

SYNTHESIS OF REFERENCE COMPOUNDS RELATED TO CHEMICAL WEAPONS CONVENTION FOR VERIFICATION AND DRUG DEVELOPMENT PURPOSES – A BRAZILIAN ENDEAVOUR

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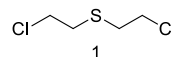
Abstract: This paper deals with challenges that Brazilian Army Organic Synthesis Laboratory has been going through to access reference compounds related to the Chemical Weapons Convention in order to support verification analysis and for research of novel antidotes. Some synthetic procedures to produce the chemicals, as well as Quality Assurance issues and a brief introduction of international agreements banning chemical weapons are also presented.

Keywords: Organization for the Prohibition of Chemical Weapons, Chemical Weapons Convention, Organic Synthesis, Reference Chemicals, Quality Assurance.

1. INTRODUCTION

The use of chemicals as weapons dates from ancient times. Nevertheless, their lethality has been improved over the years. Even though international agreements had already been signed at the end of 19th century, toxic chemicals were deployed in World War I (1914-1918), causing millions of casualties. Examples of chemicals used were gases as chlorine and phosgene, and, especially sulfur mustard, HD (**1**, figure 1).

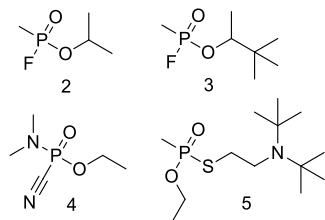
Figure 1: Sulfur mustard HD



Between Great Wars and after II World War, research on novel pesticides led to a class of very toxic pentavalent phosphorus compounds. They irreversibly inhibit acetylcholinesterase (AChE), enzyme involved in Parasympathetic Neurotransmission, which can lead to life-threatening events. Examples of these

compounds are the G-Agents sarin (**2**), soman (**3**), tabun (**4**) and V-Agent VX (**5**), all known as nerve agents (figure 2) [1-3].

Figure 2: G- Agents



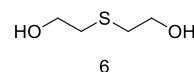
Although these organophosphorus compounds were not used in the II World War, their potential as Weapons of Mass Destruction has been demonstrated in different situations, as Iran-Iraq War (1980-1988). After this event, in 1993, the first draft of the Chemical Weapons Convention (CWC) was published. This led to foundation of “Organization for the Prohibition of Chemical Weapons (OPCW)”. The current text of CWC entered into force in 1997 [1,4]. Unfortunately, CWC has not precluded the use of chemical warfare in Japan (1994-1995) and ongoing Syrian Civil War (2013). As of February 2017, there are 192 signatories or State Parties. For its efforts throughout years to eliminate chemical arsenals, OPCW was awarded with Nobel Peace Prize in 2013 [1].

CWC has the “Annex on Chemicals”, that list the different chemicals used as weapons (Part A) or precursors (Part B) for their preparation, as well as two toxins (ricin and saxitoxin), which underlines the convergence between Chemistry and Biology. There are three Schedules of Chemicals, divided accordingly to their lethality and possibility of peaceful application. As Brazil is an OPCW State Party, these compounds cannot be produced or used as warfare. They only can be synthesized for peaceful purposes, as testing of protective equipment, detectors, forensics and research on novel medicines. This asserts the

need of synthetic capabilities, as these chemicals are not sold by chemical suppliers.

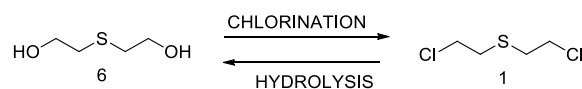
Schedule 1 contains the most toxic substances which have been developed for or used as chemical warfare agents with virtually no peaceful applications. It lists, for example, sulfur mustard (**1**), G- and V-Agents (**2-5**). Schedule 2 lists toxic chemicals that could not only be used as weapon, but also as precursors to Schedule 1 chemicals. Nonetheless, they have uses not prohibited by the CWC, as precursors for pesticides and pharmaceuticals, for example. Therefore, they have relevant industrial production. Thiodiglycol (**6**), precursor to sulfur mustards (figure 3) and used as solvent for inks, is a relevant example of this Schedule.

Figure 3: Thiodiglycol



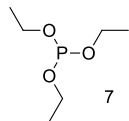
Schedule 2 is important for verification purposes, as they can be both precursors and degradation products of Schedule 1 chemicals. For example, thiodiglycol (**6**) is precursor and hydrolysis degradation product of sulfur mustard (**1**, scheme 1).

Scheme 1: Relationship between Schedules 1 and 2 chemicals



Schedule 3 refers to chemicals produced in large scale for peaceful purposes; however, they may be used such as warfare agents or precursors for chemicals of the Schedules 1 and 2. Triethyl phosphite (**7**, figure 4), is a precursor to G- and V-Agents [4], as well flame-retardant materials.

Figure 4: Triethyl phosphite



Although Schedule 3 chemicals may be easily purchased in comparison to other Schedules, the production of certified reference materials and reference materials is also restricted. There are few suppliers of reference materials certified in Brazil and also at an international level. Thus, it is extremely important that the laboratories belonging to the Defense Affairs have expertise to synthesize reference materials for CWC chemicals.

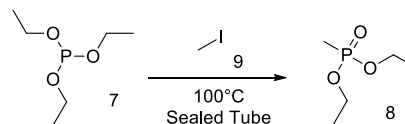
2. THE LSO EXPERIENCE IN SYNTHESIS OF CWC CHEMICALS – THE QUEST FOR THE REFERENCE STANDARDS

During the OPCW Proficiency Tests, which occurs twice a year, a forensic exercise to all laboratories which aim to be awarded with “OPCW Designated” status, LAQ has found some challenges to access reference chemicals needed [5]. Even during the ISO 17025 Accreditation process, availability of chemical references becomes a key step, because, as aforementioned, virtually all analytical targets are not sold by chemical suppliers. All Designated Laboratories have in-house procedures to afford them. LSO has been designed to carry off this problem and we have been very successful so far, even facing bureaucratic problems to access key precursors. OPCW has tried to help laboratories to overcome the synthetic limitations through its database, allowing them to take part in Proficiency Tests. This comprehensive database contains different spectroscopic and spectrometric data of chemicals from all Schedules to support laboratories’ findings.

For instance, diethyl methylphosphonate (DEMP, **8**), a Schedule 2 chemical, key precursor to some G-Agents, has been synthesized from

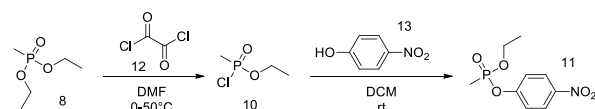
trimethyl phosphite (**7**, Schedule 3 chemical) and iodomethane (**9**, scheme 2) using Arbuzov-Michaelis Reaction:

Scheme 2: Synthesis of **8**.



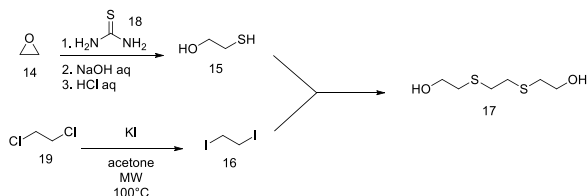
Recently, after we have sought in the literature for nerve agents’ surrogates [6], we have successfully prepared O-ethyl methylphosphonochloridate (EMPC, **10**), also a Schedule 2, structurally related to G-Agents. From EMPC, we have carried out the synthesis of O-4-nitrophenyl O-ethyl methylphosphonate (NEMP, **11**), a VX surrogate, which has been used for testing of our antidote prototypes and evaluation of detectors (scheme 2). NEMP is a potent AChE inhibitor. It is the first time that a scientific work in Brazil use nerve agents’ surrogates for *in vitro* assay of novel antidotes (scheme 3). This work has been submitted to peer-reviewed publications in Medicinal Chemistry.

Scheme 3: Synthesis of **11**.



Recently, in 41st OPCW Proficiency Test, LAQ had to confirm the presence of different degradation products of sesquimustards, some of them very challenging compounds if we consider the availability of precursors. Starting from ethylene oxide (**14**), LSO synthesized 2-mercaptoethanol (commercial, but no readily available, **15**) and 1,2-diiodoethane (**16**), to afford the synthesis of 1,2-bis(2-hydroxyethylthio)ethane (**17**) (scheme 4) [6].

Scheme 4: Synthesis of **17**.



Based on aforesaid, it is clear that LSO has all required expertise to safely undertake the synthesis CWC related compounds.

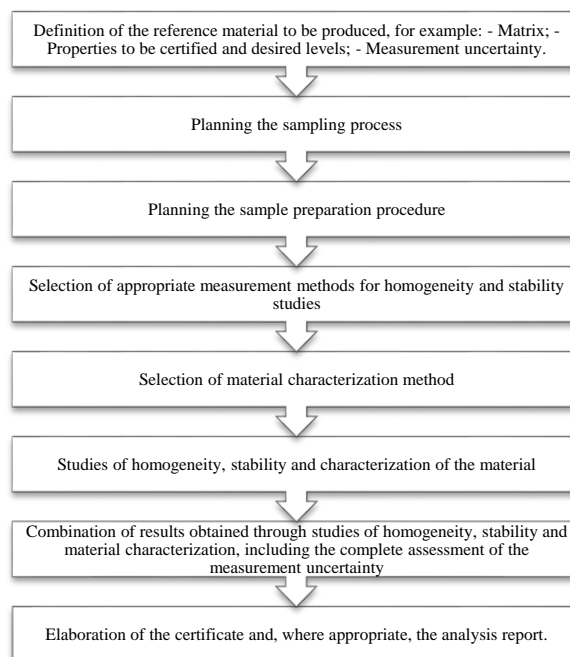
4. PERSPECTIVES

From a point of view of the Quality Assurance, LSO has striven to develop other synthetic methodologies that can fulfill the need of reference chemicals related to CWC aiming, in a future, to be a supplier of these compounds to other laboratories, complying with all requirements of ISO 17034 to produce chemical references. Naturally, all actions taken must comply with the CWC, as Brazil is a State Party.

Access to CWC Certified Reference Materials (CRM) is a challenge for many laboratories, since they are scarce and require experience in handling hazardous materials. However, their production has several advantages, such as: traceability and measurements reliability, validation of methods, calibration of equipment, quality control and improvement of performance during OPCW Proficiency Tests. Therefore, the production of CRM is one of the priorities for the LSO.

CRM production must comply the requirements of ISO 17034 and Guide 35, i.e., values for homogeneity, stability (short and long terms) characterization should be established for synthesized materials [7,8]. A flowchart to be used by the LSO, according to ISO 17034, for the production of CWC-CRM is given (chart 1).

Chart 1: Workflow



The flowchart may be explained using DEMP as example. After synthesis, purification and spectroscopic identification, the reference material should be diluted in methanol at 1000 µg/ml and stored in a suitable vial at -20°C for further studies (homogeneity, stability and characterization). If an initial batch of 100 bottles is produced, 14 of them will be separated for homogeneity studies. This percentage figure is present in the sampling criteria set out in British Standard 5309 [9]. The aliquots required for the stability and characterization studies will be drawn from the separate bottles for the homogeneity study. The choice of bottles will be made with stratified random sampling. The homogeneity of the produced material will be evaluated by analysis of variance (ANOVA), by calculating the standard deviation between the units of a reference material (s_{bb}) and the standard deviation of the repeatability of the measurement method (s_r). The uncertainty regarding homogeneity (u_{hom}) will be the greatest value between the two deviations, s_{bb} or s_r . The

calculations referring to the stability study will also be performed with analysis of variance, evaluating regression data. The stability uncertainty (u_{st}) is calculated by multiplying the standard error value (s_{bl}) by the study time [8, 10]. The short-term stability (u_{sts}), which evaluates the transport conditions of the CRM, and the long-term stability (u_{lts}), which evaluates the storage conditions of the CRM, will be calculated. The measurement reference temperature in the stability studies shall be 4°C. The short-term stability will be carried out in the period of fifteen days, with temperatures of 25°C and 50°C. Long-term stability studies will be carried out over 12 months at temperatures of 4°C and 25°C. The characterization study of the CRM will be carried out using two different analytical techniques, Gas Chromatography coupled to Mass Spectrometry (GC-MS) Nitrogen and Phosphorus Detector (GC/NPD). The methodology of analysis will be carried out according to the established in the “Blue Book”, method standardized by the OPCW [11]. The measurement of traceability of the chromatographic and spectrometric analysis procedure will be ensured using certified DEMP methanolic solution, in conjunction with calibration of the laboratory instruments used and sample preparation. The characterization uncertainty may be measured by Interlaboratory comparison if there is a population of laboratories that are equally capable of determining the characteristics of the CRM, providing results that have acceptable accuracy. In the absence of such laboratories the characterization uncertainty can be calculated by the standard deviation of the results of measurement divided by the square root of the number of replicates tested. The final expanded uncertainty (U_{CRM}) will be calculated with the values of the uncertainty of homogeneity (u_{hom}), short term stability (u_{sts}), long term stability (u_{lts}) and characterization (u_{char}) (equation 1). The coverage factor ($K = 2$) should

be considered for the calculation of the expanded final value.

Equation 1: expanded uncertainties (U_{CRM})

$$U_{CRM} = K \times \sqrt{u_{hom}^2 + u_{sts}^2 + u_{lts}^2 + u_{char}^2}$$

Finally, the certificate of the reference material will be drawn up in accordance with the requirements set out in ISO Guide 31 [12].

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