of protein. Especially enzymes and antibodies can be very sensitive and some may already significantly loose activity after two hours at +4 °C. If the application does not require biologically active protein a storage time of one or two weeks should be OK. Some storage conditions are listed in the Table.

TABLE 7: STORAGE CONDITIONS OF PROTEINS

Storage temperature	Storage conditions			
	Maximum storage time	Number of times sample can be at room temperature	Addition of antibacterial agent	Addition of protease inhibitor
In solution, +4 °C	1 day – 2 weeks	Many	Required	Required

[16] *Secondary structure*: determined by the bond lengths and angles of its primary amino acid sequence and hydrogen bonds between them, e.g. alpha helices and beta sheets. *Tertiary structure*: three dimensional arrangement of secondary structure, the active form of protein. *Quaternary structure*: protein complex of several protein molecules which function as part of this complex.

Storage temperature	Storage conditions			
	Maximum storage time	Number of times sample can be at room temperature	Addition of antibacterial agent	Addition of protease inhibitor
-20 °C, -80 °C or in liquid N_2	Several years	One time (if necessary twice)	If possible, yes	If possible, yes
Lyophilized, +4°C or -20 °C	Several years	Several times	Not required	Not required
In solution (with 50% glycerol) -20 °C	6 – 12 months	Many	Not required	Not required

3. Factors affecting the stability of a protein

Temperature. Most proteins from mammals have their optimum biological temperature at +37 °C. Temperatures above +43 °C will quickly denature most mammalian proteins. At +55 °C complete denaturation takes place within one to two hours, at +95 °C only few minutes is enough to denature proteins.

Protein denaturation and destabilisation occurs also at room temperature, but mainly due to other factors. Proteins are protected in their normal cellular environment by other proteins, so-called chaperones, but this protection does not take place in pure protein solutions. Proteins in solution lose their biological function quite quickly at room temperature. To keep proteins in their active state all work in laboratory should take place on ice at + 4 °C.

Freeze/thaw cycles. Repeated freeze/thaw cycles usually degrade proteins. The same batch of proteins should never be frozen twice. Some proteins are more stable than others, but to be sure you should make aliquots of your protein sample, once you have used one of the aliquots it should be discarded (Table).

Proteases/peptidases. Proteases and peptidases, usually also present in protein solutions, are enzymes which degrade proteins and peptides. Most of these enzymes have optimum temperature ca. +37 °C where some proteins start to degrade by proteases within minutes. Lower temperatures will only reduce the activity of some proteases but do not stop their degradation process even at +4 °C. Protein degradation can be minimised by adding protease inhibitors.

pH. Every protein has an optimal pH for its biological activity or function. Slight changes can affect this activity. Strong acidic or basic pH can quickly denature proteins.

Bacteria. In non-sterile environment the contamination of protein solutions with bacteria is possible. Antibacterial agent e.g. 0.02-0.05% (w/v) of sodium azide (NaN_3) should be added to protein solutions if working at +4 °C is planned.

Protein concentration. Protein concentration does not directly affect protein stability. However, at low concentrations (< 1 mg/ml) low-level binding of proteins to the

storage vessel may occur. Adding a carrier protein to the solution (e.g. 2 mg/ml bovine serum albumin, BSA) is helpful, if it does not interfere with the experiment.

Salt conditions. Too high or low salt concentrations can lead to precipitation of proteins, suggesting their denaturation. Phosphate Buffered Saline (PBS) is commonly used as physiological buffer for proteins. The use of pure water to dissolve proteins is avoided.

4. General handling of protein samples

Working as cleanly as possible is first and foremost important, because contamination also with proteins from the environment could prevent identification of the protein of interest. The most frequent contaminants are human keratin and BSA. The keratin comes from dust, small hairs and fingerprints. The source of BSA contamination is usually western blotting. The following should be taken into account when working with proteins:

(a) always wear gloves (powderless, rinsed with water and ethanol before use) to eliminate contamination by keratins, etc. Gloves should be changed frequently. Gloves are considered contaminated if you have touched surfaces that are handled with bare hands (for example a phone, a computer keyboard) and should be discarded;

(b) close boxes of pipette tips as well as other containers of equipment right after use to minimise keratin contamination;

(c) reserve tubes, pipette tips and containers for protein work only;

(d) use low-binding polypropylene tubes as well as low-retention tips to minimise protein loss by adsorption to a tube wall. Glass tubes are not recommended. Clean your working area every time before starting your work with proteins using a wet paper towel to remove any accumulated dust. Use 70% ethanol as wetting solution for wiping the working areas;

(e) use clean dishes in gel electrophoresis, for gel casting, as well as staining. To minimise the risk of BSA contamination never use for staining of gels containers earlier used for western blotting for staining of gels; and

(f) use fresh high purity reagents and water. Contaminants from buffers, detergents, etc. may affect the protein identification.

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