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Laboratory analysis of CBRN-substances: Stakeholder networks as clue to higher CBRN resilience in Europe

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ABSTRACT

The threat of terrorists using CBRN agents continues to pose a risk of mass casualties and severe disruption of societal functions in Europe. Standardisation of crisis management activities is one important step towards effective national and international interoperability and increased resilience. Understanding which CBRN agents are involved in an incident is vital for appropriate response measures. We applied a system's view on the process of CBRN sample analysis and see three discrete applications; Immediate incident response, Forensics, Post incident monitoring. Together with laboratory experts and policy makers from across Europe we identified needs for quality assurance measures in these three areas. Here, we suggest various harmonisation activities that can facilitate interoperability between all stakeholders concerned with CBRN sample analysis. Foremost, we recommend purpose-oriented laboratory networks, but also minimum performance requirements for First Responders' detection and sampling capabilities, best practices for sample transport and documentation.

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1. Introduction

In the last decades a number of chemical, biological or radio-nuclear (CBRN) threats have been directed at the civil society. The threat of terrorists using CBRN agents continues to develop and poses a risk of mass casualties and severe disruption of societal functions in Europe. Therefore, response capabilities to counter and mitigate such threats need to be developed and made available to the European community. By issuing the EU CBRN Action Plan [1] and approving the Decision of the European Parliament and of the Council on serious cross-border threats to health [2], the EU Commission demonstrated that it regards CBRN issues with high priority. The overall goal of the Action plan and the Decision is an all-hazard approach to reduce the threat of and damage from CBRN incidents of accidental, natural or intentional origin, including acts of terrorism.

With the aim of increasing societal resilience against CBRN incidents, recent European efforts and initiatives have been directed towards standardisation of response and preparedness activities that can facilitate effective and efficient national and international interoperability. Such efforts include initiatives like "Mandate M/487", which was led by Technical Committee 391 "Societal and Citizen Security" of the European Committee for Standardization [3] and states that some degree of international 'standardisation' will be required – both as a way to regulate ('top-down') as well as a way to learn from others and to overcome resistance/roadblocks ('bottom-up') in the CBRN domain. Another example of recent standardization initiatives is the EU project "Standardisation for Laboratory Analytical Methods" [4], a cooperation and support action under the seventh framework programme, in which the authors have been involved with, and which is the basis for this paper.

Understanding which CBRN substances are involved in an incident is vital for appropriate crisis management. The samples that are to be collected, transported and analysed are usually of either biomedical (blood, tissue, urine etc.) or environmental nature (air, water, soil, surface wipes, powder etc.). Procedures and protocols warranting the reliable and timely identification of as many agents as possible (if not all) must be in place beforehand; they need to be tested, evaluated and trained in order to ensure the necessary accuracy of the results. Many times alternative protocols (alternative technical or procedural solutions) are available for sample preparation or for the identification analysis of specific groups of CBR agents or types of samples. Different laboratories may use different protocols. It is therefore of outmost importance to allow for a comparison of results from different laboratories. Furthermore, sampling procedures, sample preparation and analysis protocols are often very complex, which further emphasises the need for standardisation in order to improve reliability, efficiency and comparability of the results. This is especially relevant for matters of international cooperation, e.g. assistance with laboratory capacity across borders or an incident affecting neighbouring countries. In this context, regulations for the transport of CBRN samples – especially across national borders – should not be forgotten, both for ensuring a safe and timely transport and for preserving the integrity of the sample(s).

We have together with laboratory experts and policy makers across Europe identified the needs for standardisation activities throughout the process of CBRN sample analysis to achieve a more resilient European society in the future. In this paper we discuss those needs and recommend activities to meet the needs. The term "standardisation" is used in this paper interchangeably with "harmonisation" to illustrate that the concept not only covers standards *per se* but also complementary processes and publications such as technical specifications, guidelines, workshop agreements, best practices etc. In the broadest sense, a standard is a document that sets out requirements for a specific item, material, component, system or service, or describes in detail a particular method or procedure. Standards bring benefits to businesses and consumers in terms of reducing costs, enhancing performance and improving quality and safety [5].

2. Methodology

Because of the complexity and the differences between the areas of C, B, and RN analyses, a common definition has been used to consider CBRN incidents and applications for laboratory procedures: "A CBRN incident is an event where substances of chemical, biological or radio-nuclear nature have been released intentionally or accidentally and which have the potential to harm people".

We applied a system's view on the need for quality assurance and control measures, i.e. the need for standards in the area of CBRN sample analysis. This system's view considers the entire processing chain, from sample collection and transport to sample preparation, analysis and interpretation of results; and structures CBRN analysis into three discrete applications:

- Immediate incident response
- Forensic investigations
- Post incident monitoring

These three applications and their specific requirements are further detailed below.

In the immediate incident response (for operational use) the aim of the analyses is to identify the threat agent as soon as possible so that the appropriate response, e.g. medical care or setting up security zones, can be adopted in a timely manner. Various numbers and types of samples will have to be analysed with low to medium resolution, with less accuracy but high speed in order to identify the threat agent. It is often argued that a silent release of pathogenic microorganisms will not require an immediate response because clinical symptoms may be the first alert of a biological incident. Nonetheless if a warning of a potential release has been received or a typical crime scene can be identified the immediate incident response will be relevant.

The aim of forensic investigation analyses is to link the agent to the cause of an accident or to the perpetrator of an attack, respectively. Few samples and sample types will have to be analysed with high resolution and accuracy in order to identify not only the agent itself but also its source. These analyses require careful continuous chain of custody (handling log) in order to undoubtedly and unquestionably preserve the integrity of the sample(s) and the entire processing chain.

The aims of post incident monitoring (mid-/long-term perspective) analyses are to i) determine the scale of the release, ii) ensure medical follow up, iii) monitor contamination levels for clearance purposes and for definition of acceptable threshold levels. Large numbers of samples of one or few types have to be analysed over a longer period of time. This kind of screening might be slower but ought to be reliable and requires higher sensitivity of the analytical method compared to those methods used during the immediate incident response. Degradation or metabolisation of the CBR agent over time poses an additional challenge.

The generic needs were identified during two international workshops and based on scenarios, which were specifically developed for the purpose of the workshops to cover a broad range of analytical requirements and issues like cross-border sample transport, or interpretation and communication of results. The workshops were arranged to engage with laboratory experts and policy makers across Europe that are involved in the development of common methods, procedures and protocols for the detection, analysis and identification of CBRN substances and to enable comparison of results from different laboratories and operators within Europe. Full details of

the workshops, experts and stakeholders that participated and conclusions are presented by Griffiths and co-workers [6,7]. Participation included both reference and accredited laboratories to obtain information on current capabilities and capacities as well as regulatory bodies to determine their required capabilities and capacities.

The gap analysis followed the chronology of the analytical process starting with sampling and sample transport through sample preparation and analysis to reporting of the findings. In the following we matched the generic needs for each application with standardisation solutions at both the operational and strategic level.

3. Needs and critical aspects

One of the key requirements for CBRN preparedness and resilience is the capability to identify CBRN agents that have been released during an incident and pose a threat to humans. The number of substances that can be included in a CBRN substance list is huge and it is therefore essential to clearly define what types of substances are important in case of a CBRN incident.

Chemical substances were selected from the US-EPA document [8] according to criteria such as toxicity, availability, ease of dissemination and stability in the environment. Drugs, explosives and household chemicals were not considered to be part of a major CBRN incident. The selected compounds were grouped into chemical warfare agents (CWA) and toxic industrial chemicals (TIC). CWA's were divided into live agents and precursors and degradation products, TIC's were divided into organic and inorganic compounds.

The biological agents relevant to CBRN incidents fulfil criteria such as contagiousness, infectious dose, stability in the environment, and probability of intentional release. We also considered the need for standardization of analytical procedures of biological agents specifically in environmental matrices [9–13].

The radionuclides of interest are those which are most likely to be a threat to humans in the case of radiological and nuclear terrorist acts, incidents or accidents and historical cases of radiological and nuclear terrorism acts and accidents which have been reported and described by the International Atomic Energy Agency (IAEA) [14–16].

Importantly, CBRN agents will have to be analysed in a variety of possible matrices. Relevant environmental sample matrices are e.g. water, soil, air and swipe samples (to measure for example surface contamination). In addition biomedical sample matrices such as blood and urine need to be covered. It should also be emphasised that effective and correct sampling is crucial for reliable identification analysis e.g. [17]. Sample size, number, and distribution, as well as the time and method of transport are crucial.

A final critical aspect is safety. Safety aspects of analysing samples in dedicated CBRN-facilities is mentioned below. But safety is also important during the steps prior to laboratory analysis, i.e. exploration of the scene, the actual sampling, packaging, transport, sample reception and unpacking. It should be stressed that safety does not only concern possible contamination, but also "weapon-related" aspects such as the presence of explosives as a mixed threat, means of dissemination or as booby-traps.

3.1. Analysis of chemical substances

Analytical methods used by chemical laboratories with capabilities for the immediate incident response, forensic investigations and post monitoring have been reviewed. Methods for the analyses of chemical substances of interest are described in databases like EPA [8], OSHA and NIOSH [18]. An overview of sampling and analysis procedures for CWA's and related compounds, is provided in the Blue Book from Verifin and the NATO-SIBCRA handbook [19,20].

The most abundant analytical techniques are: Gas chromatography- Mass Spectrometry (GC-MS) [21], Atomic Absorption Spectroscopy (AAS) [22], ICP-MS [23], X-Ray Fluorescence (XRF) [24] and Ion Chromatography (IC) [25]. For CBRN-related chemicals mass spectrometry is the most commonly used method of analysis. This technique is very useful for screening because of the MS library search function. In case the compound of interest is known, the analysis can be performed again in a more sensitive Single Ion Monitoring (SIM) mode to obtain a higher sensitivity and thus being able to determine lower concentrations. Although GC-MS offers great possibilities, better analytical techniques are available as well. Especially when the chemical of interest is known, the analytical technique can be tailored to the compound.

In the last 20 years Liquid Chromatography (LC)- MS has been developed to an affordable routine analytical technique, equipped for high throughput analyses [26–28]. For certain compounds the (LC)- MS is favoured over GC-MS, especially for larger molecules with low volatility. In some cases the detectability of the compound is also more sensitive. For example detection limits of VX in the pg/ml range can easily be obtained. Secondly, some low volatile compounds require derivatization in order to be analysed with GC-MS, while they can be analysed as original chemicals using LC-MS. Thirdly, LC-MS is more suitable for handling aqueous samples, while for GC-MS a transfer to a GC- compatible solvent is required. The progress in mass spectrometry itself has been significant in the last few decades as well. Tandem MS techniques offer high selectivity and sensitivity. Another major development in mass spectrometry is the improvement in accuracy and precision of the mass determination. New Time of Flight (TOF) or Orbitrap instruments enable accurate mass determination helping the identification of unknown chemicals by providing suggestions on the molecular formula [29,30].

Inorganic compounds can be detected using atomic absorption spectrometry or XRF techniques. These instruments operate autonomously and are dedicated to determine the total metal content in samples. They do not provide information about the chemical itself, but in most cases the toxicity of the compound is caused by the element, in this case the metal. A derivative of the technology is also available as selective detector for GC (e.g. Atomic Emission Detector) [31]. Using this technique samples can be screened for compounds with specific elements (e.g. lewisite derivatives). Finally, IC is recommended for the analysis of ionogenic compounds, since these compounds cannot be analysed with GC or LC-MS.

In the immediate phase quick identification can be possible with handheld identifiers based on Fourier Transform Infrared Spectrometer (FTIR) or Raman detection. These techniques are not equipped to analyse samples that contain multiple components. If available, more sophisticated analyses can be performed in a mobile laboratory that is often equipped with GC-MS. However these analytical techniques are more time consuming. A typical analysis of a persistent chemical agent including sample preparation will take at least one hour. Some of the samples will be sent to a laboratory for quick analysis with any of the screening techniques mentioned above. In this phase the level of identification is provisional.

During a forensic case the laboratory focus will be on the unambiguous identification of the agent. Additionally the analysis of chemical attribution signatures can provide more information on the origin of the actual agent. The requirements for this type of analysis are very high, but the identification of chemicals is considered as the weak link in the chemical analysis [32].

In most cases during the post incident monitoring phase the compounds of interest have been identified. The challenge will be in the sensitivity of the analysis that is needed to determine low levels of agent. Additionally the number of samples might be very large and could overwhelm the capacity of one laboratory. In that case samples may be sent to other laboratories.

Obviously for each chemical a number of alternative techniques can be used, that might provide equally good or even better measurements for the particular compound that is analysed, but not all methods are suitable for all analytical purposes/applications.

3.2. Analysis of biological agents

Recognition of a silent release of biological substances is particularly difficult. Infected humans or animals will develop clinical symptoms, which can function as a first alert. However this is only after an incubation period during which the carriers may move away from the site of the release. Such scenario may lead to numerous clinical samples, possibly require international cooperation and considerable screening efforts in order to localise the site of release and to identify the perpetrator. If there has been a warning of a potential release of biological agents or a typical crime scene can be identified, e.g., release of powder or a laboratory facility, sampling and analysis of environmental samples are required. In the case of an unknown sample, the focus will be on identification. If the threat agent is known, the focus can also be on quantification and the ability to analyse many samples in a certain time period.

We have reviewed established identification methods with respect to potential benefit from standardisation, harmonisation or introducing best practice. In general, identification assays must be sensitive and specific, capable of detecting low concentrations of target agents without interference from background materials, reproducible and reliable [33,34]. A number of ISO standard procedures have been developed and implemented for analysis of bacterial agents that are important for the food industry, water quality and clinical diagnostics. Some of these standards can also be of interest for the analysis of biological substances in environmental samples [35].

Laboratories able to analyse highly pathogenic bacteria and viruses require appropriate biosafety facilities including BSL3 and BSL4 as well as experience in analysing and preparing a wide range of pathogenic bacterial and viral agents. If the sampling, handling, sample processing, and storage of the samples are not correctly performed, the biological material for evidence may be lost [36,37]. NATO has produced a handbook that provides procedures and guidelines needed to sample, detect and identify CBRN agents [38]. The ISO18593 standard [39] specifies horizontal methods for sampling techniques using contact plates or swabs on surfaces in the food industry environment, with a view of detecting or enumerating viable microorganisms. The forensic investigation has focus on obtaining unambiguous evidence.

During the immediate incident response First Responders (police, rescue services, ambulance/emergency medical services etc.) can in some cases benefit from using rapid and easy-to-use hand held methods for identification of the biological substances on scene. Example of a technology to be used is the Handheld test Assay (HHA) [40], which is available from different vendors [41]. HHA is a simple, antibody-based assay that can be used for provisional identification of biological substances [42]. However, the sensitivity is often poor and varies between different tests. This may give false results when compared to other tests run in the laboratory, e. g. Enzyme-Linked Immuno Sorbent Assay (ELISA) or Polymerase Chain Reaction (PCR). A validation of HHA is needed. The HHA provides only a provisional identification and the results need further analysis to be confirmed. A mobile laboratory performing immunological assays and PCR could also be used for provisional analysis as well as for confirmation analysis [43].

Analysis in a forensic case will be focusing on unambiguous identification of the agent and bioforensic is dedicated to the characterization, analysis, and interpretation of evidence for attributional purposes from a bioterrorism act, biocrime, hoax or inadvertent agent release [44,45]. Different strain typing methods (genotyping) and whole genome sequencing can be used for bioforensic and the choice of genotyping methods depends on the genetic variance in the different strains of the particular bacteria and available resources of the laboratory [46,47]. In general, genotyping methods involve a PCR analysis step, which is a technique commonly established in laboratories.

For analysis of samples containing biological agents in the post incident monitoring phase, methods such as immunological approaches (ELISA) and real-time PCR can be used. For confirmation of viable bacteria, culturing on agar is required, which can be supplemented with molecular methods such as PCR or DNA sequencing and with immunological methods [33,48]. Another applicable method on culturable bacteria is matrix-assisted laser desorption ionization time of flight (MALDI TOF) [49,50]. The deployment of next-generation DNA-sequencing technologies (NGS) has greatly enhanced capabilities for sequencing large meta–data sets and has the capability to fully characterize the microbiological content in samples. NGS has a very high coverage rate i.e. most biological material in the sample is detected, enabling detection of even low frequency impurities such as pathogenic microorganisms [51–54].

One of the challenges for any harmonisation attempt in the field of biological analyses lies in the complexity of many procedures and, in the fact, that details in the methods have often been developed in-house. BSL-3 laboratories around Europe are constructed and organised differently. Thereby, the procedures and equipment used in the different laboratories may vary and strict standardisation might be difficult. In addition, molecular biological and immunological methods require highly agent specific reagents. Internal controls, validation of procedure and methods and evaluation of reagents and accuracy of every step of the procedures are therefore vital in order to guarantee reliable, specific, sensitive and reproducible identification of biological substances in different laboratories and countries (quality control and quality assessment).

3.3. Analysis of radio-nuclear substances

In the immediate incident response phase, fast analytical techniques requiring no or few sample preparation steps have to be used [55–57] in order to confirm on-site measurements mainly based on transportable gamma spectrometry. Such techniques consist of high resolution gamma spectrometry, gross alpha and beta counting techniques. Most of the laboratories in charge of environmental monitoring are currently using these kinds of techniques [58,59]. Furthermore, the fast analytical techniques need to be supplemented by techniques for nuclide specific analysis of alpha and beta emitters in order to better identify the RN substance involved in the accident or incident. Techniques for nuclide specific analysis of alpha and beta emitters may also be required where lower limits of detection are needed than can be obtained using gamma-spectrometry. However, those methods are generally time-consuming and challenging. All these methods and techniques are also used in the post-incident monitoring phase.

For forensic investigations, other analytical techniques have to be used to identify the origin of the substance [60]. These techniques require very skilled staff, are time-consuming and consist mainly of specific mass spectrometric techniques such as Thermo-Ionisation Mass Spectrometry (TIMS), Inductively Coupled Plasma Mass Spectrometry (ICPMS), and Secondary Ion Mass Spectrometry (SIMS) [61,62].

If an unknown sample is classified as being exclusively radiological, i.e. biological and chemical hazards can be excluded, a generic approach can be adopted. Depending on the purpose for the analysis different steps will be reached. The first step in the sequence is to use screening methods to confirm the *in situ* measurements (gamma spectrometry, gross alpha and beta counting techniques). In the next step the sample is divided into sub-samples for a non-

destructive analysis for nuclear forensic characterisation (gamma spectrometry, imaging and microanalysis techniques e.g. SEM, TEM, SIMS) or an RN specific analysis for the evaluation of health and environmental impact (gamma spectrometry, alpha spectrometry, beta liquid scintillation or ICPMS). The non-destructive analysis for the forensic characterisation is followed by destructive analysis (alpha spectrometry, TIMS, ICPMS, beta liquid scintillation) [63].

Most analytical methods are well described in national and international standards. Only for certain biomedical matrices i.e. urine and feaces, standard methods are lacking. For nuclear forensics, the standardisation of analytical methods is more challenging since nuclear forensics does not consist of routine procedures that can be universally applied to all samples but is an iterative process in which the results from one analysis are used to guide the selection of subsequent analyses. Standardised methods are therefore difficult to implement for this analytical purpose since each case can be different. However, a general approach has been adopted by the Nuclear Forensics International Technical Working Group (ITWG) [64] and consists of a series of analytical methods starting with non-destructive analyses (gamma spectroscopy, micro-analysis techniques) followed by elemental and isotopic analyses using mainly mass spectrometry [65]. Proficiency Testing Schemes (PTS) cover all types of matrices, including biological samples, and most of the radionuclides expected to be present in a RN incident. Certified Reference Materials for calibration of instruments or to be used as tracers do exist for most matrices and radionuclides. PTS are organized at an international level by the ITWG. However, creation of ITWG in 1996, only three PTS have been organized on plutonium and Highly Enriched Uranium (HEU), with only between 6 and 10 laboratories participating [66].

4. Suggestions and perspectives for standardisation activities

Our review of the requirements for analytical procedures has shown that in many cases multiple protocols (technical or procedural solutions) are available for C, B and RN sample analysis. Many times – particularly in the area of B sample analysis – methods comprise of in-house developed components. Certain steps in the analytical process, i.e. sampling, documentation and reporting, are not regulated or harmonized, yet very important for the quality of the analysis and for communicating results between the various stakeholders of a CBRN incident. A number of ISO standard procedures have been developed for quality assurance in other fields, e.g. food security, water quality, clinical diagnostics, that could be adapted for analysis of environmental CBRN samples.

While the variety in available methods offers the potential benefit of obtaining the same result by using independent, alternative methods, it requires comparability of the results. Furthermore, high-end technologies are not widely used by laboratories, not always necessary (immediate incident response) and are less suitable as recommended standards because of the high costs for equipment and staff training. In addition, flexibility in the choice of method might be needed when attempting to identify unknown threat agent(s) in a sample or for forensic purposes, in which cases the analyses are rather investigative than straightforward. In any case, the analytical method itself may need to be tailored to the type of the sample and the agent that is likely to be identified. Therefore, the method itself is less suitable for standardization, but a number of alternative methods for a specific purpose can be collected on a list of recommended techniques. Other steps of the analytical process, e.g. sampling, transport, reporting, as well as documentation (chain of custody) and staff training can and should be standardised to a higher degree (see sections 4.1. to 4.3.).

In addition, threat levels or tolerability of residual hazard levels often vary between countries. Such gaps can make the interpretation of analytical data and recommendations for operational and for clearance use difficult. Knowledge about normal background levels in the various sample matrices is scarce and complicate the interpretation of results. Research might also be necessary for more clarity on the fate or the persistence of CBRN agents in environmental and in biomedical matrices (degradation, modification, accumulation).

4.1. Immediate incident response

On scene detection of CBRN agents by the First Responders was identified as one of the critical areas. Specifically, First Responders should have the capacity to detect i) chemical substances like CWA, sulphur dioxide, chlorine, hydrogen cyanide and explosive hydrocarbons, ii) microorganisms on species level within 15 minutes (methodologies already exist for about 10 biological agents), and iii) gamma radiation. The necessary instruments should pass through regular functional checks and verification tests, and should include checking of expiration dates on antibodies and other reagents. All detection instruments should also have a standardised manufacturing calibration. The effort laid down on the equipment needs to be combined with staff training and exercises in how and when to use the equipment.

Poor communication between sampling teams, the police, administrative staff and laboratories can result in poor quality or insufficient quantities of samples being collected to render the analysis meaningless. Therefore, attention should be given to ensuring good communication between the different stakeholders during collection of samples, and accurate documentation to accompany the sample. Better standardisation would ensure information on how, where, why and when the sample was taken, and by whom. During a large incident involving the collection of different sample types, this approach will help a great deal with reporting and prioritising of work.

During a cross border incident, resilience can also be strengthened through harmonised document information and reporting, presented consistently to avoid conflicting messages between countries. One option could be the development of a sample submission form. This may be met with resistance from some countries and so a checklist approach might be a better way to proceed on a European scale, where all the necessary details can be included in the checklist but the reporting organisation is not obliged to fill in all the fields.

As the First Responders motivation for standardisation is driven by a functional conformity that contributes to a more efficient and precise response, minimum requirements are recommended for detection, sampling and transport to an off-site laboratory for confirmative analysis. Additional requirements should include a sampling checklist.

4.2. Forensic investigations

The motivation for standardisation is probably highest in this area, where law enforcement is the customer, and analytical results are intended to aid prosecution of a perpetrator. The entire sample handling will have to stand trial and a standardised chain of custody is of outmost importance as is documentation of the analytical procedures and interpretation of the results. However, because of the often investigative character of sample analyses, the analytical techniques can be chosen from a list of recommended methods.

Apart from the strict regulations and proficiency test schemes of the Organisation for the Prohibition of Chemical Weapons (OPCW) and SIBCRA, serving military purposes, a few civilian initiatives of laboratory networks in the area of forensics exist that ought to be extended and enforced: the World Anti Doping Association (WADA) [67], ITWG [64] and the national Bioforensic Analysis and Countermeasures Center in USA (NBFAC) [68].

4.3. Post incident monitoring (mid-/long-term perspective)

For a laboratory to quantify levels of contamination or the success of decontamination efforts, appropriate samples have to be collected, along with controls and blanks, under controlled conditions and using appropriate documentation procedures. This aspect of sample collection does not necessarily need to be performed to a highly standardised level that is applicable across all European Member States. A number of existing guidelines and technical documents provide guidance on appropriate sampling strategies. It would therefore be sensible to adopt recommended best sampling strategies for post incident monitoring that allow sample analysis and support the laboratory in obtaining the requested information. Such instructions can include information on instrumentation and on sample containers to use for different agents; whether or not preservatives can or should be added; at which temperature samples are to be stored in transit; matrices, volumes and numbers of samples required; as well as duration and condition of sample storage before handing over to the analytical laboratory. In addition, and similar to the need for operational use (section 4.1), we suggest accurate sample documentation at an intermediate level of standardisation i.e. best practice for this purpose.

Furthermore, the conditions under which samples are transported need to be standardised and better documented. The transport regulations that already are in place for commercial courier services can be supplemented with a documentation of transport conditions (temperature, time) [69–76]. Harmonisation of existing regulations would allow for smooth border inspection and rapid validation by the receiving country in cases of multinational cooperation. In international proficiency test schemes existing procedures are already applied, when small sample volumes are distributed to the participating laboratories. Another example of cross border transport is how the OPCW laboratories work to analyse samples possibly containing chemical warfare agents following a conflict. These examples show that well-rehearsed and acceptable mechanisms exist, which can be broadened to larger networks of laboratories and can be adapted to serve CBRN incident management purposes across national borders.

4.4. Laboratory network

Sections 3.1. to 3.3. dealing with the analytical techniques clearly show the need for laboratories that can perform quality analyses of biomedical and environmental samples under various circumstances, e.g. fast analysis for immediate incident response, mass screening during post-incident surveillance or highly specific forensic agent profiling. In addition, considering the risk of cross-border incidents or a lack of specific laboratory capacities in single EU member states, it is also important to link and to harmonize the existing capacities. This means not only that sampling and analysis procedures need to be validated, but also that there should be a common understanding of best practices, quality assurance measures, and even of organisational structures in order to allow for comparability of results and complementarity of analytical capacities in EU member states.

Throughout our work we have seen a need for a dedicated, organised and official networks of CBRN laboratories across Europe. We find it important to establish an overarching list of European laboratories capable of performing accurate analyses for specific agents and purposes.

The use of the term standardisation for analytical methods might discourage laboratories from implementing unfamiliar but validated standardised methods as standardisation is often understood as involving very strict regulations and leaving little room for individual solutions. However, as we have pointed out in the beginning there are many forms of standardisation. Therefore it is preferable to develop a set of “harmonised” alternative analytical methods for laboratories to use. The best way to determine the suitability of these methods is to perform proficiency test exercises through an established network of laboratories. Apart from exercises for military organisations, e.g. SIBCRA, exercises should also be performed for the civilian sector. Such exercises can be used to share information, learn from each other and build trust. Also, it is difficult to standardise methods for which certified reference materials are lacking. However, standardisation is useful for quality assurance and control, especially for provisional identification in the field.

Closer collaboration between military and civilian laboratories is an opportunity to improve preparedness and this could be achieved by joint proficiency test exercises. Another benefit of proficiency testing is the harmonisation of reporting formats. A model to closely consider for the successful management of large laboratory networks is the one used by the Environmental Response Laboratory Network (ERLN) in the USA [77]. This could be considered together with the framework provided by the European Reference Network for Critical Infrastructure Preparedness (ERNCIP) [78].

Whilst countries have preparedness plans which clearly define the roles and responsibilities of different national organisations and individuals in case of a CBRN incident, the stipulated procedures are often not well-rehearsed and do not always operate at the level of the decision maker. This may be due to a low perceived threat. An increase in the frequency with which preparedness plans are exercised would help reduce this risk; all stakeholders, including those at policy level, should be included regardless of exercise type (table-top, command post, coordinated practical field exercises). Multi-agency training exercises should be prioritised; laboratories, government agencies and intelligence services should all take part in the preparedness planning.

We believe that high quality laboratory capacity and interoperability can be facilitated through proficiency test schemes, multi-agency training exercises and broader accreditation according to several already existing international standards, i.e. ISO/IEC 17025 for laboratories, ISO 17043 for PTS providers and ISO Guide 34 for certified reference material producers [79–81].

5. Conclusions

In summary, we have here provided an overview of existing standards and additional requirements for CBRN sample analysis that will be necessary in order to move towards a harmonised, interoperable and resource efficient European response capability. Apart from concrete suggestions for improvements of First Responders’ detection capability, recommendations for sample transport (across national borders) and for sample documentation, we primarily suggest to establish lasting purpose-oriented networks of dedicated C, B or RN laboratories and to equip them with the mandate (and accompanying resources) to further develop the necessary standards. This mandate should include accreditation according to existing standards with proficiency test schemes as *modus operandi* for collecting and exchanging best practices and determining minimum performance requirements.

Naturally, the three capability levels of laboratory analyses, i.e. immediate incident response, forensics and post-incident monitoring, are not necessarily mutually exclusive. Depending on their expertise individual laboratories might participate in more than one network and provide different services during the life span of a CBRN event.

For any harmonisation process to succeed it is of outmost importance to recognise the customers and to integrate their needs. Often the customer will be a national public service agency (police, public health agency) or a local, regional or national government. Moreover, during incident response the customer may shift from local to national level. Therefore, the standardisation process ought to be both horizontal and vertical. In the case of standardising

laboratory CBRN sample analysis for a more resilient European society we find it necessary to engage with the European Member States. The outputs from the FP7 funded project SLAM and the experience gained by other European initiatives have demonstrated the necessity for political will to drive the standardisation approach on a policy level and to ensure implementation of European agreements.

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