Toxicology Letters 231 (2014) 352–359

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Acrylonitrile exposure assessment in the emergency responders of a major train accident in Belgium: A human biomonitoring study

A. Van Nieuwenhuyse [a,*,1], S. Fierens [a,1], T. De Smedt [a], K. De Cremer [b], C. Vleminckx [a], B. Mertens [a], I. Van Overmeire [b], M. Bader [c], P. De Paepe [d], T. Göen [e], B. Nemery [f], T. Schettgen [g], C. Stove [h], H. Van Oyen [a], J. Van Loco [b]

[a] Scientific Institute of Public Health, Department Public Health and Surveillance, J. WytsmanStraat 14, Brussels 1050, Belgium
[b] Scientific Institute of Public Health, Department Food, Medicines and Consumer Products, Brussels,Belgium
[c] BASF SE, Occupational Medicine & Health Protection, Ludwigshafen, Germany
[d] Ghent University Hospital, Department of Emergency Medicine, Ghent, Belgium
[e] University of Erlangen-Nuremberg, Institute and Outpatient Clinic of Occupational, Social and Environmental Medicine, Erlangen, Germany
[f] Katholieke Universiteit Leuven, Department of Public Health and Primary Care, Centre for Environment and Health, Leuven, Belgium
[g] RWTH Aachen University, Institute for Occupational and Social Medicine, Medical Faculty, Aachen, Germany
[h] Ghent University, Laboratory of Toxicology, Department of Bioanalysis, Ghent, Belgium

HIGHLIGHTS

- *N*-2 cyanoethylvaline (CEV) was measured after a train accident with acrylonitrile.
- 26% of the non-smoking emergency responders exceeded the CEV reference value.
- CEV concentrations were comparable with background levels for a smoking population.
- CEV concentrations remained relatively moderate as compared to the local population.

ARTICLE INFO

Article history:
Received 14 March 2014
Received in revised form 8 August 2014
Accepted 11 August 2014
Available online 13 August 2014

Keywords:
Human biomonitoring
Acrylonitrile
Exposure assessment
Emergency responders
Train accident
N-2-cyanoethylvaline

ABSTRACT

Background: On May 4, 2013, a train transporting chemicals derailed in Wetteren, Belgium. Several tanks loaded with acrylonitrile (ACN) exploded, resulting in a fire and a leakage of ACN.

Objectives: To determine exposure to ACN and to assess discriminating factors for ACN exposure in the emergency responders involved in the on-site management of the train accident.

Methods: The study population consisted of 841 emergency responders. Between May 21 and June 28, they gave blood for the determination of *N*-2-cyanoethylvaline (CEV) hemoglobin adducts and urine for the measurement of cotinine. They also filled in a short questionnaire.

Results: 163 (26%) non-smokers and 55 (27%) smokers showed CEV concentrations above the reference values of 10 and 200 pmol/g globin, respectively. The 95th percentile in the non-smokers was 73 pmol/g globin and the maximum was 452 pmol/g globin. ACN exposure among the non-smokers was predicted by (1) the distance to the accident, (2) the duration of exposure, and (3) the occupational function.

Discussion and conclusion: Emergency responders involved in the on-site management of the train accident were clearly exposed to ACN from the accident. However, the extent of exposure remained relatively moderate with CEV concentrations staying within the ranges described in literature as background for a smoking population. Moreover, the exposure was less pronounced in the emergency responders as compared to that in the local population.

Abbreviations: ACN, Acrylonitrile; CART, Classification And Regression Tree; CEV, *N*-2-cyanoethylvaline; IARC, International Agency for Research on Cancer; WIV-ISP, Wetenschappelijk Instituut Volksgezondheid – Institut Scientifique de Santé Publique.

*Corresponding author. Tel.: +32 2 642 57 50.

[1] These authors have contributed equally.

http://dx.doi.org/10.1016/j.toxlet.2014.08.013

1. Introduction

In the night of Saturday May 4 2013, a train transporting butadiene, triethylaluminium and acrylonitrile (ACN) derailed in the village of Wetteren (Belgium). Several rail tank cars with ACN exploded and a fire developed. Toxic vapors of ACN as well as hydrogen cyanide and nitrogen oxides were released due to the fire-induced decomposition of ACN. To avoid explosion of the rail tank cars with butadiene and triethylaluminium, water was used to extinguish the fire and to cool the intact rail tanks. This water has partly joined the stream located along the railway track and ended up in the sewers which resulted in a further distribution of ACN. More than 2000 residents living in the close vicinity of the accident and along the sewage system were evacuated. One resident living next to the sewage system died and two other residents experienced life-threatening symptoms. In total, around two hundred inhabitants of Wetteren presented themselves at the emergency services of the surrounding hospitals. The disaster plan was triggered. It was estimated that, in total, more than 2000 emergency responders were involved in the on-site management of the train accident.

ACN (C_3H_3N) is a monomer used as an intermediate in the manufacturing of acrylic fibres, styrene plastics, and adhesives. At room temperature, ACN is a volatile, flammable, water-soluble, colorless liquid with a garlic or onion-like odor (European Commission, 2004). ACN is highly reactive and may induce explosion. The vapors of ACN are heavier than air and may thus spread along the ground over a long distance. After inhalation, ACN is readily and almost completely absorbed. Metabolism and toxicity of ACN have been described and reviewed elsewhere (ATSDR, 1990; European Commission, 2004; DFG Deutsche Forschungsgemeinschaft, 2007). Briefly, signs of acute toxicity include respiratory tract irritation and central nervous system dysfunction, resembling cyanide poisoning, which may lead to loss of consciousness or even death. With regard to chronic toxicity, ACN has been classified by IARC (IARC, 1999) in the group of possible carcinogens (2B) on the basis of sufficient evidence in experimental animals, but inadequate evidence in humans.

Due to their electrophilicity, ACN and its epoxide readily react with nucleophilic sites in DNA or other macromolecules to form adducts (SCOEL, 2003). *N*-2-cyanoethylvaline (CEV) is the adduct formed by reaction of ACN with the N-terminal valine in human globin (Tornqvist et al., 1986). This adduct is highly specific for exposure to ACN and has a long half-life corresponding to 0.5 times the lifespan of the erythrocytes (126 days in humans) (Granath et al., 1992). Other biomarkers of exposure exist for ACN but they have shorter half-lives (like N-acetyl-S-(2-cyanoethyl) cysteine, CEMA) or are less specific (like N-acetyl-S-(2-hydroxyethyl) cysteine, HEMA) (Schettgen et al., 2012; Wu et al., 2012). Hence, the measurement of CEV in blood allows to carry out a biomonitoring study specifically for ACN in a longer delay. Consequently, CEV has been recommended as the biomarker of choice for chronic as well as for acute ACN exposure (Osterman-Golkar et al., 1994; Van Sittert et al., 1997; Bader and Wrbitzky, 2006).

On May 15, the Belgian Minister of Social Affairs and Public Health advised to perform a biomonitoring study to assess the exposure to ACN in the populations with highest suspected exposure, i.e., the residents of Wetteren and the emergency responders. The specific aims of this study are (1) to determine exposure to ACN by means of CEV adducts in the blood of the emergency responders involved in the on-site management of the train accident of Wetteren, and (2) to assess discriminating factors for ACN exposure in this group of emergency responders. The results of the residents of Wetteren, are reported elsewhere (De Smedt et al., 2014, this issue).

2. Materials and methods

2.1. Study population

The eligible population consisted of all the emergency responders involved in the on-site management of the train accident between May 4–13. Emergency planning in Belgium distinguishes different disciplines involved in the on-site management of accidents and disasters, belonging to different policy levels and administrations, e.g., fire-fighters, police, medical staff, communication services, civil protection, army, etc.

Practically, rescue organizations that had been operating on-site received a mailing with information on the biomonitoring study. They were asked to pass a list with the number and the names of the persons within their organization that were willing to participate. After that, they received the necessary sampling material from the WIV-ISP (Scientific Institute of Public Health). The blood samples themselves were taken by the occupational health physician of each organization. In addition, an e-mail address was opened (biomonitoring@wiv-isp.be) for any questions related to the biomonitoring study in Wetteren. Emergency responders who presented themselves spontaneously but were not on the lists, were also accepted for the study.

2.2. Data collection

The study protocol was approved by the Ethical Committee of the Ghent University Hospital and an informed consent was signed by all participants prior to their participation in the study. The sampling took place from May 21 until June 28, i.e., days 17–55 after the train accident. The data collection was organized in collaboration with the occupation health services. Each participant provided venous blood, collected in a tube filled with EDTA for the determination of *N*-2-cyanoethylvaline (CEV). Urine samples were collected for the measurement of cotinine because smoking may influence the CEV concentration. All emergency responders also filled in a short questionnaire, including (i) demographic information, i.e., name, address, gender and date of birth; (ii) smoking status (non-smoker, ex-smoker, occasional smoker and daily smoker); (iii) some specific variables related to the sampling, i.e., the day and the hour at which blood and urine sampling took place; (iv) a table with detailed information on where participants had been in the night of and in the days following the train accident, i.e., <50 m, 50–250 m, 250–500 m, 500–1000 m, and >1000 m away from the train accident; by day between May 4–10; and (v) the use of respiratory protection (yes/no) in the night of and in the days following the train accident, by day between May 4–10. The function of the participants was provided by the emergency responder organizations.

In total, 1054 emergency responders participated in the biomonitoring. Persons with missing value in either blood CEV measurements, urinary cotinine measurements, questionnaire (spatial and temporal information of the presence on-site between May 4–10), or transmission of the function, were omitted from the analyses of this article. The final study population consisted therefore of the 841 emergency responders.

2.3. Adduct and cotinine analyses

Blood samples were pre-treated within 24 h to obtain a lysate of erythrocytes. The pretreated samples were stored at −20 °C. Because of the need for substantial analyzing capacity, blood samples were sent on dry ice to three different laboratories specialized in CEV analyses where a modified Edman degradation was used for adduct dosimetry (Tornqvist et al., 1986; Van Sittert et al., 1997). All three laboratories applied N-2-cyanoethyl-valineleucine-anilide (Bachem, Bubendorf, Switzerland) for the calibration of the quantitative Edman procedure. Moreover all three laboratories participated successfully in the G-EQUAS inter-laboratory comparison before (Göen et al., 2012). The LLOQ's (lower limit of quantification) were respectively 0.5 (Lab I), 4.0 (Lab II) and 2.0 (Lab III) pmol/g globin. When receiving the first results from the labs at the end of July, some CEV concentrations showed to be strongly increased, especially in the residents (>1000 pmol/g globin, De Smedt et al. (2014), this issue). To verify the results, we decided to carry out an extra inter-laboratory performance test at that moment on a sub sample of the residents and emergency responders who participated in the human biomonitoring study. Therefore, 10 samples per laboratory were chosen, i.e., the 5 highest concentrations and 5 randomly lower concentrations. The 10 samples of the Lab I batch were sent to Lab II, the 10 samples of the Lab II batch were sent to Lab III, and finally, the 10 samples of the Lab III batch were sent to Lab I. The additional inter-laboratory test revealed comparable results among the three labs. The estimate for the total error due to inter- and intra-laboratory variance was 11% and the estimate for the mean standard deviation within a laboratory was 6.5%. For the detailed results on the additional inter-laboratory comparison, the reader is referred to De Smedt et al., (2014), this issue.

Smokers and non-smokers were identified based on cotinine in urine (De Cremer et al., 2013). Cotinine is a metabolite of nicotine and is generally accepted as the optimal biomarker for tobacco smoke exposure (Benowitz et al., 2009). We measured cotinine to account for individual smoking status. Indeed, tobacco smoke is a major source of ACN exposure and may thus interfere with the interpretation of the CEV measurements. Based on urinary cotinine measurements, the participants were classified as smoker or non-smoker according to Benowitz (1996). Persons with urinary cotinine >100 μg/L (n = 198) were classified as smokers and persons with urinary cotinine <25 μg/L (n = 628) were classified as non-smokers. For those in between (n = 15), the smoking status was determined based on the self-reported questionnaire: self-reported 'smokers' (n = 1) and 'occasional smokers' (n = 7) were classified as 'smokers', whereas self-reported 'non-smokers' (n = 5) and 'ex-smokers' (n = 2) were classified as 'non-smokers'.

2.4. Statistical analyses

Based on the CEV concentrations measured in the blood, values were extrapolated by back-calculation to the concentration that was to be expected at the time of the accident, i.e., May 4. The extrapolation is based on the zero-order elimination kinetic of CEV hemoglobin adducts, depending of the lifespan of the erythrocytes that is 126 days. The following formula was used for the extrapolation: extrapolated CEV = measured CEV/$(1 - t \times 0.008)$, where "t" is the number of days between the accident and the blood sampling (Granath et al., 1992; Bader and Wrbitzky, 2006). The extrapolation method firstly requires to subtract, from the measured CEV value, the background CEV value, which is supposed to be stable over time. Without subtracting this background value, a correct back-calculation of the exposure to the time of the accident is not possible. In this study, the background CEV level is unknown. In non-smokers however, the background CEV value is supposed to be so small that it can be neglected for back-calculation. But in smokers, the background CEV value is substantial and depends on the extent of tobacco consumption in the population. A precise evaluation of the ACN exposure from the accident by the extrapolation method was therefore only possible for non-smoker emergency responders.

We calculated the proportions of CEV concentrations above the reference value, which corresponds to the 95th percentile in the general population that is not exposed to ACN. For the non-smokers, the reference value is clearly defined in the literature, i.e., 10 pmol/g globin (Kraus et al., 2012). In contrast, for smokers, the reference value in the general population is less unequivocal (Kraus et al., 2012). The reported 95th percentiles range between 146 pmol/g globin and 332 pmol/g globin with the maximum being 607 pmol/g globin, mainly determined by the extent of tobacco consumption (Kraus et al., 2012) For the present study, a reference value of 200 pmol/g globin was used for the smokers.

Discriminating factors for CEV concentrations were identified by the classification and regression tree (CART) methodology (Breiman et al., 1984). CART incorporates two different types of tree-based methods: classification trees for categorical variables, and regression trees for continuous variables. CART can use the same predictor variable in different places in the tree, allowing for complex interdependencies between different predictor variables to unfold. We used the algorithm provided by the PARTY package in R for building the classification or regression tree (Hothorn et al., 2006). To estimate the misclassification of each possible sub-tree, cross-validation was used with the optimal tree being the one with the lowest misclassification. The following predictor variables (Table 1) were included: (i) gender; (ii) age; (iii) smoking status; (iv) occupational function; (v) use of respiratory protection per day between May 4–10; (vi) the zone of presence on-site in the night of the train accident and by day between May 4–10; (vii) the cumulative number of days in each of the three predefined zones between May 4–10; and (viii) the closest zone of presence on-site between May 4–10. The response variable were the (log-transformed) CEV concentrations, extrapolated to the day of the train accident, i.e., May 4. The variable 'function' was categorized into five groups for the analyses, i.e., (i) fire-fighters, (ii) police, (iii) civil protection workers, (iv) army, and (v) a group named 'others'. This last category included the functions that were less prevalent in the study population, including journalists, medical staff, wastewater management teams, and soil remediation teams. Finally, the five zones of presence on-site from the questionnaire were regrouped into three zones in the analyses: <50 m (immediately on the site of the train accident); 50–250 m; and >250 m. This last category corresponded to the perimeter of the evacuation zone that was determined for the residents.

2.5. Communication of the results

To facilitate an efficacious medical assistance to the emergency responders after the biomonitoring study, a communication plan was established in close collaboration with the communication departments of the WIV-ISP and of the Federal Public Service Health, Food Chain Safety and Environment. Apart from a mailing to each participant with their individual value, it envisaged an all-embracing communication to the other stakeholders including recommendations to authorities and various information sessions for the individual participants and their occupational physicians. In addition, the plan provided that participants with a high CEV value got a home visit by a medical practitioner to discuss their results.

3. Results

3.1. General characteristics of the study population

In total, there were 841 emergency responders (Table 2) with measures of CEV (blood), cotinine (urine), spatial and temporal information of the presence on-site between May 4–10 (questionnaire), and for whom the function was known. This study population was mainly composed of fire-fighters (54%) and police

Table 1
Description of the predictor variables in the emergency responders participating in the human biomonitoring study following the train accident.

Variable description	Values
Gender (i)	1 = men 2 = women
Age (ii)	Years
Smoking status (iii)	0 = non-smoker 1 = smoker
Function (iv)	Fire fighters, police, civil protection, army, others
Use of respiratory protection in the night of the train accident and by day between May 4–10 (v)	Night of the train accident: 0 = no; 1 = yes May 4: 0 = no; 1 = yes May 5: 0 = no; 1 = yes . . . May 10: 0 = no; 1 = yes
Zone of presence on-site in the night of the train accident and by day between May 4–10 (vi)	Night of the train accident: <50 m; 50–250 m; >250 m May 4: <50 m; 50–250 m; >250m May 5: <50 m; 50–250 m; >250 m ... May 10: <50 m; 50–250 m; >250 m
Cumulative number of days in each of the three predefined zones between May 4–10 (vii)	<50 m: 0,1,2, . . . ,7 days 50–250 m: 0,1,2, . . . ,7 days >250 m: 0,1,2, . . . ,7 days
Closest zone of presence on-site between May 4–10 (viii)	<50 m; 50–250 m; >250 m

(34%); the three other groups (army, civil protection and ‘others’) together representing only 12%. The majority (89.5%) of the participants were men, with the highest proportions (95% or more) in the fire-fighters, the civil protection, and the army. In the police workers and in the group ‘others’, men were somewhat less represented (70.8% till 78.3%). Median ages were comparable among the different functions, varying between 35 and 46 years. Of the 841 emergency responders, 206 (24.5%) were classified as ‘smokers’. The proportion of smokers was comparable among the different functions, ranging between 22.7% and 25.3%.

3.2. CEV adduct concentrations

3.2.1. Extrapolated CEV concentrations in the non-smokers

Table 3 presents the CEV concentrations in the non-smokers, after extrapolation to the time of the accident, i.e., May 4. Twenty-six percent of the non-smokers exceeded the reference value of 10 pmol/g globin. The overall distribution of CEV concentrations in the non-smokers, however, remained within the ranges as described for smokers in the literature, the 95th percentile and the maximum value being 73 and 452 pmol/g globin, respectively. CEV levels differed clearly according to function with median values ranging from 2.6 pmol/g globin among the army till 15 pmol/g globin among the civil protection workers. The civil protection workers appeared to be the mostly exposed with almost 60% of results above the reference value, which is two times more than the proportion of increased CEV levels in fire-fighters or the group ‘others’. In the group ‘others’, 11 (30.6%) of the 36 non-smokers exceeded the reference value. Of these 11 persons, 7 belonged to the soil remediation and wastewater management teams.

3.2.2. Measured CEV concentrations in the smokers

As discussed in the methodology, the method of extrapolation of exposure to May 4 may not be applied in a valid way in the smokers. Therefore, the results presented for the smokers are limited to the CEV concentrations that were measured in the blood samples as such, i.e., the CEV concentration at the day of the blood sampling (Table 4).

Of the 206 smokers, 27% exceeded the reference value. CEV levels were different among the functions. The fire-fighters were the most exposed group with 33% of the CEV concentrations above the reference value.

3.3. Discriminating factors for CEV concentrations among non-smokers (Fig. 1)

The major discriminant factor among the non-smokers was the presence in the <50 m zone between May 4–10. As compared to colleagues without presence in the <50 m zone, emergency responders who had been less than 50 m away from the train accident showed higher CEV concentrations. In this last group, the cumulative number of days within the <50 m zone was important: CEV concentrations were higher in participants who had been

Table 2
Characteristics of the emergency responders participating in the human biomonitoring study following the train accident (n = 841).

	Fire-fighters	Police	Civil protection	Army	Others	Total
N (% of total study population)	450 (54%)	286(34%)	35(4.2%)	22(2.6%)	48(5.7%)	841(100%)
Men (n, %)	439 (97.6%)	224 (78.3%)	35(100%)	21(95.5%)	34(70.8%)	753 (89.5%)
Age (median, IQR)	40.0(33–46)	35.0(29–44)	46.5(41–49)	35.5(31–49)	39.0(31–46)	38.0(32–46)
Smokers (n, %)	114 (25.3%)	67 (23.4%)	8(22.9%)	5(22.7%)	12 (25.0%)	206 (24.5%)

Table 3
CEV concentrations, extrapolated to the time of the train accident (pmol/g globin), in the non-smoking emergency responders participating in the human biomonitoring study following the train accident (n = 635).

	Fire-fighters	Police	Civil protection	Army	Others	Total
N	336	219	27	17	36	635
Median (IQR)	4.4 (2.6–17)	2.9 (2.6–5.1)	15 (6.1–47)	2.6 (1.3–5.1)	5.1 (2.9–10)	3.2 (2.6–10)
95th percentile	91	26	110	11	217	73
Maximum	452	117	147	11	379	452
N (%) >ref value[a]	106 (31.5%)	29 (13.2%)	16 (59.3%)	1 (5.9%)	11 (30.6%)	163 (25.7%)

[a] 10 pmol/g globin.

more than two days in the <50 m zone (median: 42, IQR between 7.7 and 76 pmol/g globin) vs. those being present 2 days or less (median: 8.0, IQR between 2.7 and 22 pmol/g globin). In the first group, i.e., the emergency responders without presence in the <50 m zone, the function turned out to be the most important determinant. The police and the army (median: 2.9, IQR between 2.5 and 4.2 pmol/g globin) showed clearly lower CEV concentrations as the other three groups, i.e., the fire-fighters, the civil protection workers and the group 'others'. Finally, among these last three groups, two factors were predictive for the CEV concentrations, i.e., the 'closest zone of presence on-site between May 4–10′ and 'the cumulative number of days of presence in that zone between May 4–10'. Similar CEV concentrations were observed in those who had been present in the 50–250 m zone for more than one day (median: 10.8, IQR between 3.3 and 23 pmol/g globin) as well as in workers who had been present in the zone >250 m for more than 5 days (median: 7.7, IQR between 3.2 and 26 pmol/g globin). The median CEV concentration was lower (median: 2.7, IQR between 2.5 and 6.2 pmol/g globin) in fire-fighters, civil protection workers, and 'other' workers who were present in the zone farther than 250 m from the train accident, although several outliers were observed in this group (maximum 379 pmol/g globin) .

4. Discussion

4.1. Principal findings of the study

This study describes the results of the largest human biomonitoring study performed to date in order to assess accidental ACN exposure in occupational populations. The basis of exposure in this case was a train derailment at Wetteren, Belgium, on May 4, 2013, after which the Belgian Minister for Social Affairs and Public Health advised a large scale biomonitoring to assess ACN exposure in the emergency responders involved in the on-site management of the accident. Results indicated that emergency responders were clearly exposed to ACN from the accident as 26% of the non-smokers had CEV concentrations above the reference value of 10 pmol/g globin. However, the extent of the overexposure in the emergency responders remained moderate. First, while a substantial proportion of the emergency responders exceeded CEV values above what is observed in a background population, the median values observed in both smokers and non-smokers in our population are comparable to what is described in the literature for a non-exposed population (Kraus et al., 2012). Second, even the higher CEV concentrations in the non-smokers (95th percentile of 73 pmol/g globin and maximum of 452 pmol/g globin) remained within the ranges as described for smokers in the literature. Third, the higher CEV concentrations in smokers (95th percentile of 342 pmol/g globin and maximum of 811 pmol/g globin) exceeded only slightly what has been reported in a non-exposed population in Germany (95th percentile of 332 pmol/g globin and maximum of 607 pmol/g globin) (Kraus et al., 2012). The difference of CEV concentrations between smokers and non-smokers is also similar in the study population to what is reported in non-exposed populations, smokers having CEV concentrations largely above the concentrations observed in non-smokers. The CEV contribution due to tobacco smoking is therefore preponderant in the CEV concentrations of smokers.

CART methodology was used to assess factors predictive of the CEV concentration in the non-smokers. CART offers the advantage of using variables multiple times in different branches of the classification and regression trees, allowing to uncover complex interdependencies between variables. CART can easily incorporate a large number of both numerical and categorical predictor variables, although care should be given to potential overly complex trees as a result of overfitting. Three discriminating factors were identified, i.e., (1) the distance to the accident, (2) the duration of exposure, and (3) the occupational function. The increased CEV concentrations in function of proximity to the accident and exposure duration are in accordance with a direct exposure from the accident and the cumulative character of the CEV biomarker that was used, respectively. The interpretation of 'function' as predictive determinant is more complicated. First, the 'function' turned out to be the most important determinant in the emergency responders without presence in the <50 m zone, with the fire-fighters, the civil protection workers and the group 'others' having higher CEV levels than the police and the army. Second, among this group of fire-fighters, civil protection workers and 'others', higher CEV concentrations were observed in those who had been present on the field within the 50–250 m zone or further away. Third, fire-fighters and civil protection workers were the main disciplines involved in the management of the sewage. Fourth, among the group 'others', the majority of the workers with CEV concentrations above the reference value

Table 4
CEV concentrations, as measured at the day of blood sampling (pmol/g globin), in the smoking emergency responders participating in the human biomonitoring study following the train accident (n = 206).

	Fire-fighters	Police	Civil protection	Army	Others	Total
N	114	67	8	5	12	206
Median (IQR)	156 (76–225)	105 (57–175)	153 (83–228)	132 (130–159)	100 (53–169)	140 (69–209)
95th percentile	403	282	331	323	284	342
Maximum	811	394	331	323	284	811
N (%) >ref value[a]	38 (33.3%)	12 (17.9%)	2 (25%)	1 (20%)	2 (16.7%)	55 (26.7%)

[a] 200 pmol/g globin.

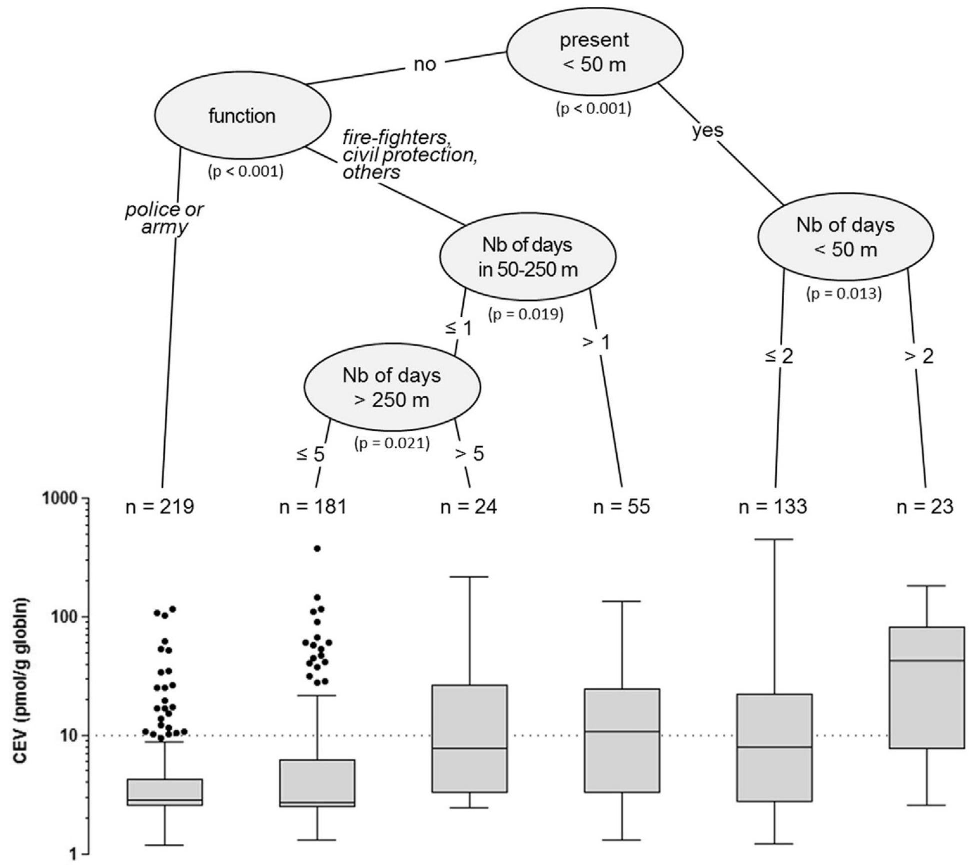

Fig. 1. Determinants of the CEV concentrations* by CART-modeling in the non-smoking emergency responders participating in the human biomonitoring study following the train accident (n = 635).
*CEV concentrations extrapolated to the time of the train accident (pmol/g globin).

belonged to companies involved in the waste water management of the accident. All these observations together may be suggestive of exposure via the sewage system. This remains, however, speculative because no information was available on the specific tasks that were carried out, and that may be different for a same function.

The use of respiratory protection did not appear as a determinant of the CEV concentrations among the non-smokers in this study. The question included was respiratory protection (yes/no) per day between May 4–10. More detailed information on the continuous or effective use of the respiratory protection material was not available. A potential effect of this factor may thus remain undetected because of the less precise question and, as such, no interpretations on the usefulness of respiratory protection may be deduced from this observation. Other routes of exposure may have played a role, but given the circumstances of the accident (fire) and the nature of the substance (highly volatile), inhalation appears to have been the major route of exposure.

4.2. Comparison with other human biomonitoring studies

Biological monitoring following chemical disasters has been recommended as part of disaster management in order to objectivate the internal human exposure (Scheepers et al., 2011). To the authors' knowledge, two previous studies have reported on biological monitoring of CEV following accidental ACN exposure in occupational populations. Following the death of a cleaning worker after decontamination of an ACN containing tank wagon, Bader and Wrbitzky (Bader and Wrbitzky, 2006) reported CEV concentrations of 679 pmol/g globin (non-smoker) and 768–2424 pmol/g globin (smokers) in the co-workers. In the rescue workers and medical staff who tried to resuscitate the person, no increased CEV concentrations were observed. In another German study (Leng, 2014), CEV monitoring was carried out on 600 persons from fire brigades, police and rescue organizations after a fire in an ACN tank of a chemical plant in 2008. In 99% of the sampled population, body burden was <40.8 pmol/g globin for non-smokers and <612 pmol/g globin for smokers.

In another paper (De Smedt et al., 2014, this issue), we have reported on the results of the human biomonitoring study following the train accident of May 4 in the residents of Wetteren with the highest suspected exposure to ACN. In summary, we concluded that: (1) ACN overexposures, as determined by the CEV biomarker, were high in the residents with 37.3% of the non-smokers and 40.0% of the smokers exceeding the reference values of 10 and 200 pmol/g globin, respectively; (2) the more extreme CEV concentrations were observed in the street along the railway and particularly in the streets corresponding to the sewage system, with maxima obtaining 4951 and 12 615 pmol/g globin; and (3) the CEV concentrations in the 250 m zone (95th percentile of 36 pmol/g globin in non-smokers), which was evacuated in the night of the train accident itself, were substantially lower than the CEV concentrations along the sewage system, which was evacuated later, i.e., in the days following the train accident. More specifically, along the sewage system, the 95th percentiles amounted to 2761 and 340 pmol/g globin in the residents who were known to have presented at the emergency services and those who did not, respectively. In the present study, ACN overexposure was also clearly present in the emergency responders involved in the on-site management of the train accident. In comparison with the residents, ACN exposures in the emergency responders remained moderate, with 26% of the non-smokers and 27% of the smokers exceeding the CEV reference values of 10 and 200 pmol/g globin, respectively. In addition, the ranges of CEV concentrations in the non-smokers stayed within the ranges described in the literature as background for a smoking population (Kraus et al., 2012). As to the mechanism of ACN overexposure, essentially the sewage system and to a lesser extent the vicinity of the accident were important in the residents, while in the emergency responders it was mainly the presence close to the train accident that emerged.

4.3. Limitations of the study

This biomonitoring study illustrates a rapid response in a crisis situation following an accident. The decision to carry out a biomonitoring study was taken 11 days after the accident. Two days later, invitations to participate were launched to the emergency responders. This investigation was carried out to provide answers to authorities about the extent of exposure following the accident. From a scientific point of view, this approach has some limitations regarding the study design. For example, it may have been useful to undertake a pre-sampling before the accident to determine background values for the purpose of comparison. While this could be a relevant point-of-attention in future studies, pre-sampling was per definition not possible anymore in this study.

The biomonitoring has been carried out in the emergency responders involved in the on-site management of the Wetteren train accident. Participation to the study was on a voluntary basis. Age gender, and smoking characteristics of the study population corresponded to what may be expected in occupational populations. However, it is unclear whether the study population is representative of the whole group of emergency responders involved in the on-site management of the Wetteren train accident because of the lack of exhaustive lists of persons occupationally involved in the on-site management of accidents or disasters in Belgium. Consequently, neither participation rates, nor potential selection biases may be estimated. It may be advisable to develop such lists in order to manage more effectively future accidents and disasters.

N-2-cyanoethylvaline is considered as the best biomarker to assess ACN exposure. It allows performing blood sampling several weeks after the accident given the long lifespan of CEV adducts, up to 126 days in humans. The measured CEV concentrations could be extrapolated to the CEV concentrations expected on the day of the accident, based on the well-known toxicokinetics of the CEV adducts. For emergency responders, the time between accident and blood sampling was generally longer than for residents. Accordingly, difference between measured and extrapolated CEV concentrations was more pronounced for emergency responders than for residents. The extrapolation method is adequate when the CEV background in the blood is negligible, i.e. in the case of non-smokers. For smokers, we cannot use this formula as such because we need to take into account the background CEV concentrations due to tobacco smoking. Indeed, acrylonitrile from tobacco smoke has a significant influence on the CEV levels in globin (Lewalter, 1996; Schettgen et al., 2002). While CEV is usually close to the detection limit in the blood of non-smokers, a background value between 50 pmol/g globin and 300 pmol/g globin is typically found in smokers, depending on their tobacco consumption (Bader and Wrbitzky, 2006). In this study, the background CEV level of the smokers is unknown. Without this value, a correct extrapolation of the exposure to the time of the accident is not possible. And without extrapolation we cannot take into account the decrease in CEV concentrations due to elimination of CEV adducts between accident and sampling date. A precise evaluation of the ACN exposure from the accident was therefore only possible for non-smoker emergency responders.

5. Conclusion

This human biomonitoring study is among the first published examples of large-scale investigations carried out promptly after a crisis, in this case a severe train accident with leakage of ACN. An increased exposure to ACN was found in emergency responders involved in the on-site management of the train accident with more than a quarter of the non-smokers exceeding the reference value of the non-exposed and non-smoking general population. The extent of the exposure remained, however, relatively moderate as it corresponded to what may be observed as background levels in smokers. In addition to smoking, ACN exposure was influenced by the distance to the accident, the number of days spent on-site, and the occupational function of the participants. The exposure in the emergency responders was less pronounced than the exposure in the local population. Thus, the present study demonstrates that human biomonitoring is an efficient tool in the exposure assessment of certain chemicals released following accidents and disasters.

Conflict of interest

The authors declare no conflict of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

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 emergency responders for their participation in the study and the occupational health physicians and the local physicians for their assistance in the sampling and the 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 organization 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 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.

References

Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for acrylonitrile. 1990. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=447&tid=78

Bader, M., Wrbitzky, R., 2006. Follow-up biomonitoring after accidental exposure to acrylonitrile—implications for protein adducts as a dose monitor for short-term exposures. Toxicol. Lett. 162 (2–3), 125–131. doi:http://dx.doi.org/10.1016/j.toxlet.2005.09.034.

Benowitz, N.L., 1996. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol. Rev. 18 (2), 188–204.

Benowitz, N.L., Hukkanen, J., Jacob III, P., 2009. Nicotine chemistry, metabolism, kinetics and biomarkers. Handb. Exp. Pharmacol. 192, 29–60. doi:http://dx.doi.org/10.1007/978-3-540-69248-5_2.

Breiman, L., Friedman, J., Olshen, R., Stone, C., 1984, 1984. Classification and Regression Trees. Chapman & Hall/CRC Press, London.

De Cremer, K., Van Overmeire, Van Loco, J., 2013. On-line solid-phase extraction with ultra performance liquid chromatography and tandem mass spectrometry for the detection of nicotine, cotinine and trans-3′-hydroxycotinine in urine to strengthen human biomonitoring and smoking cessation studies. J. Pharm. Biomed. Anal. 76, 126–133. doi:http://dx.doi.org/10.1016/j.jpba.2012.12.018.

De Smedt, T., De Cremer, K., Vleminckx, C., Fierens, S., Mertens, B., Van Overmeire, I., Bader, M., De Paepe, P., Göen, T., Nemery, B., Schettgen, T., Stove, C., Van Loco, J., Van Oyen, H., Van Nieuwenhuyse, A., 2014. Acrylonitrile exposure in the general population following a major train accident in Belgium: a human biomonitoring study. Toxicol. Lett. 231, 352–359.

DFG Deutsche Forschungsgemeinschaft, 2007. Acrylonitrile. The MAK-Collection for Occupational Health and Safety. Part I: MAK Value Documentations, 24. Wiley-VCH, Weinheim doi:http://dx.doi.org/10.1002/3527600418.mb10713e0024 pp. 1–40.

European Commission. European Union Risk Assessment Report: acrylonitrile, Volume 32. EUR 20857 EN.2004. Environment and quality of life series

Granath, F., Ehrenberg, L., Tornqvist, M., 1992. Degree of alkylation of macromolecules in vivo from variable exposure. Mutat. Res. 284 (2), 297–306.

Göen, T., Schaller, K., Drexler, H., 2012. External quality assessment of human biomonitoring in the range of environmental exposure levels. Int. J. Hyg. Environ. Health 215, 229–232.

Hothorn, T., Hornik, K., Zeileis, A., 2006. Unbiased recursive partitioning: a conditional inference framework. J. Comput. Graph. Statist. 15 (3), 651–674.

IARC 1999 Monograph Vol. 71. Evaluation of carcinogenic risks to humans. Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide: Acrylonitrile. Part I. pp. 43–108.

Kraus T, Angerer J, Bader M, Schettgen T. 2012. Acrylnitril, Addendum [BAT Value Documentation in German language, 2011]. The MAK Collection for Occupational Health and Safety. 17–26. 10.1002/3527600418. bb10713d0017.

Leng, G., 2014. Biomonitoring following a chemical incidence with acrylonitrile and ethylene. Toxicol. Lett. 231, 360–364.

Lewalter, J., 1996. N-alkylvaline levels in globin as a new type of biomarker in risk assessment of alkylating agents. Int. Arch. Occup. Environ. Health 68 (6), 519–530.

Osterman-Golkar, S.M., MacNeela, J.P., Turner, M.J., Walker, V.E., Swenberg, J.A., Sumner, S.J., Youtsey, N., Fennell, T.R., 1994. Monitoring exposure to acrylonitrile using adducts with N-terminal valine in hemoglobin. Carcinogenesis 15 (12), 2701–2707.

Scheepers, P.T., Bos, P.M., Konings, J., Janssen, N.A., Grievink, L., 2011. Application of biological monitoring for exposure assessment following chemical incidents: a procedure for decision making. J. Expo. Sci. Environ. Epidemiol. 21 (3), 247–261. doi:http://dx.doi.org/10.1038/jes.2010.4.

Schettgen, T., Broding, H.C., Angerer, J., Drexler, H., 2002. Hemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile and acrylamide-biomarkers in occupational and environmental medicine. Toxicol. Lett. 134 (1–3), 65–70.

Schettgen, T., Bertram, J., Kraus, T., 2012. Accurate quantification of the mercapturic acids of acrylonitrile and its genotoxic metabolite cyanoethylene-epoxide in human urine by isotope-dilution LC-ESI/MS/MS. Talanta 98, 211–219. doi:http://dx.doi.org/10.1016/j.talanta.2012.06.074.

SCOEL 2003. Recommendation from the Scientific Committee on Occupational Exposure Limits for acrylonitrile. European Commission. Employment, Social Affairs and Inclusion. SCOEL/SUM/104.

Tornqvist, M., Mowrer, J., Jensen, S., Ehrenberg, L., 1986. Monitoring of environmental cancer initiators through hemoglobin adducts by a modified Edman degradation method. Anal. Biochem. 154 (1), 255–266.

Van Sittert, N., Angerer, J., Bader, M., Blaszkewicz, M., Ellrich, D., Krämer, A., Lewalter, J., 1997. 2-Cyanoethylvaline, N-2-Hydroxyethylvaline, N-Methylvaline (as evidence of exposure to acrylonitrile, ethylene oxide and methylating agents). Analyses of hazardous substances in biological materials. Deutsche Forschungsgemeinschaft. VCH Verlagsgesellschaft, Weinheim.

Wu, C.F., Uang, S.N., Chiang, S.Y., Shih, W.C., Huang, Y.F., Wu, K.Y., 2012. Simultaneous quantitation of urinary cotinine and acrylonitrile-derived mercapturic acids with ultraperformance liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 402 (6), 2113–2120. doi:http://dx.doi.org/10.1007/s00216-011-5661-4.