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Assessment of long-term health risks after accidental exposure using haemoglobin adducts of epichlorohydrin

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HIGHLIGHTS

- A human biomonitoring study was performed to evaluate internal exposure after accidental release of epichlorohydrin.
- Haemoglobin adducts of epichlorohydrin, the *N*-(3-chloro-2-hydroxypropyl)valine (CHPV) and the *N*-(2,3-dihydroxypropyl)valine (DHPV), were measured in blood.
- In 6 out of 628 samples, CHPV adduct levels above the LOQ of 25 pmol/g ranged from 32.0 to 116.4 pmol/g globin.
- DHPV was not detected above the LOD of 10 pmol/g globin in any of the blood samples.
- Based on the quantified CHPV adduct values, estimates of the cumulative additional lifetime cancer risks range from 2.61×10^{-8} to 9.48×10^{-8}.

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ABSTRACT

On September 9th, 2002, two goods trains collided in Bad Münder, Lower Saxony, causing the release of more than 40 metric tonnes of epichlorohydrin (1-chloro-2,3-epoxypropane) into the environment. A human biomonitoring study was performed to evaluate the accidental exposure to epichlorohydrin and to assess the possible long-term, i.e. carcinogenic health effects. This was done on the basis of a biochemical effect monitoring using the *N*-(3-chloro-2-hydroxypropyl)valine and the *N*-(2,3-dihydroxypropyl)valine haemoglobin adducts of epichlorohydrin in blood to respond to missing ambient monitoring immediately after the crash. *N*-(3-chloro-2-hydroxypropyl)valine adduct levels above the LOQ (25 pmol/g globin) ranged from 32.0 to 116.4 pmol/g globin in 6 out of 628 samples. The *N*-(2,3-dihydroxypropyl)valine adduct was not detected above the LOD (10 pmol/g globin) in any of the blood samples. Based on the quantified *N*-(3-chloro-2-hydroxypropyl)valine adduct values, the body doses after two days of exposure were estimated to be in the range of 1.7–6.2 nmol/kg body weight. The reverse estimation of the external exposure leads to cumulative additional lifetime cancer risks ranging from 2.61×10^{-8} to 9.48×10^{-8}. The estimated excess lifetime cancer risks have to be assessed as extremely low. Our biomonitoring study facilitated the dialogue between individuals and groups concerned and authorities, because suspected or occurred exposures and risks to human health could be quantified and interpreted in a sound manner.

1. Introduction

On September 9th, 2002, two goods trains collided head-on on the outskirts of Bad Münder, Lower Saxony, causing the release of more than 40 metric tonnes (MT) of epichlorohydrin (1-chloro-2,3-epoxypropane, ECH, CAS No. 106-89-8) into the environment. The emissions of ECH can be described as a thriphasic process: (a) the

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continuous phase of combustion which was steadily fed from leaking ECH and accompanied by a first minor burst, (b) a release during the second and significantly larger explosion, and (c) the residual release during the fire-fighting, which started immediately after the larger explosion. The fire was under control after about 4 h. From the originally 49.4 MT of ECH about 5 MT drained away into the subsurface of the close-up range of the collision site and 6–10 MT polluted the nearby river Hamel (Lilienblum et al., 2003; Lilienblum and Müller, 2004; Wollin et al., 2008). Further 29–33 MT of burnt, vapourised or hydrolysed ECH were released into the atmosphere and approximately 5 MT of ECH remained in the tank wagon. In the early phase of the accident, temporally and spatially dispersed systematic measurements of ECH were not performed and hence especially ambient air concentrations of ECH were virtually unknown. Police officials, firemen, officials of the Federal Railway Authority, other task force members, employees of a neighbouring firm and residents were potentially exposed to unburnt ECH as well as the hazardous gases and aerosols of its combustion. During the first twenty days after the accident, about 540 health practitioner consultations concerning temporary lachrymatory effects, irritation to throat and respiratory tract, headache and notable discomfort were registered (Lilienblum et al., 2003). The health impairments that obviously occurred widespread in the residents during the first days after the accident are not consistent with the observed plausible meteorological behaviour of a thermally exaggerated fire cloud (Lilienblum and Müller, 2004). However, these health complaints can partially be explained by low passing plumes in the main sector of the emission probably resulting from cooling/quenching effects of the fire water and hydrogen chloride formed. With regard to possible health outcomes of exposure that may be delayed in onset, an increase of excess lifetime cancer risk attributable to inhaled ECH or dermal uptake of ECH ranked first. This was the formative aspect in risk communication with those individuals or groups who were accidentally exposed. Admittedly, the extent of the individual exposure was unknown.

Due to its chemical reactivity, ECH is locally toxic if inhaled, swallowed and in contact with skin (IPCS, 1984; U. S. EPA, 2008; DFG, 2012; HSDB, 2014). It causes delayed erythema, oedema, and papules along with burning, and itching when the liquid comes into contact with the skin (U. S. EPA, 2008; HSDB, 2014). Severe eye irritation, skin irritation, and delayed contact skin sensitisation in animals have been noted after topical application of undiluted or diluted ECH (U. S. EPA, 2008). Human volunteers showed significant cardiotoxic effects when they were exposed to vapours of ECH (IPCS, 1984). Burning of the eyes and nasal mucosa were reported along with throat irritation (HSDB, 2014; U. S. EPA, 2008). Systemic toxicity (damage of liver, kidney, adrenal gland and CNS and annoyance of reproduction) besides local effects was observed after long-term exposure in animal studies (HSDB, 2014; U. S. EPA, 2008). Genotoxicity in vitro and in vivo has been demonstrated in numerous studies (IPCS, 1984; DFG, 2012; U. S. EPA, 2008). Because of its alkylating properties, the mechanism of primary genotoxicity via formation of DNA adducts (Singh et al., 1996; Koskinen and Plná, 2000; Sund and Kronberg, 2006) and DNA interstrand cross-linking (Romano et al., 2007) is relevant for ECH. Human carcinogenicity data indicates possible weak effects that require validation (IARC, 1999; AGS, 2012). ECH acts via the electrophilic carbon of the chloromethylene group and the C3 of the epoxy ring as a bifunctional directly alkylating epoxide. Because of the higher reactivity of the C3 in the epoxy moiety, the predominant reaction of ECH with macromolecules in cells such as DNA and haemoglobin is the formation of open-chain chlorohydroxypropyl adducts which can be transformed into the corresponding dihydroxypropyl adducts by hydrolysis. The formation of the ultimate dihydroxypropyl adduct of ECH can however also result from the ECH metabolite glycidol, respectively.

According to the CLP Regulation (2008), ECH is categorised into the health hazard classes Carcinogenicity 1B (presumed to have carcinogenic potential for humans, classification is largely based on animal evidence), Acute Toxicity 3 (hazard statement H331, toxic if inhaled), Acute Toxicity 3 (H311, toxic in contact with skin), Acute Toxicity 3 (H301, toxic if swallowed), Skin Corrosion 1B (H314, causes severe skin burns and eye damage) and Skin Sensitization 1 (H317, may cause an allergic skin reaction).

The combustion of ECH and/or hydrolysis in the presence of fire-fighting water or air moisture produces irritant and toxic gases. Combustion by-products include hydrogen chloride, chlorine, and phosgene. Quantitatively, hydrogen chloride is the most important secondary product of ECH in case of fire or from hydrolysis, exhibiting a pronounced irritant potency. Remarkably, the lowest acute exposure guideline level value (AEGL-1) of hydrogen chloride (U. S. EPA, 2013a) is lower if compared to ECH's AEGL-1 (U. S. EPA, 2013b) (1.8 ppm vs. 5.7 ppm (interim value)); both AEGLs (as airborne concentrations) are based upon the respective substance-related no-effect level for irritation in humans.

This study aims: (1) to evaluate the extent of the accidental exposure to epichlorohydrin of the persons concerned and (2) to assess the possible long-term, i.e. carcinogenic health effects on the basis of a biochemical effect monitoring using the chlorohydroxypropyl- and dihydroxypropyl haemoglobin adducts of ECH to respond to missing ambient monitoring immediately after the crash. The dihydroxypropyl haemoglobin adduct of ECH has already been used as quantitative biomarker of the internal body burden in humans and in the Wistar rat model (Hindsø Landin et al., 1996) and to investigate long-term exposure at the workplace (Hindsø Landin et al., 1997), but the chlorohydroxypropyl adduct precursor was not considered comprehensively so far in biological monitoring. In general, the chlorohydroxypropyl- as well as the dihydroxypropyl adducts should also be suitable for evaluating the individual internal body burden in case of acute exposure after accidental release of ECH. Moreover, haemoglobin adduct data is considered more relevant for assessing internal exposure than extrapolations from chemical concentrations, e.g. in soil, water or air. Haemoglobin adducts are surrogates of DNA adducts, and as such, they provide a measure of both exposure and biochemical effect. Due to their high specificity and the sensitivity of the detection methods, haemoglobin adducts are preferable to the analysis of genotoxic substances and their metabolites in human body fluids (Angerer et al., 2007; Pavanello and Lotti, 2012). Used in such a way, they have the advantages of an individual health assessment which integrates the inhalational, dermal and – if applicable – the oral route of exposure. If exposure to ECH is proven by haemoglobin adduct data, it would be possible to utilise the individual internal ECH dose for extrapolating to airborne ECH exposures. A reasonable approach to estimate the excess lifetime cancer risk of people involved in the emergency could then be based on established quantitative estimates of the carcinogenic risk from inhalation exposure.

2. Material and methods

2.1. Sampling

The management of blood sampling was conducted by the public health department of the administrative district Hameln-Pyrmont and the Institute for Occupational Medicine of Hannover Medical School. Samples were obtained from Lower Saxony state policemen, employees of the federal police, railway officials and

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persons who were participating in the 'Public Health Service Programme' (PHSP). The haemoglobin adducts measurements were a voluntary offer, but they were ultimately demanded by the potentially exposed subjects. In the case of the relief units, the human biomonitoring was part of the public employers' obligation to provide for welfare of their employees.

Participants of the programme completed a standardised medical questionnaire and signed an informed consent sheet, which applied normative bioethical principles (Harrison, 2008; Reis et al., 2008; Morello-Frosch et al., 2009; Quigley, 2012). Informed consent covered aspects of sample collection and the storage as well as processing of data. The guarantee of confidentiality of the data collected was an essential part of the informed consent framework. Besides informed consent prior to the study, participants were fully informed of their individual results. This appeared to be an important incentive for participation, particularly with regard to the preservation of evidence of possible late sequelae, incapacity for work or claims for compensation. In order to be able to communicate individual results the participants' identities cannot be completely anonymised, but names were coded prior to data processing and analysis. The tracing of individual measurement results reported in this study and assignment to specific participants is excluded.

Specific questions regarding the onset time, the mean distance from the accident site and personal protection measures were part of the medical questionnaire. Sampling was performed from 17th September, 2002 to 15th November, 2002.

2.2. Haemoglobin adduct measurements

In order to perform the ECH-haemoglobin adduct measurements, new applicable bioanalytical methods had to be developed and validated in the context of the project for both the *N*-(3-chloro-2-hydroxypropyl)valine (CHPV) and the *N*-(2,3-dihydroxypropyl) valine (DHPV) in blood as haemoglobin adducts of ECH and glycidol, respectively. The new methods first published by Müller et al. (2005) (DHPV) and Bader et al. (2009) (CHPV) were later adopted by the working group "Analyses in Biological Materials" of the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) (Müller, 2013; Bader et al., 2013).

Generally, in order to determine the respective haemoglobin adduct, the erythrocytes are separated from whole blood and then lysed. Subsequently, the globin is isolated from the haemoglobin solution by precipitation. The globin preparation was conducted immediately after receiving the blood samples in the laboratory. The dried globins were subsequently stored at −20 °C (DHPV) and −80 °C (CHPV), respectively, prior to analysis. Müller (2013) demonstrated that globin samples stored at −20 °C for up to 2 years exhibit no noteworthy changes in DHPV adduct levels produced in vitro with racemic glycidol. Bader et al. (2009, 2013) stated that the dry globin can be stored up to 12 months at −27 °C without noteworthy CHPV adduct losses. As regards the storage of the material to be analysed, it is necessary, to carry out isolation of the globin and its subsequent storage in the freezer as soon as possible after specimen collection. There are no indications that a degradation of the CHPV adduct occurs under these conditions.

Törnqvist (1990) investigated the long-term stability of the reaction products of ethylene oxide with haemoglobin under different storage conditions and different methods of isolating red cells and preparing hemolysate as well as globin. According to Törnqvist (1990), haemoglobin adduct samples should be stored as precipitated globins at ≤−20 °C. Under these conditions, Törnqvist found the examined *N*-(2-hydroxyethyl)valine levels to be stable during a 4-year period.

The latest CHPV and DHPV analyses were conducted in mid-2007. Under the specific storage conditions as applied in this study, a significant adduct loss should not be assumed within the time of sampling and time of analysis.

After derivatisation of the globin with pentafluorophenyl isothiocyanate, the selective cleavage is carried out via a modified Edman degradation (Törnqvist et al., 1986) to yield the corresponding thiohydantoin derivatives. After a further derivatisation step with acetic anhydride (CHPV) or acetone (DHPV) to form the acetyl derivate and a ketal, respectively, the quantitative analysis is carried out using GC–MS/MS in the selected reaction monitoring (SRM) mode (CHPV) or in the selected ion monitoring (SIM) mode after negative chemical ionisation (NCI) (DHPV). The performance characteristics of the analytical tests are shown in Table 1.

Details of the methods used are available from Müller (2013) and Bader et al. (2013). The validation of both methods demonstrated that the newly developed analytical procedures

Table 1
Performance characteristics of the analytical tests of *N*-(3-chloro-2-hydroxypropyl)valine and *N*-(2,3-dihydroxypropyl)valine.

Parameter	*N*-(3-chloro-2-hydroxypropyl)valine (CHPV)	*N*-(2,3-dihydroxypropyl)valine (DHPV)
Within day precision		
Standard deviation (rel.) sw	12.4% or 9.8%	6.9% or 4.6%
Prognostic range *u*	27.6% or 21.8% at a spiked concentration of 25 or 100 pmol CHPV per gram globin and where $n = 10$ determinations	17.7% or 11.9% at a spiked concentration of 50 or 450 pmol DHPV per gram globin and where $n = 6$ determinations
Day to day precision		
Standard deviation (rel.) sw	15.0%	6.0% or 10.1%
Prognostic range *u*	33.4% based on the variance of the calibration graph slope and where $n = 10$ determinations	15.4% or 32.1% at a spiked concentration of 40 or 100 pmol DHPV per gram globin and where $n = 5$ or 4 determinations
Accuracy		
Recovery rate *r*	99.8 ± 12.5% at a nominal concentration of 100 pmol CHPV per gram globin and where $n = 10$ determinations	98% or 102% at a spiked concentration of 50 or 450 pmol DHPV per gram globin and where $n = 8$ or 6 determinations
Limit of detection (LOD)	10 pmol CHPV per gram globin	10 pmol DHPV per gram globin
Limit of quantitation (LOQ)	25 pmol CHPV per gram globin	25 pmol DHPV per gram globin

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are suitable for the detection and quantitation of globin adducts of ECH in blood with very high specificity and sensitivity.

2.3. Estimation of the individual excess lifetime cancer risk

ECH reacts directly with the terminal valine of the human haemoglobin chains forming chemically stable adducts that are not removed by repair. Experimental evidence for the chemical stability of the DHPV adduct of ECH in vivo is given by Hindsø Landin et al. (1999). The life time of haemoglobin adducts depends upon their chemical stability in vivo. Stable haemoglobin adducts are eliminated by linear, zero-order kinetics determined by the live-span of the erythrocytes (Fennel et al., 1992; Troester et al., 2001). Deviations occur in the case of unstable haemoglobin adducts. They are eliminated more rapidly because they are simultaneously lost to zero-order haemoglobin turnover and first-order chemical instability. For the haemoglobin adducts of ECH, the kinetics of removal is determined primarily by removal of the erythrocytes from circulation. Following a single, accidental exposure in absence of haematotoxicity, the elimination kinetics of haemoglobin adducts are zero-order and can be described by a simple linear equation. The adduct levels decline linearly, reaching background or zero concentrations at the lifetime of the erythrocytes due to the fact that the haemoglobin of the erythrocytes of all ages has been alkylated to a similar degree (Fennell et al., 1992). Because an exposure to ECH from other sources in the study population is not to be assumed, the haemoglobin level at the end of the lifespan of erythrocytes declines to zero. Therefore, the extrapolation of the haemoglobin adduct values measured at time of sampling to time of exposure can be carried out using the linear equation c haemoglobin adducts$_{t0}$ = c haemoglobin adducts$_{ts}$ × (c haemoglobin adducts$_{ts}$/($t_{lterythroc} - t_s$)), where c haemoglobin adducts$_{t0}$ = haemoglobin adducts dose at the time of single exposure referring to the accident, c haemoglobin adducts$_{ts}$ = haemoglobin adducts dose at the time of sampling, t_s = difference from date of sampling and date of exposure, and $t_{lterythroc}$ = lifetime of erythrocytes. The average lifespan value of human erythrocytes is 120 days; upper limits of about 126 days (Bader and Wrbitzky, 2006; Hindsø Landin et al., 1996) or 128 days (Tannenbaum et al., 1985), respectively, have also been reported.

The individual haemoglobin adduct concentrations were estimated using an overall haemoglobin content in the human of 750 g and 128 days as the lifetime value of erythrocytes (Tannenbaum et al., 1985). The recalculation of the haemoglobin adduct doses to an external exposure was done by means of a simplified reverse dosimetry approach using the data from Hindsø Landin et al. (1999) and Miraglia et al. (2001). Internal haemoglobin adduct doses were extrapolated to airborne ECH with the presumption that 0.1% of the body dose has bound to globin (Hindsø Landin et al., 1999) and 20% of this fraction are N-terminal adducts (Miraglia et al., 2001). A total of 2 days of accidental exposure to ECH was assumed. The resulting excess lifetime cancer risk after exposure was calculated by annualising the inhalational dose for the period of 2 days and finally converting the annual dose into a lifetime dose. To estimate the individual excess lifetime cancer risk an inhalation unit risk of 1.2×10^{-6} ($\mu g/m^3$)$^{-1}$ (U. S. EPA, 2013c) was used. The unit risk represents the upper-bound excess lifetime cancer risk estimated to result from continuous exposure to an agent – in this case ECH – at a concentration of 1 μg/L in water, or 1 μg/m^3 in air.

2.4. Statistical analyses

Microsoft Office Professional Plus Excel 2010 (Version 14.0.7106.5003) statistical software was used for all analyses.

3. Results

A total of 560 samples from four different groups of potentially exposed subjects of the relief units were analysed. They included 54 haemolysates of Lower Saxony state policemen and 172 haemolysates of federal policemen. Further 328 subjects (mostly firefighters or residents) were participating in the 'Public Health Service Programme' and further 6 samples came from railway officials. The results of the adduct measurements differentiated by group and the estimated excess lifetime cancer risks are given in detail in the Tables 2–5. Haemoglobin adduct concentrations were detectable, i.e. they exhibited values >LOD – in 36 out of 560 (6.4%) of the blood samples measured (Table 6). CHPV adduct levels above the LOQ of 25 pmol/g globin were quantified in 6 out of 560 samples from all sub-groups of the forces. The adduct values of these samples ranged from 30 to 80 pmol/g globin (measured values at the time of sampling) and from 32.0 to 116.4 pmol/g globin (extrapolated values for the time of exposure), respectively. The maximum value of 116.4 pmol/g globin was determined in a sample which belonged to a federal policeman who was according to his self-report in charge on September 12th, 2002, thus 3 days after the accident and at a distance of about 150–200 m from the centre of the contamination. The value was confirmed by two measurements in each independent series.

Table 2
Adduct concentrations measured in the 'Public Health Service Programme' sub-group and corresponding individual excess lifetime cancer risks.

Number of subjects (n)	Days after exposure (n)	Type of adduct	Concentration at time of sampling (pmol/g globin) c [$(c-u)$–$(c+u)$]	Extrapolated concentration at time of exposure (pmol/g globin) c [$(c-u)$–$(c+u)$]	Excess lifetime cancer risk (range)
1	60	CHPV	35 [23–47]	65.9 [43.3–88.5]	5.37×10^{-8} ($3.53 \times 10^{-8} - 7.21 \times 10^{-8}$)
1	46	CHPV	45 [30–60]	70.2 [46.8–93.7]	5.72×10^{-8} ($3.82 \times 10^{-8} - 7.63 \times 10^{-8}$)
1	59	CHPV	38 [25–51]	70.5 [46.4–94.6]	5.75×10^{-8} ($3.78 \times 10^{-8} - 7.71 \times 10^{-8}$)
11	67	CHPV	<25[a] [17–33]	52.5 [35.7–69.2]	4.28×10^{-8} ($2.91 \times 10^{-8} - 5.64 \times 10^{-8}$)
314	67	CHPV	<10[b]	n/a	n/a
328	67	DHPV	<10[c]	n/a	n/a

All concentrations represent single determinations. Values >LOD were confirmed by one repeat measurement. Range of concentration is given as follows: c – prognostic range u (day to day precision: 33.4%, cf. Table 1) and $c+u$, respectively.

[a] Adduct concentrations were measured between LOD and LOQ. For the purpose of risk assessment under the assumption of the worst case, haemoglobin adduct levels >LOD and <LOQ have been combined with the largest difference of sampling date after exposure with a concentration that corresponds to the LOQ (25 pmol/g globin). The individual values ranged from 11 to 17 pmol/g globin with the median at 12 pmol/g globin.

[b] CHPV adduct levels <LOD (10 pmol/g globin).

[c] DHPV adduct levels <LOD (10 pmol/g globin).

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Table 3
Adduct concentrations measured in the federal police sub-group and corresponding individual excess lifetime cancer risks.

Number of subjects (n)	Days after exposure (n)	Type of adduct	Concentration at time of sampling (pmol/g globin) c [$(c-u)$–$(c+u)$]	Extrapolated concentration at time of exposure (pmol/g globin) c [$(c-u)$–$(c+u)$]	Excess lifetime cancer risk (range)
1	40	CHPV	80 [53–107]	116.4 [77.1–155.6]	9.48×10^{-8} ($6.28 \times 10^{-8} - 1.27 \times 10^{-7}$)
14	60	CHPV	<25[a] [17–33]	47.1 [32.0–62.1]	3.84×10^{-8} ($2.61 \times 10^{-8} - 5.06 \times 10^{-8}$)
157	60	CHPV	<10[b]	n/a	n/a
172	60	DHPV	<10[c]	n/a	n/a

All concentrations represent single determinations. Values >LOD were confirmed by one repeat measurement. Range of concentration is given as follows: c – prognostic range u (day to day precision: 33.4%, cf. Table 1) and $c+u$, respectively.
[a] Adduct concentrations were measured between LOD and LOQ. For the purpose of risk assessment under the assumption of the worst case, haemoglobin adduct levels >LOD and <LOQ have been combined with the largest difference of sampling date after exposure with a concentration that corresponds to the LOQ (25 pmol/g globin). The individual values ranged from 10 to 22 pmol/g globin with the median at 14 pmol/g globin.
[b] CHPV adduct levels <LOD (10 pmol/g globin).
[c] DHPV adduct levels <LOD (10 pmol/g globin).

Table 4
Adduct concentrations measured in Lower Saxony state policemen sub-group and corresponding individual excess lifetime cancer risks.

Number of subjects (n)	Days after exposure (n)	Type of adduct	Concentration at time of sampling (pmol/g globin) c [$(c-u)$–$(c+u)$]	Extrapolated concentration at time of exposure (pmol/g globin) c [$(c-u)$–$(c+u)$]	Excess lifetime cancer risk (range)
1	8	CHPV	30 [20–40]	32.0 [21.3–42.7]	2.61×10^{-8} ($1.74 \times 10^{-8} - 3.48 \times 10^{-8}$)
1	8	CHPV	31 [21–41]	33.1 [22.7–43.7]	2.69×10^{-8} ($1.83 \times 10^{-8} - 3.56 \times 10^{-8}$)
3	8	CHPV	<25[a] [17–33]	26.7 [18.1–35.2]	2.17×10^{-8} ($1.48 \times 10^{-8} - 2.87 \times 10^{-8}$)
1	49	CHPV	<25[a] [17–33]	40.5 [27.5–53.5]	3.30×10^{-8} ($2.24 \times 10^{-8} - 4.36 \times 10^{-8}$)
1	50	CHPV	<25[a] [17–33]	41.0 [27.9–54.2]	3.34×10^{-8} ($2.27 \times 10^{-8} - 4.41 \times 10^{-8}$)
46	51	CHPV	<10[b]	n/a	n/a
1	100[c]	CHPV	<10[b]	n/a	n/a
54	51	DHPV	<10[d]	n/a	n/a

All concentrations represent single determinations. Values >LOD were confirmed by one repeat measurement. Range of concentration is given as follows: c – prognostic range u (day to day precision: 33.4%, cf. Table 1) and $c+u$, respectively.
[a] Adduct concentrations were measured between LOD and LOQ. For the purpose of risk assessment under the assumption of the worst case, haemoglobin adduct levels >LOD and <LOQ have been combined with the largest difference of sampling date after exposure with a concentration that corresponds to the LOQ (25 pmol/g globin). The individual values ranged from 10 to 20 pmol/g globin with the median at 11 pmol/g globin.
[b] CHPV adduct levels <LOD (10 pmol/g globin).
[c] Registering late.
[d] DHPV adduct levels <LOD (10 pmol/g globin).

Table 5
Adduct concentrations measured in the officials of the Federal Railway Authority sub-group and corresponding individual excess lifetime cancer risks.

Number of subjects (n)	Days after exposure (n)	Type of adduct	Concentration c at time of sampling (pmol/g globin)	Extrapolated concentration c at time of exposure (pmol/g globin)	Excess lifetime cancer risk
1	14	CHPV	<10[a]	n/a	n/a
1	15	CHPV	<10[a]	n/a	n/a
1	21	CHPV	<10[a]	n/a	n/a
2	20	CHPV	<10[a]	n/a	n/a
1	42	CHPV	<10[a]	n/a	n/a
6	42	DHPV	<10[b]	n/a	n/a

[a] CHPV adduct levels <LOD (10 pmol/g globin).
[b] DHPV adduct levels <LOD (10 pmol/g globin).

CHPV adduct levels which comply with measuring signals between the LOD and the LOQ have been evaluated as non-quantitative data and they are given in Tables 2–5 for the purpose of comparison only. In these cases, the chemical analyses did not comply with the analytical quality criteria as listed above and therefore the quantification was not reasonable due to the large margin of error.

By contrast, the DHPV adducts could not be detected in any blood sample analysed despite the fact that the sensitivity of the DHPV method is equal to that of the CHPV measurements.

Table 6 summarises the number of CHPV measurements related to all sub-groups and the percentage of results above the limit of quantification as well as above the limit of detection and additionally presents the results regarding blood samples from

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Table 6
CHPV measurements related to all sub-groups and the percentage of results above LOQ and LOD.

Subgroup	Total number of samples	Number of samples >LOQ (%)	Number of samples >LOD and <LOQ (%)
'Public Health Service Programme'	328	3 (0.9)	11 (3.4)
Federal policemen	172	1 (0.6)	14 (8.1)
Lower Saxony state policemen	54	2 (3.7)	5 (9.2)
Officials of the Federal Railway Authority	6	0 (0)	0 (0)
Sum of forces	**560**	6 (1.1)	30 (5.4)
Privately financed requests[a]	**68**	0 (0)	0 (0)
Sum of all subjects	**628**	6 (0.96)	30 (4.8)

[a] Proprietary data that cannot reported in detail.

privately financed requests. Noteworthy is the low percentage of less than 1% of subjects in the PHSP and in the group of federal police. The group of the Lower Saxony state policemen in turn reveals the highest percentage of samples with adduct levels >LOQ (3.7%) as well as samples with adduct levels ranging from the limit of detection to the limit of quantification (9.2%). A detectable, but not quantifiable exposure has likewise been observed in 8.1% of the federal police group. Finally, if also considering the number of samples from privately financed requests, the CHPV adduct was quantifiable in only 6 out of 628 samples. This corresponds approximately to a proportion of 1% (0.96%) of all samples tested, whereas 30 of the total of 628 samples (4.8%) had non-quantifiable haemoglobin adduct values >LOD and <LOQ. For the accidental ECH release discussed here, the extrapolation of an adduct level of 25 pmol/g globin and exposure over 48 h would have resulted in a mean airborne ECH concentration of 0.218 mg/m^3 per day. As described above, for a CHPV adduct level of 25 pmol/g globin that corresponds to the limit of quantification, an excess lifetime cancer risk of 2.04×10^{-8} can be calculated. In case of the quantified CHPV adducts exceeding the LOQ (Tables 2–4), the resulting estimates of the cumulative additional lifetime cancer risks then range from 2.61×10^{-8} to 9.48×10^{-8}. This corresponds to body doses from ECH exposure over two days ranging from 1.7 to 6.2 nmol/kg body weight. Estimates of the additional cancer risk that have been made with adduct concentrations between the LOD and LOQ on the basis of an assigned maximum level of 25 pmol/g globin lead to excess lifetime cancer risks which are in the same order of magnitude and do practically not differ from those with levels >LOQ. In fact, these risk estimates would still be up to a factor of maximal 2.5 below the values listed in Tables 2–4 because the related adduct values measured were within the range of LOD and LOQ but were not equal to the limit of quantification.

4. Discussion

To our knowledge, this is the first time that the CHPV adduct has been demonstrated to be the primary haemoglobin adduct of ECH exposure in humans. Hindsø Landin et al. (1996, 1997, 1999) did not differentiate between the CHPV- and DHPV-adducts in their studies with volunteers or workers and focused on the quantitative determination of *N*-(2,3-dihydroxypropyl)valine. They presumed from experiments with female Wistar rats administered single doses of 0.11–0.97 mmol ECH/kg body weight by i.p. injection that the levels of CHPV adducts decline as a result of turnover of the erythrocytes and by rearrangement of the adducts (Hindsø Landin et al., 1999). Twenty-four hours after administration the major measured adduct was the unstable S-(3-chloro-hydroxypropyl) cysteine and DHPV was a minor product. The possibility to identify exposure by using the DHPV adduct was considered as limited due to the relatively high background of unknown origin (Hindsø Landin et al., 1997).

In our study, the DHPV adduct could not be detected above the achieved LOD of 10 pmol/g globin in any of the globin samples of all the analysed sub-groups. By contrast, DHPV adduct levels of 21.1 ± 17.1 pmol/g globin (smokers, $n = 7$) and 7.3 ± 2.7 pmol/g globin (non-smokers, $n = 8$) have been reported for continuous exposure to ECH at the workplace (air concentrations were 0.4–0.86 mg/m^3 for 12 h/day with peak levels of 1.5–10 mg/m^3 for 45 min/day as well as 4 days/week) (Hindsø Landin et al., 1997). Compared to the exposed workers, mean DHPV adduct levels in German controls were 13.1 ± 12.4 pmol/g globin (smokers, $n = 9$) and 6.8 ± 3.2 pmol/g globin (non-smokers, $n = 5$). The corresponding values in Swedish controls were 9.5 ± 2.2 pmol/g globin (smokers, $n = 4$) and 2.1 ± 1.1 pmol/g globin (non-smokers, $n = 6$). The reported detection limit was approximately 2 pmol/g globin (Hindsø Landin et al., 1997). The origin of the ECH burden in the controls, in particular in the subset of samples from non-smokers, does not seem to be fully understood and remains unclear. Above all, the data of the controls (particularly of non-smokers) raises concerns about their analytical reliability because they are very close to the detection limit of 2 pmol/g globin; the LOQ was not stated. The DHPV adduct levels from the workplace study (Hindsø Landin et al., 1997) refer to long-term exposure and airborne ECH concentrations that at this level are unlikely as a result of the accidental release, if we consider our estimate of 4.7 mg ECH/m^3 which corresponds to the extrapolated maximum haemoglobin adduct value of 116.4 pmol CHPV/g globin (Table 2). The orientating measurement of the air concentration performed by the firefighters 6 h after the accidental ECH emission by means of field screening methods was comparatively insensitive with values of <5 ppm (<19.2 mg/m^3) and only permitted monitoring ECH's Acute Exposure Guideline Levels.

Our human biomonitoring study was part of the tiered approach to assess the health effects resulting from the emissions of ECH after the crash of the goods trains. Following an offer of the local public health department within its immediate action programme and in order to exclude a considerable exposure of the forces and residents to ECH and its toxic breakdown products, a broad programme of individual health checks on a voluntary basis was performed in three phases including biomonitoring and effect monitoring investigations (such as liver enzymes in blood or cytogenetic analyses) (Toedt et al., 2003; Umweltbundesamt, 2006). As parameters of liver injury, ALT, AST and gamma-GT were examined in 1747 subjects (firemen and self-notifiers of the residential population) during the first phase in September 2002. The liver enzyme values were compared with those of two local, non-exposed reference populations. Whether their partially observed increase was in fact due to the uptake of ECH could not be established beyond all doubt (Toedt et al., 2003). To what extent methodological aspects of the investigating laboratories might play a role with respect to their internal laboratory reference values remained unclear. Similar equivocal data were obtained in the follow-up in March 2003 (phase three) (Toedt et al., 2003).

Scheduling the measurements of haemoglobin adducts began in the middle of September 2002. Unlike the insensitive methods for ECH exposure such as the liver enzyme determinations in

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blood, the haemoglobin adduct measurements could undoubtedly confirm if an external exposure had taken place and had led to a measurable internal burden. In comparison to the initially presumed relevant intake of ECH and its metabolites, which was supported by the high number of physician consultations, the determination of the ECH adducts CHPV and DHPV in blood demonstrates that ECH had led both to only a marginally internal exposure and a lower number of affected subjects than expected. The resulting excess lifetime cancer risks have to be assessed as extremely low. Usually, excess lifetime cancer risks in the range of approximately 10^{-5}–10^{-6} (Directive, 2004; Directive, 2008) have been set as a reasonable level of environmental health protection in regulations of ambient air quality in the European Union. Our estimates of the excess lifetime cancer risks are about two to three orders of magnitude below those cancer risks that are associated with the EC limit values for carcinogenic substances in ambient air.

It is an important finding that the measured haemoglobin adducts of those subgroups such as the forces, which could have most probably been exposed to ECH proved to be noticeably lower than they themselves had initially feared. We have found no significant association between adduct concentrations and case history regarding the exposure parameters like average distance of the place of action from the accident centre and duration of the presence on-site. 50% of all adduct values >LOD have been analysed in samples of forces which were within the first 8 h at the accident location.

The maximum value of 116.4 pmol CHPV/g globin (Table 2), as extrapolated from the actually measured adduct concentration to the time of the accident, corresponds to an airborne ECH concentration of 4.7 mg/m^3, which is even below the U.S. EPA's proposed AEGL 1 values which are constantly 5.7 ppm (21 mg/m^3, endpoint: no effect level for irritation) for 10-min to 8-hexposures. Thus, other compounds than ECH could be responsible for the observed irritations of the eye and respiratory tract and other acute health effects, formed from combustion and by environmental abiotic degradation of ECH. For example, ECH hydrolyses in distilled water to yield hydrogen chloride (irritant to the eyes and airways) or the skin-irritating 1-chloro-2,3-propanediol, having a half-life of 8.2 days (HSDB, 2014). The half-life for the reaction of ECH with water at room temperature was found to be 148, 79, and 62 h, respectively, in neutral, acidic, and alkaline solutions containing 9 mg ECH/L, initially (IPCS, 1984). This degradation pathway might have been substantial because of fire-fighting and the intense rain 24 h after the ECH emission. Volatilisation from moist soil surfaces is expected to be an important fate process based upon an estimated Henry's Law constant of 3.0×10^{-5} (atm $\times$ m^3)/mole. Vapour-phase ECH will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals (HSDB, 2014). It is conceivable that the perceived health effects in residents and forces with symptoms that partly persisted for several days or longer originated rather from combustion products and metabolites of abiotic degradation than from ECH itself.

The low ECH burden of involved firefighters was indirectly confirmed by cytogenetic analyses. The results from a study with a total of 229 subjects demonstrated that both the mean chromosomal aberrations rates and mean SCE rates as well showed no statistically significant differences compared with the control groups (Umweltbundesamt, 2006). First follow-up results from a prospective cohort study of the Epidemiological Cancer Registry of Lower Saxony referring to the ECH accident in Bad Münder showed no elevated incidence rates of cancer cases overall (ICD-10 C00 – C97 without C44) or lung cancer (ICD-10 C33 + C34) neither for men nor for women (Urbschat et al., 2012).

ECH's ADME characteristics could explain the firstly surprising finding that despite the considerable ECH release no significant internal exposure was observed particularly in the forces. In the rat, ECH is absorbed via the inhalational and oral pathway to about 90% and is distributed in the organism within a few hours. The excretion of more than 90% of ECH is accomplished within 72 h, mainly via urine and exhalation (approximately 50% and 25–40%), and only in small amounts (about 4%) via faeces (DFG, 2012; IARC, 1999). In a pharmacokinetic study, male Fischer 344 rats were exposed to 1 or 100 ppm of ECH-1,3-^{14}C vapour for 6 h, and about 72% of the dose was excreted in the first 24 h and about 83% of the dose was excreted within 72 h regardless of the exposure level (Smith et al., 1979). About 46 and 54% of the radioactivity was recovered from urine, 34 and 27% in expired air, and 3% from faeces after exposure to 1 and 100 ppm, respectively. Excretion of ECH was biphasic; the half-life for the slower fraction was about 24 h. The half-life for elimination from plasma was 26 h, which was comparable to that for excretion. The authors concluded that ECH is not extensively sequestered in a deep body compartment (Smith et al., 1979).

Although haemoglobin adduct levels usually cannot directly reflect the genotoxic and carcinogenic potential of a substance, they are nevertheless a quantitative parameter that is closer to the biologically active dose. Conclusions about the possible cancer risk on the basis of haemoglobin adducts must take into account the principle that erythrocytes represent a surrogate tissue as blood components and do not represent the critical target organ of DNA adduct formation and/or tumour formation. The ability of ECH to alkylate macromolecules in animal models in vivo appears to be generally less despite the greater chemical reactivity than that of the structurally similar oxiranes propylene oxide and ethylene oxide (Koskinen and Plná, 2000), which can be interpreted as a rapid metabolism/elimination of ECH. The IUCLID data (European Commission, 2000) to ECH documents in vitro studies of the covalent binding of ECH to macromolecules of various tissue homogenates of rats and humans. Thus, the covalent binding in human tissues was either similar to or lower than in rat tissues. In vivo human data is apparently not available.

N-(2,3-dihydroxypropyl)valine (DHPV) is the putative haemoglobin adduct of ECH formed via different pathways. It could be formed from the CHPV adduct by hydrolysis, but the intermediate stages glycidol and 1-chloro-2,3-propanediol, respectively, have also been discussed as precursors of DHPV. That the secondary DHPV adduct could not be measured may be caused by differences in the toxicokinetics at higher dose level as they were observed at the workplace (Hindsø Landin et al., 1997) and in the present experimental animal data (Hindsø Landin et al., 1999) if compared with low doses from the accidental exposure.

This study has some uncertainties that especially relate to the input data of the simplified reverse dosimetry approach. There was no data accessible with regard to the uncertainty referring to the percentage of 0.1% of the body dose that has bound to globin (Hindsø Landin et al., 1999) and the 20% of this fraction that are N-terminal haemoglobin adducts (Miraglia et al., 2001). By contrast, the studies on the covalent binding of ECH to macromolecules of various tissue homogenates of rats and humans are not suggestive of considerably differences between species (European Commission, 2000). The presumption of two days exposure to ECH represents a worst case scenario resulting in an overestimation of the excess lifetime cancer risk.

5. Conclusion

To sum up, haemoglobin adduct measurements are a valuable tool for estimating the actual uptake of carcinogens like ECH and determining the individual response connected with single or accidental acute exposure. The new developed GC–MS/MS method for *N*-(3-chloro-2-hydroxypropyl)valine and GC/NCI-MS method

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for *N*-(2,3-dihydroxypropyl)valine ensured the reliable measure of both adducts at low levels in blood. In respect thereof, both human biomonitoring methods should also be suitable to monitor airborne ECH exposure in workplaces. In the present case, the determined CHPV haemoglobin adduct values demonstrate that the internal exposure of forces and residents was actually low in spite of the considerable release of ECH during the course of the accident and furthermore that a considerable lower number of subjects than supposed were affected. The advanced methodical approach of using the haemoglobin adducts CHPV and DHPV, respectively, has led to a new quality of risk communication with forces and residents. Interpreting biomonitoring in a risk communication context optimises its value and impact by capacitating public health professionals to communicate results to individuals and groups in terms of their health concerns (Dourson et al., 2013). Our haemoglobin adduct measurements facilitated the dialogue between people concerned and authorities, because suspected or occurred exposures and risks to human health could be quantified and interpreted in a sound manner. Thus, each individual risk estimate could be communicated in a rational and transparent way. The measurement of the haemoglobin adducts and the joined risk assessment has contributed to a noticeable objectification of risk communication.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

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

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