An Overview of Biological Markers of Exposure to Chemical Warfare Agents

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Abstract

An overview is given of biological markers of exposure to chemical warfare agents. Metabolites, protein, and/or DNA adducts have been identified for most nerve agents and vesicants and validated in experimental animals or in a small number of human exposures. For several agents, metabolites derived from hydrolysis are unsatisfactory biomarkers of exposure because of background levels in the human population. These are assumed to result from environmental exposure to commercial products that contain these hydrolysis products or chemicals that are metabolized to them. In these cases, metabolites derived from glutathione pathways, or covalent adducts with proteins or DNA, provide more definitive biomarkers. Biomarkers for cyanide and phosgene are unsatisfactory as indicators of chemical warfare exposure because of other sources of these chemicals or their metabolites.

Introduction

The analysis of biomedical samples, such as urine and blood, can provide qualitative and quantitative evidence of exposure to chemical warfare (CW) agents. The detection of free metabolites and covalent adducts with macromolecules provides forensic evidence in cases of allegations of military or terrorist use of CW agents. Such biomarkers may also be useful for diagnostic purposes, to ensure the administration of appropriate medical countermeasures, and for monitoring exposure in workers engaged in demilitarization and other defensive activities. This paper provides a short overview of the biomarkers that have been identified and discusses their advantages and limitations. Only selected references are given; comprehensive listings, together with more detailed discussions of analytical methods, are provided in recent reviews (1–4).

Types of Biomarker

The CW agents of most concern are nerve agents and vesicants (blister agents); the incapacitant 3-quinuclidinyl benzilate (BZ) is considered of lower importance. Some structures are shown in Figure 1. Phosgene and hydrogen cyanide, both used as CW agents in World War I, remain of concern as toxic industrial chemicals.

Organophosphorus nerve agents, vesicants, and phosgene are electrophiles with varying degrees of reactivity and selectivity towards nucleophiles. In the body they react with a range of biological nucleophiles, for example, with water and glutathione to form free metabolites, with SH, OH, NH, and CO_2^- amino acid residues on proteins to form protein adducts, and, in the case of mustards, with NH and -P(O)O$^-$ residues on DNA. Because of their chemical reactivity, most CW agents have short lifetimes in the body and therefore have very limited utility as markers of exposure. The major fraction of an absorbed dose is metabolized or chemically hydrolyzed and eliminated as more polar metabolites, predominantly in the urine but with a small fraction in the feces. The remainder of the absorbed dose is accounted for by covalent reactions with nucleophilic sites on macromolecules, mainly proteins (e.g., hemoglobin, albumin, cholinesterases), and DNA in the case of mustards.

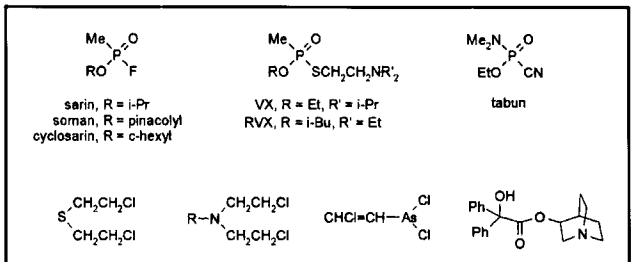

Figure 1. Structures of the major chemical warfare agents.

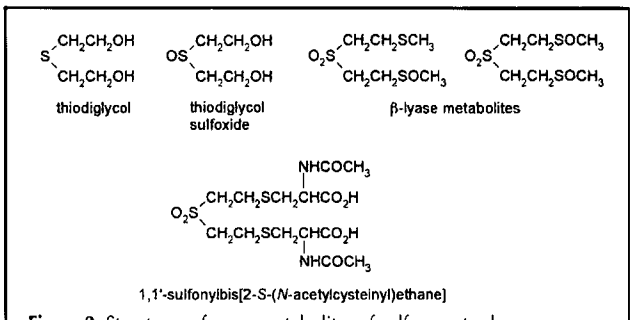

Figure 2. Structures of some metabolites of sulfur mustard.

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Biomarkers of Vesicants and Nerve Agents

Most attention has been focused on sulfur mustard and nerve agents, for which detailed metabolism studies have been undertaken (5,6).

Sulfur mustard has two relatively non-selective electrophilic centers. Initial reactions with water and glutathione, plus oxidation on sulfur, lead to the formation of many metabolites that are excreted in urine, 10 of which were identified in the rat (5). Chemical structures of some sulfur mustard metabolites

Table I. Metabolites as Biomarkers of CW Agents

Agent	Sample*	Biomarker	Comments
Sulfur mustard	urine, blood	thiodiglycol, thiodiglycol sulfoxide	TDG (minor) and TDGO (major) are urinary metabolites in the rat. TDG occurs usually at < ~2 ng/mL and TDGO at < 10 ng/mL in urine of non-exposed human subjects†, but with occasional outliers. Significantly higher levels have been detected in human casualties of mustard poisoning.
	urine	β-lyase metabolites (two)	Major metabolites in the rat. No background levels (> ~0.1 ng/mL) in > 130 human samples. When analyzed, have been detected in all samples from human accidental and CW casualties. The bis-sulfoxide may be partially formed by oxidation of the monosulfoxide during sample preparation.
	urine	1,1'-sulfonylbis [2-*S*-(*N*-acetylcysteinyl)ethane]	Major urinary metabolite in the rat but appears to be a minor excretion product in humans, based on samples from three accidental casualties.
HN-1	urine	*N*-ethyldiethanolamine	Minor urinary metabolite in the rat. No background levels detected (> 1 ng/mL) in 120 human urine samples; no samples from human exposures. Has commercial uses.
HN-2	urine	*N*-methyldiethanolamine	As above. HN-2 still in limited use as an anticancer agent.
HN-3	urine	triethanolamine	Urinary metabolite in the rat. Detected in 47% of urine samples from 120 non-exposed subjects, sometimes at high levels. Widespread use in domestic and industrial products.
Lewisite 1	urine	chlorovinylarsonous acid (CVAA)	Urinary metabolite in guinea pigs. No background levels detected in 120 human samples; no samples from human exposures.
Sarin	urine, blood	isopropyl methylphosphonic acid	Major metabolite in animals. Detected in samples from human casualties in Matsumoto and Tokyo.
	urine, blood	methylphosphonic acid (MPA)	Secondary hydrolysis product that may result from metabolism or slow hydrolysis of iPrMPA in the sample. Detected in samples from human casualties in Matsumoto and Tokyo. Could also arise from other nerve agents (soman, cyclosarin, VX) and from fire retardants.
Soman	urine, blood	pinacolyl MPA	Metabolite in animals; no samples from human exposures.
Cyclosarin	urine, blood	cyclohexyl MPA	Metabolite in animals; no samples from human exposures.
VX	urine, blood	ethyl MPA	Metabolite in animals. Detected in a human assassination victim. Could arise from other V agents or the EtO analogue of sarin. Confirmatory for VX when detected with $MeSCH_2CH_2N(iPr)_2$.
	blood	$MeSCH_2CH_2N(iPr)_2$	Detected in serum of a human assassination victim. Confirmatory for VX when detected with ethyl MPA.
Tabun	urine	Me_2N-P(O)(OEt)OH, HO-P(O)(OEt)CN	Appear to be too unstable to be useful biomarkers.
	urine	EtO-P(O)$(OH)_2$	Ubiquitous occurrence in human urine. A metabolite common to some pesticides and plasticizers.
BZ	urine	benzilic acid, 3-quinuclidinol	Assumed to be metabolites but no animal studies reported; no samples from human exposures.
Phosgene	urine		No urinary metabolites identified.
HCN	urine	2-aminothiazoline-4-carboxylic acid	Metabolite in animals. Background levels found in urine from 40/40 human subjects; sources include tobacco smoke and food constituents.

* Biomedical sample in which they have been shown or are assumed to occur.
† All reported background studies have been in U.S. or Western European populations.

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are shown in Figure 2. Reactions with nucleophilic sites on hemoglobin (N-terminal valines, histidines, and aspartic and glutamic acid residues) (7,8), albumin (cysteine-34) (9), keratin (aspartic and glutamic acids) (10), and DNA (11) provide at least six biomarkers that can be detected in appropriate samples. Eleven biomarkers of sulfur mustard (metabolites and adducts) have been detected in cases of accidental or deliberate human exposure (1,12). Much debate has concerned the validity of thiodiglycol (TDG) and its sulfoxide (TDGO) as biomarkers, derived from hydrolysis of sulfur mustard and subsequent oxidation. Both occur at trace levels (Table I) in Western human populations (13,14), source unknown, and in abnormally high concentrations in subjects who have been exposed to sulfur mustard (15,16). In contrast, no background levels or interferences have been detected for β-lyase metabolites (13,17), which are derived from an initial reaction of sulfur mustard with glutathione. These are regarded as unequivocal biomarkers of exposure to sulfur mustard and have the added advantage of sensitive analytic methods using both gas chromatography–tandem mass spectrometry (GC–MS–MS) (13,17) and liquid chromatography (LC)–MS–MS (18). 1,1'-Sulfonylbis[2-*S*-(*N*-acetylcysteinyl)ethane], also derived from an initial reaction with glutathione, is a major metabolite in the rat (5) but appears to be a minor metabolite in humans based on the analysis of samples from three accidental exposures (19,20).

Much less is known on the biological fate of nitrogen mustards and Lewisite; detailed metabolism studies have not been reported. Hydrolysis products of the three nitrogen mustards, *N*-methyldiethanolamine, *N*-ethyldiethanolamine, and triethanolamine (Figure 3), have been detected as excretion products in rats exposed to the agents, but appear to be minor metabolites (21). No background levels of *N*-methyl and *N*-ethyldiethanolamines were detected [limit of detection (LOD) ~1 ng/mL] in urine from 120 subjects but both have limited commercial/industrial uses. Triethanolamine, the hydrolysis product of HN-3, occurs at variable and sometimes very high levels in human urine (21). Nitrogen mustards will undoubtedly alkylate a number of residues on proteins, but to date, only the formation of the HN-2 adduct with the cysteine-34 residue on albumin has been identified (22). HN-2 reacts with the N-7 of deoxyguanosine residues in DNA as does sulfur mustard (23).

The hydrolysis product of Lewisite 1,2-chlorovinylarsonous acid (CVAA) (Figure 3) has been detected as its derivative with 1,2-ethanedithiol in urine from exposed guinea pigs (24). Residues have been liberated from human hemoglobin in vitro and guinea pig blood ex vivo by treatment with 1,3-dimercapto-2-propanol (BAL) (25).

Nerve agents are more selective in their reactions.

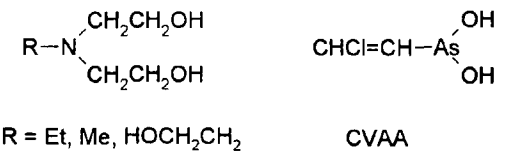

Figure 3. Structures of metabolites derived from hydrolysis of nitrogen mustards and Lewisite 1.

Metabolism is dominated by chemical or enzyme mediated hydrolysis (6); the alkyl methylphosphonic acids so produced are generally regarded as unequivocal biomarkers of exposure as there are no known alternative sources. Methylphosphonic acid (MPA), the secondary hydrolysis product and minor excretion product, could possibly arise from fire retardants or their precursors. Isopropyl MPA, the hydrolysis product of sarin, was detected in blood and urine from casualties of the Matsumoto and Tokyo terrorist attacks (26). Tabun poses a problem because its primary hydrolysis products are unstable, and there may be high background levels of its secondary hydrolysis product ethyl phosphoric acid (27). Some metabolites of phosphonofluoridate and V-type nerve agents that are used as biomarkers are shown in Figure 4.

Major reactions with proteins appear to be limited to the serine residues in the active sites of acetylcholinesterase, the biochemical target of nerve agents, and the related butyrylcholinesterase present in plasma (28) and, for the more reactive nerve agents (phosphonofluoridates and tabun), tyrosine-411 on albumin (29,30). The butyrylcholinesterase adduct of sarin was detected in Matsumoto and Tokyo casualties as a phosphonylated peptic nonapeptide (28) and as sarin after reactivation with fluoride (31). A disadvantage of cholinesterases as biomarkers is that the organophosphorus residues are prone to aging, in which the *O*-alkyl group is cleaved, thus losing key structural information; this occurs particularly rapidly with soman. The Me_2N substituent in tabun is prone to cleavage during enzymatic digestion if detected as a nonapeptide. Also the entire organophosphorus moiety may be partially displaced from non-aged residues if therapeutic oximes have been administered. Albumin adducts, which are much more stable with regard to aging or oxime displacement, have been identified in experimental animals but appear to be valuable biomarkers at higher exposure levels in comparison to butyrylcholinesterase adducts (29). One point of ambiguity that arises from all these nerve agent biomarkers is that the organophosphorus residue could be derived from a sarin type nerve agent or a V-type nerve agent because the fluoride or 2-(dialkylamino)ethylthio groups are displaced (other leaving groups are much less likely). A blood metabolite derived from the 2-(diisopropylamino)ethylthio side chain of VX was identified in a human subject assassinated by percutaneous exposure to VX (32); it is not known if this metabolite is further elaborated before excretion in urine.

Desirable Properties of Biomarkers

If biomarkers are to be used for forensic purposes, usually as unequivocal qualitative indicators of exposure, they need to meet a number of requirements.

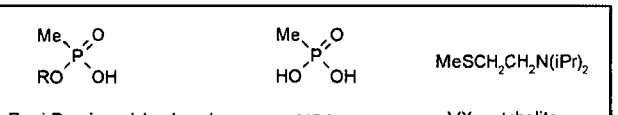

Figure 4. Structures of some nerve agent metabolites.

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Lifetime

In cases of alleged military use of CW agents, historical precedence (1,12) suggests that samples are likely to be collected several days, possibly longer, after the incident, depending on how quickly an investigating team can gain access to the casualties. Biomarkers therefore need to be detectable at least up to a week after the exposure, preferably longer. In most cases this excludes the use of the original agent as a biomarker (unless large quantities are absorbed into fatty tissue). The period of detectability of a biomarker will depend on a number of factors, including its inherent stability, the elimination profile, and the LOD of the analytical method.

A disadvantage of urinary metabolites as biomarkers is that the major portion (~ 90%) of the metabolites formed are excreted within the first 72 h after exposure. For this reason, LODs < 1 ng/mL are desirable for application in investigations into alleged CW use. Adducts with proteins generally provide much longer lived biological markers, in favorable cases up to the lifetime of the macromolecule. For example, the abundant blood proteins hemoglobin and albumin have half-lives in the body of ~6 and 3 weeks, respectively. DNA adducts have a more limited lifetime because of the intervention of repair processes.

In the event of terrorist use of CW agents, samples are likely to be collected much sooner after the event, and demands on both the lifetime of the biomarker and the LODs of analytical methods may be less. This was demonstrated following the terrorist release of sarin in the Tokyo subway, when most samples were collected within 24 h and biomarkers were detectable in some casualties at relatively high concentrations.

Chemical stability

Biomarkers should have good stability in the biological matrix, and not be subject to hydrolysis or oxidation. This is a particular problem with the initial hydrolysis products of the nerve agent tabun, which further hydrolyse to ethyl phosphoric acid, a ubiquitous excretion product (Figure 5). Some metabolites of sulfur mustard are prone to oxidation on sulfur.

Adducts with butyrylcholinesterase may be subject to slow reactivation (e.g., VX) or aging. The latter occurs very rapidly with the soman adduct and more slowly with other nerve agents. Fortunately, the fluoride reactivation method is so sensitive that it still shows good "retrospectivity" for butyrylcholinesterase adducts (33).

Non-CW sources of biomarkers

The most important factor for forensic-type applications is that the biomarker should preferably be an unequivocal indicator of exposure to the CW agent. A number of CW agent hydrolysis products are excreted in the urine of a large percentage of the human population who have not been exposed to the CW agent. Background levels of ethyl phosphoric acid (Figure 5) (27) result from exposure to organophosphorus pesticides and plasticizers that are hydrolyzed to a common excretion product. Thiodiglycol and triethanolamine have commercial uses and are present in urine from much of the Western population (in which background studies have been undertaken). Background levels of triethanolamine, which is widely used in industrial and domestic cleaning products and cosmetics, can be very high (21). Metabolites derived from glutathione pathways (β-lyase metabolites, *N*-acetylcysteine conjugates), and adducts with proteins and DNA, have the advantage as biomarkers in that their formation requires the reaction of an electrophilic species with a biological nucleophile, and are much less likely to occur from non-CW sources.

In the case of hydrogen cyanide, smoke (including that from tobacco) and some foods are sources of exposure. 2-Aminothiazoline-4-carboxylic acid, a metabolite of cyanide, is found in the urine of a large percentage of the population (34). Phosgene metabolism is complicated because it has two labile chlorines, forms metabolites in common with chloroform, and may form protein adducts that could arise from reaction of amino acid residues with carbon dioxide (35).

The advantages, limitations, and occurrence of currently known biomarkers for nerve agents, vesicants, phosgene, hydrogen cyanide, and BZ are summarized in Tables I and II (for full references and discussions of analytical methods, see references 1–4).

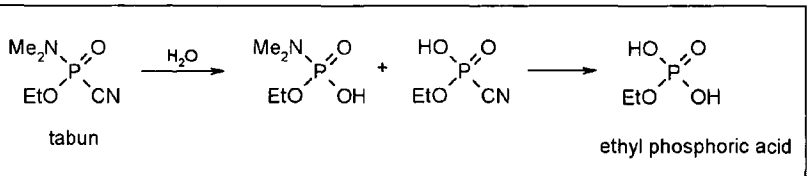

Figure 5. Hydrolytic pathway for tabun.

Analytical Considerations

Techniques

In most situations for which biomedical sample analysis is required, analytes are likely to be present at low or sub-parts-per-billion concentrations. MS is the only spectrometric technique that universally provides the requisite combination of sensitivity and selectivity, usually in combination with GC or LC, or less commonly with capillary electrophoresis. For analysis down to mid to low parts-per-billion levels, single-stage GC–MS or LC–MS using selected ion monitoring (SIM) (full scanning on some instruments) may provide adequate limits of detection. For detection at low to sub-parts-per-billion levels, and with a higher degree of confidence, tandem MS is required (or high-resolution single-stage MS).

For most urinary metabolites, analysis can be performed using less expensive benchtop instrumentation by GC–MS or GC–MS–MS (ion traps or quadrupole instruments). Some thermally labile metabolites (e.g., *N*-acetylcysteine conjugates) require LC–MS–MS (19), as do most methods for identifying protein and DNA adducts (3), unless the agent residue can be chemically liberated from the protein (25,31,36–38).

Immunoassays are useful for rapid screening of large numbers of samples, but unless antibodies for the analyte are available, they require a considerable effort to develop (39).

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Selectivity

A feature of most analytical methods for CW agent biomarkers is that they are targeted at individual or chemically specific classes of biomarker. The history of the incident, including eyewitness accounts, symptoms in casualties, and results of any environmental analysis, is therefore important in guiding the analysis. A more generic method has been reported for identifying butyrylcholinesterase inhibited by exposure to any organophosphorus nerve agent or pesticide (but without identifying the agent) (40).

Table II. Protein and DNA Adducts as Biomarkers of CW Agents

Agent	Sample*	Biomarker	Comments
Sulfur mustard	blood: hemoglobin	N-terminal valine NH-HETE* adduct	Demonstrated in animals. No background levels in a limited number of human samples. Detected in samples from human accidental and CW casualties.
	blood: hemoglobin	histidine N-HETE adduct	As above.
	blood: albumin	cysteine-34 S-HETE adduct	Demonstrated in animals. No background levels detected in 80 human samples. Detected in samples from human accidental and CW casualties.
	blood: albumin and hemoglobin	aspartic acid/glutamic acid CO_2-HETE	Detected as TDG released by hydrolysis. Demonstrated in animals. No significant background levels detected in a limited number of human samples. Detected in a single accidental human exposure.
	blood and skin: DNA	N7-(HETE)-2'-deoxyguanosine	Demonstrated in animals. Detected in blood from human CW casualties. N7-(HETE)-guanine demonstrated as a short-lived urinary metabolite in animals.
HN-2	blood: albumin	cysteine-34 S-HETE adduct	Formed in human blood in vitro; no samples from human exposures.
HN-2	blood: DNA	N7-2'-deoxyguanosine adduct	Formed in human blood in vitro.
Lewisite 1	blood	CVAA (free and bound to hemoglobin)	Demonstrated in the rat; no samples from human exposures.
Sarin	blood: BuChE/AChE	iPrMP-serine† adduct	Demonstrated in animals. No background levels in a limited number of human samples. Detected in samples from human casualties in Matsumoto and Tokyo. May be detected as a phosphonylated nonapeptide, by hydrolytic displacement as iPrMPA, and most sensitively as sarin after fluoride displacement.
	blood: albumin	iPrMP-tyrosine adduct	Demonstrated in animals and human plasma in vitro; no samples from human exposures.
Soman	blood: BuChE/AChE	MP(OH)-serine adduct‡	Demonstrated in animals and human plasma in vitro; no samples from human exposures. Indicative of an aged nerve agent residue but does not identify the agent. Not displaced by fluoride.
	blood: albumin	pinacolylMP-tyrosine adduct	Demonstrated in animals and human plasma in vitro; no samples from human exposures. Does not rapidly age.
Cyclosarin	blood: BuChE/AChE	cyclohexylMP-serine adduct	Demonstrated in animals and human plasma in vitro; no samples from human exposures.
	blood: albumin	c-HexMP-tyrosine adduct	Demonstrated in animals and human plasma in vitro; no samples from human exposures.
VX	blood: BuChE/AChE	EtMP-serine adduct	Demonstrated in animals and human plasma in vitro. Could arise from other V agents or EtO analogue of sarin. Confirmatory for VX when detected with $MeSCH_2CH_2N(iPr)_2$.
Tabun	blood: BuChE/AChE	$Me_2N(EtO)P(O)$-serine adduct	Demonstrated in animals and human plasma in vitro; no samples from human exposures. Tends to lose Me_2N-P substituent during digestion when detected as a nonapeptide.
	blood: albumin	$Me_2N(EtO)P(O)$ -tyrosine adduct	Demonstrated in animals and human plasma in vitro; no samples from human exposures.

* 2-Hydroxyethylthioethyl, $HOCH_2CH_2SCH_2CH_2^-$.
† MP = $MeP(O)^-$.
‡ Non-aged adduct was apparently detected in guinea pigs by fluoride displacement but was probably derived from phosphonylated albumin (50); low concentrations of non-aged adduct were detected in rhesus monkeys by fluoride displacement, but BuChE in this species ages more slowly than in humans; some interaction with the less active P(+) isomer may be a contributing factor (33).

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Sample preparation

Sample preparation is a key component of biomedical sample analysis, particularly for protein adducts that require digestion to smaller molecules. Some proteins (albumin, butyrylcholinesterase) can be selectively isolated from blood by affinity-based solid-phase extraction (SPE) prior to digestion (9,40); alternatively blood can be crudely fractionated using precipitation techniques. Some analytes can be extracted directly from urine, blood, plasma, or digests using liquid–liquid extraction, but SPE is more commonly employed. Early methods tended to use C_{18} or C_8 bonded silica cartridges, or anion exchange for acidic analytes such as alkyl methylphosphonic acids. In recent years, there has been increasing use of hydrophobic-hydrophilic polymeric cartridges, which are often more efficient for extracting partially polar analytes such as thiodiglycol (14,41). In a few examples, solid-phase microextraction has provided very low LODs (42). Molecularly imprinted polymers (MIPs) are currently under investigation for affinity-based SPE (43).

Most metabolites, particularly those derived from hydrolysis, are polar and require derivatization for GC–MS analysis (44). This can be a source of error in trace analysis. Derivatization usually requires isolation from aqueous media, which can result in significant loss of analyte, and extraneous material or traces of water remaining in the residue may suppress derivatization. An advantage of derivatization is that it can be used to enhance detection. Conversion to perfluorinated derivatives and detection by negative ion chemical ionization MS provides very low LODs for thiodiglycol (13,38,45) and alkyl methylphosphonic acids (46). LC–MS–MS usually avoids the need for derivatization, and in favorable cases, it can also provide low LODs (18,47).

A number of strategies can be applied to the isolation and identification of protein adducts. Detection of the entire protein adduct is possible with modern MS techniques, using electrospray or desorption ionization methods, for example, but LODs are modest. A common approach is to selectively digest the protein with enzymes, such as trypsin, pepsin, or other protease, to produce short-chain peptides and detect the alkylated or phosphylated peptide using LC–MS–MS (9,28). Alternatively, the protein may be digested to its constituent amino acids using a protease (29) or 6M hydrochloric acid (37), although these methods tend to produce a high chemical background. In some cases, particularly where ester linkages are formed, the bound moiety may be displaced from the protein by hydrolysis (e.g., sulfur mustard adducts with glutamic/aspartic acid residues) (38), or fluoride ion in the case of nerve agent residues on the serine of AChE/BuChE (31). The 2-hydroxyethylthioethyl residue from sulfur mustard on the N-terminal valines of hemoglobin can be liberated by selective derivatization with a modified Edman reagent (36,37). In each of these examples the liberated moiety can be detected using simpler methodology (GC–MS or GC–MS–MS).

Analytical standards

One of the obstacles to the broader application of biomedical sample analysis is the acquisition of analytical standards. The advantage of urinary metabolites is that most are relatively simple molecules and are easily synthesized. Analytical standards for protein adducts, particularly if the analyte is an alkylated or phosphylated peptide, are more demanding. An expedient is to use an incubate of the CW agent with whole blood, plasma or blood protein as a crude analytical standard, although this requires access to the agent.

Criteria for identification of biomarkers

Criteria for the identification of biomarkers of CW agents in a forensic context are currently being discussed in a number of international and national fora. The Laboratory of the Organisation for the Prohibition of Chemical Weapons (OPCW) has to date only defined and exercised criteria for analysis at ppm levels in environmental samples, at which concentrations full scan data are obtainable. Identification by two independent techniques is required by the OPCW. It is likely that any agreed set of criteria for identification of CW biomarkers at trace levels will be broadly consistent with criteria defined by regulatory bodies in other contexts, drug testing in sports and banned substances in animal products, for example. The World Anti-Doping Agency (WADA) accepts identification by GC–MS or LC–MS by selected ion monitoring of three ions, provided they are structurally characteristic and meet defined tolerances for retention time and ion ratios (48). Alternatively, GC/LC–MS–MS is accepted if two transitions are monitored using multiple reaction monitoring, again subject to defined tolerances for retention time and ion ratios. The European Commission directive for banned substances in animal products (49) uses a system of identification points which is broadly consistent with the WADA criteria but with some differences in tolerances. If any individual method cannot meet the requirements (e.g., if only two selected ions or one MRM transition is available), both systems allow identification by more than one analytical method (e.g., by using two derivatives, two ionization methods, or GC and LC, subject to a number of restrictions). Where the question is "has a subject been exposed to a particular CW agent?", the identification of more than one biomarker should offer an added degree of flexibility for identification, provided the identification of each biomarker meets a minimum set of criteria.

Conclusions

Metabolites and/or protein or DNA adducts have been identified for most nerve agents and vesicants and validated in experimental animals or in a small number of human exposures. Urinary metabolites and protein adducts derived from nitrogen mustards, and urinary metabolites derived from the side chain of V agents are knowledge gaps.

For several agents, metabolites derived from hydrolysis are unsatisfactory biomarkers of CW exposure because of background levels in the human population. In these cases, metabolites derived from glutathione pathways, or covalent adducts with proteins or DNA, generally provide unequivocal biomarkers.

No satisfactory biomarkers have been identified for phosgene

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and cyanide because of background levels of metabolites or adducts from other sources of exposure.

Further consideration needs to be given to criteria for unequivocal identification of biomarkers of exposure to CW agents in forensic-type analysis.

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