

Analysis of Biomedical Samples

[dstl]



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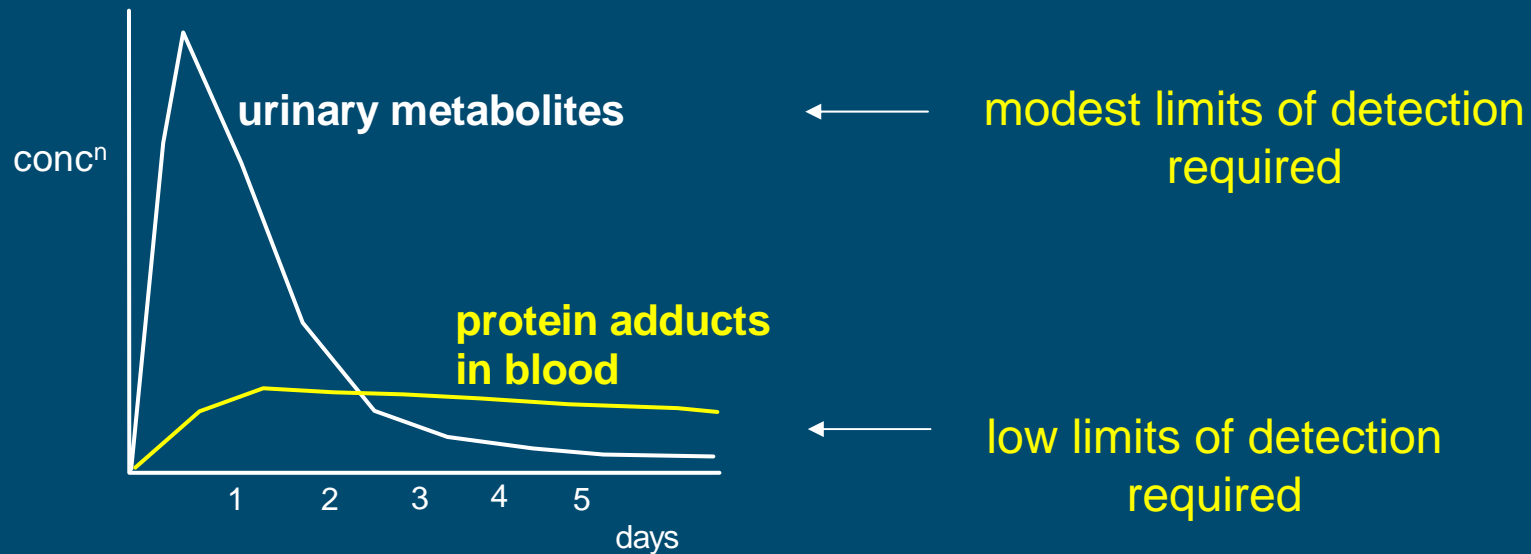
CW agents of concern

Declarations to the OPCW (Dec 2000)

	Agent	Tonnes
vesicants	sulphur mustard	17375
	Lewisite	6745
nerve agents	GB (sarin)	15048
	GD (soman)	9175
	VX	4032
	R-33 (Russian V)	15538

Biomarkers of exposure

- Urinary excretion accounts for the major part (~50-90%) of an absorbed dose *but*
- Bulk of metabolites (typically 70-90%) excreted in first 48-72 hrs
- Protein adducts account for much less but have longer half-lives



Biomedical samples

Sample types

- Urine is a simpler matrix
 - but is inherently more variable than blood
- Blood is a complex heterogeneous matrix
 - sample preparation can be tedious
 - but is less variable in composition than urine
- Blood requires invasive collection
 - medical skills required
- High levels of safety required for handling blood

Biomedical samples

Analysis of biomedical samples

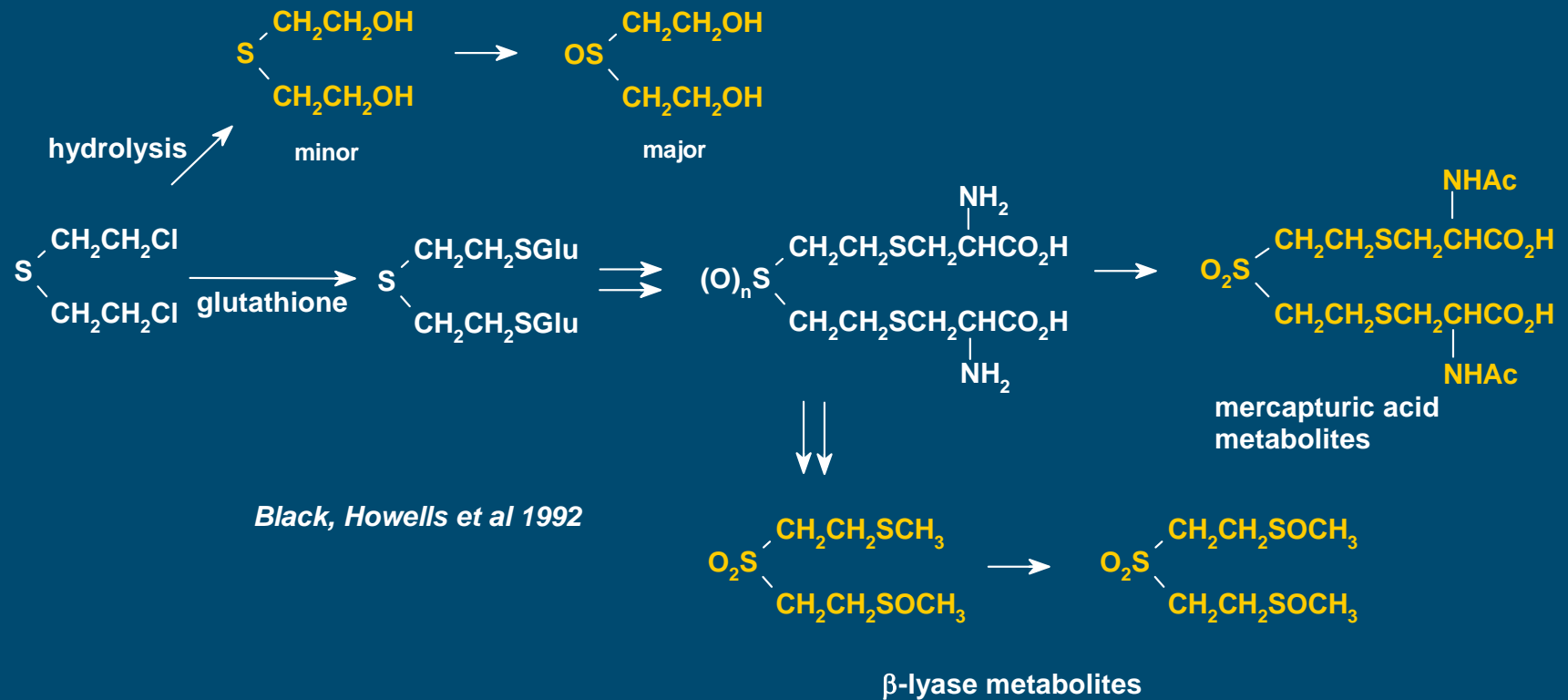
- Investigations of alleged use
 - several examples
 - Iran – Iraq war, Iraq Kurds, Tokyo subway + assassination
- EOD operations
 - recent sulphur mustard exposure in US
- Accidental exposures
 - sulphur mustard
 - recent VX exposure in US
- Monitoring of workers in destruction facilities

Urinary metabolites

- Urinary metabolites
 - If urine is collected within ~5 days, LODs 1-10 ng/ml are usually sufficient
 - depending on agent and severity of exposure
 - If urine collected > ~5 days, LODs down to ~0.1 ng/ml desirable
 - or if exposure is low

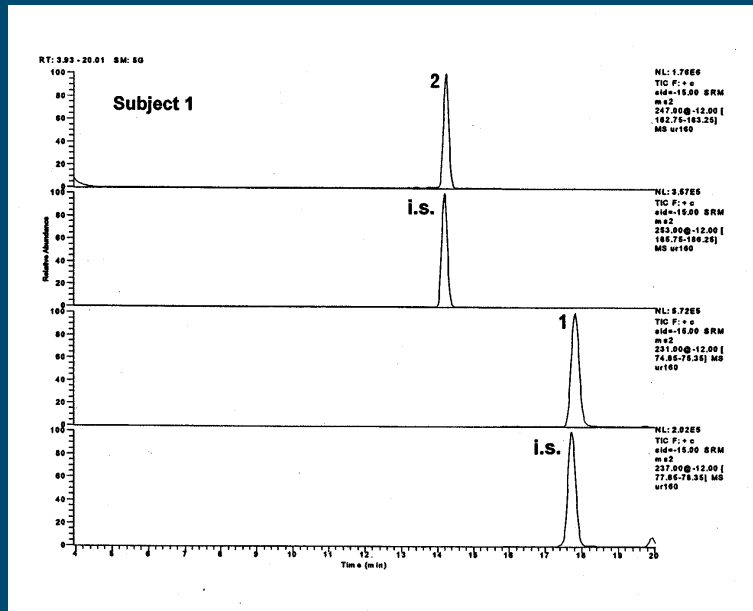
Urinary metabolites of sulphur mustard

Metabolic pathways of sulphur mustard in the rat

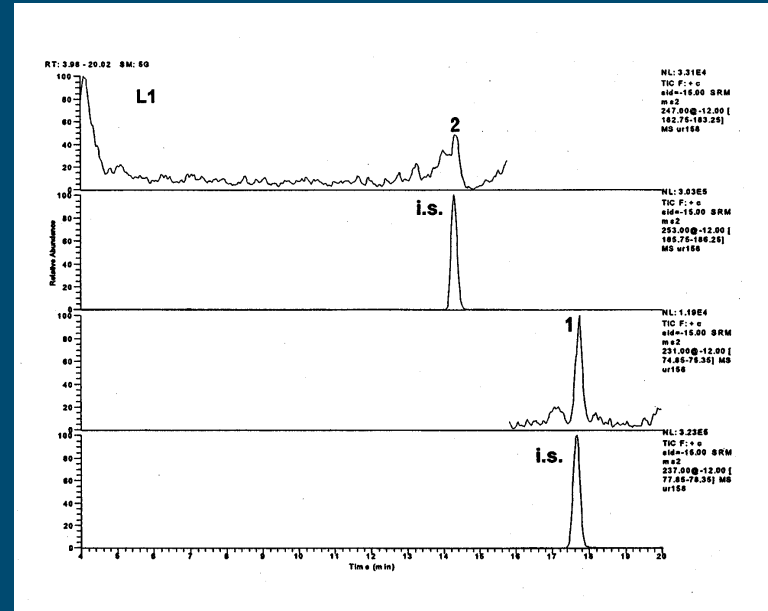


Urinary metabolites of sulphur mustard

LC-MS-MS analysis



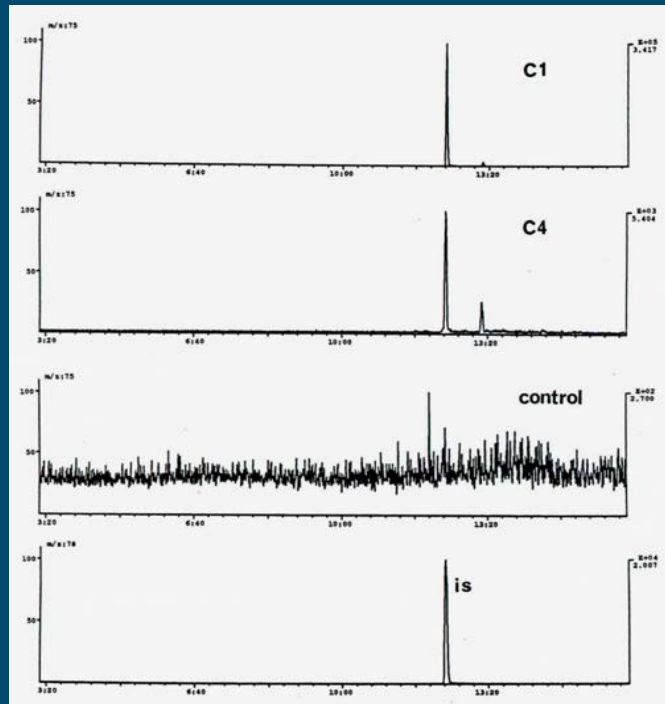
urine from a casualty
accidentally exposed from a
WW1 munition; urine collected
2-3 days after exposure, stored
11 years -20°C



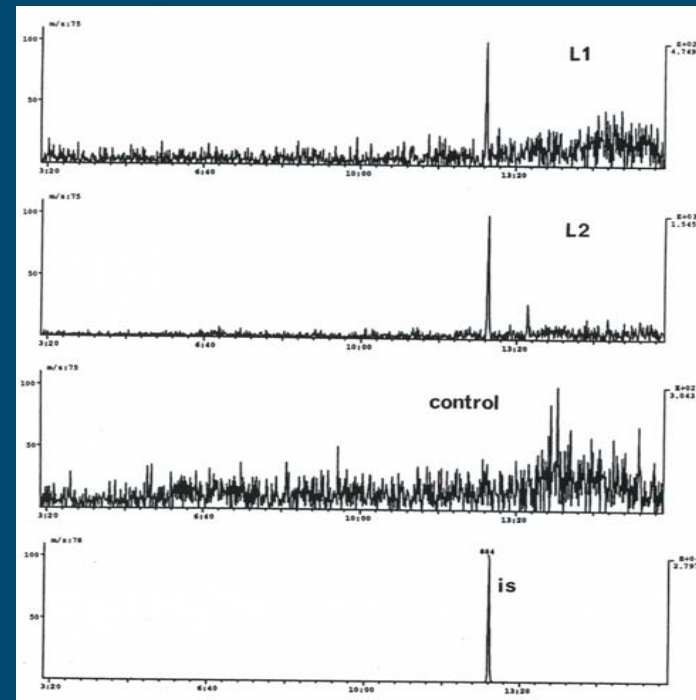
urine from a Kurdish CW
casualty treated in London;
urine collected 13 days after
exposure, stored 15 years
-20°C

Urinary metabolites of sulphur mustard

GC-MS-MS analysis



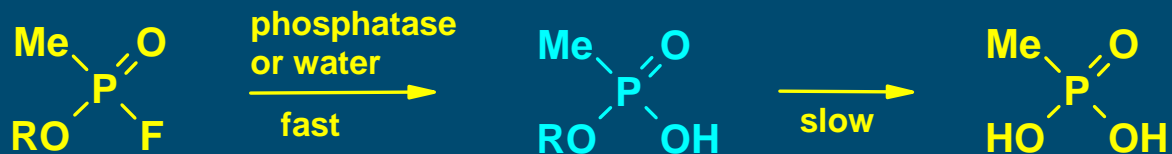
urine from Iranian casualties
treated in Ghent; **collected 10 days
after exposure**, stored 10 years -20°C



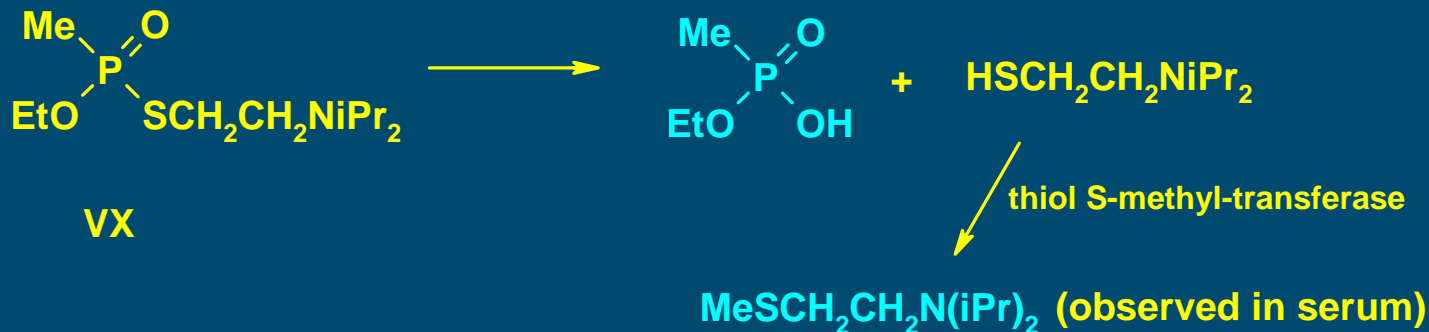
urine from Kurdish casualties
treated in London; **collected 13 days
after exposure**, stored 6 years -20°C

Urinary metabolites of nerve agents

- Metabolism dominated by hydrolysis



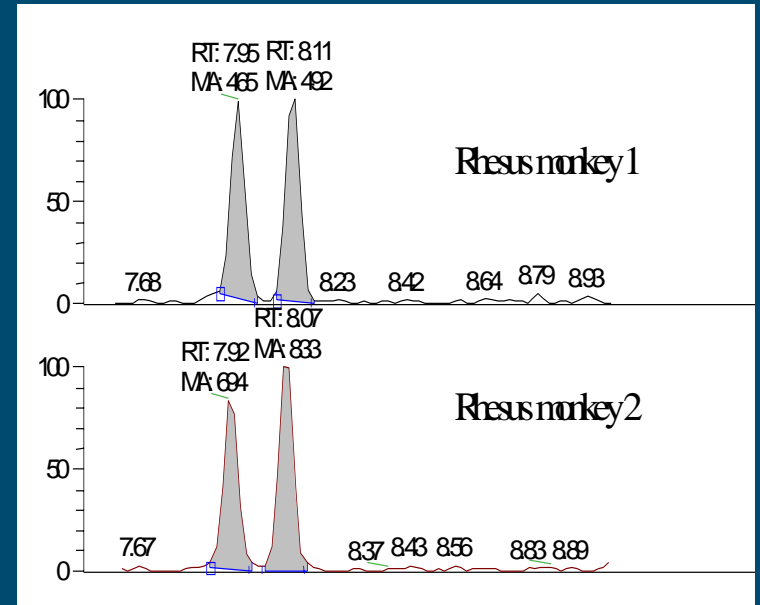
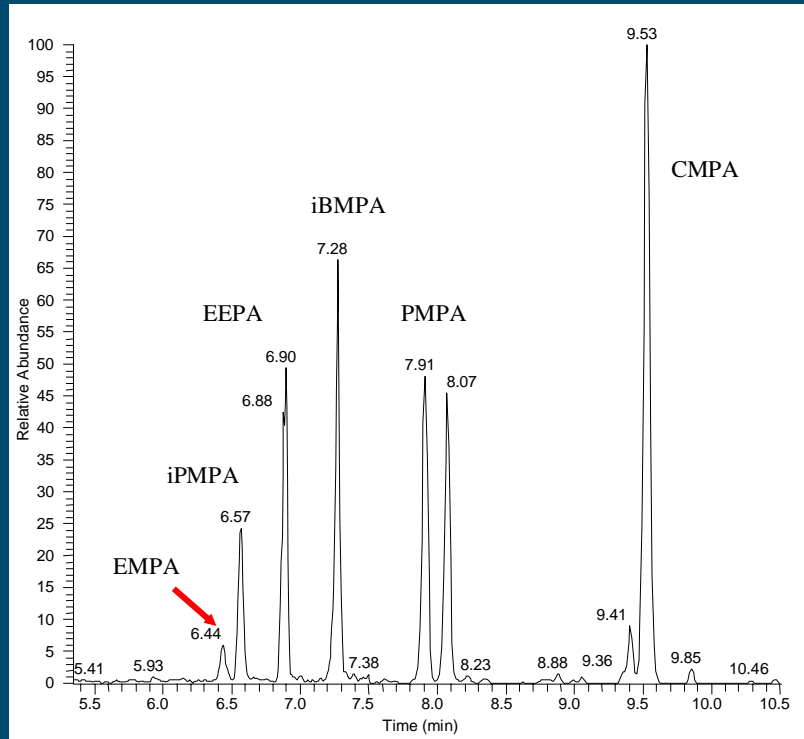
- Additional metabolite from side chain with V agents



Tsuchihashi et al 1998

Urinary metabolites of nerve agents

GC-MS-MS analysis of alkyl alkylphosphonic acids



Urine of Rhesus monkey, 11 days after 0.5xLD₅₀ dose im soman

Reconstructed ion current, 1 ng/ml spiked urine, PFB derivs, NICI, MRM.

GCQ ion trap

Protein adducts

Protein adducts

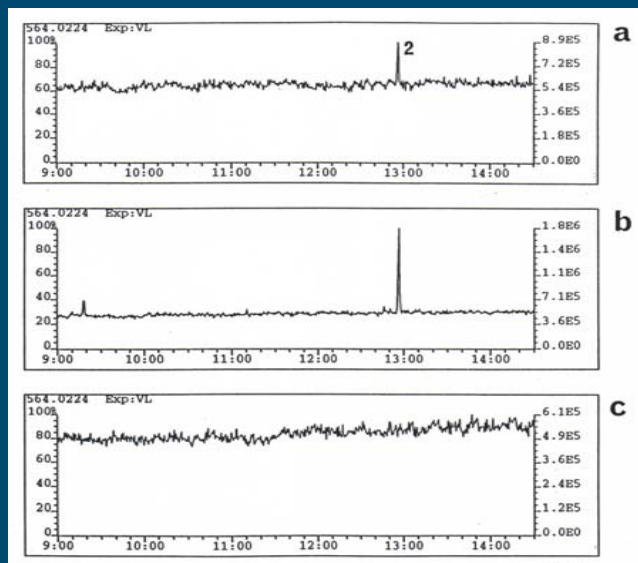
- adducts with proteins longer lived than urinary metabolites
 - up to biological lifetime of the protein
- proteins in blood are usual analytical targets
 - haemoglobin, albumin, cholinesterases
- High sensitivity analytical methods always required
 - LOD <0.1 ng/ml

Protein adducts of sulphur mustard

- Sulphur mustard has been shown to alkylate the abundant blood proteins haemoglobin & albumin, and keratin in skin
- Reacts with NH, CO₂H and SH (OH?) groups on amino acids
- Produces an adduct **HOCH₂CH₂SCH₂CH₂-amino acid**
 - N-terminal valine, histidine, glutamic and aspartic acids, lysine, cysteine

Protein adducts of sulphur mustard

Haemoglobin adducts detected in human casualties



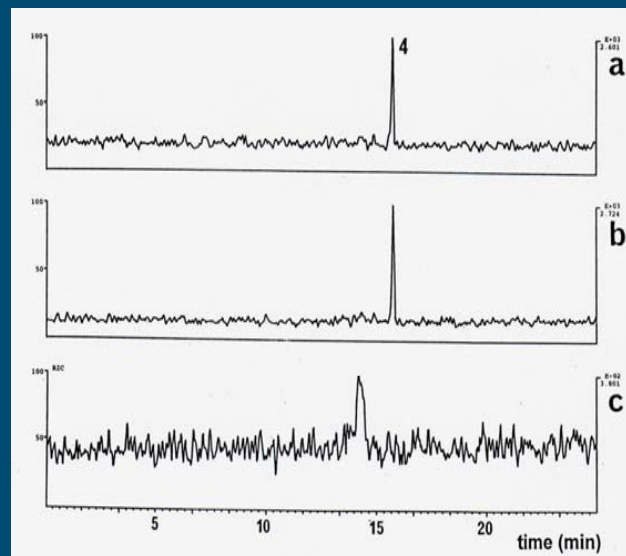
⇐ Alkylated N-terminal valine

GC-HRMS, samples collected 2 & 10 days after exposure

a. accidental exposure; b. Iranian CW casualty; c. control

Alkylated histidine ⇒

LC-MS-MS, samples collected 2 & 10 days after exposure



Protein adducts of nerve agents

Cholinesterase adducts

- Acetyl & butyrylcholinesterase are specific targets for nerve agents
 - BuChE much more abundant in blood
- Three methods have been developed for trace analysis
 - displacement of phosphyl moiety with fluoride
 - in animals can detect down to 0.01% inhibition of BuChE in plasma
 - applicable to GB, GA, VX but low sensitivity for agents that 'age'
 - hydrolytic displacement of phosphyl moiety
 - pepsin digestion to a phosphylated nonapeptide
 - contains active site serine
 - probably not applicable following oxime therapy
- All three were applied to blood samples from Japanese terrorist incidents

Protein adducts of nerve agents

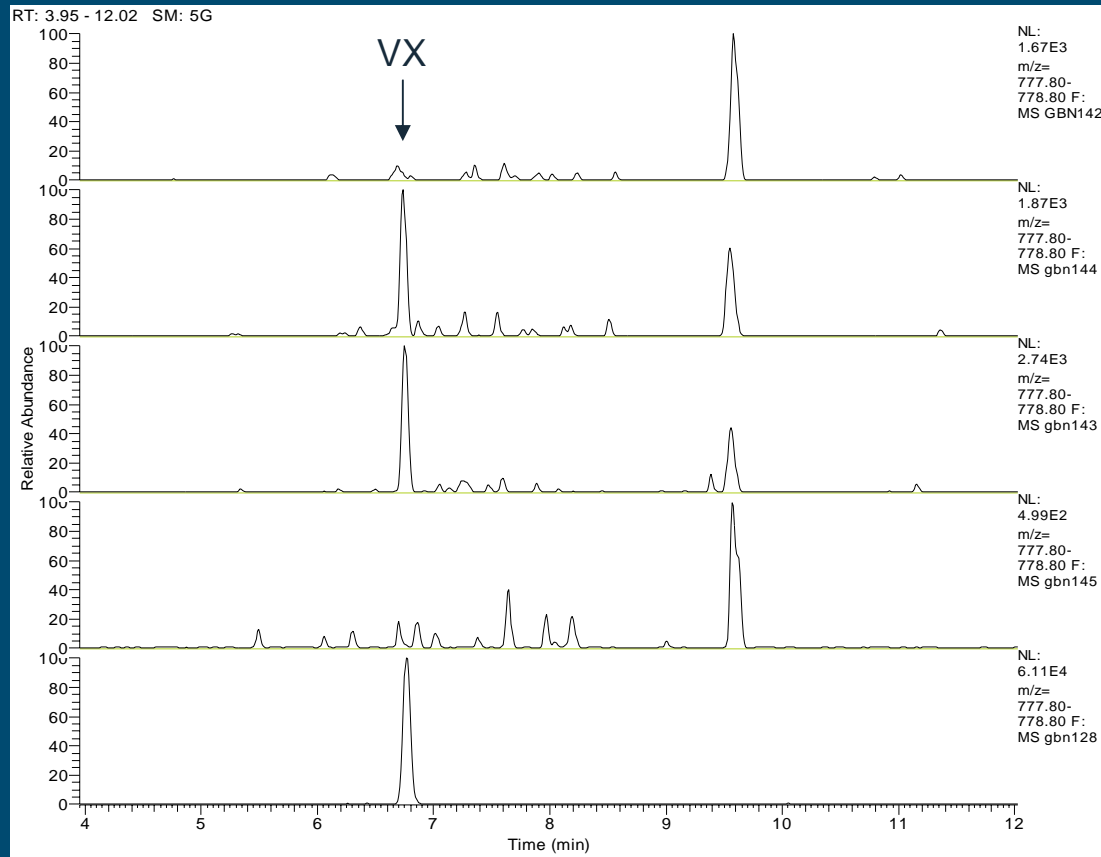
Blank guinea pig

2xLD₅₀, 3 hr

2xLD₅₀, 24 hr

0.5xLD₅₀, 48 hr

Human plasma
VX incubate

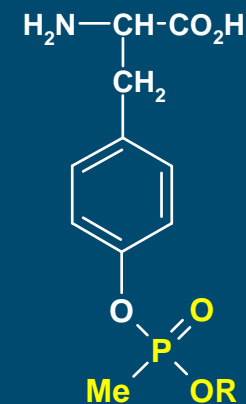


VX butyrylcholinesterase nonapeptide in guinea pigs (no therapy)

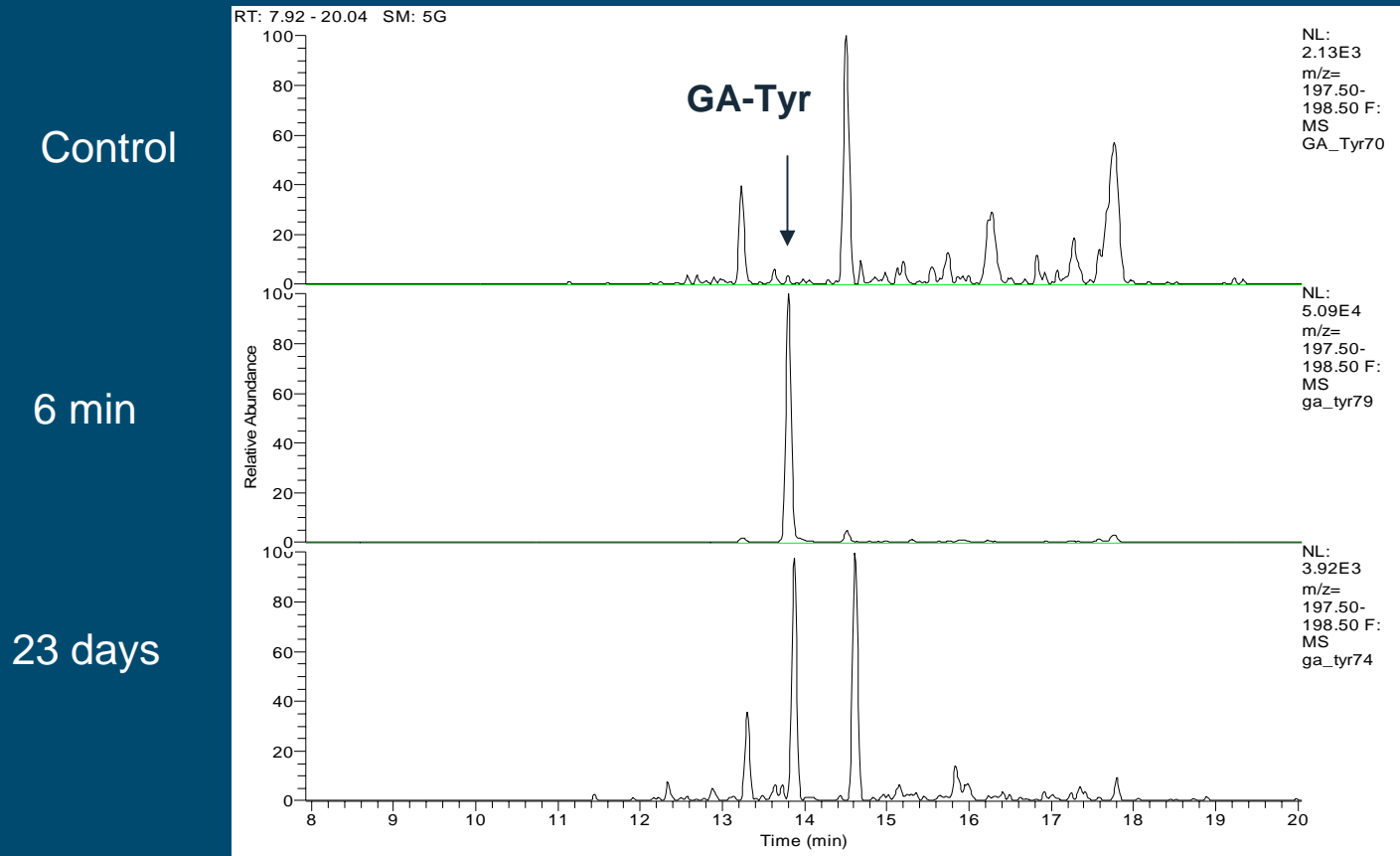
Protein adducts of nerve agents

Phosphonylated tyrosine

- Phosphonylated tyrosine detected in protease digests of human plasma incubated with sarin, soman, cyclosarin and tabun
 - tyrosine-411 in albumin
- No indication of rapid 'ageing' with soman
- Probably a chemical reaction & applicable only to moderate exposures
- Detected in marmoset plasma up to 23/24 days after doses of $2 \times LD_{50}$ G-agents
 - in presence of oxime therapy
 - particularly useful for tabun



Protein adducts of nerve agents



Tyrosine adduct in marmosets, 2 x LD₅₀ GA

Alternative approaches

- Cholinesterase monitoring for nerve agent exposure
 - cheap but not very sensitive
 - requires baseline measurement
- Lewisite exposure
 - blood arsenic measurement possible
 - other arsenicals, e.g. irritants

Summary

- Current methods developed for unequivocal identification of exposure
 - exposures resulting in obvious symptoms
 - single samples often obtained several days after exposure
 - sensitive analytical methods usually necessary
- Monitoring exposure during destruction operations
 - in most cases the agent will be known
 - exposure may result in obvious symptoms
 - or exposure may result in no symptoms
 - samples could be obtained earlier and over longer time scales
 - for exposures with no symptoms most current analytical methods probably not sufficiently sensitive