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Acrylonitrile – Addendum for re-evaluation of EKA and BAR

Assessment Values in Biological Material – Translation of the German version from 2021

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Keywords

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the exposure equivalents for carcinogenic substances (EKA) of acrylonitrile [107-13-1]. The equivalence values in biological material for the acceptable and tolerable concentrations of the German "Risk-related concept of measures for activities involving carcinogenic hazardous substances" (TRGS 910) were added to the existing EKA and the adduct concentrations were converted into the internationally common unit [pmol/g globin]. The biological reference value (BAR) for the adduct concentrations was also converted to 12 pmol N-(2-cyanoethyl)valine/g globin.

In this context, a BAR for acrylonitrile, considering N-acetyl-S-(2-cyanoethyl)-L-cysteine (S-(2-cyanoethyl)mercapturic acid, CEMA) in urine to characterize the internal exposure, was additionally evaluated. In a number of human biomonitoring studies, the excretion of CEMA in urine of individuals occupationally not exposed to acrylonitrile was investigated. Available publications are described in detail. Smoking status of the individuals affects the CEMA concentration in urine significantly; therefore, non-smokers and smokers have to be considered separately. Taking the results of studies of occupationally non-exposed individuals into consideration, which calculate a 95th percentile, a BAR of 15 µg CEMA/g creatinine was derived for non-smokers. This value is supported by median and maximum values of other available studies. Sampling time is at the end of exposure or the end of shift and for long-term exposures at the end of the shift after several shifts.

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EKA (2018)

The following correlation between external and internal exposure is obtained:

Air Acrylonitrile		Erythrocyte fraction of whole blood N-(2-Cyanoethyl)valine	
[ml/m³]	[mg/m³]	[pmol/g globin]	[µg/l blood]
0.12	*0.26*	*650*	16
0.23	0.5	1400	35
0.45	1	2450	60
1.2	*2.6*	*6500*	160
3	7	17 000	420

Sampling time: after exposure for at least 3 months

BAR (2018)

12 pmol N-(2-cyanoethyl)valine/g globin[a)]

Sampling time: after exposure for at least 3 months

BAR (2018)

15 µg S-(2-cyanoethyl)mercapturic acid/g creatinine[a)]

Sampling time: end of exposure or end of shift; for long-term exposures: at the end of the shift after several shifts

MAK value	**not established**
Absorption through the skin (1958)	H
Carcinogenicity (1977)	Category 2

[a)] evaluated for non-smokers

In 1993, acrylonitrile was evaluated for the first time; however, the data available at that time did not yet permit the establishment of exposure equivalents for carcinogenic substances (EKA) (Bolt and Lewalter 1995). In 1996, EKA were established for the parameter N-(2-cyanoethyl)valine (Lewalter and Bolt 2010). In 2009, a biological reference value (BAR) for N-(2-cyanoethyl)valine was additionally derived (Kraus et al. 2021). In the present addendum, the two equivalence values for the acceptance and tolerance concentrations (TRGS 910) were added to the existing EKA, and the concentrations of the EKA were converted into the internationally common unit of adduct analysis [pmol/g globin].

In the meantime, a number of studies have been published on the background exposure of S-(2-cyanoethyl)mercapturic acid in urine as a biomarker for acrylonitrile exposure, which can be used to derive a BAR value for this biomarker.

Conversion of the EKA and Addition of the Equivalence Values for the Acceptance and Tolerance Concentrations

The adduct level of N-(2-cyanoethyl)valine is usually given in publications in pmol/g globin. For reasons of practicability, the determination is often carried out using the unit "µg/l blood" in laboratories. A conversion of the two units is possible assuming an average globin concentration of 144 g/l (Bunn 1992). Furthermore, it is assumed that the contribution of the four haem groups to the total molar mass of haemoglobin of 64 kDa can be neglected.

The conversion is based on the following formula:

$$\text{adduct level in blood}\left[\frac{\text{pmol}}{\text{g}}\right] = \frac{\text{adduct level in blood}\left[\frac{\mu\text{g}}{\text{l}}\right]}{\text{molar mass adduct}\left[\frac{\mu\text{g}}{\text{pmol}}\right] \times \text{globin level in blood}\left[\frac{\text{g}}{\text{l}}\right]}$$

molar mass N-(2-cyanoethyl)valine): 170.2 g/mol = 170.2 $\times 10^{-6}$ µg/pmol; globin level in blood: 144 g/l

In addition, the EKA are supplemented by the value pairs of the acceptance and tolerance concentrations of 0.12 ml/m³ and 1.2 ml/m³, respectively, published in TRGS 910 (AGS 2019).

By using the air concentrations of the EKA, the following values are obtained:

Air Acrylonitrile		Erythrocyte fraction of whole blood N-(2-Cyanoethyl)valine	
[ml/m³]	[mg/m³]	[µg/l blood]	[pmol/g globin]
0.12	*0.26*	*16*	*650*
0.23	0.5	35	1400
0.45	1	60	2450
1.2	*2.6*	*160*	*6500*
3	7	420	17 000

BAR for N-(2-Cyanoethyl)valine

Since the adduct level of N-(2-cyanoethyl)valine is also to be given in pmol/g globin in analogy to the EKA correlation, the BAR of 0.3 µg N-(2-cyanoethyl)valine/l blood derived in 2009 for non-smokers is converted accordingly. The concentration corresponds to a

BAR of 12 pmol N-(2-cyanoethyl)valine/g globin.

BAR for S-(2-Cyanoethyl)mercapturic acid (CEMA)

A specific biomarker for acrylonitrile is N-(2-cyanoethyl)valine, for which EKA and a BAR for the evaluation of the test results are available. In addition to this parameter, an increasing number of studies have been published in which the CEMA in urine was investigated as a biomarker for acrylonitrile. Due to the short elimination half-life, the determination of CEMA in urine reflects the current exposure situation to acrylonitrile and thus supplements the long-term biomarker N-(2-cyanoethyl)valine. Additional advantages are non-invasive sampling and significantly less time-consuming analysis.

Detailed information on the metabolism and kinetics of acrylonitrile is contained in the MAK and BAT documentations of acrylonitrile (Bolt and Lewalter 1995; Greim 2007). Jakubowski et al. (1987) investigated the elimination kinetics of CEMA in six male humans without physical exercise after eight hours of inhalation exposure to 5 and 10 mg acrylonitrile/m³. Under these conditions, 52% was absorbed via the lungs and about 22% of the absorbed dose was excreted as CEMA in urine. For CEMA, Sumner et al. (1997) determined a share of 41% and 25% of the total renally excreted metabolites in the rat and mouse, respectively. The elimination half-life of CEMA was estimated by Jakubowski et al. (1987) to be approximately eight hours. Despite different elimination kinetics of the individual volunteers, a correlation (r = 0.83) was found between the acrylonitrile taken up from the air and the CEMA concentration in urine collected six to eight hours after the exposure (standardised to specific gravity).

Background exposure

Table 1 shows published background values for CEMA in the urine of adults. Exposure to tobacco smoke is a major cause of CEMA excretion, so a differentiation between smokers and non-smokers is necessary.

In four studies (CDC 2019; Hou et al. 2012; Schettgen et al. 2009, 2012) the 95th percentile was given separately for non-smokers and smokers. These publications are described in detail below:

- Schettgen et al. (2009) determined the CEMA concentration in the urine of 210 subjects without occupational exposure to acrylonitrile and categorised the persons with respect to cigarette smoke exposure into four categories based on the measured cotinine levels: non-smokers (n = 73), smokers (n = 81), and persons with low (n = 38) or high (n = 18) passive smoke exposure. The median CEMA concentration for non-smokers (cotinine level: < 5 µg/l) was 2.0 µg/l (range: < 1–21.3 µg/l). The 95th percentile was 5.9 µg/l. The study observed a correlation between the cotinine level and the CEMA concentration in urine as well as a significant difference between passive smokers or smokers and non-smokers. The 95th percentile of CEMA excretion in urine was 12.6 µg/l for persons with low passive smoke exposure (cotinine level: < 10 µg/l), 37.7 µg/l for persons with high passive smoke exposure (cotinine level: 10–60 µg/l) and 870 µg/l for smokers (cotinine level: 77–4300 µg/l).
- In another study by Schettgen et al. (2012), samples from 47 non-smokers (cotinine level: < 1–34 µg/l) and 36 smokers (cotinine level: 56–4940 µg/l) without occupational exposure to acrylonitrile were analysed. Among the non-smokers, 79% of the measurements were above the limit of quantification of 1 µg/l. The 95th percentile in the case of non-smokers was 6.0 µg CEMA/l urine (median: 1.9 µg/l, maximum value: 16.4 µg/l) and 5.9 µg CEMA/g creatinine (median: 1.5 g/g creatinine, maximum value: 7.8 µg/g creatinine), respectively. For smokers, the 95th percentile was 412 µg CEMA/l urine (median: 184 µg/l; maximum value: 907 µg/l) and 393 µg CEMA/g creatinine (median: 148 µg/g creatinine; maximum value: 485 µg/g creatinine), respectively.
- In a study by Hou et al. (2012), the CEMA concentration was determined in 24-hour urine. A total of 58 samples from 58 non-smokers and 246 samples from 82 smokers were examined. The 95th percentile was 9.5 µg CEMA/24-hour urine (median: 3.9 µg/24-hour urine; range: < LOQ–13.1 µg/24-hour urine) for non-smokers and 116.1 µg CEMA/24-hour urine (median: 37.8 µg/24-hour urine, range: 0.9–204.1 µg/24-hour urine) for smokers.
- The CDC Fourth Report on Human Exposure to Environmental Chemicals (CDC 2019) presents the results of the National Health and Nutrition Examination Survey (NHANES) from the years 2011/12 and 2013/14. The 95th percentile of CEMA excretion in 2011/12 was 13.5 µg/g creatinine for non-smokers (n = 1317) (median: 1.49 µg/g creatinine; geometric mean (GM): 1.82 µg/g creatinine) and 537 µg/g creatinine for smokers (n = 869) (median: 166 µg/g creatinine; GM: 127 µg/g creatinine). In 2013/14, the 95th percentile of CEMA excretion for non-smokers (n = 1350) was 32.2 µg/g creatinine (median: 1.53 µg/g creatinine; GM: 2.01 µg/g creatinine) and for smokers (n = 872) 516 µg/g creatinine (median: 132 µg/g creatinine; GM: 98.9 µg/g creatinine).

Further studies on background exposure are listed in Table 1.

Tab. 1 CEMA excretion in urine of the adult general population

Collective	Statistical parameters	CEMA concentration in urine		References
		Non-smokers	Smokers	
2011/2012: 2186 persons (USA) (1317 non-smokers, 869 smokers)	geometric mean	1.82 µg/g creatinine	127 µg/g creatinine	CDC 2019
	median	1.49 µg/g creatinine	166 µg/g creatinine	
	95th percentile	13.5 µg/g creatinine	537 µg/g creatinine	
2013/2014: 2222 persons (USA) (1350 non-smokers, 872 smokers)	geometric mean	2.01 µg/g creatinine	98.9 µg/g creatinine	
	median	1.53 µg/g creatinine	132 µg/g creatinine	
	95th percentile	32.2 µg/g creatinine	516 µg/g creatinine	
140 persons (China) (58 non-smokers, 82 smokers (246 samples)) 24-hour collection	median	3.9 µg/24 h[a)]	37.8 µg/24 h[a)]	Hou et al. 2012
	95th percentile	9.5 µg/24 h[a)]	116.1 µg/24 h[a)]	
	range	< LOQ–13.1 µg/24 h[a)]	0.86–204.1 µg/24 h[a)]	
190 persons (Germany) (50 non-smokers, 140 smokers)	median	1.1 µg/24 h	75.4 µg/24 h (1 mg tar) 140.2 µg/24 h (4 mg tar) 186.6 µg/24 h (10 mg tar)	Minet et al. 2011
	mean ± SD	1.3 ± 0.7 µg/24 h	96.6 ± 81.8 µg/24 h (1 mg tar) 139.3 ± 72.1 µg/24 h (4 mg tar) 214.8 ± 113.8 µg/24 h (10 mg tar)	
50 persons (Germany) (25 non-smokers, 25 smokers)	median	0.46 µg/g creatinine	53.6 µg/g creatinine (LS) 72.5 µg/g creatinine (HS)	Pluym et al. 2015
	range	0.23–8.6 µg/g creatinine	28.6–140.4 µg/g creatinine (LS) 28.6–140.4 µg/g creatinine (HS)	
154 persons (Germany) (73 non-smokers, 81 smokers)	median	2.0 µg/l	240 µg/l	Schettgen et al. 2009
	95th percentile	5.9 µg/l	870 µg/l	
	range	< 1–21.3 µg/l	2.0–1382 µg/l	
83 persons (Germany) (47 non-smokers, 36 smokers) without occupational exposure spontaneous urine samples	median	1.9 µg/l 1.5 µg/g creatinine	184 µg/l 148 µg/g creatinine	Schettgen et al. 2012
	95th percentile	6.0 µg/l 5.9 µg/g creatinine	412 µg/l 393 µg/g creatinine	
	maximum	16.4 µg/l 7.8 µg/g creatinine	907 µg/l 485 µg/g creatinine	
33 persons (Taiwan) (31 non-smokers, 2 smokers) spontaneous urine samples	geometric mean	2.59 µg/g creatinine[b)]		Wu et al. 2012
	range	< LOQ–111.70 µg/g creatinine[b)]		
126 persons (China) (30 non-smokers, 96 smokers (different brands))	mean	0.641 µg/l	26.3 µg/l (8 mg tar) 30.4 µg/l (10 mg tar) 38.9 µg/l (13 mg tar)	Xiaotao et al. 2014
	range	0–7.02 µg/l	0.551–162 µg/l (8 mg tar) 4.91–66.7 µg/l (10 mg tar) 9.17–110 µg/l (13 mg tar)	
140 persons (China) (58 samples from non-smokers, 246 samples from 82 smokers)	mean	3.47 µg/l	50.69 µg/l	Zhang et al. 2014
	range	< LOQ–12.4 µg/l	1.58–198.69 µg/l	

[a)] calculated from nmol/24 h (MM: 216.255 g/mol); [b)] collective of smokers and non-smokers
HS: heavy smokers; LOQ: limit of quantification; LS: light smokers; SD: standard deviation

Evaluation

For the derivation of a BAR, the above four studies are used in which the 95th percentile of CEMA excretion was determined (CDC 2019; Hou et al. 2012; Schettgen et al. 2009, 2012; see Table 2).

Tab. 2 Studies reporting the 95th percentile of CEMA excretion in urine of non-smokers (extract from Table 1)

n, non-smokers	95th percentile of CEMA excretion in urine [µg/l]	[µg/g creatinine]	References
n = 1318 (µg/l urine), n = 1317 (µg/g creatinine) (2011/2012)	16.6	13.5	CDC 2019
n = 1351 (µg/l urine), n = 1350 (µg/g creatinine) (2013/2014)	39.9	32.2	
n = 58	44 nmol/24-h-urine		Hou et al. 2012
	6.9[a)]	*5.75*[a)]	
n = 73, without passive smoke exposure (cotinine level < 5 µg/l urine)	5.9	*4.9*[a)]	Schettgen et al. 2009
n = 38, light passive smokers (cotinine level < 10 µg/l urine)	12.6	*10.5*[a)]	
n = 18, heavy passive smokers (cotinine level 10–60 µg/l urine)	37.7	*31.3*[a)]	
n = 47	6	5.9	Schettgen et al. 2012
arithmetic mean of 95th percentiles	**17.94**	**14.86**	

[a)] calculated with molar mass of 235,26 g/mol, 1,2 g creatinine/l urine and 1,5 l urine/day

From the available data a

BAR for non-smokers of 15 µg S-(2-cyanoethyl)mercapturic acid/g creatinine

is established. Other studies on the background exposure of CEMA in urine in which the 95th percentile was not reported and only median, mean and/or maximum values were given, support these results (Figure 1).

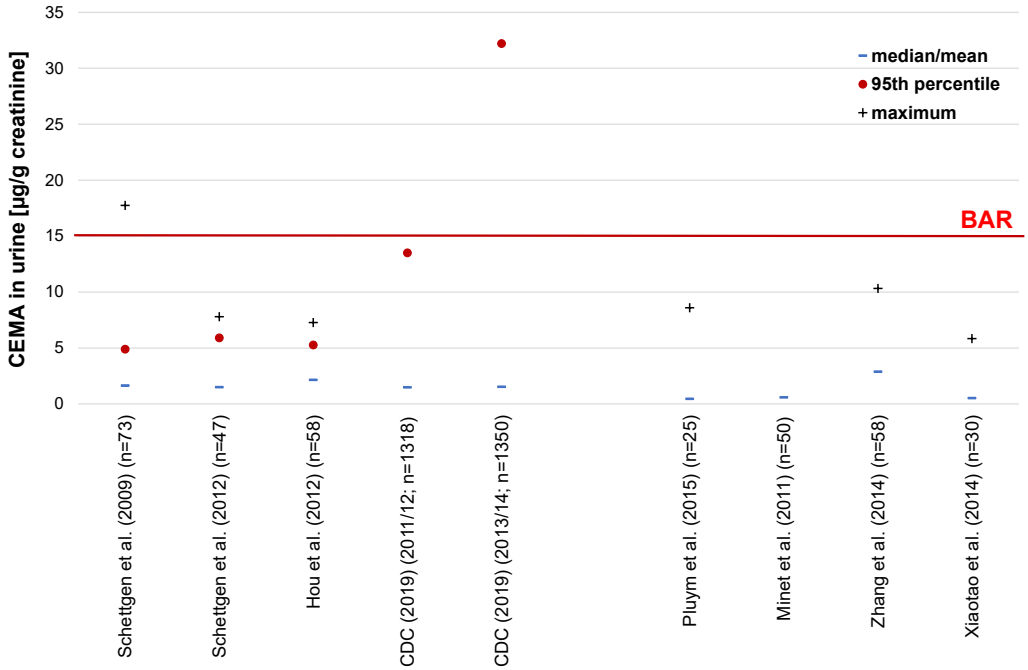

Fig. 1 CEMA concentrations (mean, 95th percentile and maximum values) in urine of non-smokers

In general, the baseline excretion of CEMA in the urine of non-smokers shows only slight variability. Smokers and also passive smokers were found to have significantly higher CEMA concentrations in urine than non-smokers. The study of the elimination kinetics of CEMA by Jakubowski et al. (1987) revealed an elimination half-life of eight hours. Therefore, it is recommended that sampling should be performed at the end of exposure or at the end of a shift, as for other mercapturic acids (for example S-phenyl mercapturic acid), and for long-term exposures at the end of a shift after several shifts.

Interpretation of results

When interpreting the results, personal influencing factors, especially smoking behaviour, as well as passive smoke exposures should be taken into account (see also Section "Background exposure"). The BAR for CEMA refers to normally concentrated urine, in which the creatinine concentration should be in the range of 0.3–3.0 g/l. As a rule, where urine samples are outside these limits, a repetition of the measurement in normally hydrated test persons is recommended (Bader et al. 2016).

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