ANALYSIS OF PROTEIN ADDUCTS AS BIOMARKERS OF SHORT-TERM EXPOSURE TO ETHYLENE OXIDE AND RESULTS OF FOLLOW-UP BIOMONITORING*

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An accidental exposure of six workers to ethylene oxide (EO) provided the rationale for a biomonitoring and follow-up study, whose aim was to analyse protein adduct kinetics and examine the differentiation between accidental and environmental exposure, e.g., from tobacco smoke. For this purpose, the decrease in the concentration of the haemoglobin adduct N-2-hydroxyethylvaline (HEV) was followed during a five-month period after the accident, together with N-2-cyanoethylvaline (CEV) and urinary cotinine, two well-established biomarkers for smoking. The follow-up study showed that EO adduct concentrations significantly increased after a short but presumably high exposure. Initial biomonitoring revealed HEV levels above 500 pmol g⁻¹ globin in all cases, with a maximum of about 2,400 pmol g⁻¹ globin. This compares to a German EKA value (exposure equivalent for carcinogenic substances) for a daily 8-h-exposure to 1 ppm EO of 90 μg L⁻¹ blood (~3,900 pmol g⁻¹ globin). The adduct levels dropped in accordance with the expected zero-order kinetics for a single exposure. After the five-month observation interval, the HEV concentrations in blood reflected the individual background from tobacco smoking. The results of this study show that even a short exposure to ethylene oxide may result in a significant rise in haemoglobin adduct levels. Although protein adducts and their occupational-medical assessment values are considered for long-term exposure surveillance, they can also be used for monitoring accidental exposures. In these cases, the calculation of daily ‘ppm-equivalents’ may provide a means for a comparison with the existing assessment values.

KEY WORDS: accidental exposure, haemoglobin adduct kinetics, occupational exposure

Ethylene oxide (EO) is an important chemical intermediate mainly used for the synthesis of ethylene glycol and its ethers, ethoxylates, ethanolamines, polyoles, and polyesters, or for sterilisation purposes (1). Its acute toxic properties comprise moderate irritation of the eye, the mucous membranes, and the upper respiratory tract with typical symptoms of exposure being headache, dizziness, nausea, and vomiting. EO is a known animal carcinogen and a suspected human carcinogen (2, 3). Due to its hazardous potential, ethylene oxide is usually handled and reacted in closed systems. Its use for sterilisation purposes, e.g., in hospitals and in the production of sterile disposable medical equipment, is strictly controlled in Germany by technical guidelines, and biological monitoring is recommended for health surveillance (4-7).

Ethylene oxide is readily absorbed either by inhalation or through the skin (skin notation). The main metabolic pathway of EO involves a hydrolysis
of the epoxide to yield ethylene glycol and a subsequent sequential oxidation to oxalate, formiate and carbon dioxide, or an enzymatically mediated conjugation to glutathione to form S-2-hydroxyethyl mercapturic acid (8, 9). In addition, ethylene oxide binds spontaneously to nucleophilic acids in proteins, namely to haemoglobin in blood. One of these covalently bound addition products, or 'adducts', is the N-terminal N-2-hydroxyethylvaline (HEV), a well-established biomarker for ethylene oxide exposure (10-12). In Germany, the Senate Commission for the Investigation of Health Hazards in the Work Area of the German Research Foundation (Deutsche Forschungsgemeinschaft - DFG) has established so-called 'exposure equivalents for carcinogenic substances' (EKA), which describe the correlation between ethylene oxide concentrations in air and HEV concentrations in blood (13). A confounder for this biomarker is smoking, as ethylene oxide is a metabolite of ethene in tobacco smoke (14-16). While nonsmokers usually show HEV levels below 75 pmol g⁻¹ globin, levels up to 550 pmol g⁻¹ globin are observed in blood samples from smokers (17). One specific aspect in the interpretation of adduct biomonitoring is the fact that assessment and reference values are based on steady-state concentrations. These are achieved only after a regular exposure for at least 120 days, which corresponds to the average life-span of the erythrocytes (18-21). Under constant exposure conditions, the adduct levels continue to increase during this interval until the equilibrium between daily increment of adducts and erythrocyte breakdown has been reached. While protein adducts are valuable markers of a long-term integrated dose, measurement and interpretation of adduct levels after intermittent or single exposures have not yet been well established. In the context of isolated accidental exposures, it would also be interesting to see if the decline of adduct levels follows the expected linear function until the baseline level, e.g., associated mainly with regular smoking habits, has been reached after 120 days. In this case, an extrapolation of adduct concentrations, which were measured weeks or even months after the exposure, would be both possible and advantageous, as it offers a broader time-frame for sample collection than most other biomarkers.

In a study following an accidental exposure of six workers to ethylene oxide in a chemical plant, we investigated the degradation kinetics of the haemoglobin adduct HEV during a period of five months. Furthermore, we calculated the additional exposure following the accident and developed an approach for the interpretation of adduct levels after single or short-term exposures. As the individual background levels from tobacco smoking are to be considered for HEV interpretation, urinary cotinine and N-2-cyanoethylvaline (a haemoglobin adduct of acrylonitrile in tobacco smoke) were analysed (17, 22, 23).

MATERIALS AND METHODS

Study group and ethylene oxide exposure

Six male workers from a chemical plant were accidentally exposed to ethylene oxide outside the building. About 40 kg of liquid EO were released through an open valve and evaporated into the surroundings. While there were no specific data available on the concentration of EO in air, the nearby gas alert system was activated, thus indicating that several hundred ppm of EO were detected in the vicinity of the building. Taking into account that the open valve was shut after 2 to 3 min and that a mobile measuring station did not detect any airborne EO after about 15 min, the maximum exposure time of six workers was estimated to be 15 minutes. Immediately after the accidental exposure, the workers were taken care of in the out-patient clinic of the Occupational Medicine & Health Protection Department of the company.

Biomonitoring

Blood and urine samples were collected in the out-patient clinic one day after the accident and thereafter on a monthly basis over a five-month interval. Blood samples were drawn into regular EDTA-containing disposable syringes (Monovettes®, Sarstedt, Germany). The erythrocytes were separated from the plasma fraction by centrifugation (800 × g, 5 min) and washed twice with isotonic saline (addition of 0.9 % sodium chloride, centrifugation, removal of saline) until the supernatant was colourless and clear. The original sample volume was then restored by addition of ultrapure water, while the erythrocytes were lysed by this procedure. To monitor exposure to ethylene oxide and to the tobacco smoke contaminant acrylonitrile, the haemoglobin adducts N-2-hydroxyethylvaline (HEV) and N-2-cyanoethylvaline (CEV) were quantified in these samples. The protein adduct analyses were carried out in an accredited and
certified contract laboratory (Currenta, Leverkusen, Germany) essentially following a procedure described by van Sittert et al. (17). Globin was isolated from the haemolysates by fractionated precipitation. The dried protein was then subjected to the so-called ‘modified or N-Alkyl-Edman method’ (10): the adduct-bearing N-terminal amino acid (HEV, CEV) was cleaved off of the protein chain and simultaneously derivatised with pentafluorophenyl isothiocyanate into a thiohydantoin. It was then extracted with diethyl ether, evaporated to dryness, and reconstituted in toluene. Subsequently, the samples were analysed by gas chromatography-mass spectrometry (GC-MS) in the electron impact mode.

Spot urine samples were collected in parallel to the blood specimens in 100 mL polystyrene containers. The nicotine metabolite cotinine was analysed in these samples in the biomonitoring laboratory of the Occupational Medicine & Health Protection Department at BASF SE, Ludwigshafen, Germany, according to a method described by Müller et al. (24). Urine samples were alkalised with sodium hydroxide solution and extracted with dichloromethane after the addition of 2-benzylpyridine as internal standard. The extract was then analysed by GC-MS in the electron impact mode using selected ion monitoring. The method has been tested and certified within several round-robin tests of the German External Quality Assessment Scheme (G-EQUAS, c/o Institute of Occupational, Social and Environmental Medicine of the University of Erlangen-Nuremberg). To correct for diuretic variance, urinary creatinine was analysed by an HPLC-UV method (25) in the BASF laboratory. This parameter was also successfully certified within the G-EQUAS program.

### RESULTS

Four of the six workers exposed to ethylene oxide reported moderate acute effects: mild irritation of the mucous membranes and the upper respiratory tract, dizziness, shortness of breath, cough, and headache. Lung function testing of one worker revealed a slightly reduced peak flow. Two workers with pulmonary symptoms received intravenous corticoids for lung oedema prevention according to the established medical guidelines (26) and stayed in a clinic overnight for observation. Four days after the accidental exposure, two workers still reported general weariness and one of them felt a sunburn-like itchy feeling on some parts of his skin. All observed effects had completely resolved by the time the first follow-up biomonitoring investigation was carried out, four weeks after the accident.

As individual smoking habits are an important factor for further interpretation of the adduct levels of exposure to ethylene oxide, two biomarkers of tobacco smoke, urinary cotinine and haemoglobin adduct CEV, were analysed in order to confirm the self-reported smoker status of the employees (Table 1). Cotinine biomonitoring results for all self-reported smokers (study participants 1, 2, 3, 5, and 6) were always high above the reference values (95th percentiles) of 16 μg g⁻¹ creatinine for never-smokers and 53 μg g⁻¹ creatinine for smokers. Data processing and calculations

All calculations were carried out with either the Microsoft® Office Excel 2003 software package or with IBM® SPSS® Statistics 19.0.

### Table 1 Results of N-2-cyanoethylvaline and cotinine analyses

<table>
<thead>
<tr>
<th>Worker</th>
<th>Biomarker</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
<th>Sample 5</th>
<th>Sample 6</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (S)</td>
<td>CEV / pmol g⁻¹ globin</td>
<td>212</td>
<td>192</td>
<td>204</td>
<td>94</td>
<td>--</td>
<td>106</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>cotinine / μg g⁻¹ creatinine</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1,459</td>
<td>--</td>
<td>2,649</td>
<td>2,054</td>
</tr>
<tr>
<td>2 (S)</td>
<td>CEV / pmol g⁻¹ globin</td>
<td>114</td>
<td>102</td>
<td>94</td>
<td>69</td>
<td>106</td>
<td>90</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>cotinine / μg g⁻¹ creatinine</td>
<td>--</td>
<td>2,086</td>
<td>1,766</td>
<td>2,126</td>
<td>1,989</td>
<td>2,247</td>
<td>2,043</td>
</tr>
<tr>
<td>3 (S)</td>
<td>CEV (pmol g⁻¹ globin)</td>
<td>147</td>
<td>147</td>
<td>106</td>
<td>53</td>
<td>114</td>
<td>69</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>cotinine / μg g⁻¹ creatinine</td>
<td>--</td>
<td>6,244</td>
<td>1,577</td>
<td>752</td>
<td>1,132</td>
<td>3,879</td>
<td>2,717</td>
</tr>
<tr>
<td></td>
<td>cotinine / μg g⁻¹ creatinine</td>
<td>--</td>
<td>16</td>
<td>15</td>
<td>10</td>
<td>15</td>
<td>--</td>
<td>14</td>
</tr>
<tr>
<td>5 (S)</td>
<td>CEV / pmol g⁻¹ globin</td>
<td>200</td>
<td>167</td>
<td>155</td>
<td>49</td>
<td>188</td>
<td>131</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>cotinine / μg g⁻¹ creatinine</td>
<td>--</td>
<td>1,073</td>
<td>678</td>
<td>432</td>
<td>709</td>
<td>2,045</td>
<td>987</td>
</tr>
<tr>
<td>6 (S)</td>
<td>CEV / pmol g⁻¹ globin</td>
<td>131</td>
<td>118</td>
<td>94</td>
<td>69</td>
<td>139</td>
<td>82</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>cotinine / μg g⁻¹ creatinine</td>
<td>--</td>
<td>--</td>
<td>734</td>
<td>181</td>
<td>893</td>
<td>1,485</td>
<td>823</td>
</tr>
</tbody>
</table>
creatinine for ex-smokers (Third German Environmental Survey) (27). Many samples showed cotinine levels above 1,000 μg g⁻¹ creatinine, these results being well in the range of typical smokers. The German Federal Environment Agency reports a cotinine median of 998 μg g⁻¹ creatinine and a 95th percentile of 3,340 μg g⁻¹ creatinine for a representative sample of smokers (n=1,605) (27). The individual smoking status was further confirmed by the CEV analyses: all adduct levels were above 50 pmol g⁻¹ globin, whereas nonsmokers normally show CEV levels below 10 pmol g⁻¹ globin (13). Only one worker (study participant 4) declared himself a nonsmoker and biomonitoring results confirmed this: CEV was not detectable in blood samples of this employee and the cotinine levels were between 10 μg g⁻¹ and 14 μg g⁻¹ creatinine. Both tobacco smoke related biomarkers showed a distinct intra-individual variation during the study interval, with a larger variability of the short-term biomarker cotinine (average: 53 %) as compared to the protein adduct CEV (average: 30 %).

The results of the initial and follow-up biomonitoring of HEV in the six workers are summarised in Table 2. The adduct levels observed one day after the accidental exposure (four days in one case) ranged between 522 pmol g⁻¹ and 2,396 pmol g⁻¹. Every worker provided five more samples in the following four months (one sampling time was missed by study participant 1), however not always strictly according to the scheduled 30-day intervals. Nevertheless, the follow-up data showed the expected decline in individual HEV concentrations until the sixth sampling, which took place after 162 days (166 days in one case). The smokers still showed HEV levels in a range between 151 pmol g⁻¹ and 276 pmol g⁻¹ globin at the end of the study; this is consistent with typical smoker values as reported, for example, by van Sittert et al. (17), Bader et al. (16) and Schettgen et al. (22). This also confirms the results of biomonitoring for urinary cotinine and CEV. In contrast, the nonsmoking employee revealed an HEV level of only 30 pmol g⁻¹ globin. According to Törnqvist et al. (28), background levels of HEV in nonsmokers are probably associated with ethene production by intestinal bacteria. Typical HEV levels in nonsmokers are below 50 pmol g⁻¹ globin (16, 17, 22).

Due to the average life-span of human erythrocytes of about 120 days, the final HEV concentration of every individual more than 160 days after the accidental exposure was regarded as the tobacco smoke and intestinal ethene related background. This value was therefore subtracted from the initially measured adduct concentration in order to calculate the impact of the additional accidental exposure on HEV levels (Table 3). According to the German EKA values for ethylene oxide, a daily exposure to 1 ppm (1830 μg m⁻³) EO corresponds to an HEV level of approximately 3,900 pmol g⁻¹ globin under steady-state conditions (13), which in turn reflects the average exposure of 60 working days (12). Therefore, an increment of 3,900/60=65 pmol g⁻¹ per 8-h-exposure day can be calculated and used to derive ‘ppm-equivalents’ from the adduct concentrations related to accidental exposure. Following this approach, the adduct levels of the exposed workers correspond to 5 to 36 ‘ppm-equivalents’.

To analyse adduct kinetics, the initial HEV results were individually adjusted to 100 % after subtraction of the respective background values (Figure 1). A cubic fit (R²=0.950, p<0.001) was applied in this case.
to visualise the reduction of adduct concentrations and its confidence interval. It is noteworthy that after more than 120 days the HEV values were still higher than the final background values (>160 days). This was also the case with the nonsmoking study participant, thus pointing to a fraction of exposed erythrocytes with a longer than average life-span. According to the assumption of a constant adduct decline following zero-order kinetics during the first 120 days (12, 21), all adjusted HEV results between initial biomonitoring and 130 days post-exposure were subjected to a linear regression analysis (Figure 2). The resulting linear fit was \( y = -0.81 \times \text{(days post-exposure)} + 95 \) (with \( R^2=0.865, \ p<0.001 \), numbers in brackets = 95% confidence intervals). According to this equation, the concentration of HEV after one-time exposure decreases constantly at a rate of 0.81% of the initial value per day until the background level is reached after \( 95/0.81 = 117 \) days (or 123 days, in case only the slope of the curve is considered).

### DISCUSSION

Six workers of a chemical plant were exposed during a period of approximately 15 min to vaporous ethylene oxide. Although no data on the exposure intensity were available, a rough estimate was made that about 40 kg of liquid EO were released and vapourised rapidly. Ethylene oxide is heavier than air and its distribution within a radius of 50 m and up to a height of 5 m (≈39,270 m³) would result in a mean concentration of about 1,000 mg m⁻² or 500 ppm.
respectively. This estimated concentration is far above
the previous German Technical Guideline Concentra-
tion (TRK) for EO of 1 ppm and would explain why the
gas alert system in the vicinity of the leak was
triggered. Also, the irritative effects and pulmonary
symptoms experienced by four workers are consistent
with a short but high exposure to ethylene oxide (2).

First of all, ethylene oxide adducts were monitored
in order to identify exposed workers and to provide a
quantitative measure for the exposure intensity. As the
initially measured adduct concentrations were
considered to be fairly high in relation to the short
exposure time, follow-up biomonitoring was
implemented and adduct kinetics was studied to
confirm the association of adduct concentrations with
the accidental exposure. It was assumed that additional
adduct levels would attenuate in a linear fashion down
to background levels within 120 days, in accordance
with zero-order kinetics (12, 21, 29). While the
constant decrease of haemoglobin adducts after a
single, or accidental, exposure can be estimated on
the basis of the known life-span of erythrocytes,
reports on this aspect from human in vivo studies are
scarce (30, 31). In theory, a single exposure to ethylene
oxide during one work shift leads to the formation of
covaingly bound and chemically stable adducts to
erthrocyte haemoglobin in the blood. The total
erythrocyte population in humans at a given point in
time comprises an equally distributed number of cells
between one day and a maximum of 120 days of age,
which is the typical life-span of an erythrocyte. After
the exposure event, the adducts are removed
together with the haemoglobin and erythrocytes at a
theoretical rate of 1/120x100=0.83 percent per day until,
after about 120 days, all adducts associated with the single
exposure have been eliminated. In the case of ethylene
oxide adducts, nonsmokers as well as smokers reveal
a distinct adduct background due to endogenously
formed ethene from intestinal bacteria or from ethene
in tobacco smoke, respectively (10, 28, 32). Follow-up
biomonitoring in this study has provided new in vivo
human data to support the above-mentioned toxico-
kinetic considerations. As can be seen in the
Figures 1 and 2, the elimination curve can reasonably
be described with a linear fit during the first three
post-exposure months, while the attenuation curve
seems to level off near the end of and beyond the 120-
day interval. As the erythrocytes’ life-span may vary
to a certain degree, the asymptotically extended curve
may be due to a fraction of older erythrocytes with
haemoglobin adducts from the accidental exposure.

Nevertheless, the linear decline during the first post-
exposure months points to an almost complete
elimination after about 120 days (range: 101 to 140
days), which is in very good accordance with expected
kinetics. In an earlier study by Bader and Wrbitzky
(31), a somewhat longer average elimination phase of
148 days was observed for acrylonitrile adducts.
However, the database in that investigation was
smaller than in the study presented here, as it
comprised only samples from four individuals and
three sampling times during the 120-day interval.

In this study, urinary cotinine and the acrylonitrile
adduct CEV were analysed alongside with the ethylene
oxide adducts. Both biomarkers were monitored in
order to confirm the self-reported smoker status, but
also to provide a measure for constant or changing
smoking behaviours of the study participants. While
both biomarkers showed a relatively large variability,
the results can be interpreted in the way that individual
smoking habits of the participants did not change
significantly during the study interval. Therefore, the
results of the last HEV analyses after about 160 days
can reasonably be regarded as the individual adduct
background values of the study participants. In one
case (worker no. 1), the result of one HEV determination
was available from a general survey three months
before the accidental exposure. This earlier adduct
level was 224 pmol g⁻¹ globin as compared to the
164 pmol g⁻¹ globin found 166 days after the accidental
exposure. The variation of the mean value of these
results (30 pmol g⁻¹ globin=15 % of the 194 pmol g⁻¹
globin mean value) is within the range of the analytical
uncertainty of the adduct method. It seems reasonable
to assume that both values reflect the same adduct
background associated with cigarette smoking and the
endogenous exposure.

Another important aspect of linearity of the adduct
attenuation curve is that it allows for a back-calculation
of the initial post-accidental adduct levels from
samples collected several days or weeks after the
exposure. The validity of this approach relies,
however, on the exposure intensity. As the analytical
method has an imprecision of about 12 % (17), the
additional adduct formation related to exposure should
be at least higher than ~25 % of the background value,
otherwise it may go undetected. Therefore, it seems
advisable in the case of an accidental exposure to
collect at least two samples, one in connection with
the exposure and one after the 120-day interval in
order to distinguish between additional and background
exposure. If the first sample is significantly higher
than the background value, considering the analytical imprecision, an extrapolation to the initial adduct concentration is warranted. Although increased adduct levels may be observed still weeks and even months after the exposure, in particular when the exposure was high, the collection of blood should be carried out as promptly as feasible.

A particularly difficult and yet untackled question is the assessment of adduct concentrations after single or accidental exposures. Current assessment values for adducts such as the EKA of the German DFG are based on the assumption of a steady-state condition, which is reached only after at least 120 days of exposure. Provided, e.g., that an individual is exposed to a similar ethylene oxide concentration for 8 hours a day, the adduct levels will accumulate and rise with a certain daily increment which is reflective of the daily absorbed dose. However, older erythrocytes are also removed constantly from the blood stream. Therefore, a steady-state is achieved only after one life-span of the erythrocytes (120 days) has elapsed, and the corresponding adduct level reflects the cumulative dose of 60 exposure days, or 60 daily increments (12, 21). The German EKA value for a daily exposure to 1 ppm ethylene oxide is 90 μg L⁻¹, or a rounded 3,900 pmol g⁻¹ globin (13). Therefore, a daily (8 h exposure) increment or ‘ppm-equivalent’ of 3,900/60 = 65 pmol g⁻¹ globin can be calculated and used for the assessment of single or accidental ethylene oxide exposures.

In the current study, the initial adduct levels are equivalent to an 8-h-exposure of workers to 1 ppm and 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents’. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents'. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents'. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents'. Considering the 15-min exposure interval (1/32 of an 8-h-shift), these equivalents correspond to a short-term exposure of 160 ppm to 1152 ppm (300 mg m⁻³ to 2000 mg m⁻³) a value well equivalent to an 8-h-exposure of workers to 5 to 36 ‘ppm-equivalents'.
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Sažetak

ANALIZA PROTEINSKIH ADUKATA KAO BIOMARKERA KRATKOTRAJNE IZLOŽENOSTI ETILEN OXIDU I REZULTATI BIOMONITORINGA

U radu su prikazani rezultati biomonitoringa provedenog neposredno nakon akcidentalnog izlaganja šestorice radnika etilen oksidu i studije praćenja (follow up) provedene u cilju procjene kinetike razgradnje proteinskih adukata i utvrđivanja razlika nakon kratkotrajne izloženosti i izlaganja čimbenicima iz okoliša kao što je duhanski dim. U tu smr se svrhu tijekom petomjesečnog razdoblja nakon nezgode pratili smanjenje koncentracije hemoglobinskog adukta N-2-hidroksietilvalina usporedo s mjerenjem razina N-2-cijanoetilvalina i kotinina u mokraći, koji su pouzdaniji biomarkeri za dokazivanje pušenja duhana. Studija praćenja je pokazala da su koncentracije adukata etilen oksida značajno porasle nakon kratkotrajnoga izlaganja visokoj razini etilen oksida. U početnom biomonitoringu svih radnika izmjerene su razine N-2-hidroksietilvalina iznad 500 pmol g⁻¹ globina, s maksimalnom vrijednošću od oko 2400 pmol g⁻¹ globina. Ti su podaci usporedivi s vrijednostima njemačkih normi ekvivalenata izlaganja kancerogenim tvarima (EKA) od 90 μg L⁻¹ krvi (~3900 pmol g⁻¹ globina) kroz osnosatno dnevno izlaganje koncentraciji od 1 ppm etilen oksida. Razine adukata smanjile su se u skladu s očekivanom kinetikom nultoga reda za jednokratno izlaganje. Koncentracije N-2-hidroksietilvalina izmjerene u krvi radnika nakon petomjesečnoga praćenja mogu se povezati s njihovim osobnim pušačkim navikama. Rezultati toga istraživanja pokazuju da čak i kratkotrajna izloženost etilen oksidu može znatno povisiti razine adukata hemoglobina. Premda se u zdravstvenom nadzoru u okviru medicine rada proteinski adukti i njihove vrijednosti razmatraju u procjeni dugotrajnoga izlaganja, oni se mogu koristiti i za praćenje akcidentalnih izlaganja. U tim slučajevima izračun dnevnih vrijednosti (tzv. ppm-ekvivalenta) može poslužiti za usporedbu s postojećim procijenjenim vrijednostima.

KLJUČNE RIJEČI: akcidentalna izloženost, kinetika adukata hemoglobina, profesionalna izloženost

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