Acrylonitrile exposure in the general population following a major train accident in Belgium: a human biomonitoring study

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Introduction

- May 4 2013 (2h30 AM): rail road accident in Wetteren: derailment of freight cars, explosion, and fire

- Three different substances in freight cars:
  - Butadiene
  - Triethylaluminium
  - Acrylonitrile (ACN)

- Water was used to extinguish the fire
Acrylonitrile metabolism

Acrylonitrile (=vinylcyanide, VCN)

- **Conjugation**
  - VCN-glutathione

- **CYP2E1: Oxidation**
  - Cyanoethylene oxide (CEO)
    - **Conjugation**
      - CEO-glutathione
    - **Epoxide Hydrolase (EH)**
      - Epoxide hydrolase
    - **Rhodanese**
      - Thiocyanate (SCN)
        - Eliminated in the urine
First impressions on site…
Although acrylonitrile is heavier than air it can ascend in the sewer system of houses that have either no or a failing isolation from the main sewer system.
Introduction

- One resident was found deceased in his house with his dead dog
- One resident experienced cardiac arrest, but was successfully resuscitated
- One resident developed deep coma
- Around 200 residents were hospitalised
- More than 2000 residents were evacuated
"TOESTAND ECHT ALARMEREND"

Expert waarschuwt na treinramp: Verhoogde kans op longkanker’

"Bloed laten invriezen als bewijs voor later"

08/05/13, 07u59
MODERN COMMUNICATION ON THE CHEMICAL DISASTER IN WETTEREN
Introduction

Human biomonitoring (HBM)

- Pressure from the media and public opinion “to do something”
- Pro
  - Every individual has the right to know whether he or she has been exposed
  - Proof of exposure can be used in judicial/insurance matters when developing e.g. cancer
  - Interesting from a scientific viewpoint
  - Information can be used in management of future incidents
- Con
  - Psychological impact
  - High cost
  - Exposure does not mean risk
Introduction

Decision to carry out HBM:
Need for a fast study setup & start

- Train accident: 4/5
- Decision HBM: 15/5
- Invitation to participate: 17/5
- Sampling residents: 18-25/5
- Sampling rescue workers: 21/5-28/6
- Sample Analysis
- Presentation results residents: 31/8
Objectives

General Objective

To assess the human exposure to acrylonitrile (ACN) in the populations with highest suspected exposure, i.e. the residents of Wetteren and the persons that assisted professionally in the accident.
Objectives

Specific Objectives in the residents

1. To determine human exposure to ACN by means of HBM

2. To assess the distribution pattern of human ACN exposure
Methods

1. Study population and area
2. Data collection
3. Biomonitoring
Train accident

Railroad

Sewerage system

Prevailing wind directions at the moment of and in the days following the accident

Zone 1 (EZ1): 250m perimeter of the evacuation zone that was evacuated at night in the hours following the accident

Zone 2 (EZ2): streets parallel with the sewage system and downwind of the train accident that were evacuated later, i.e. in the days following the accident

Zone of ‘Controls’: the commune of Wetteren, EZ1 and EZ2 excluded
Study population and area

The **eligible population** consisted of:

- **EZ1**: All the residents living within EZ1
- **EZ2**: All the residents living in EZ2 who presented at the emergency services
- **EZ2** (evacuated, but did not present at the emergency services): 10% sample of the residents living in EZ2 who were evacuated, but did not present at the emergency services
- **OUT EZ**: All the residents of Wetteren living outside the EZ who presented at the emergency services

Children below the age of 10 were not included in the study.
## Study population and area

<table>
<thead>
<tr>
<th></th>
<th>EZ1</th>
<th>H</th>
<th>EZ2</th>
<th></th>
<th>H</th>
<th>OUT EZ</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eligible (n)</td>
<td>40</td>
<td>99</td>
<td>219</td>
<td>116</td>
<td></td>
<td></td>
<td>474</td>
</tr>
<tr>
<td>Participants (n, %)</td>
<td>26  (65.0)</td>
<td>47 (47.5)</td>
<td>124 (56.6)</td>
<td>45 (38.8)</td>
<td></td>
<td>242 (51.1)</td>
<td></td>
</tr>
<tr>
<td>Participants (n)</td>
<td>26</td>
<td>47</td>
<td>124</td>
<td>45</td>
<td></td>
<td>242</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.5 (39.3-68.5)</td>
<td>47.0 (34.0-57.3)</td>
<td>48.0 (33.3-61.0)</td>
<td>34.0 (24.5-46.5)</td>
<td>45.0 (32.0-58.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men n (%)</td>
<td>13  (50)</td>
<td>19 (40.4)</td>
<td>51 (41.1)</td>
<td>17 (37.8)</td>
<td>100 (41.3)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Methods

1. Study population and area
2. Data collection
3. Biomonitoring
Data collection

**Sampling**: from May 18 till May 25 2013 – at some local places

**Coordination**: FPS Health, Food Chain Safety and Environment + collaboration with the Province, the commune of Wetteren, and local general practitioners

**Ethics**: study protocol approved by UZ Ghent
Informed consent signed by all participants
Data collection

• **Blood**: N-2-cyanoethylvaline (CEV)
  → Biomarker for exposure to ACN

• **Urine**: cotinine
  → Biomarker for tobacco smoke exposure

• **Questionnaire**: Demographic variables, smoking status, sampling day and hour, detailed info about presence near the accident (time-space), symptoms.
Data collection

*N-2-cyanoethylvaline (CEV)*

- CEV is an adduct resulting from the binding of a metabolite of acrylonitrile to haemoglobin
- CEV is not toxic
- Highly sensitive and specific for exposure to acrylonitrile
- CEV level declines linearly to the background value during the lifespan of the circulating erythrocytes (126 days)
- Cigarette smoke contains acrylonitrile
Methods

1. Study population and area
2. Data collection
3. Biomonitoring
Biomonitoring

CEV analyses

• Pretreatment at WIV-ISP → lysate of erythrocytes

• Because of need of substantial analyzing capacity
  → 3 German labs (G-Equas – additional interlaboratory comparison: comparable results among labs)

• Adduct dosimetry: modified Edman degradation
  (Von Sittert et al, 1986; Tornqvist et al, 1997)
## Biomonitoring

### Cotinine analyses
- online-SPE-UPLC-MS/-MS (De Cremer *et al*, 2013)

<table>
<thead>
<tr>
<th>Urinary cotinine (µg/L)</th>
<th>EZ1</th>
<th>H</th>
<th>EZ2</th>
<th>H</th>
<th>OUT EZ</th>
<th>Total (n=242)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;100</td>
<td>6 (23.1)</td>
<td>14 (29.8)</td>
<td>35 (28.2)</td>
<td>19 (42.2)</td>
<td>74 (30.6)</td>
<td></td>
</tr>
<tr>
<td>≤100</td>
<td>20 (76.9)</td>
<td>33 (70.2)</td>
<td>89 (71.8)</td>
<td>26 (57.8)</td>
<td>168 (69.4)</td>
<td></td>
</tr>
<tr>
<td>Non-smokers n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>20</td>
<td>32</td>
<td>87</td>
<td>25</td>
<td>164</td>
<td></td>
</tr>
<tr>
<td>25-100</td>
<td>1*</td>
<td>2**</td>
<td>1***</td>
<td></td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

*1 self-reported ‘ex-smoker’; **1 self-reported ‘ex-smoker’ and 1 self-reported ‘non-smoker’; ***1 self-reported ‘non-smoker’
Results

1. CEV concentrations in- versus outside the EZ
2. CEV concentrations in the different EZ zones
3. Spatial distribution of CEV concentrations
# CEV(*) in- vs. outside the EZ

<table>
<thead>
<tr>
<th>Non-smokers</th>
<th><strong>EZ FULL</strong></th>
<th></th>
<th></th>
<th></th>
<th><strong>OUT EZ</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(n=142)</strong></td>
<td>(n=26)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td>206.7</td>
<td>(1163.4)</td>
<td></td>
<td></td>
<td>71.3</td>
</tr>
<tr>
<td><strong>Median (IQR)</strong></td>
<td>6.9</td>
<td>(3.4-16.8)</td>
<td></td>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td><strong>P95</strong></td>
<td>634.6</td>
<td></td>
<td></td>
<td></td>
<td>21.9</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>12614.8</td>
<td></td>
<td></td>
<td></td>
<td>1725.5</td>
</tr>
<tr>
<td><strong>&gt;ref value n(%)</strong></td>
<td>53 (37.3)*</td>
<td></td>
<td></td>
<td></td>
<td>3 (11.5)</td>
</tr>
</tbody>
</table>

* CEV concentrations extrapolated at the moment of the train accident (pmol/g globin)

†10 pmol/g globin
### CEV(*) in- vs. outside the EZ

<table>
<thead>
<tr>
<th>Non-smokers</th>
<th>EZ FULL (n=142)</th>
<th></th>
<th></th>
<th></th>
<th>OUT EZ (n=26)</th>
<th>OUT EZ (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>206.7 (1163.4)</td>
<td></td>
<td></td>
<td>71.3 (337.4)</td>
<td>4.3 (3.3)</td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>6.9 (3.4-16.8)</td>
<td></td>
<td></td>
<td>4.0 (2.3-6.6)</td>
<td>3.0 (2.3-5.7)</td>
<td></td>
</tr>
<tr>
<td>P95</td>
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<td></td>
<td></td>
<td>21.9</td>
<td>8.2</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>1725.5</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>&gt;ref value n(%)†</td>
<td>53 (37.3)*</td>
<td>3 (11.5)</td>
<td>1 (4.2)*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†10 pmol/g globin  # Corrected for localisation
* P value = 0.003
* CEV concentrations extrapolated at the moment of the train accident (pmol/g globin)
Results

1. CEV concentrations in versus outside the EZ
2. CEV concentrations in the different EZ zones
3. Spatial distribution of CEV concentrations
CEV(*) in the different EZ zones

<table>
<thead>
<tr>
<th>Non-smokers</th>
<th>EZ FULL (n=142)</th>
<th>EZ1 (n=20)</th>
<th>EZ2 (n=122)</th>
<th>OUT EZ (n=26)</th>
<th>OUT EZ (n=24#)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (SD)</strong></td>
<td>206.7 (1163.4)</td>
<td>13.7 (15.4)</td>
<td>238.9 (1253.0)</td>
<td>71.3 (337.4)</td>
<td>4.3 (3.3)</td>
</tr>
<tr>
<td><strong>Median (IQR)</strong></td>
<td>6.9 (3.4-16.8)</td>
<td>9.9 (4.0-14.4)</td>
<td>6.9 (3.4-18.1)</td>
<td>4.0 (2.3-6.6)</td>
<td>3.0 (2.3-5.7)</td>
</tr>
<tr>
<td><strong>P95</strong></td>
<td>634.6</td>
<td>35.9</td>
<td>1151.7</td>
<td>21.9</td>
<td>8.2</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>12614.8</td>
<td>64.8</td>
<td>12614.8</td>
<td>1725.5</td>
<td>16.2</td>
</tr>
<tr>
<td><strong>&gt;ref value n(%)</strong></td>
<td>53 (37.3)*</td>
<td>10 (50.0)^</td>
<td>43 (35.2)^</td>
<td>3 (11.5)</td>
<td>1 (4.2)*</td>
</tr>
</tbody>
</table>

* P value = 0.003; ^P value = 0.310
* CEV concentrations extrapolated at the moment of the train accident (pmol/g globin)

†10 pmol/g globin    # Corrected for localisation
### CEV(*) in the different EZ zones

<table>
<thead>
<tr>
<th></th>
<th>Non-smokers (n=142)</th>
<th>EZ1 (n=20)</th>
<th>EZ2 (n=122)</th>
<th>EZfull (n=33)</th>
<th>OUT EZ (n=89)</th>
<th>OUT EZ (n=26)</th>
<th>OUT EZ (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (SD)</strong></td>
<td>206.7 (1163.4)</td>
<td>13.7 (15.4)</td>
<td>238.9 (1253.0)</td>
<td>662.8 (2325.0)</td>
<td>80.9 (317.0)</td>
<td>71.3 (337.4)</td>
<td>4.3 (3.3)</td>
</tr>
<tr>
<td><strong>Median (IQR)</strong></td>
<td>6.9 (3.4-16.8)</td>
<td>9.9 (4.0-14.4)</td>
<td>6.9 (3.4-18.1)</td>
<td>8.0 (5.7-67.6)</td>
<td>6.8 (3.4-15.8)</td>
<td>4.0 (2.3-6.6)</td>
<td>3.0 (2.3-5.7)</td>
</tr>
<tr>
<td><strong>P95</strong></td>
<td>634.6</td>
<td>35.9</td>
<td>1151.7</td>
<td>2760.6</td>
<td>339.5</td>
<td>21.9</td>
<td>8.2</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>12614.8</td>
<td>64.8</td>
<td>12614.8</td>
<td>12614.8</td>
<td>2128.5</td>
<td>1725.5</td>
<td>16.2</td>
</tr>
<tr>
<td><strong>&gt;ref value n(%)</strong></td>
<td>53 (37.3)*</td>
<td>10 (50.0)^</td>
<td>43 (35.2)^</td>
<td>13 (39.3)$</td>
<td>30 (33.7)$</td>
<td>3 (11.5)</td>
<td>1 (4.2)*</td>
</tr>
</tbody>
</table>

*10 pmol/g globin  # Corrected for localisation

* P value = 0.003; ^P value = 0.310 ; $ P value = 0.711

* CEV concentrations extrapolated at the moment of the train accident (pmol/g globin)
Results

1. CEV concentrations in- versus outside the EZ
2. CEV concentrations in the different EZ zones
3. Spatial distribution of CEV concentrations
Extrapolated CEV concentration ≤ 10 pmol/g globin
Extrapolated CEV concentration > 10 pmol/g globin
Has been in the EZ at the moment of or in the days following the train accident
Extrapolated CEV concentrations of 4951 and 12615 pmol/g globin
Conclusions

In the **non-smokers**, some clear patterns with regard to ACN exposure following the train accident were seen in function of the subgroups.

1° The evacuation zone (EZ) seems to have been determined well by the Crisis Management Team.
In the non-smokers, some clear patterns with regard to ACN exposure following the train accident were seen in function of the subgroups.

1° The evacuation zone (EZ) seems to have been determined well by the Crisis Management Team.

Outside the EZ, CEV concentrations above the reference level were only observed in 4.2% of the non-smokers, which is in line with what is to be expected on the basis of the definition of the reference value, i.e. the 95th percentile in a non-exposed population.
Conclusions

In the non-smokers, some clear patterns with regard to ACN exposure following the train accident were seen in function of the subgroups.

1° The evacuation zone (EZ) seems to have been determined well by the Crisis Management Team.

2° The timing of evacuation seems to have had an effect on the CEV concentrations, especially on the occurrence of higher concentrations.
Conclusions

In the non-smokers, some clear patterns with regard to ACN exposure following the train accident were seen in function of the subgroups.

2° The timing of evacuation seems to have had an effect on the CEV concentrations, especially on the occurrence of higher concentrations.

In EZ1, 50.0% of the non-smokers exceeded the reference level with a maximum of 65 pmol/g globin. In EZ2, 35.0% had values above the reference level with maxima in the order of magnitude of several thousands of pmol/g globin. Whether these higher values reflect a more intense exposure or rather a more prolonged exposure (leading to accumulation of the biomonitoring parameter) is not known.
Conclusions

In the non-smokers, some clear patterns with regard to ACN exposure following the train accident were seen in function of the subgroups.

1° The evacuation zone (EZ) seems to have been determined well by the Crisis Management Team.

2° The timing of evacuation seems to have had an effect on the CEV concentrations, especially on the occurrence of higher concentrations.

3° The CEV concentrations above the reference value were observed in the street along the railway and particularly in the streets corresponding to the sewerage system.
Conclusions

In the **non-smokers**, some clear patterns with regard to ACN exposure following the train accident were seen in function of the subgroups.

3° The CEV concentrations above the reference value were observed in the street along the railway and particularly in the streets corresponding to the sewerage system.

The differences in the two populations along the sewerage system may be due to (i) differences in connection to the sewerage system and/or (ii) different places of dwelling in the building of the individuals.
Conclusions

In contrast to the non-smokers of the EZ, no clear pattern could be distinguished among the different subgroups of the smokers in the EZ.

Ideally, for every individual smoker, a personal background value should be known to draw conclusions and still then, it is likely that the CEV background imposed by tobacco exposure will mask a mild exposure to ACN. Hence, no formal conclusions can be inferred from the CEV values observed in smokers.
Conclusions

Biological monitoring following chemical disasters has been recommended as part of disaster management in order to objectivate the internal human exposure.

The results found in this study are in excellent accordance with duration and assumed intensity of exposure, respectively. Thus, the recent study confirms the applicability of the biomonitoring approach for risk assessment and studying the causality of effects of the victims of such a chemical disaster.
Acknowledgments

This study has been financed by the FPS Health, Food Chain Safety and Environment, following an advice of the Belgian Minister of Social Affairs and Public Health. The authors thank the inhabitants of Wetteren for their participation in the study and the local practitioners for their assistance in the sampling and their close involvement throughout the whole study. The authors thank Geert Gijs, crisis coordinator of the FPS Health, Food Chain Safety and Environment, and his team for the logistical organisation of the study. The authors are grateful to Wesley Van Dessel and Jan Eyckmans, respective heads of the communication services of the WIV-ISP and of the FSP Health, Food Chain Safety and Environment, and their team members, for the continuous support in the communication of the study and its results. The authors also want to thank Stéphanie Fraselle and her colleagues (WIV-ISP) for the preparation of the blood samples before sending them to the German labs. Finally, the authors thank Sabine Janssens and Tadek Krzywania and his team (WIV-ISP) for the enormous efforts with regard to data input, data processing and administrative support.
Thank you for your attention!