



Detection of nerve agent and chlorine biomarkers using dried blood spots

Mirjam de Bruin-Hoegée (TNO, The Netherlands)

[Start presentation](#)

Introduction

- Chemical weapons are still being used in conflict zones and to assassinate individuals
- Novichok **nerve agents** have been used for various poisonings, but have been scarcely studied
- Limited knowledge about mechanism of action and biomarkers of **chlorine gas** exposure for early risk assessment
- Respiratory disease is more likely to affect military personnel compared to the civilian population^{1,2}



[1] Sudhakar, D., Claggett, C. L., & Zacher, L. L. (2014). Military service and lung disease. *Journal of Occupational and Environmental Medicine*, 56(10), S13–S17.

[2] Pugh, M. J., Jaramillo, C. A., Leung, K. W., Faverio, P., Fleming, N., Mortensen, E., Amuan, M. E., Wang, C. P., Eapen, B., Restrepo, M., & Morris, M. J. (2016). Increasing prevalence of chronic lung disease in veterans of the wars in Iraq and Afghanistan. *Military Medicine*, 181(5), 476–481.

Introduction

- Innovative exposure detection methods are needed that adapt to operational circumstances
 - Fast diagnosis is required for appropriate treatment
 - Biomarkers can be used for verification purposes
- Stability in field conditions is a challenge
- Advantages DBS and microsamplers
 - preserving signature,
 - reducing sample volume,
 - less invasive,
 - Less stringent storage conditions



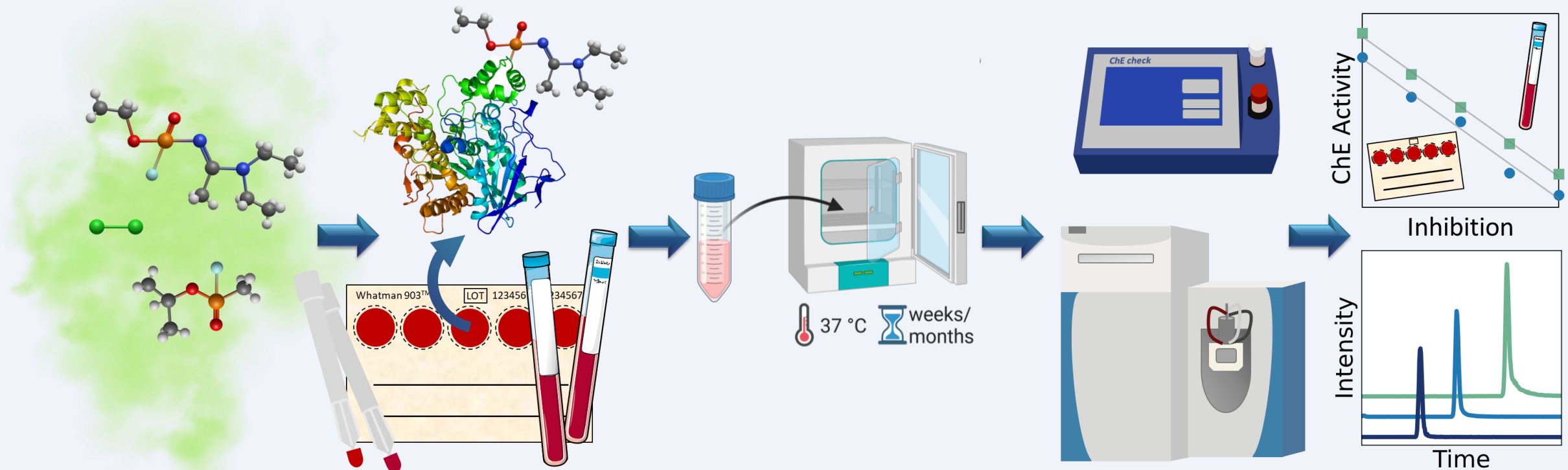
[3] M.T. Ackermans, et al., Hematocrit and standardization in DBS analysis: A practical approach for hormones mainly present in the plasma fraction, Clin. Chim. Acta. 520 (2021) 179–185.

[4] J.W. Perez, et al., Enhanced stability of blood matrices using a dried sample spot assay to measure human butyrylcholinesterase activity and nerve agent adducts, Anal. Chem. 87 (2015) 5723–5729.

[5] N.L. Koryagina, et al., Determination of Phosphonylated Tyrosine as a Marker of Exposure to Nerve Agents in Dried Blood Plasma Spots by HPLC–HRMS/MS, J. Anal. Chem. 74 (2019) 1341–1348.

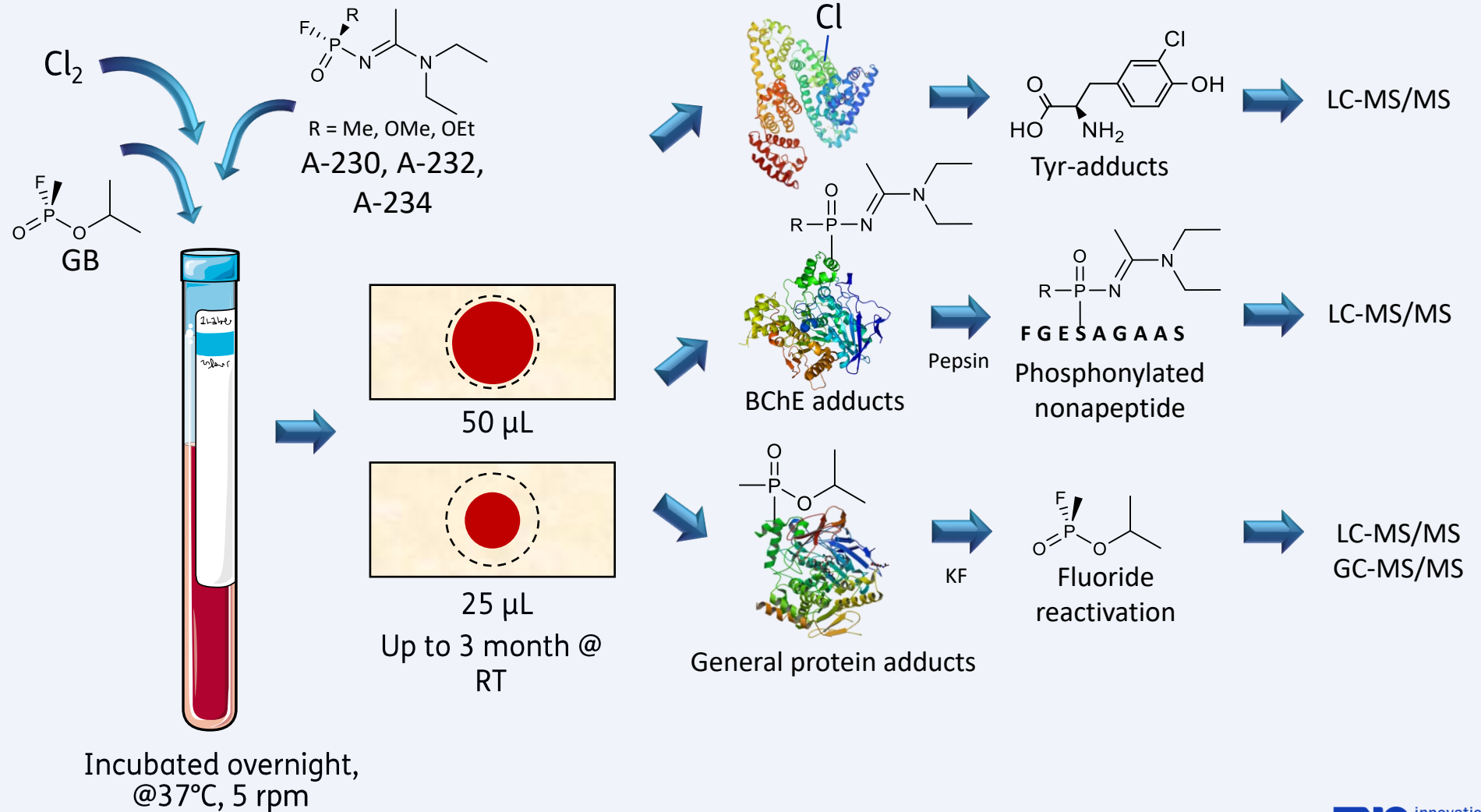
Aim of the research

- Investigating the feasibility of using dried blood spots and microsampling for detection of organophosphate and chlorine poisoning
 - Comparison of sampling methods (blood, dried blood spots and microcuvettes), storage conditions (RT, -20 °C) and 3-4 exposure levels



Method

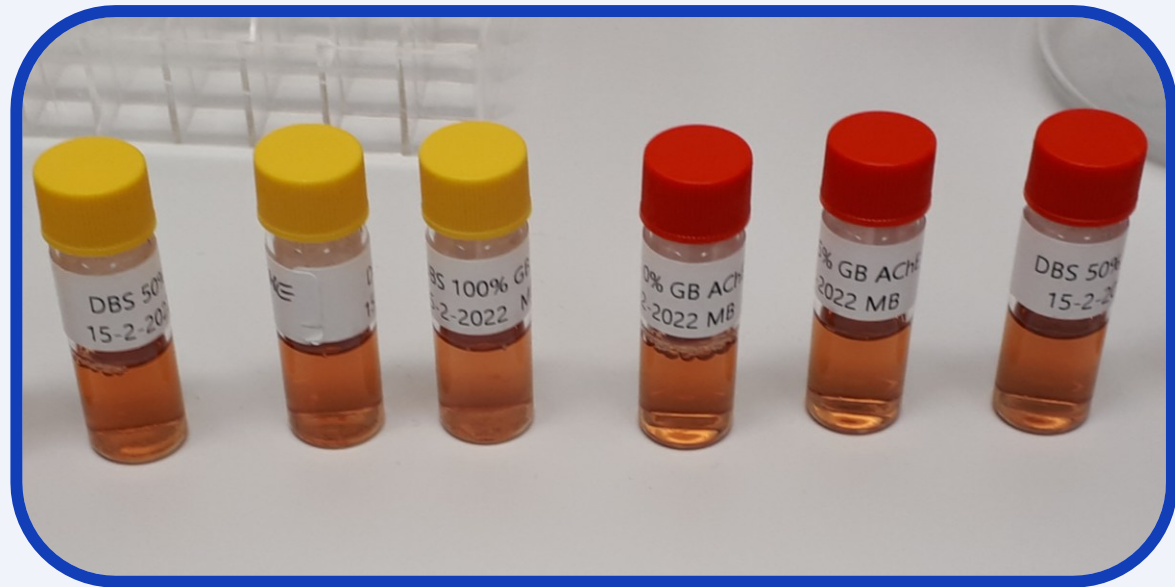
- Laboratory detection



Method

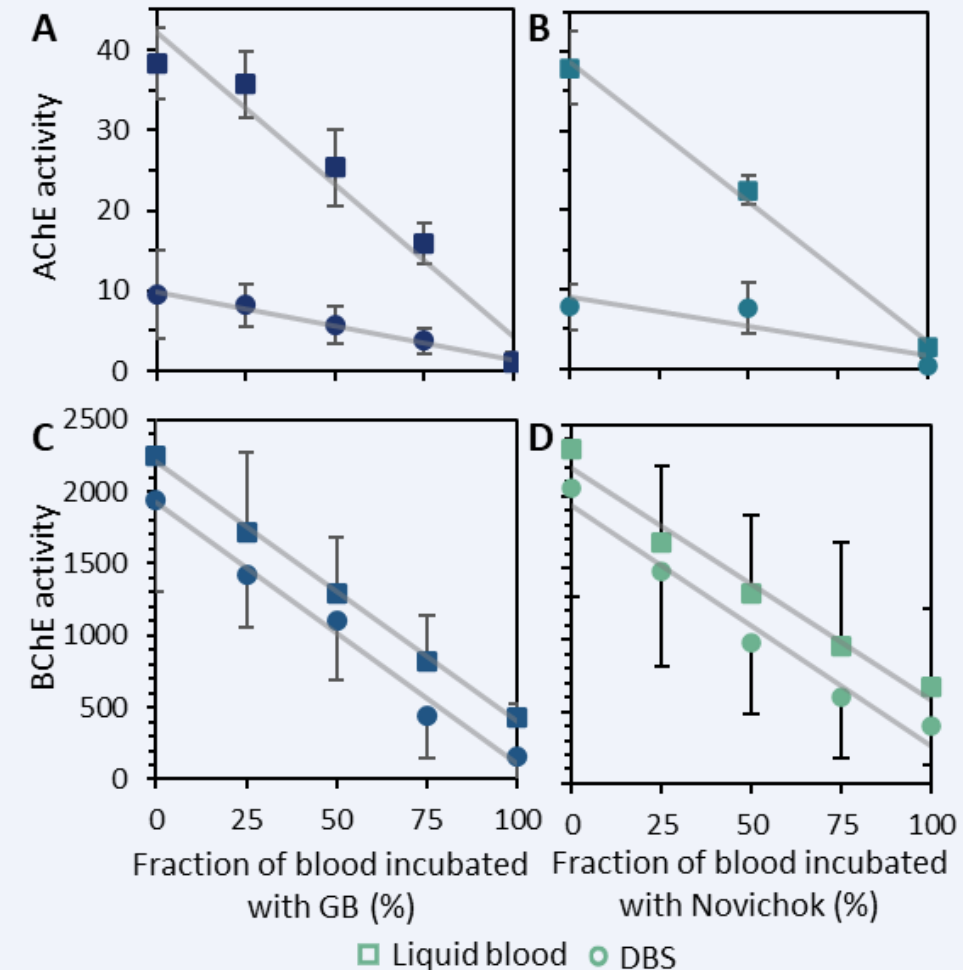
On-site detection

- Ellman photometric assay produces a yellow color after reaction of organophosphates with AChE or BChE
- User-friendly cholinesterase (ChE) mobile test kit designed for whole blood (discontinued product)



Results nerve agents

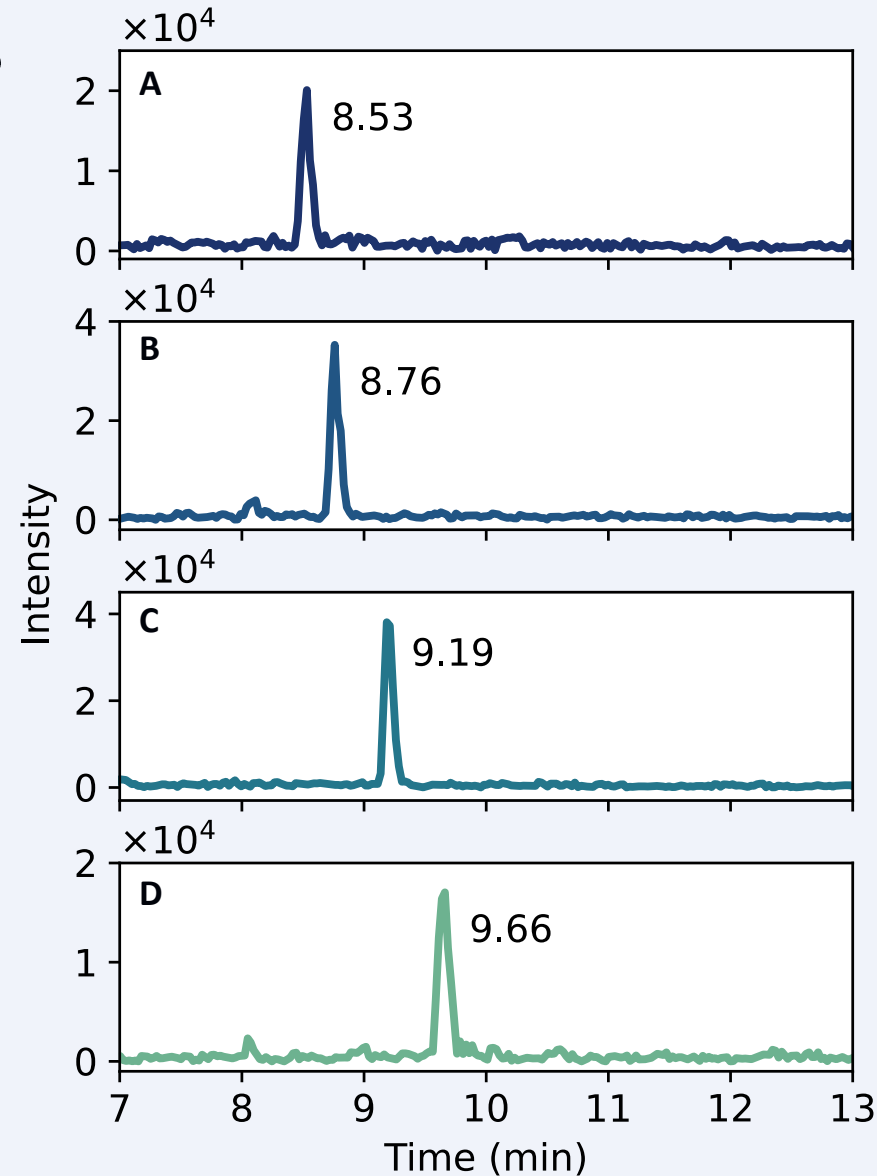
- Comparable results BChE for 10 μ L liquid blood or $\frac{1}{2}$ 25 μ L-based DBS
- Reduction of ChE activity after GB and Novichok exposure
- Stability > 1 months after storage of dried spots
- AChE in DBS is not a reliable indicator of CWA exposure (possible AChE loss in vesicles during concentration of red blood cells)
- Disadvantage: not specific
 - Organophosphorus pesticides have same mechanism of action



Results nerve agents

Targeted analysis with LC-MS/MS

- BChE nonapeptide adducts
- 25 μL spots, 100% incubation
- Verification with reference standards
- Sensitivity: 50% was clearly identified
- Stability: 1 month after storage of DBS



m/z

m/z

m/z

m/z

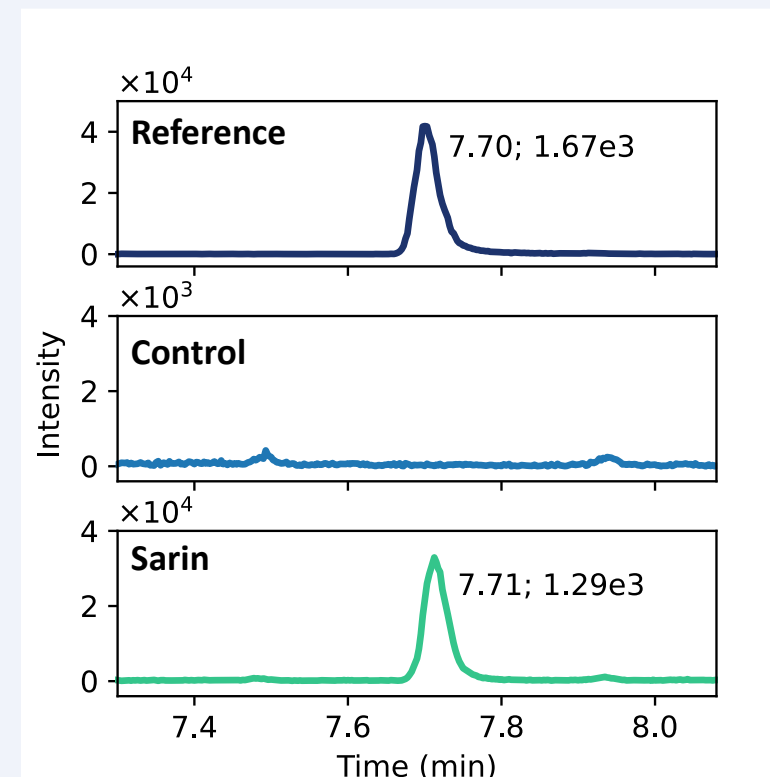
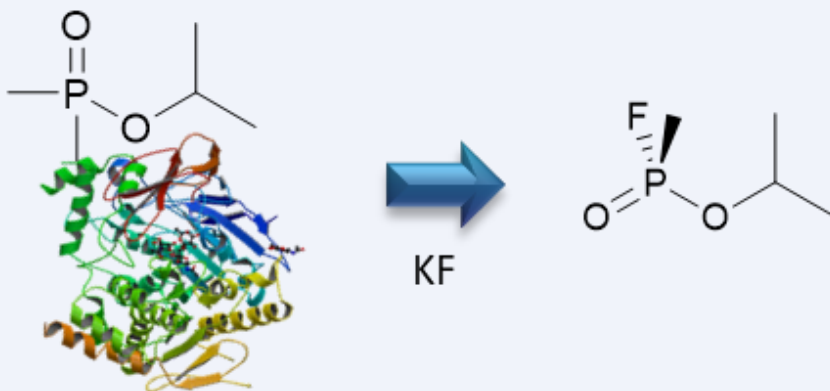


Results nerve agents

- Unambiguous verification requires identification of multiple biomarkers or two analytical techniques

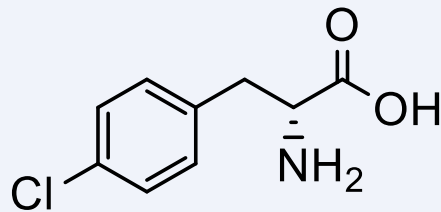
Targeted analysis with GC-MS/MS

- Regenerated sarin was detected in liquid whole blood and DBS (no intact sarin was detected)
- Regenerated Novichok could not unambiguously be determined (intact nerve agent still present in control without KF addition)
- Storage of 3 days

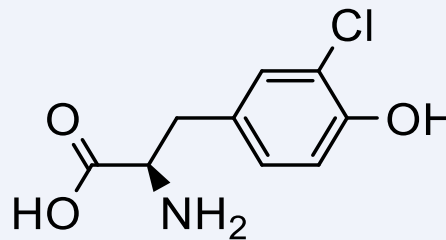


Results chlorine gas

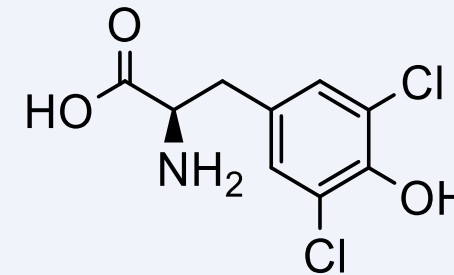
- Targeted LC-MS/MS analysis of 7 biomarkers
 - Chlorodopamines not detected in plasma
 - DNA-adduct Cl-Cyt detected in plasma (near LOD), not detected in blood
 - Cl-Phe, Cl-Tyr and 2Cl-Tyr identified in blood, DBS, and microcuvettes
 - Cl-Cyt and Cl-Phe for the first time identified as biomarker for chlorine gas exposure



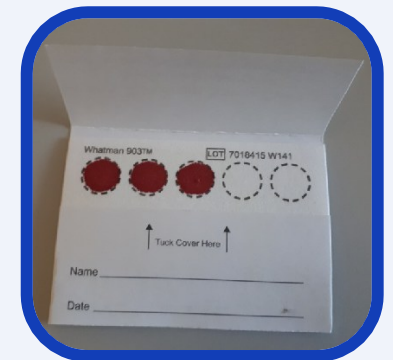
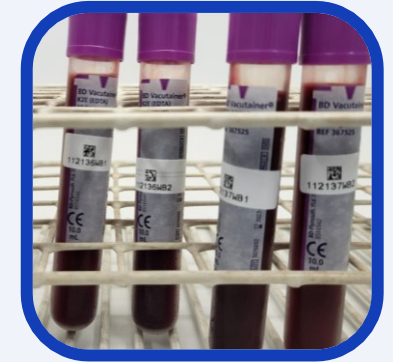
Chlorophenylalanine



Chlorotyrosine



Dichlorotyrosine

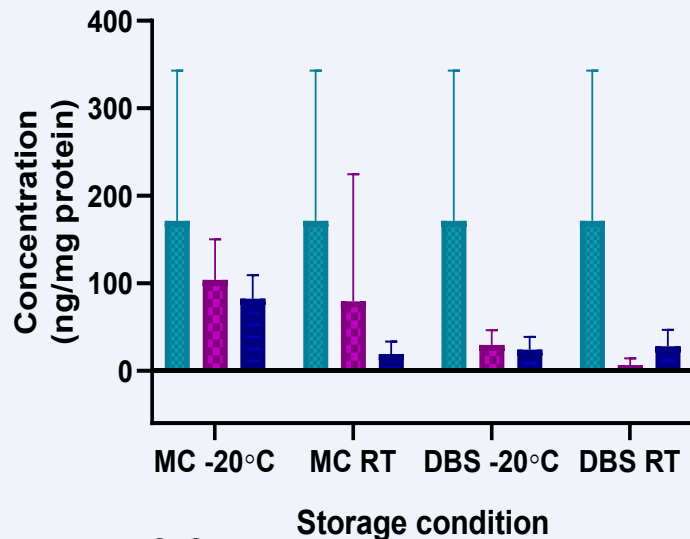


Results chlorine gas

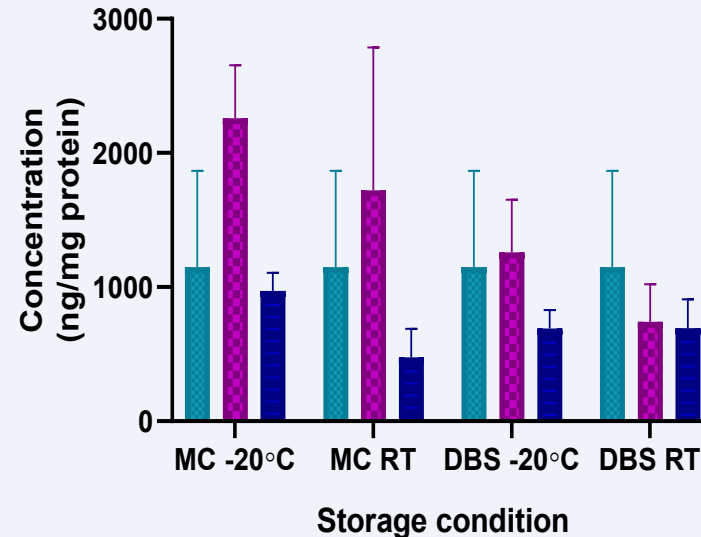
Storage conditions

- Biomarkers still detected after four weeks
- Highest stability in microsampling (MC) devices at -20 °C (-52%, -17%, -29%)
- Limited difference between DBS at different storage temperatures (-85%, -38%, =64%)
- Challenge: statistically significant variation between donors (3 Caucasian donors, 2 female, 1 male)

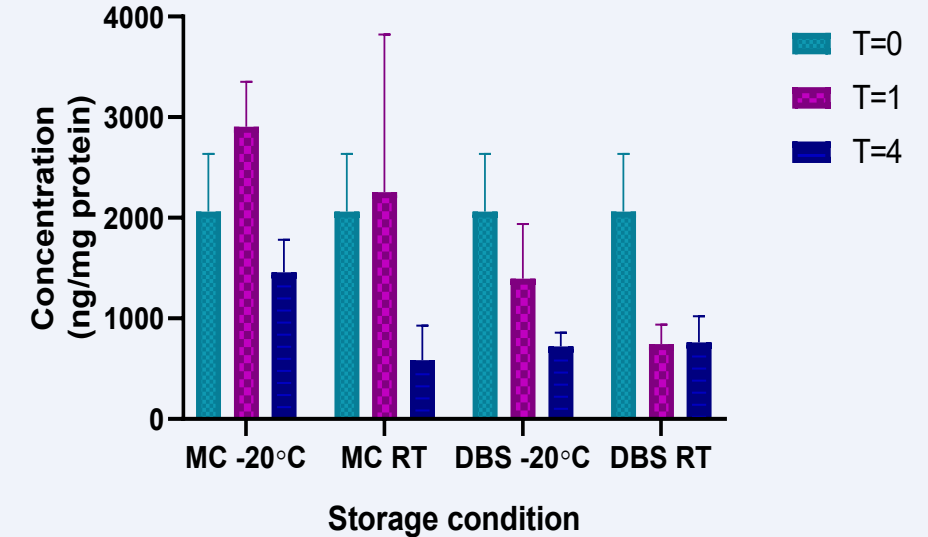
Chlorophenylalanine



Chlorotyrosine



Dichlorotyrosine



Conclusions

- Biomarkers could still be detected after storage for at least 1 month
- Less invasive sample collection required and possibility to store sample under ambient conditions

Nerve agents

- The cholinesterase activity was measured by a mobile test kit months after exposure
- Both LC-MS/MS and GC-MS/MS could be used for unambiguous verification

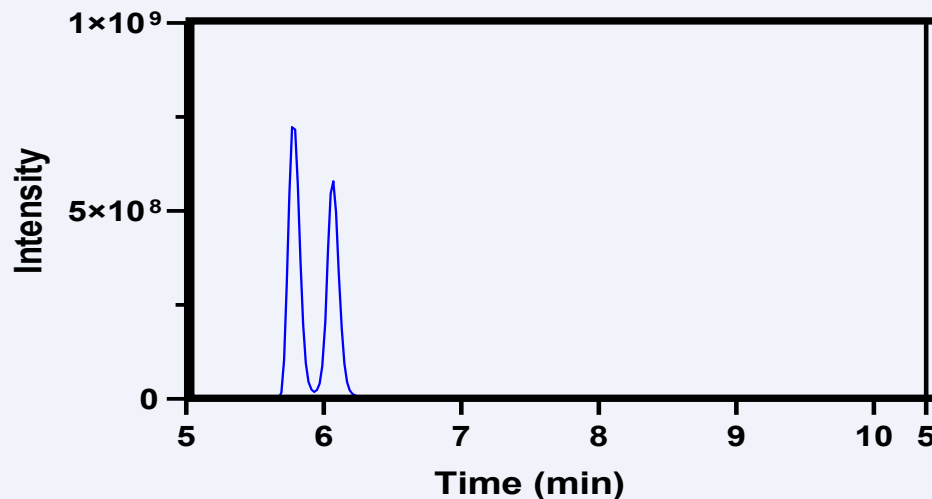
Chlorine gas

- Cl-Phe for the first time identified as biomarker for chlorine gas exposure



Outlook

- LC-TIMS-TOF-MS of 2, 3 and 4-chlorophenylalanine
- Compare Cl-biomarker concentrations with animals exposed to chlorine gas
- Improvement of sample preparation methods to lower detection limit
- Chemical profiling of nerve agents, search for synthesis specific compounds such as DIMP



Faster and more reliable biomarker detection will ultimately save lives!



**THANK YOU FOR
YOUR ATTENTION**

ACKNOWLEDGEMENTS

TNO

MARCEL VAN DER SCHANS

ALEX FIDDER

TOMAS VAN GRONINGEN

UNIVERSITY OF AMSTERDAM

ARIAN VAN ASTEN

OPCW

DAAN NOORT

WAGENINGEN UNIVERSITY &
RESEARCH

FLEUR VAN DIJK

DUTCH MINISTRY OF DEFENCE