Bioanalytical methods for monitoring of tricresylphosphate exposure

Harald John, Horst Thiermann

Bundeswehr Institute of Pharmacology and Toxicology, Munich
bleed air might contain TCP

TCP
- exposure
- isomers
- analysis

Biotransformation

Protein-adducts

Biomonitoring studies

Summary

ToCP:
- flame retardant in jet engine oil (approx. 0.03-0.06%)
- non-volatile (vapor pressure 5-10 exp-5 Pa)

http://www.injuredonflight.com/?tag=aerotoxic-syndrome
Tri-cresylphosphates

**structures and toxicity**

- **TCP**
  - exposure
  - isomers
  - analysis

**Biotransformation**

**Protein-adducts**

**Biomonitoring studies**

**Summary**
TCP detection by GC-MS

cockpit air and glare shield

Content

TCP
- exposure
- isomers
- analysis

Biotransformation

Protein-adducts

Biomonitoring studies

Summary

http://1080.plus/Ku0jmPRbPLE.video

TiBP: tri-iso-butyl-phosphate; TnAP: Tri-n-amyl-phosphate; TnBP: tri-n-butyl-phosphate; TPP: tri-phenyl-phosphate


Biotransformation in male cats


**Content**

TCP

Biotransform.
- analysis

Protein-adducts

Biomonitoring studies

Summary

**Diagram:**

- TOCP
- hydroxymethyl TOCP
- saligenin cyclic-o-toloyl phosphate
- CBDP
  - cresyl-benzodioxa-phosphorin-oxide

**Chemical Structures:**

- TOCP
- hydroxymethyl TOCP
- saligenin cyclic-o-toloyl phosphate
- CBDP
  - cresyl-benzodioxa-phosphorin-oxide

**CYP450:**

- 1A2
- 3A4

**Summary:**

- Biotransform.
- analysis
- Protein-adducts
- Biomonitoring studies
Detection of TOCP and biotransformation products

analysis of in vitro samples by LC-UV

**Content**

TCP

Biotransform. analysis

Protein-adducts

Biomonitoring studies

Summary

**Fig. 2.** Separation of TOCP and nine of its possible metabolites by reverse-phase HPLC, peaks:

1. o-cresyl dihydrogen phosphate
2. di-o-cresyl hydrogen phosphate
3. o-hydroxybenzyl alcohol
4. salicylic acid
5. o-cresol
6. salicylaldehyde
7. saligenin cyclic-o-tolyl phosphate
8. hydroxymethyl-TOCP
9. TOCP
10. dihydroxymethyl-TOCP.

Biotransformation in male cats

Adduct formation with proteins

**Protein-adducts**
- BChE analysis
- HSA analysis

**Biotransformation**

**TCP**

**Biomonitoring studies**

**Summary**

Poisoning by chemical warfare agents

incorporation and bioanalytical targets

Content
TCP
Biotransformation
Protein-adducts
- BChE analysis
- HSA analysis
Biomonitoring studies
Summary

Koller, Thiermann, John Wehrmed. Mschr. 58 (2014) 310-315
Analysis of protein adducts

general experimental procedure

Content

TCP
Biotransformation
Protein-adducts
- BChE analysis
- HSA analysis
Biomonitoring studies
Summary

John, Thiermann
Challenge 1 (2012) 9-13 (modified)
Butyrylcholinesterase adducts

human butyrylcholinesterase

BChE

EC 3.1.1.8 (P06276)

MW: 85 kDa (monomer)

AA: 574

$Conc_{blood}$: 3.5 µg/ml

Protein-adducts
- BChE analysis
- HSA analysis

Biotransformation

TCP

Biomonitoring studies

Summary
immunomagnetic separation (IMS) for extraction

TCP
Biotransformation

Protein-adducts
- BChE analysis
- HSA analysis

Biomonitoring studies

Summary

https://www.youtube.com/watch?v=VMw4Qzpfuo4

Fig. 1. Immunomagnetic bead separation of BChE coupled to LC-MS/MS analysis. This protocol efficiently purifies human BChE from 100 µl of plasma or less in one single step, and is readily adaptable for high-throughput analytical protocols. Samples are digested on-beads with chymotrypsin (CHY) and analyzed by LC-MS/MS.

Phosphorylated BChE as biomarker of exposure

BChE from plasma by LC-ESI MS/MS

enzymatic cleavage by pepsin

LC-ESI MS/MS

Content
- TCP
- Biotransformation

Protein-adducts
- BChE analysis
- HSA analysis

Biomonitoring studies

Summary
Phosphorylated BChE as biomarker of exposure

nonapeptide analyzed via LC-ESI MS/MS

liquid chromatography (LC)

tandem-mass spectrometry (MS/MS)

Serine adducts of BChE

MS/MS for sequence identification

\[
\text{TCP} \\
\text{Biotransformation} \\
\text{Protein-adducts} \\
\begin{itemize}
  \item BChE analysis
  \item HSA analysis
\end{itemize}
\text{Biomonitoring studies} \\
\text{Summary}

**Protein-adducts**
- BChE analysis
- HSA analysis

**Biomonitoring studies**

**Summary**

**Human Serum Albumin (HSA)**

- **P02768**
- **MW:** 68 kDa
- **AA:** 585
- **Conc**\(_{\text{blood}}\): 40 mg/ml
Phosphylated HSA as biomarker of exposure

HSA from plasma by LC-ESI MS/MS

TCP
Biotransformation
Protein-adducts
  - BChE analysis
  - HSA analysis
Biomonitoring studies
Summary

Content

TCP
Biotransformation
Protein-adducts
  - BChE analysis
  - HSA analysis
Biomonitoring studies
Summary

enzymatic cleavage by pepsin

enzymatic cleavage by trypsin

[Chemical structure and sequences]
Phosphylated HSA as biomarker of exposure

hexadecapeptide analyzed via MALDI TOF MS


TCP
Biotransformation
Protein-adducts
  - BChE analysis
  - HSA analysis
Biomonitoring studies
Summary

diverse peptides analyzed by LC-ESI MS/MS


enzymatic cleavage by pepsin

enzymatic cleavage by trypsin
CBDP might also react with other proteins than BChE and HSA

- military aircraft...48/78 < LOD of TCP...all < LOD of TOCP
- first BChE assay...6/12 passengers positive...exposure very low
- 15 healthy F-16 pilots...none had evidence of exposure
- KLM 737...no TOCP present...health effect unlikely due to TOCP
- TCP (50-100 ng/m³) does not exceed provisional tox thresholds
Summary

- TCP exists in different isomers
- Isomers are analyzed by GC-MS
- Biotransformation products (small molecules) analyzed by LC
- Protein-adducts (large molecules) are long-term markers
- Adducts are analyzed by LC-ESI MS/MS after proteolysis
- BChE-adduct allows monitoring of exposure
Thank you for your attention
Serine adducts of BChE

diverse adducts detected by µLC-ESI MS/MS

Table 1. LC-ESI MRM analysis of nonapeptides derived from phosphorylated BChE

<table>
<thead>
<tr>
<th>Adduct of</th>
<th>( t_r ) [min]</th>
<th>Precursor ion [M+H]⁺ at m/z</th>
<th>Qualifying ion (1)</th>
<th>Qualifying ion (2)</th>
<th>Qualifying ion (3)</th>
<th>Ion ratio (%b)qual (2)/qual (1)</th>
<th>Ion ratio (%b)qual (3)/qual (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-type nerve agents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>24.3</td>
<td>931.4</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>87</td>
<td>73</td>
</tr>
<tr>
<td>GA aged⁴</td>
<td>22.3</td>
<td>904.4</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>70</td>
<td>73</td>
</tr>
<tr>
<td>GB</td>
<td>21.7</td>
<td>916.4</td>
<td>778.3</td>
<td>673.3</td>
<td>602.2</td>
<td>92</td>
<td>64</td>
</tr>
<tr>
<td>d7-G8</td>
<td>21.5</td>
<td>923.4</td>
<td>778.3</td>
<td>673.3</td>
<td>602.2</td>
<td>84</td>
<td>57</td>
</tr>
<tr>
<td>GD</td>
<td>27.6</td>
<td>958.3</td>
<td>778.3</td>
<td>673.3</td>
<td>602.2</td>
<td>66</td>
<td>41</td>
</tr>
<tr>
<td>GF</td>
<td>26.3</td>
<td>956.3</td>
<td>778.3</td>
<td>673.3</td>
<td>602.2</td>
<td>80</td>
<td>42</td>
</tr>
<tr>
<td>d11H-GF</td>
<td>26.2</td>
<td>967.3</td>
<td>778.3</td>
<td>673.3</td>
<td>602.2</td>
<td>50</td>
<td>29</td>
</tr>
<tr>
<td>crotyl-G8⁵</td>
<td>n.d.</td>
<td>928.4</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>71</td>
<td>68</td>
</tr>
<tr>
<td>DF²</td>
<td>n.d.</td>
<td>876.4</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>71</td>
<td>68</td>
</tr>
<tr>
<td>G-agent aged⁴</td>
<td>20.9</td>
<td>874.4</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>71</td>
<td>68</td>
</tr>
<tr>
<td>V-type nerve agents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVX</td>
<td>24.4</td>
<td>930.3</td>
<td>778.3</td>
<td>673.3</td>
<td>602.2</td>
<td>87</td>
<td>58</td>
</tr>
<tr>
<td>RVX</td>
<td>24.3</td>
<td>930.3</td>
<td>778.3</td>
<td>673.3</td>
<td>602.2</td>
<td>91</td>
<td>71</td>
</tr>
<tr>
<td>VX</td>
<td>20.1</td>
<td>902.4</td>
<td>673.3</td>
<td>778.3</td>
<td>602.2</td>
<td>92</td>
<td>73</td>
</tr>
<tr>
<td>V-agent aged⁴</td>
<td>20.9</td>
<td>874.4</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>71</td>
<td>68</td>
</tr>
<tr>
<td>Organophosphate pesticides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMP</td>
<td>19.4</td>
<td>904.3</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>83</td>
<td>73</td>
</tr>
<tr>
<td>d6-DMP</td>
<td>19.4</td>
<td>910.3</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>77</td>
<td>71</td>
</tr>
<tr>
<td>DMP aged</td>
<td>21.1</td>
<td>890.3</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>77</td>
<td>75</td>
</tr>
<tr>
<td>d6-DMP aged</td>
<td>21.1</td>
<td>893.3</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>77</td>
<td>75</td>
</tr>
<tr>
<td>DEP</td>
<td>22.6</td>
<td>932.3</td>
<td>673.3</td>
<td>778.3</td>
<td>602.2</td>
<td>95</td>
<td>73</td>
</tr>
<tr>
<td>d10-DEP</td>
<td>22.6</td>
<td>942.3</td>
<td>778.3</td>
<td>673.3</td>
<td>602.2</td>
<td>98</td>
<td>67</td>
</tr>
<tr>
<td>DEP aged</td>
<td>22.3</td>
<td>904.3</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>70</td>
<td>73</td>
</tr>
<tr>
<td>d10-DEP aged</td>
<td>22.5</td>
<td>909.3</td>
<td>673.3</td>
<td>602.2</td>
<td>778.3</td>
<td>70</td>
<td>73</td>
</tr>
</tbody>
</table>

⁴Loss of NMe₂
⁵RSD of ion ratios was typically about ± 4%
⁶only detected as aged product
⁷only detected as hydrolyzed product being identical to aged G-agent adducts
⁸aged G-agent adduct is common for aged GB, GB(d7), GD, GF, GF(d11) and crotyl-G8 and identical to aged adducts of VX, CVX, RVX as well as to hydrolyzed adduct of DF crotyl-G8, crotylsarin; CVX, Chinese VX; DEP, diethyl-phosphate pesticide; d10-DEP, 10-fold deuterated DEP; DF, difluoromethyl phosphonate; DMP, dimethyl-phosphate pesticide; d6-DMP, 6-fold deuterated DMP; GA, tabun; GB, sarin; GB(d7), sevenfold deuterated GB; GD, soman; GF, cyclosarin; GF(d11), elevenfold deuterated GF; qual, qualifier ion, qual (1) is the most intense followed by qual (2) and qual (3); RVX, Russian VX

Serine adducts of BChE

μHPLC-UV/ESI MS/MS of BChE-OP adducts

Content
- TCP
- Biotransformation
  - Protein-adducts
    - BChE analysis
    - HSA analysis
- Biomonitoring studies
- Summary