From symptoms of intoxication to identification of the noxious agents: challenges in ambient and biomonitoring analyses

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7th International Workshop How to handle imported containers safely, Berlin, 22-23 05-2014

Pesticides used as fumigants are often at least as poisonous to humans as to the pests against which they are used” IMO, 2005 (International Maritime Organisation).

The fumigation of transported goods was originally introduced to protect plants and foodstuffs from infestation and destruction by pests 

FAO (Food and Agriculture Organization) edict, ISPM No. 15, requires fumigation by methyl bromide or heat treatment of the wooden packaging and flooring material (FAO, 2007). environmental reason (spreading alien species)
Container-Air-Measurements in Hamburg and Rotterdam
> 4000 Container air measurements (TD-GCMS-Analyses)
Studies: 2007-2013

**Aim:** to determine the fumigant residues in containers arriving harbor areas

*ISPM No. 15,* requires fumigation by methyl bromide or heat treatment of the wooden packaging and flooring material (FAO, 2007).

**Practice:**
- Fumigation is becoming a standard procedure, especially in south-east Asia
- **Other fumigants than methyl bromide (bromomethane) are used**
- There is no labeling (< 3%)

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Data showed that additionally to the declared hazardous cargoes, more than 20% of the containers were contaminated with various industrial chemicals not declared as "dangerous".

*Baur X, Poschadel B, Budnik LT. Occup Environ Med (2010)*

Container-Air-Measurements in Hamburg and Rotterdam
> 4000 Container air measurements (GCMS, Flow tube mass spectrometry, TD-GCMS-Analyses) Studies: 2006-2013

1. Study in Hamburg and Rotterdam 2006-2007 (n=2013 random samples)
*Published in: Baur X, Poschadel B, Budnik LT. Occup Environ Med (2010)*

Out of 2113 sampled import containers, 1478 were chemically contaminated above chronic reference exposure levels (RELs) and 761 encompass more highly contaminated containers above acute RELs

RELs (US Office of Environmental health Hazard Assessment and National Institute for Occupational Safety and Health, NIOSH) are independent science-based recommendations rather than legally applicable standards.*
Container-Air-Measurements in Hamburg and Rotterdam
> 4000 Container air measurements

1. Study in Hamburg and Rotterdam 2006-2008 (2113 samples)
Published in: Baur X, Poschadel B, Budnik LT. Occup Environ Med (2010)

REL Values in ppb (µL/m³): formaldehyde 2.44, 76; benzene 18, 410; bromomethane (methyl bromide) 1.28, 1000; hydrogen phosphide 0.6, 300; 1,2-dichloroethane (ethylene dichloride) 98, 1000; trichloronitromethane 0.06, 100; ethylene oxide 16.6, 100; sulphuryl difluoride 5.13, 5000; hydrogen cyanide 8.2, 300.

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Container-Air-Measurements in Hamburg and Rotterdam
> 4000 Container air measurements (TD-GCMS-Analyses)

2. Study in Hamburg (additional 1201 random samples)

Methyl Bromide (Bromomethane), MeBr, Ethylene dichloride 1,2.dicholoethane) EDC
Container-Air-Measurements in Hamburg and Rotterdam

> 4000 Container air measurements

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Methyl Bromide (Bromomethane), MeBr, Ethylene dichloride 1,2.dichloethane) EDC

Fumigants above acute Reference Exposure Limits (community)
Fumigants above occupational exposure limits (OEL)

% of total Containers

>acute RELs

>occupat. exp. limits

2006 2007 2008
MeBr EDC

Container air measurements Studies: 2007-2013

3/4. Study in Hamburg (additional 53+1000 random samples)

Fahrenholtz S, Hünerfuss H, Baur X, Budnik LT. J Chromatography A 2010,
Fahrenholtz et al., in preparation

Industrial chemicals found:
Solvents and fumigant additives
(high concentrations)

benzene
toluene
ethyl benzene
dichloromethane
tetrachloromethane

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Off gassing from container transported products

Example
Toys: (doll’s playhouse)

Container and other transport units are un-loaded deep within the country sides outside the harbour areas (those un-loading are often migrant workers, without medical support or coverage)

Workers and Bystanders can be exposed to fumigant residues in storage rooms
How to detect exposure?

Human Biomonitoring

- Ambient-monitoring
- Exposure-Biomonitoring
- Effect-Biomonitoring
- Diagnosis

inhalative

dermal

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Major problem: sampling time

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Short-term high exposure symptoms: (latency period of 2-48 h - 3 days)
- Central nervous system depression, neuropsychiatry abnormalities
  (headache, nausea, dizziness)
- Inflammation of the bronchi and lung, Pulmonary edema
  (flu like symptoms, phlegm, chest pain, shortness of breath)
Exposure to methyl bromide

- Incorporation
  - Lung (exhalation MeBr/ CO₂) ~40%
  - Skin
  - Kidneys (elimination in urine) ~40%
  - Biotransformation in the liver (i.e. GST/ CYP)
  - ~10% persists in the body

- Found in: bile, liver, adipose tissue, serum and urine (post mortem)


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Only extremely high concentrations can be detected late

Max. elimin. time described

<table>
<thead>
<tr>
<th>elimination time (days)</th>
<th>methyl bromide in blood (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>300</td>
</tr>
<tr>
<td>10</td>
<td>200</td>
</tr>
<tr>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Usually</td>
<td>1-3 days after exposure possible</td>
</tr>
</tbody>
</table>

Own study: 164 individuals with presumed intoxication to fumigants, plus 30 controls.
78% male and 22% female subjects at median age of 44; 66% smoker, 34% non-smoker

The exposure assessment was performed with human biomonitoring (methyl bromide, ethylene dichloride, dichloromethane, other halo-alkanes).

86 patients with confirmed exposures to halo-alkanes above the non-cancer reference doses (RfD), but (for many) lower than the occupational exposure limits

Published in: Budnik et al., PLoS ONE (2013)
Mitochondrial DNA has a potential to serve as a biomarker recognizing vulnerable risk groups after exposure to toxic/carcinogenic chemicals

Published in: Budnik et al., PLoS ONE (2013)

intox. target organs release cell-free DNA into the bloodstream

Mitochondrial DNA has a potential to serve as a biomarker recognizing vulnerable risk groups after exposure to toxic/carcinogenic chemicals

Published in: Budnik et al., PLoS ONE (2013)

Enhanced cell free mtDNA levels in patients exposed to halogenated hydrocarbon pesticides (methyl bromide)

Published in: Budnik et al., PLoS ONE (2013)
The relative amounts of mtDNA-79 in serum were positively associated with the lag-time after intoxication of these chemicals ($r=0.99$, $p<0.0001$).

Published in: Budnik et al., PLoS ONE (2013)

Patient subgroups
- P1 current intoxication
- P2 short-term past exposure (weeks, up to 4 months)
- P3 long-term past exposure (interim time at least 5 months)

The specificity for this biomarker increased from 30% to 97% several months post-exposure in patients with intoxication symptoms.
Case report

European medium size company importing electronic construction parts from South America and South East Asia.

6 storage room workers were un-packaging wooden pellets and/or paper boxes covered with plastic containing with construction parts.

3 Workers were claiming on:
- itchy skin
- very red eyes
- headache
- pins and needles in the legs
- dizziness
- breathing difficulties
- increasing irritability

Patients
1. f, ns
2. f, s+
3. m, s++
4. f, s+
5. f, s++
6. m, ns

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Long lag time post exposure

Day 2
Industrial physician is collecting blood samples (Serum/EDTA-Blood, NaF-Blood, Urine) and send samples to the commercial clinical chemistry laboratory to proof for the intoxication parameter.

No bromide or methyl bromide measurements or measurements for other intoxication parameter were performed.
(Only differential blood picture was performed, the samples were destroyed)

Governmental industrial hygienist is taking air samples.

Results:
2.5 ppm-200 ppm methyl bromide

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It is still possible to extrapolate the exposure from the hemoglobin adduct levels (for accidental exposures)

**N-Methylvalin**
NS = 320 pmol/g Ggobin, S = 390 pmol/g globin

**N-Hydroxyethylvalin**
NS = <75 pmol/g Globin, S = 150 pmol/g globin

Existing problems in many small or medium size companies through Europe

- no risk assessment
- no (or not sufficient) air measurements (no correct measurements performed)
- possible intoxication after chronic exposure from off gassing products, packing materials in storage facilities is not considered!
- No human biomonitoring samples collected to confirm the exposures (or wrong sampling time, wrong matrices, wrong samples etc.)
- Destroying valuable human sample in the clinical chemistry labors
Conclusions

In an emergency, appropriate protection measures can be undertaken only if the type of chemical that has been released is known precisely.

Personal air samples should be taken along the supply chain, e.g. when unloading the containers for reloading at harbor ports and human-biomonitoring should be performed for vulnerable groups.

Contribution
Co-workers & Research Partners

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- ............
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New chemical health risks in transportation and warehousing due to the process of globalization

Thank you