Chemical Exposure

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1. EXECUTIVE SUMMARY

1.1 Problem and Task

This study was commissioned by the FBI and IARPA as a follow-up to two related JASON products: (i) a preliminary 2011 JASON study (Letter Report JSR-11-511); and (ii) a full 2012 JASON study entitled "Exposure Status" (JSR-12-450). These studies focused on signatures of human exposure to biological agents. The current study focuses on signatures of human exposure to chemical warfare (CW) agents. The Statement of Work for the present study (Appendix I) requested that JASON address the following questions:

1) Are there any new and unique biomarkers and signatures of CW agent exposure that can be used to determine unambiguously if an individual was exposed, and to reveal the identity of the agent?
2) Is there a temporal evolution of biomarkers and signatures associated with CW agents that can provide a time stamp to determine when an individual was exposed?
3) Are there impacts of dosage and/or route of entry on detectable signatures of CW agent exposure?
4) What are the optimal samples to take, and at what time point post-exposure do they yield optimal signatures of exposure?
5) What are the best technologies to detect exposure? Relevant issues include: portability, time to answer, logistical requirements, detection specificity, detection sensitivity, and availability and need for confirmatory analyses.
6) What categories of biomarkers are the most promising targets for detection of exposure signatures under realistic operational conditions?

The sponsors also requested that the report make recommendations on further scientific research and development needed to devise robust methods for interrogating an individual's past exposures to chemical agents.

To address these issues, JASON examined the known modes of action of several key classes of CW agents and their biochemical effects on humans. In seeking possible sources of new biomarkers, all major areas of the molecular life sciences were considered, including those relevant to the metabolome, genome, transcriptome, epigenome, proteome, immunome, and microbiome. Of particular interest were novel signatures that have the potential to expand current capabilities for analysts, especially

those that enable determination of exposure after long periods of time have elapsed. It was also important that biomarkers be amenable to analysis by a future field-deployable test that could reveal the identity of the CW agent.

1.2 Background

Schedule 1 CW agents are typically fast-acting toxic compounds that selectively or broadly target biological processes and that rapidly incapacitate, and often kill, humans. Certain organophosphorus (OP) compounds, such as sarin and VX, constitute a major CW agent class. These molecules target a key enzyme for the degradation of a neurotransmitter whose resulting accumulation can lead to muscle spasms and death. OP compounds are highly reactive, and as a result form covalently linked byproducts (adducts) with specific proteins and metabolites in addition to their primary enzyme target. Another major CW agent class includes the sulfur and nitrogen mustards, whose targets of modification are much less specific. Mustard compounds spontaneously transform into extremely reactive electrophiles that form adducts with many metabolites and biopolymers, including DNA.

Exposure to OP or mustard compounds produces adducts with distinctive chemical structures and with patterns that are likely to be specific for each CW agent. In addition to these adducts, perturbation of cell states and human physiology can yield a diversity of biomarkers with different decay rates, and these offer tremendous opportunities for chemical detection of chemical exposure. For example, protein- or metabolite-based biomarkers might be used to evaluate recent CW agent exposure, whereas nucleic acid-based biomarkers might be used to evaluate CW agent exposure, even decades after the event.

Other Schedule 1 chemicals (e.g. Lewisite, saxitoxin) or incapacitating agents of concern do not form covalent adducts with biomolecules as their primary mode of action. Adduct-based biomarkers for these compounds will be less common or even non-existent. Therefore, additional methods and biomarkers must be developed to evaluate exposure to non-adduct-forming compounds.

1.3 Conclusions

JASON concludes that there are numerous candidate biomarkers that could be used to improve substantially the assessment of human exposure to CW agents. Especially promising signatures are proteins that form covalent adducts with agents of interest,

including the large classes of OP agents and mustards. CW agents that function via other mechanisms pose a more challenging problem, but there are opportunities to improve methods for their evaluation as well. Each class of biomarkers could be targeted by analytical systems that would be derived, for the most part, from existing technology platforms. If such systems were developed, the resulting data would reveal fundamental facts about CW agent exposure events, including the agent or agent class, the dose, the time post-exposure, and perhaps even the route of entry.

The different classes of signatures will likely provide the most informative conclusions when used in combinations, but they also have associated advantages and disadvantages. The technologies necessary to evaluate each biomarker class are at different stages of technical development, and therefore certain biomarker classes should be ranked higher in terms of technological maturity, and their development pursued accordingly. JASON assesses that protein adduct biomarkers currently offer the most promising combination of capability features and maturity, although immunological and genomic signatures also appear to be very promising.

The Designated Laboratories that are approved by the OPCW (Organization for the Prohibition of Chemical Weapons) to examine samples for potential CW agent exposure are currently using analytical chemistry procedures that are highly sensitive and state-of-the-art. However, they focus on only a few primary biomarkers and chemical signatures. The preferred markers tend to be short-lived, and this property limits the time available for post-exposure analysis to just a few days or weeks.

The discovery and validation of new biomarkers is only the first step in expanding capabilities for the evaluation of CW agent exposure. Additional or improved technological platforms will need to be created to exploit new biomarkers that best permit identification of the CW agent and expand the time window within which prior exposure to CW agent can be detected. JASON proposes several such platforms. The new biomarkers and their corresponding analytical platforms will ultimately require approval for use by the OPCW to enable stakeholder laboratories to use them for CW forensic analysis under the Chemical Weapons Convention. However, their use in a forensic context by the U.S. Intelligence Community is similarly constrained. Thus, JASON also identifies cultural changes that should be implemented to advance the ability to detect chemical exposure in humans, both in the laboratory and in the field.

1.4 Recommendations

JASON recommends that the number and diversity of biomarkers evaluated for signatures of CW agent exposure be greatly expanded. The discovery of additional biomarkers and signatures can be achieved using existing research methods and technologies. Although there is tantalizing potential for biomarker discovery and validation across many major areas of biology, we assess that protein adduct-based, immunological, and nucleic acid-based biomarkers offer the best combination of mature technologies and broad capabilities to identify the CW agent and the time since exposure. Other biomarker types should be regarded as either long-term research projects, or as areas to monitor for further developments.

In particular, JASON proposes strategies to discover new protein-adduct biomarkers that are tissue- and agent-specific. The human proteome generates more than 20,000 different proteins, and each protein contains a constellation of hydrophobic and hydrophilic surfaces as well as nucleophilic groups in a unique three-dimensional arrangement. The entire proteome therefore provides a vast diversity of reactive surfaces that will yield adducts with patterns that are agent-dependent. Because proteins differ greatly in their characteristic rates of turnover, some of these new biomarkers are likely to provide signatures that are relevant over longer time scales than those in use today. These new adduct biomarkers can be discovered by using mass spectrometry (MS) or activity-based protein profiling (ABPP) of tissues exposed to CW agents.

New biomarkers that provide sensitive and relatively long-lived signatures for CW agents could be exploited, in principle, to create an inexpensive, field-deployable system that provides a rapid readout of the specific CW agent and yields other relevant information. One possible configuration, described in this report, relies on the creation of many adduct-specific antibodies, each binding to one member of a large set of protein adducts, and their subsequent use in an Enzyme-Linked Immunosorbent Assay (ELISA). The envisioned system would harness existing biosensor technologies, be just as sensitive as state-of-the-art analytical laboratory methods, such as MS, but could be deployed as miniaturized, portable, disposable, and rapid-readout device for use in the field.

JASON also proposes methods to exploit immunologic, genomic, and transcriptomic methods that could be used to evaluate CW agent exposure, regardless of whether that exposure occurred recently or many decades in the past. Additional ideas are presented on how more traditional analyses could be enhanced to evaluate long-lasting biological

reservoirs for the presence of a CW agent, agent metabolites, and corresponding biomarkers.

Finally, JASON regards the laboratory "confidence-building" exercises and trials for OPCW certification as being somewhat too restrictive. In particular, these laboratory drills do not especially foster innovation, or the ready adoption of new methods, for evaluating CW signatures. By complementing short-duration multi-agency exercises with longer-term "innovation exercises", one could promote the development of new detection methods and the discovery and validation of new signatures. Law enforcement agencies also would benefit from the adoption of new methods that were rigorously validated by this process. The IC might serve as an excellent partner for technology development, and perhaps an early adopter of new technologies, given their distinct needs.