OPCW

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SUMMARY OF THE SECOND MEETING OF THE SCIENTIFIC ADVISORY BOARD'S TEMPORARY WORKING GROUP ON CHEMICAL FORENSICS 3 – 5 JUNE 2024

1. AGENDA ITEM ONE – Opening of the meeting

1.1 The Temporary Working Group (TWG) on Chemical Forensics of the Scientific Advisory Board (SAB) held its second meeting from 3 to 5 June 2024. The meeting was chaired by Dr Anne Bossée on behalf of the SAB, with Dr Simon Ovenden as Vice-Chairperson.

1.2 Dr Bossée opened the first in-person meeting of the TWG by warmly welcoming its members and thanking the external speakers for accepting the invitation to make presentations. She remarked that she had briefed the SAB at its Thirty-Eighth Session on the work conducted to date by the TWG and noted that the Director-General had highlighted the significance of the work of the Group in his opening address to the Board. Dr Bossée then shared some specific feedback from the SAB session with the Group.

(a) First, she stressed the importance of carefully explaining the difference between "verification analysis" and "chemical forensics analysis" as discussions among the SAB members had highlighted that this important difference was not clearly understood.

(b) There could be value in exploring the possibility of exploiting biomedical samples in the chemical forensics process or integrating a clinical dimension (covering signs and symptoms, for example).

(c) The TWG should take the impact of decontamination processes on chemical forensics into consideration during its review.

(d) Staff from the OPCW Laboratory had informed the Board that, regarding generating data for a chemical forensics database, the Laboratory would not have the capacity to conduct extensive synthesis and would not be the primary source of the requisite data. It was additionally clarified that data from authentic samples stored at the OPCW Laboratory could not be added to the database owing to the confidentiality issues associated with them.

1.3 Finally, all participants at the meeting were invited to introduce themselves to their colleagues. A list of participants appears in the Annex to this report.

2. AGENDA ITEM TWO – Adoption of the agenda

As no objections or comments were raised in response to the proposed programme of work for the three days of the meeting, the following agenda was adopted:

1. Opening of the meeting
2. Adoption of the agenda
3. Updates from subgroups[1]
4. Forensic science and "chemical forensics"
5. The hunt for chemical attribution signatures – Chemical forensics at DSTG
6. Chemical impurity profiling – Linkage of starting materials and an intermediate synthesis product
7. Everything everywhere all at once – An introduction to total correlation mass spectrometry (TOC-MS)
8. Cracking the chemical code: A data-science approach to deciphering the chemical information stored in environmental samples
9. The interface between analytical chemistry and fire and explosion investigation
10. Chemical forensic approaches at DGA CBRN Defence
11. Subgroups 2 and 4 breakout sessions[2]
12. Subgroups 1 and 3 breakout sessions[3]
13. LLNL approaches to chemical forensics
14. Subgroup 1 deep dive[4]
15. Subgroup 2 deep dive[5]
16. Subgroup 3 deep dive[6]
17. Subgroup 4 deep dive[7]
18. Plenary discussion[8]

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1 While agenda items 3, 11, 12, and 14 to 18 were discussed separately during the meeting, they are reported herein under a single agenda item for clarity purposes and to minimise duplication.

2 Ibid.

3 Ibid.

4 Ibid.

5 Ibid.

6 Ibid.

7 Ibid.

8 Ibid.

19. Meeting recap, identification of remaining gaps, and next steps

20. Any other business

21. Closure of the meeting

3. AGENDA ITEMS THREE – Updates from subgroups

Subgroups – Agenda items Eleven, Twelve, Fourteen, Fifteen, Sixteen, Seventeen, and Eighteen – Discussions on subgroup topics

3.1 Subgroup 1, led by Dr Crister Åstot, is focusing on the state-of-the-art of chemical forensics and considering the areas of batch-matching, impurity profiling, synthesis route, geographical and environmental factors, and isotope ratios. Initially, the members discussed the value of the end-of-mandate report from the TWG on Investigative Science and Technology as a foundation for the work of the current TWG.

3.2 The overall work of this TWG on Chemical Forensics was considered to determine whether or not the analysis and identification of Schedule 1 chemicals is within scope. The subgroup noted that the identification of illicit drugs and explosives is a key component in chemical forensics analysis within the broader chemical forensics community. However, in an investigation of alleged use of a chemical weapon, identification of the chemical itself would be conducted within the OPCW network of designated laboratories, using existing methods and procedures. Chemical forensics techniques would be applied following confirmed identification of a chemical warfare agent and would exploit intrinsic and/or extrinsic information to provide the OPCW with a deeper understanding of the origin of the toxic chemical. The subgroup therefore agreed that the identification of Schedule 1 chemicals was outside the scope of the TWG and had already been addressed by the TWG on Investigative Science and Technology.

3.3 In the scientific literature, it has been proposed that the chemical analysis of biomedical samples could provide additional information on the production route of a chemical. The subgroup recognised that the extra layer of complexity owing to the effects of human metabolism on chemical profiles and the very low level of biomarkers coming from impurities are likely to limit the practical use of such an approach.

3.4 Information available from other areas of chemical forensics applicable to chemical warfare agents was discussed at length. Several key areas were identified as being potential sources of useful information, such as:

(a) illicit drug chemical forensics and its role in anticipating novel routes of production;

(b) environmental forensics and the use of chemical profiles to link chemical agents released into the environment with suspected sources;

(c) counterfeit food products;

(d) metabolomics as a source of knowledge on analytical methods for chemical profiling, including peak picking and data handling; and

(e) the use of artificial intelligence (AI) to augment the information that can be extracted from chemical samples.

3.5 Subgroup 1 noted that as a result of the large numbers of investigations in these areas, chemical forensics methods have been tested extensively with authentic samples. The subgroup members further noted that, in most chemical forensics areas, these authentic samples do not contain highly reactive and unstable chemicals which are a common challenge in the chemical forensics analysis of chemical warfare agents.

3.6 Subgroup 1 explored the possibility of leveraging the expertise of students at the University of Amsterdam to gain additional insights into some of the specific areas under review. This work would comprise the preparation of in-depth, literature-based theses and be facilitated by TWG member Prof Arian van Asten. It was agreed that there was value in this approach, which would significantly bolster the work of the subgroup, although it was also acknowledged that a suitable set of questions would need to be identified and care taken to ensure they are not too wide in scope. A set of questions based around the steps of a chemical warfare agent investigation will be drafted and agreed by early September. It was recognised that while the contributions of the students will be helpful, the subgroup will need to further process and consider the information provided during the course of the TWG's mandate.

3.7 Through both plenary and subgroup discussions, it was established that the work of Subgroup 1 will inform and be complementary to that of Subgroup 2. Led by Dr Grégoire Delaporte, Subgroup 2 is focusing on future capabilities, specifically considering the analysis of large or limited datasets, dataset creation or expansion, and model validity. The input from Subgroup 1 will provide an understanding of the impact of the implementation of new AI-related data analysis methods and how these methods might be applied to chemical forensics. It will also enable the identification of any critical gaps.

3.8 Subgroup 2 discussed the impact of machine learning and deep learning on chemical forensics. The members determined a number of topics to be considered during its work including:

(a) adapting AI-related methods to the specificity of chemical forensics (including the translation of analytical data to fit into these methods);

(b) how results from a machine learning model can be converted to evidence (i.e. qualify and quantify the confidence in a result from a data analysis workflow);

(c) use of AI and machine learning to merge data from different sources (that is, from different analytical techniques);

(d) use of AI-related methods to indirectly assist chemical forensics investigations (for example, by generating an in silico database); and

(e) risks and threats posed to chemical forensics by AI methods.

3.9 While considering the future of the field in the coming decade, Subgroup 2 noted the significant impact of computer science on established forensics fields in the last five years, exemplified by the establishment of dedicated research and development teams at the Netherlands Forensic Institute. It was agreed that chemical forensics will be similarly impacted over the coming years. However, given the specificity of the chemical forensics field, namely limited data availability and the uniqueness of each case, as well as the difficulty of bringing together competences in chemical warfare agent reactivity, chemistry, and data science, a time lag could be expected before AI methods expand fully into chemical forensics.

3.10 During discussions on presenting its findings in the end-of-mandate report, Subgroup 2 members decided that their section should be divided into two principal parts. The first part will consist of an introduction that will be accessible to a non-expert audience. This introduction will emphasise that chemical forensics, and data analysis in particular, is only one piece in a much large investigative puzzle; that no single data analysis method is a universal solution; and that a collection of diverse data analysis methods should be available for different contexts. The second part will discuss the technical findings of the Subgroup and provide the main conclusions. A glossary of AI terms will also be included in the end-of-mandate report.

3.11 In the intersessional period, assisted by input from Subgroup 1, Subgroup 2 plans to compile tables of AI methods and their applications and commence developing the introductory text.

3.12 Subgroup 3, led by Ms Ang Lee Hwi, is focusing on methods and procedures in chemical forensics and considering issues such as standardisation, information sharing, reproducibility, and best practices, among others. In the first meeting of the TWG in March 2024, the subgroup discussed the six questions posed in the terms of reference and categorised them into three main tasks:

(a) establishing a framework for database building and sharing;

(b) developing standard operating procedures and building capability in labs; and

(c) developing on-site sampling protocols.

3.13 For the first task, the subgroup discussed data sensitivity and expressed the importance of appointing an analytical data custodian, such as the OPCW, to encourage contributions from laboratories. The Subgroup also discussed the possible concerns of the contributing laboratories, including access to and management of the database, revealing laboratory and consequently State Party identities through unique signatures generated, and the possibility of revealing the synthesis routes of Schedule 1 chemicals. To address these concerns, the subgroup proposed solutions, including studying open-source or AI-predicted synthesis routes, as well as conducting the synthesis at the OPCW Centre for Chemistry and Technology (ChemTech Centre). The idea of curating a database from open-source profiling data was also noted as a potential short-term outcome, and an interim solution until a framework is put in place for collecting data from experimental studies to develop a more comprehensive database.

3.14 The subgroup discussed the importance of including data from authentic samples in the database, as well as realistic, artificially generated samples with complex matrices. It was proposed that the interlaboratory confidence-building exercises could be a useful means to collect data on samples prepared by the OPCW and sent to designated laboratories. These samples could stem from increasingly challenging scenarios, incorporating variables such as storage containers, dissemination devices, and complex matrices. The data provided by the participating laboratories would subsequently be organised into useful output for synthesis route determination, in addition to being "cleaned up" and anonymised to remove sensitive signatures. To incentivise participation, the subgroup proposed that the participating laboratories could be given access to these data. This proposed approach would be discussed and considered further by the entire Group at its next meeting.

3.15 For the second task, the subgroup learned that in the drug domain—while there are no common methods or procedures—data, libraries, best practices, and advice are shared relatively freely through informal forums given the low levels of data sensitivity. More information about other domains such as environmental forensics and food analysis will be useful references. Subgroup 3 also discussed the harmonisation of methods and procedures, and proposed that review of past data, such as the recent evaluation of a chemical profiling method for a nerve agent precursor performed by eight analytical laboratories[9] as well as confidence-building exercises will be a useful means to assess the need for and the extent of harmonisation.

3.16 The possibility of adapting current proficiency tests for the exercises was discussed and considered to be of low utility given the different objectives and scope of analysis for verification and chemical forensics purposes. However, confidence-building exercise samples could be dispatched with proficiency test samples to minimise additional costs and to encourage designated laboratories to participate. This would provide laboratories with the option of participating in the exercise and those laboratories that submit confidence-building exercise results would gain access to the data acquired. An important task for the subgroup will be proposing forensic chemical criteria and a template for reporting the data, which will help steer contributions from participating laboratories. These could be further refined by the OPCW Laboratory as the exercises progress. Suggestions were also made to extend the confidence-building exercises to other forensics laboratories.

3.17 For the third task, the subgroup proposed to review the output of the Generic Integrated Forensic Toolbox for CBRN Incidents project, and to hear from the OPCW Investigation and Identification Team to assess if there are current gaps that need to be addressed.

3.18 Subgroup 4, led by Dr Hanna Hakulinen, is focusing on how chemical forensics capabilities at the OPCW can be augmented. Subgroup members emphasised the importance of highlighting the difference between verification analysis and chemical forensics, and the need to carefully define chemical forensics as it could have different meanings for different people. Furthermore, the distinction between chemical forensics and attribution should also be made clear.

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9 Karin Höjer Holmgren, Hanna Hakulinen, Rikard Norlin, Mirjam de Bruin-Hoegée, Marie Spiandore, Samantha Qi, Renee Webster, et al. 2023. "Interlaboratory Comparison Study of a Chemical Profiling Method for Methylphosphonic Dichloride, a Nerve Agent Precursor." Forensic Chemistry 33 (May): 100473–73. https://doi.org/10.1016/j.forc.2023.100473.

3.19 Subgroup members discussed identifying sources of expertise that could be leveraged and recognised and that would likely be found outside the network of designated laboratories. They agreed that engaging with forensics laboratories and participating in conferences is key to staying informed about established methodologies and connecting with other experts in the field. Literature reviews and collaborative efforts across different subgroups would also be beneficial.

3.20 Discussions identified the augmentation of the capabilities of designated laboratories with expertise from other non-designated laboratories, the creation of a roster of non-designated laboratories with relevant forensic expertise, and the determination of criteria for selecting laboratories as important considerations. It remains unclear how appropriate forensic laboratories would be selected and how, and by whom, they would be tasked.

3.21 While non-designated laboratories will play an important role in augmenting capabilities, Subgroup 4 recognised that any results from such laboratories presented in court could be subject to additional scrutiny and the capabilities of the laboratories questioned.

3.22 Subgroup 4 agreed that exploring applications of chemical forensics beyond chemical warfare agents is a valuable approach and that combining knowledge from designated and non-designated laboratories, while preserving specific chemical warfare agent expertise (synthesis, reactivity, and analysis), is vital. Overlap between national and international interests should be considered—for example, how designated laboratories collaborate with crime laboratories.

3.23 Subgroup 4 noted that the current proficiency testing designation framework (chemical scope and reporting criteria) is not directly applicable to chemical forensics and that confidence-building exercises will be critical to developing the capabilities of designated laboratories. These exercises could enhance teamwork as well as capacity building, through data analysis, harmonisation, and fusion of different analytical techniques such as gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) spectroscopy. This approach would address challenges in data analysis and ensure quality control and comparability of chemometric methods between laboratories. It is expected that laboratories will not need to perform every aspect of chemical forensics analysis, and that sufficient time will be provided to carry out the required tasks.

3.24 Additionally, exploring the utilisation of existing ground truth data (that is, the raw data from samples whose provenance is known) within designated laboratories was considered essential by the Subgroup. The potential role of the OPCW as a data custodian and the value of a voluntary network of laboratories engaged in chemical forensics without significant funding highlight the importance of collaboration and data sharing in this field. To this end, the subgroup views inclusion of and collaboration with the Chemical Forensics International Technical Working Group (CFITWG) as important.

3.25 It was agreed that a road map, with appropriate steps to the requisite tools (analytical, statistical, reporting, and others) is essential. Reporting was discussed specifically, and one option could be to start with a "freestyle" report for confidence-building exercises. With involvement from the OPCW Laboratory and designated laboratories, this could subsequently transition to a standard forensic report. This methodology has been employed for the official OPCW exercises for the analysis of biotoxins. The OPCW should define the necessary additional capabilities that the designated laboratories need to have or achieve, such as a matrix of impurity profiles, samples batch-matching, and isotope ratios, and what the requirements are beyond the work of the Investigation and Identification Team in the Syrian Arab Republic. Owing to the low expected concentration of impurities only one identification method might be available and semi-quantitative analysis might be required, for which ways to achieve relative abundance need to be considered.

3.26 In addition to these discussions, the following points were also raised by Subgroup 4:

(a) inclusion of biomedical samples in chemical forensics and challenges thereof;

(b) the impact of decontamination measures (from initial collection at the incident scene to analysis and subsequent disposal);

(c) building databases with data from microsyntheses (challenging because signatures are very different from large-scale production);

(d) the potential of simulated data;

(e) the importance of excluding certain trace chemicals or elements;

(f) AI tools (for example, toxicity prediction when no experimental data is available);

(g) issues with sharing data (political sensitivities concerning the raw data of Schedule 1 chemicals);

(h) how to encourage and incentivise designated laboratories to perform the optional chemical forensics analysis in proficiency tests; and

(i) the importance of training inspectors on sampling procedures.

4. AGENDA ITEM FOUR – Forensic science and "chemical forensics"

4.1 Prof Niamh Nic Daéid from the University of Dundee in the United Kingdom of Great Britain and Northern Ireland is an experienced analytical chemist specialising in forensic science. She outlined several key challenges faced by forensic scientists, including the impact of preconceived notions shaped by books and television, as well as the complexities of their working environments, which span crime scenes, laboratories, and courtrooms.

4.2 Throughout her presentation, Prof Nic Daéid emphasised the challenges of effectively communicating forensic science techniques and analytical results to law enforcement and court officials who lack a scientific background. She stressed that scientists need to adjust their messaging, concentrating less on detailed results and more on the key findings and their implications. By focusing on the entire end-to-end process, from the crime scene to the courtroom, the scientific perspective can be integrated more seamlessly making it more impactful and useful.

4.3 Demonstrating the scientific validity of analysis and interpretation poses another challenge. Prof Nic Daéid noted that in recent years forensic science has faced increased scrutiny. Notable reports, such as the Annual Report of the Government Chief Scientific Adviser 2015 and the 2016 report by the President's Council of Advisors on Science and Technology, have highlighted the lack of rigorous scientific methodology in certain forensic disciplines. This deficiency makes it difficult to ascertain the validity and reliability of the data presented in court.

4.4 Another significant challenge in forensic science is the availability and quality of datasets. While robust datasets exist for DNA and fingerprint analysis, other areas lack comprehensive data. Often, there are few international datasets, and national or local datasets are limited or non-existent, especially for drugs and ignitable liquids. The existing data is fragmented and uncoordinated and collected in varied ways that complicate integration and exploitation, particularly for machine learning activities. This fragmentation hinders the full and effective use of the data.

4.5 Prof Nic Daéid shared insights from her group's experience with methamphetamine profiling, highlighting key considerations throughout the sample collection and analysis workflows. These considerations include the quantity and physical characteristics of the sample, sample stability, extraction strategies, and analytical techniques. Clandestine drug laboratories, ranging from small to large-scale operations, could source precursors from legitimate suppliers or synthesise them from uncontrolled "pre-precursors". This process usually comprises multiple laboratories for the synthesis of precursors and the drug itself, and then a final bulking or mixing laboratory. Each laboratory could distribute its products to several other laboratories across the country. The complexities of both the chemistry and the supply chain create a highly intricate matrix that influences the chemical profiles. Experimental work from her group has demonstrated that even the same chemical produced in the same way, by the same chemist with the same equipment can exhibit significant batch-to-batch variation. They have shown that principal component analysis (PCA) can effectively differentiate between synthesis routes, with hierarchical clustering yielding even better results. Additionally, they have obtained promising results using artificial neural networks, which allowed for the combination of datasets from GC-MS and IR-MS data, ultimately identifying the specific synthetic route and solvent used for extraction.

4.6 Prof Nic Daéid then briefly discussed their work using high-resolution FT-IR spectroscopy to analyse six commercially available sources of peroxide, a common explosives precursor. By applying a neural network and deconvolution software, they identified a specific wavelength region where all six samples produced distinct results, allowing for differentiation. They are currently investigating the potential of this approach to develop tuneable FT-IR scanners for use in airports.

4.7 Lastly, Prof Nic Daéid emphasised that, aside from the environmental science and food analysis sectors already being considered by the TWG, other sectors are also significantly advancing AI adoption. Health services and drug discovery in particular, as well as finance and technology, insurance, transportation, and marketing are leveraging AI extensively. She noted the potential to apply to forensic science algorithms and techniques from data-rich fields such as animal biometrics and human genetics, as in many cases these algorithms are achieving what forensics scientists want to achieve.

4.8 Following this, TWG members discussed several elements of Prof Nic Daéid's presentation. There was significant focus on the relevant data analysis tools and strategies, including questions regarding the optimal use of IR-MS, ICP-MS, and GC-MS in the context of obtaining reliable forensic results and the required degree of orthogonality. Prof Nic Daéid clarified that IR-MS was better at identifying precursors in a finished product, while GC-MS was better at route specificity. She noted that the solvent used made an unexpected difference to the chemical profiles, especially in the isotope ratio analysis, and postulated that this could be attributable to encapsulation of the solvent in one of the samples. It was emphasised that chemometrics models encounter difficulties when classifying samples that contain volatile chemicals. Subtle changes to the methamphetamine manufacturing processes also affected the impurity profiles and, consequently, the classification models always evolve with new curated data. The Group agreed that these observations highlighted the need for recommended operating procedures in this type of work.

4.9 Discussions related to data analysis extended to the complexity of certain datasets and the use of machine learning tools, which in turn were also discussed in the context of presenting chemical forensic data in a court of law. Scientists have to learn how to present complex forensic results clearly to a non-scientific audience. It was further clarified that as new technologies or methods emerge, and enter public consciousness or broader discursive awareness, this can complicate judicial proceedings.

5. AGENDA ITEM FIVE – The hunt for chemical attribution signatures – Chemical Forensics at DSTG

5.1 Dr Simon Ovenden from the Defence Science and Technology Group (DSTG), Australia, described studies to understand the impurity profile of fentanyl and to identify chemical attribution signatures. Impurity profiling, a chemical forensics methodology, utilises impurities and their relative abundances detected in an unknown sample to classify the sample to its corresponding class. Establishing links between samples provides highly valuable information in an investigation of the use of toxic chemicals for illicit and nefarious purposes. This work explored multiple synthetic routes for fentanyl and its precursor 4-anilino-N-phenethylpiperidine (ANPP).

5.2 A common synthetic route (Valdez method) for the formation of ANPP was used to synthesise fentanyl. Final acylation was undertaken using either the Siegfried or Janssen methods, which use different acylation agents. A total of 66 impurities were identified during the synthesis of fentanyl, with 21 specific to the Siegfried method and 22 specific to the Janssen method. As an extension to this, five methods for ANPP production were studied: Valdez, Siegfried-Valdez, Siegfried, one-pot, and Dieckmann. Not all these combinations for ANPP production yielded unique impurity

profiles. However, it was shown that only two carbamate impurities were produced during the Valdez and Siegfried methods of ANPP synthesis, providing a link to the specific reagents used in these two methods.

5.3 Impurity profiling of three bulk VX samples was undertaken using NMR, liquid chromatography-high-resolution mass spectrometry (LC-HRMS), and GC-MS. All three bulk samples were synthesised using the same production route and stored in an identical way. The bulk agents were synthesised in 2014, 2017, and 2018. In total, 44 impurities were identified and the structures of 37 were elucidated. Two observations were of particular interest. The first was the identification of the isopropyl analogues of both VX and bis-2-(N,N-diisopropylaminoethyl)disulfide. Analysis of the precursor compound 2-(N,N-(diisopropylamino)ethyl chloride showed that a minor amount of an isopropyl impurity (2-(N-isopropylamino)ethylchloride) was present. This may have been the source of the corresponding impurities identified in the bulk stored VX agent stocks. The second observation was the identification of O,O-diethyl S-(2-diisopropylaminoethyl) phosphonothioate. It was proposed that this could have been formed through reaction with small quantities of phosphorus trichloride remaining in the distilled VX precursor P-methylphosphonous dichloride. As these two chemicals have very similar boiling points (77 – 79 °C for P-methylphosphonous dichloride and 76 °C for phosphorus trichloride), it is unlikely that all traces of phosphorus trichloride would be removed through distillation. The impact of the age of the stored VX on the chemical profile was also studied and it was found that after one year at room temperature, 55% of the previously identified chemical attribution signatures could still be detected.

5.4 Several studies on the impurity profiles arising from different synthetic routes for VX production were discussed. These routes varied in the precursors and the reaction conditions used. All batches of VX were analysed by LC-HRMS and GC-MS, and were subjected to PCA and partial least squares discriminant analysis (PLS-DA). A total of 39 impurities were identified. All synthetic routes had at least one impurity identified that was specific to the method of production. A fifth method was also studied in collaboration with DSO National Laboratories in Singapore. This was a literature-based method that uses n-butyllithium as a reagent. On analysis of the VX samples produced, both laboratories identified two impurities containing butyl groups. As there were no other chemicals containing butyl moieties involved in the reaction, these impurities could only have been formed through reaction with n-butyllithium. Furthermore, no other synthetic method of VX studied produced butyl-containing impurities. This observation indicates that these impurities with butyl moieties are specific to this reaction pathway.

5.5 Following the presentation, members of the TWG had a series of questions regarding Dr Ovenden's presentation and work. This included discussions on the variability in the microreactors used for VX synthesis. Given the same precursors and temperature, the variability mainly serves to control for pipetting variability. The rigorous standardisation of purification and equipment use between the relevant labs was also discussed, as was the difficulties in sharing materials. Additional points touched on included the differences in analytical results between genuine stockpiles emerging from different sources and laboratory-generated compounds and the current work being done on assessing precursors for newly scheduled chemicals.

6. AGENDA ITEM SIX – Chemical impurity profiling – Linkage of starting materials and an intermediate synthesis product

6.1 Dr Hanna Hakulinen, of VERIFIN, Finland, presented a recent study[10] in which impurity profiles had been extracted from different starting materials for an intermediate synthesis product for Schedule 1 carbamate chemical warfare agents. In the first part of the study, samples were collected from the synthesis reaction mixtures. In the second part, samples were collected from the crude synthesis products where the reaction mixtures were extracted with a work-up procedure to remove some impurities. All synthesis batches and the different starting materials were analysed using a gas chromatography-high-resolution mass spectrometry (GC-HRMS) Orbitrap instrument and quality control samples were used before and during analyses. After data deconvolution with Compound Discoverer software, SIMCA® was used to perform multivariate data analysis.

6.2 The statistical procedure PCA was conducted to view unsupervised trends in the data and orthogonal projections to latent structures. Orthogonal partial least squares discriminant analysis (OPLS-DA) was used to extract chemical attribution signatures and to build supervised classification models. The test-set data was produced using the same starting materials by two different chemists in another laboratory, and it was used for the assessment of the prediction power of the OPLS-DA classification models.

6.3 A classification model was developed to distinguish between the six combinations of starting material suppliers in the reaction mixture samples. It accurately classified all samples except one from an external test set based on the first starting material. Accurate classification based on the second starting material was not achieved, likely because of higher purity. For the extracted samples, a classification model was constructed based on the first starting material. The model was able to classify all 12 test-set samples into their correct classes.

6.4 This work demonstrated the ability to link synthesis samples to the correct starting material supplier. However, higher purity starting materials and purification processes post-syntheses limit the possibility for classification.

6.5 The members of the TWG asked a series of questions related to different elements of the impurity analysis described, including how newly scheduled chemicals could be derivatised, the advantages of using OPLS as opposed to PCA, the sequencing of the models utilised, and the forensic value of impurities identified as emerging from different points of the synthesis procedure.

7. AGENDA ITEM SEVEN – Everything everywhere all at once – An introduction to total correlation mass spectrometry (TOC-MS)

7.1 Dr Tim Wilson, of Verdel Instruments Limited, gave a presentation on total correlation mass spectrometry (TOC-MS), an emerging technique which may be useful for the work of the OPCW. While two-dimensional mass spectrometry (2D-MS) has been

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10 Säde, Solja, Lina Mörén, Karin Höjer Holmgren, Hanna Hakulinen, Andreas Larsson, Magnus Engqvist, Linnea Ahlinder, et al. 2024. “Chemical Impurity Profiling: Linkage of Starting Materials and an Intermediate Synthesis Product of a Carbamate Chemical Warfare Agent.” Forensic Chemistry 39 (July). doi:10.1016/j.forc.2024.100581.

available in research laboratories using super-cooled magnet-based Fourier-transform ion cyclotron resonance (FT-ICR) instruments for many years, Verdel's TOC-MS technology seeks to bring this capability to benchtop instruments found in most analytical labs. TOC-MS produces up to 2,000 individual tandem mass spectra (MS/MS) from a given sample in a single experiment. These spectra are correlated to create a three-dimensional "data cube", providing a rich source of information about the sample as a whole.

7.2 TOC-MS could prove especially useful when there is no prior knowledge of the sample (data-independent acquisition); when contributions from all ionisable species are useful (fingerprinting); or when chromatographic separation is difficult. The technique also makes fragmentation using ultraviolet lasers more effective, providing additional insight into molecular structures. While TOC-MS has significant limitations and is not a silver bullet solution, it may be a useful addition to the analytical repertoire of the OPCW and deliver otherwise unobtainable insights. Having outlined the technique, Dr Wilson reviewed some data obtained from organophosphorus pesticides, chemical weapons analogues.

7.3 In the question-and-answer session that followed, Dr Wilson discussed limitations of the technique, namely the need for pre-analysis separation of a sample in the case it has varied concentrations of species. He explained that a full run at 1 Hertz takes 30 minutes, and that higher frequencies can be used but at the expense of sensitivity. Equipment is selected for its affordability and flight tubes with low resolution are used. Cluster analysis has clear applications for non-target analysis, although unexplained clusters would be a problem. Responding to a question on how chemical fingerprinting techniques may make use of a similar algorithm as used by the Shazam music identification tool, Dr Wilson noted that this is a possibility but that the library of chemical fingerprints, and especially of chemical warfare agents, is very limited compared to the library of music.

8. AGENDA ITEM EIGHT – Cracking the chemical code: A data-science approach to deciphering the chemical information stored in environmental samples

8.1 Prof Gerrad Jones, from Oregon State University, United States of America, outlined the value of multivariate statistical analysis for chemical forensics with a focus on environmental chemistry and non-target data. Three projects were undertaken by Prof Jones' team to test the following hypotheses: (a) all biological, chemical, and physical processes leave behind distinct chemical signatures; and (b) the probability that any two sources or processes produce identical chemical signatures is virtually zero given the diversity of molecules in nature.

8.2 The first project featured non-polar molecules extracted from samples taken from point sources of pollution and analysed using high-resolution liquid chromatography-quadrupole time-of-flight (LC-QTOF). Employing various machine learning classification algorithms, chemical features were ranked based on the extent to which they characterised each sample. The 10 non-target chemical features that best predicted each source were retained, achieving a cross-validation accuracy in predicting the source identity of greater than 97%. The most diagnostic features were largely unique to each source. Environmental water samples were screened for these 10 chemical features to determine the presence or absence of each source within surface waters. The presence of a wastewater treatment plant and its location were successfully detected

using the diagnostic chemical fingerprints associated with this source. This work demonstrated that screening for the presence or absence of a chemical source can be done instead of testing for the presence or absence of a specific chemical, and that only a small quantity of sample is required.

8.3 While the first project resulted in a chemical fingerprint diagnostic of a specific source, evidence suggests that these sources have relatively little influence on the overall chemical composition, indicating that sources with a disproportionately larger influence may be missing. To investigate whether runoff from landscapes was a major contributor to the overall chemical composition in surface waters, a second project was conducted. Four local creeks were sampled across three seasons at five locations per creek, and major landscape gradients (such as land use and land cover) were expected to predict chemical gradients in the water. However, no evidence was found to support this hypothesis. Instead, seasonal processes and microbial communities explained a larger proportion of the variation. Nevertheless, a relatively small fraction of the variation within the chemical dataset could be explained by environmental variables such as temperature, precipitation and pH, and landscape variables such as tree cover and agricultural land. An important conclusion from this work is that any a priori assumptions about what is important can bias the forensic approach.

8.4 The final project examined a creek time series where daily samples were composited over a week for 78 consecutive weeks. Seeking to identify the sources and processes with the largest contribution to the overall chemical composition, an unsupervised approach with non-negative matrix factorisation (NMF) was used. Five distinct chemical factors with varying influence on the chemical composition throughout the study period were identified. While NMF effectively identified distinct patterns, satellite imagery, bulk water, hydrologic characteristics, and suspect screening were used to interpret these patterns. Each chemical factor, with varying degrees of confidence, was interpreted as having been influenced by wastewater, agriculture, groundwater, snowmelt and soil hydrology, and natural processes such as microbial respiration. For this analysis, multiple approaches including unsupervised learning, supervised learning, suspect screening, and correlation were combined. This approach proved more effective at identifying the processes and sources affecting the overall chemical composition, even unknown ones. Information on the fate and transport of pesticides in watersheds, which may be transferable to chemical warfare agents, was raised as a potential topic for student literature review projects.

8.5 Asked about the transition between non-targeted analysis and more targeted, diagnostic approaches, Prof Jones stressed the need for systems thinking and explained the potential for the high-resolution data approach of his team to be translated to a more standard triple-quad approach. This would make fingerprinting more accessible to local municipalities and water quality laboratories—although the complexities of pollution attribution mean that regulators have varied reactions to Prof Jones' research. Suspect screening is a middle ground between non-targeted analysis and narrower target analysis which misses the real-world complexity of data.

8.6 Prof Jones clarified that the support vector clarification models used to analyse watershed data ignore non-classified data, which motivated the decision to switch from a supervised to a non-supervised machine-learning approach. The research community working with microbial markers in wastewater is beginning to combine metabolomics,

genomics, and other similar fields into this type of analysis. Asked how to improve the quality and accuracy of chemometric tools, Prof Jones emphasised awareness of the assumptions of certain techniques and the role of cross-validation activities, though his laboratory currently relies on internal corroboration rather than external corroboration with other laboratories. Six machine learning tools have been tuned and may be combined to improve classification models and, in turn, confidence in the chemometrics results. Regarding the quality of the tools in general, Prof Jones noted the importance of taking their assumptions into account.

9. AGENDA ITEM NINE – The interface between analytical chemistry and fire and explosion investigation

9.1 Prof Roland Goertz, from the University of Wuppertal, Germany, is a firefighter and analytical chemist. His research on fires and fire extinguishing spans 30 years and includes work on chemical safety, particularly relating to extinguishing foams and hazardous materials, as well as hazard defence management. Prof Goertz briefly described his group's laboratory facilities and recent work on evaluating the performance of fluorinated fire suppression foams. Using cluster analysis—a type of multivariate statistical analysis—they evaluated a total of 600 chemicals. This analysis technique meant that only 15 experiments with just 15 chemicals had to be carried out to provide the information required. From this, they concluded that the toxic fluorinated surfactants in foams are not essential to their performance.

9.2 Prof Goertz stressed the importance of transforming questions on a particular fire scenario to specific analytical questions, determining an appropriate sampling plan, and identifying the most suitable analytical method. The quality of the analytical results and their interpretation is wholly dependent on these steps. The interpretation of the analytical results, which also draws on plausibility and the specific circumstances, is vital in fire and explosion investigations. Prof Goertz provided a series of real-world examples of fire investigations, highlighting how analytical results in some cases disproved the working hypotheses of law enforcement.

9.3 For example, the presence of ethyl tert-butyl ether (ETBE), an additive in gasoline, indicated that a household fire may have been the result of arson, using gasoline as a fire accelerant. Prof Goertz however postulated that the ETBE may have been produced by the large number of burning polyethylene terephthalate (PET) plastic bottles found in the building. In his laboratory, he was able to confirm that ETBE was produced as a decomposition product of PET, and the absence of aliphatic hydrocarbons further corroborated that gasoline was not used. He noted that this was nevertheless challenging to explain to court officials owing to their lack of chemical knowledge and spatial awareness of the fire scene.

9.4 Prof Goertz also described the investigation of the Fremantle Highway, a cargo ship carrying electric cars whose lithium batteries caused a week-long fire to burn on board. This investigation required drawing on a variety of information, such as witness statements and fire alarm information, to determine the origin of the fire. In the laboratory, they studied thermal runaway reactions of lithium-ion batteries and analysed the toxic emissions. They noted that several toxic gases had been produced, including hydrogen fluoride, phosphoryl fluoride, and phosphorus pentafluoride. A number of mechanisms that could trigger a thermal runaway reaction, including external heating and mechanical stress, were investigated.

9.5 The members of the TWG had several questions regarding the cases described and the operational procedures of the forensic scientists and analytical chemists involved. These included discussions regarding sampling locations, collection, and procedures in different environments and the identification of different fire accelerants and the possibility of batch-matching. Prof Goertz noted that the process for identifying fire accelerants is not standardised across Germany and the 16 different police laboratories each use a different method. Considering this and, given that every fire is different, it was his view that the National Fire Protection Association (NFPA) guide, NFPA 921, is the most useful reference document for scientific-based investigations and analyses of fire and explosion incidents.

10. AGENDA ITEM TEN – Chemical forensic approaches at DGA CBRN Defence

10.1 Dr Delaporte described the two principal chemical forensics projects that are being carried out at DGA CBRN Defence, France. The first, on chlorpyrifos, aims to assess the usefulness of metabolomics tools and strategies for chemical forensics. Funded by DGA, this project is carried out at INRAE-TOXALIM laboratory, which is specialised in metabolomics. Chlorpyrifos, an organophosphate pesticide, was used as an organophosphate chemical warfare agent model compound. Samples were synthesised in triplicate using seven two-step synthetic routes, proceeding via a common synthesis intermediate, producing a total of 21 samples. Following optimisation, the best analytical conditions were determined to be liquid chromatography-electrospray-high-resolution mass spectrometry (LC-ESI-HRMS) in positive mode. Data analysis was carried out using open-source tools embedded in a standard metabolomics approach. While unsupervised data analysis (PCA) gave a first overview of variability in samples and correlation between features, a supervised approach (PLS-DA) was then applied to classify samples. The performance of seven available classification models was not found to be satisfactory. Consequently, two models were developed to make predictions at two steps of the synthetic process: one from the primary precursors to the main intermediate, and the second from the main intermediate to the final product. To annotate discriminating features, various data sources such as MS/MS spectra and collision cross-section measurement by ion mobility mass spectrometry were combined, enabling structural elucidation.

10.2 The second project featured the chemical forensics analysis of ricin conducted during the official OPCW Exercise on the Analysis of Biotoxins in 2021. Challenges of this project included the low volume of sample available, the unknown concentrations of ricin in the samples, the lack of statistical power given the low number of samples, and the free reporting criteria for such data. An NMR fingerprint of each sample was acquired and visually inspected, and the lectins in each sample were profiled. The profiles were subsequently analysed by k-nearest neighbours. This confirmed the observation previously made during inspection of the NMR spectra that one sample had a richer impurity profile than the others and may be a crude ricin extract. A manual review of lectin profiles revealed that one sample likely contained highly purified ricin D isoform, a second sample contained a mix of highly purified ricin D and E isoforms, and the crude sample appeared to contain only D isoform. However, it was not possible to assign a specific cultivar to the samples owing to the lack of existing databases.

10.3 In the question-and-answer session that followed, the tendency for metabolomics to rely on NMR, the difficulty of using NMR signals on a large scale for building databases and the potential for GC-MS to be used as an orthogonal technique were noted. The decision to use PLS-DA rather than linear discriminant analysis was explained. Asked whether small peptide biomarkers featured in the ricin attribution work, Dr Delaporte replied that they did not, perhaps owing to the methods used not being adapted to do so.

10.4 The relative advantages and disadvantages of NMR and hyphenated mass spectrometry data were discussed. Data from NMR analysis tend to be remarkably reproducible across multiple extractions and comparisons, while LC-MS data tend to have the most variation between laboratories. Under agreed pH and solvent conditions, instruments and manufacturers do not matter as much, with the downside that higher concentrations are needed. Shifting peaks are problematic when building databases. While many algorithms have been developed to correct for retention time shift in hyphenated mass spectrometry data, less attention has been paid to this issue in NMR data. In fact, NMR spectroscopy is rarely used in forensics laboratories. Accounting for matrix effects, which Dr Delaporte's laboratory has attempted to do with the use of soil extracts and water samples with lower concentrations and replicate samples, will be an important consideration moving forward.

11. AGENDA ITEM ELEVEN – Subgroups 2 and 4 breakout sessions

See agenda item three.

12. AGENDA ITEM TWELVE – Subgroups 1 and 3 breakout sessions

See agenda item three.

13. AGENDA ITEM THIRTEEN – LLNL approaches to chemical forensics

13.1 Dr Audrey Williams, from the Lawrence Livermore National Laboratory's (LLNL) Forensic Science Center (FSC) in the United States of America, gave a presentation on the FSC's approach to chemical forensics and the focuses which the FSC prioritise as one of the OPCW designated laboratories for environmental and biomedical samples in the United States. The dual mission of the FSC is conducting research and providing operational support to CBRNE-related forensic science. The work of the laboratory addresses what Dr Williams describes as "the complete threat timeline", to wit: acquisition of materials, synthesis and formulation, distribution and smuggling, use and release, and postmortem forensics. A wide range of forensic research and sample testing exists within this framing, including work on assessing the supply chain of precursors, route assessments, chemical signatures, medical countermeasures, and biomarkers.

13.2 Dr Williams described the importance of chemical attribution signatures to the chemical forensics work of the laboratory, particularly in terms of identifying pathways, sophistication of the target under investigation, use timelines, and sources. These aspects of the laboratory's work tend to develop in tandem with one another. Dr Williams discussed the work that her laboratory had carried out on fentanyl synthesis, identifying several synthesis routes and attribution signatures which could be used to identify samples and pinpoint their origins. She highlighted methods used to exploit these signatures for attribution, including isotope ratio analysis, batch-matching (and the identification of duplicate batches), as well as the current development of a complementary high-resolution mass spectrometry capability.

13.3 The LLNL's complex sample exploitation operates using multiple analysis techniques and dozens of preparations. For chemical threat agents, matrix-dependent analyses include swab, environmental, or biomedical readings, while target-dependent goals focus on the agents, degradation products, metabolites, adducts, or foreign signature. Time, collection, and storage are crucial to all these analyses, and successful identification is reliant on integrated research in conjunction with sample analysis. There are two main approaches taken in this regard: a focused analysis approach where a sample is screened for specific elements and then target compounds are identified, and an unknown analysis approach, which simply screens for target compounds by searching databases. Failing a database identification, near-neighbours are identified.

13.4 Dr Williams cited emerging fentanyl analogues as an example of the laboratory's work focusing on near-neighbours, similar compounds which emulate pre-existing illegal substances. The laboratory's model worked as a data triage tool, identifying compounds related or closer to fentanyl based on mass-spectral fragments. Work was also carried out on plant biomarkers, using vegetation as a bio collector for volatile chemical warfare agent signatures. This focused on chlorine use, which typically presents forensic challenges because of its rapid rate of dissipation. Finally, Dr Williams concluded by highlighting steps that the laboratory is taking to improve its models and analytical techniques. Improved limits of detection have benefits and drawbacks, with greater analytical precision coming at the cost of more noise. New methods are also being developed, particularly the integration of advanced data handling with more complex analytical methods. Related specifically to chemical warfare agents, Convention-related analysis continues to be broad, and maintaining expertise on a broad threat landscape was emphasised, including competence in synthesis, analysis, pharmacology, forensics, and persistence.

13.5 Members of the TWG posed a series of questions to Dr Williams regarding the equipment used for the laboratory's analysis, including on the applicability of NMR impurity analysis to the LLNL's work and the collection of data and the use of machine learning. Also discussed was the near-neighbours concept as applied to fentanyl and the applications of a similar analysis to chemical warfare agents, as well as how purification impacts the efficacy of GC-MS analysis.

14. AGENDA ITEM FOURTEEN – Subgroup 1 deep dive

See agenda item three.

15. AGENDA ITEM FIFTEEN – Subgroup 2 deep dive

See agenda item three.

16. AGENDA ITEM SIXTEEN – Subgroup 3 deep dive

See agenda item three.

17. AGENDA ITEM SEVENTEEN – Subgroup 4 deep dive

See agenda item three.

18. AGENDA ITEM EIGHTEEN – Plenary discussion

See agenda item three.

19. AGENDA ITEM NINETEEN – Meeting recap, identification of remaining gaps, and next steps

Dr Bossée briefly summarised the key points discussed during the meeting and presented a list of tasks to be completed by the third meeting of the TWG scheduled for 25 to 27 September 2024.

20. AGENDA ITEM TWENTY – Any other business

The TWG members did not wish to raise any additional points under this agenda item.

21. AGENDA ITEM TWENTY-ONE – Closure of the meeting

The Chairperson ended the meeting at 15:52 (CET) on 5 June 2024.

ACKNOWLEDGEMENTS

The TWG members thank the guests and members of the Secretariat who participated in discussions. The TWG also wishes to acknowledge Ms Ernesa Ademagić of the OPCW Office of Strategy and Policy for her support and contributions to the meeting and its preparations. Lastly, the TWG thanks the OPCW Director-General for his establishment and support of the TWG, and acknowledges the generous contribution of the European Union that helps to cover the costs of the Group's work.

Annex: List of Participants at the Second Meeting of the Scientific Advisory Group's Temporary Working Group on Chemical Forensics

Annex

LIST OF PARTICIPANTS AT THE SECOND MEETING OF THE SCIENTIFIC ADVISORY BOARD'S TEMPORARY WORKING GROUP ON CHEMICAL FORENSICS

	Participant	**Institution**
1.	Prof Arian van Asten	University of Amsterdam, Netherlands
2.	Dr Crister Åstot*	Swedish Defence Research Agency (FOI), Sweden
3.	Dr Khaldoun Bachari	Center for Scientific and Technical Research in Physical and Chemical Analysis (CRAPC), Algeria
4.	Capt. Elma Biscotti*	Institute of Scientific and Technical Research for Defense (retired), Argentina
5.	Dr Anne Bossée* (Chairperson of the TWG)	DGA CBRN Defence, France
6.	Dr Grégoire Delaporte	DGA CBRN Defence, France
7.	Ms Anne-Marie Fortin	United Nations Office on Drugs and Crime
8.	Dr Hanna Hakulinen	Finnish Institute for Verification of the Chemical Weapons Convention (VERIFIN), Finland
9.	Ms Ang Lee Hwi	DSO National Laboratories, Singapore
10.	Prof Imee Su Martinez*	University of the Philippines Diliman, Philippines
11.	Dr Simon Ovenden (Vice-Chairperson of the TWG)	Defence Science and Technology Group, Australia
12.	Dr Meehir Palit*	Defence Research and Development Organisation, India
13.	Mr Günter Povoden*	CBRN Defence Centre, Austrian Armed Forces, Austria
14.	Prof Ines Primožič*	University of Zagreb, Croatia
15.	Dr Sarah Stubbs	Defence Science and Technology Laboratory, United Kingdom of Great Britain and Northern Ireland
16.	Dr Hongmei Wang	State Key Laboratory of NBC Protection for Civilian, China
17.	Dr Audrey Williams	Lawrence Livermore National Laboratory, United States of America
	External Speakers	**Institution**
18.	Prof Niamh Nic Daéid	University of Dundee, United Kingdom of Great Britain and Northern Ireland
19.	Prof Roland Goertz	University of Wuppertal, Germany
20.	Prof Gerrad Jones	Oregon State University, United States of America
21.	Dr Tim Wilson	Verdel Instruments Limited
	Technical Secretariat Staff	**Division**
22.	Dr Peter Hotchkiss (Secretary to the SAB)	Office of Strategy and Policy

* Member of the SAB.

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