APPENDIX A6.1.3.5 ACRYLONITRILE - FACTSHEET

	HPHC	BoExp assessed by PMI
IUPAC name	2-propenenitrile	(2R)-2-acetamido-3-(2-cyanoethylsulfanyl) propanoic acid
Usual abbreviation	ACN	CEMA [1]; CYMA; CMA
Other names	Acrylonitrile, 2-propenenitrile, vinyl cyanide, Cyanoethylene	2-cyanoethylmercapturic acid; N-acetyl-S-(2-cyanoethyl)cysteine, N-acetyl-3-(2-carboxyethylthio)alanine bis(dicyclohexylamine) salt; N-acetyl-S-(2-carboxyethyl)cysteine bis(dicyclohexylamine) salt
CAS number	107-13-1	74514-75-3
Formula and molecular weight	C_3H_3N MW: 53.06	$C_8H_{12}N_2O_3S$ MW: 216.26

[1] CEMA is also a common abbreviation of 2-carboxyethyl-N-acetylcysteine (carboxyethyl mercapturic acid), a metabolite of acrolein

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Main characteristics

Chemical class	Acid derivative, carboxylic acid nitrile
HPHC lists	FDA-93 [1]; FDA-18 [2]; HC [3, 4]; PMI-58; WHO-39 [5]; WHO-18 [6]
Source in cigarettes	Amount: Main source of exposure for the general population; 3 to 15µg/cigarette [7] Amount in mainstream smoke (3R4F): 26.4µg/cigarette (ISO); 67.0µg/cigarette (HCI) [8] Smoke phase: gas/vapor phase [9] Presence in tobacco / pyrosynthesis: Not present in uncombusted tobacco [10] / Described as an incomplete combustion product during and between puffs [11]. Main generation range is between 500-800°C. The availability of NO (mainly derived from nitrate) is important, since ammonia (derived from tobacco proteins and pigments) is an intermediate in the formation of acrylonitrile in smoke [12].
Other sources [13]	Acrylonitrile is released into the environment primarily from chemical production in the chemical and plastic products industries (>95% in the sample country). There are no known natural sources. Acrylonitrile is distributed largely to the environmental compartments to which it is principally released (i.e., air or water), with movement to soil, sediment, or biota being limited; reaction and advection are the major removal mechanisms. Acrylonitrile has been detected in the general environment only in the vicinity of industrial sources [13].
Toxicity class (FDA) [1]	CA, RT
Toxicity – CA	IARC: group 2B: possible carcinogenic to humans [14] Meta-analysis of published data suggests a 25% increase in lung cancer risk attributable to occupational acrylonitrile exposure [15]. The California Environmental Protection Agency suggests, after having compared yields in the smoke of 26 common brands of conventional cigarettes that acrylonitrile, together with acetaldehyde and 1,3-butadiene, is associated with the largest calculated cancer risks [16]. For acrylonitrile, this, however, remains somewhat controversial, as stated in a review looking at the four most informative cohort studies that concluded that so far results do not support a causal relationship between

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	acrylonitrile and all cancers or any specific type of cancer [17]. Acrylonitrile is one of the few compound that was downgraded from classification in group 2A (probably carcinogenic to humans) to group 2B (possibly carcinogenic to humans) by IARC [17].
Toxicity – RT	In humans, acute poisoning following inhalation exposure: throat and respiratory tract irritation [13]
Toxicity – CT	-
Toxicity – RDT	Consistent effects on the reproductive organs of male or female rats and mice have not been observed in repeated-dose toxicity and carcinogenicity studies conducted to date [13].
Toxicity – AD	-
Toxicity - others	All available toxicity data can be found on the Website Aggregated Computational Toxicology Resource (ACToR) [18].

ADME properties

Absorption and distribution [13] - Acrylonitrile is rapidly absorbed via all routes of exposure and distributed widely in all tissues. In inhalation studies with human volunteers, 50% of acrylonitrile was absorbed [19]. There is little potential for significant accumulation in any organ, with most of the compound being excreted primarily as metabolites in the urine within the first 24–48 h following administration. Available data are consistent with conjugation to glutathione being the major detoxification pathway, while oxidation to 2-cyanoethylene oxide is considered an activation pathway.

Metabolism [13, 20, 21] - Acrylonitrile is metabolized primarily by two pathways: conjugation (or conjunction) with glutathione to form N-acetyl-S-(2-cyanoethyl) cysteine (CEMA) and oxidation by cytochrome P-450 to form remaining urinary metabolites (Figure 1). The highly reactive metabolite cyanoethylene-epoxide (CEO) is formed by liver cytochrome P450 2E1. The formation of CEO is considered to be responsible for the carcinogenic properties of acrylonitrile in animal experiments. CEO was shown to bind covalently to DNA at much higher rates than acrylonitrile itself and was shown to bind to the phosphate moiety of nucleosides, inducing DNA strand breaks. Both acrylonitrile as well as the intermediate metabolite CEO may react with glutathione via enzymatic glutathione-S-transferases (GSTs) as a detoxification reaction. In case of direct reaction with glutathione, 2-cyanoethylmercapturic acid (CMA or CEMA) is formed and excreted via urine (Figure 1). Reaction of CEO with glutathione might lead to the formation of cyanohydroxyethyl mercapturic acid (CHEMA) as well as to the formation of an intermediary cyanohydrin metabolite, which is unstable and finally leads to the excretion of hydroxyethyl mercapturic acid (HEMA) after elimination of cyanide (Figure 1). Conversion of acrylonitrile to cyanide is a P450-dependent pathway, in which CYP2E1 plays a major role [22]. Cyanide is rapidly converted to thiocyanate (SCN).

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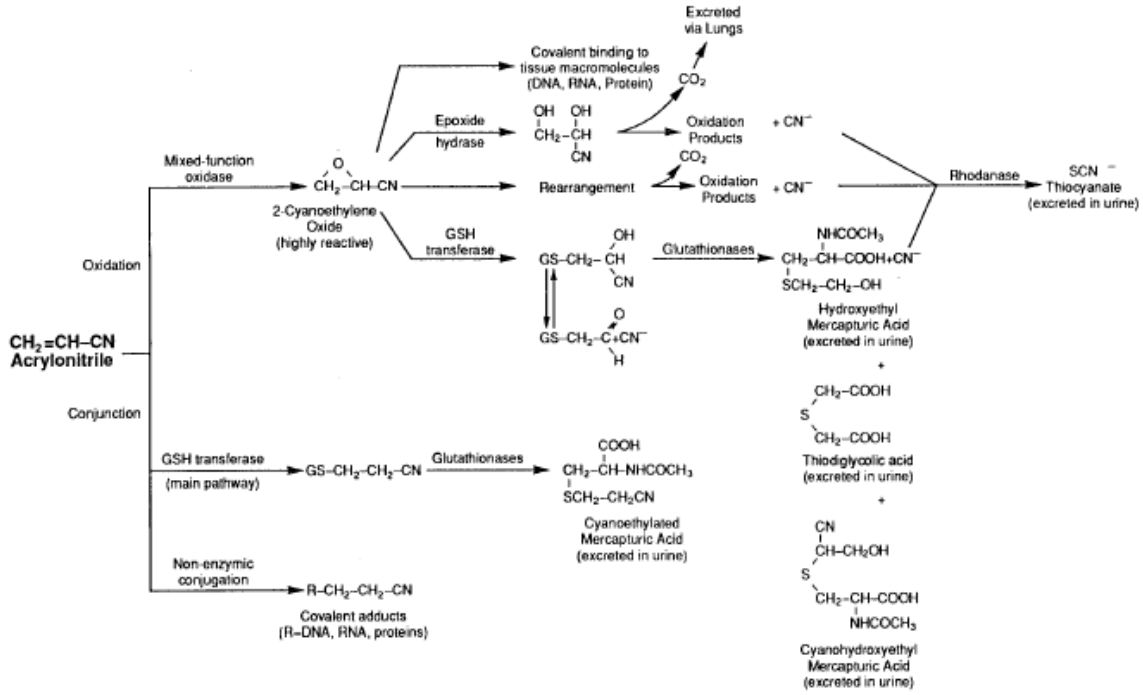


Figure 1 Proposed metabolic scheme for acrylonitrile [21]

Figure source: Agency for Toxic Substances and Disease Registry.

In humans, about 52% of acrylonitrile in the lungs is retained, and about 22% of the retained acrylonitrile is metabolized to CEMA [19]. Experiments in rats showed that CEMA and HEMA account for 40 and 30% of the dose excreted via urine (after oral administration), while CHEMA makes up for another 13% of the excreted dose [20, 23].

Acrylonitrile can also be metabolized by the epoxidation to glycidonitrile, which in turn can form DNA and protein/hemoglobin adducts such as 2-cyanoethylvaline (CEVal) [24]. The levels of these hemoglobin adducts is not affected by GSTM1 and GSTT1 genotypes [25], unlike what is observed for hemoglobin adducts of 1,3-butadiene, THBVal (N-(2,3,4-trihydroxybutyl)valine) [26].

A study indicates that L-2-oxothiazolidine-4-carboxylic acid (OTCA), a glutathione precursor, increases the capacity for detoxification via the glutathione pathway thereby partially correcting the nonlinearity between the administered dose of ethylene oxide, dibromoethane, and acrylonitrile and the amount of certain urinary metabolites [27].

Excretion [20] - As HEMA is also a metabolite of ethylene oxide as well as ethylene with varying urinary background levels, excretion of HEMA is no longer considered specific for exposure to acrylonitrile and cyanoethylene-epoxide, respectively.

The excretion of CHEMA is the only specific urinary biomarker of internal exposure to CEO, the cancer initiating metabolite of acrylonitrile. As human biomonitoring should always be aimed to determine the internal dose of the most harmful agent within metabolism of hazardous substances, the quantification of CHEMA in urine would provide valuable data for an accurate risk assessment of human exposures to acrylonitrile.

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This is especially true as the formation as well as detoxification of CEO is influenced by a number of different (polymorphic) enzymes whose influence is yet unknown. So far, CHEMA has only been identified in urine of highly exposed rats and mice by GC/MS and 13C–NMR. Most recently, CHEMA as well as CEMA was quantified in human urine [28]. However, a labelled internal standard for CHEMA was lacking in that study and human data on excretion of CHEMA and the fraction of oxidative metabolism in humans are still sparse.

In a controlled study using human volunteers, urinary CEMA data suggests that the elimination of acrylonitrile follows first-order kinetics, with a half-life of seven to nine hours [19]. The kinetics of excretion is not uniform, with a maximum rate of excretion occurring at different times after the beginning of exposure. This was, however, only a short-term study (i.e., 31 hours), which means that half-life may have been underestimated [29]. In rats, CEMA measured in urine represents about 50 to 60% to the orally absorbed dose of acrylonitrile [23]. Exhalation of volatile material related to acrylonitrile is negligible [30]. In humans, CEMA levels in urine represent about 20% of the inhaled acrylonitrile dose [7]. Jakubowski et al. reported quite variable individual urinary profiles for the kinetics of CEMA [19].

Exposure levels in smokers and non-smokers

Urinary excretion of CEMA has been shown to be consistently higher in smokers than in non-smokers (Table 1). This difference varies from study to study, but ranges in non-smokers are usually below 2 ng/mL [7, 20, 31, 32] and increase to levels of more than 20 to 205 ng/mL in smokers [7, 20, 31-33]. Smokers excreted 187 ± 181 µg/L (mean ± SD) or 184 µg/L of CEMA, while non-smokers only excreted 4.6 ± 35 µg/L or 1.9 µg/L ([34] and [20], respectively). CEMA excretion was highly correlated with serum cotinine levels [28, 34]. Another study reported a CEMA excretion of 71 µg/day [35]. CEMA levels in smokers are significantly correlated with ISO tar yield, daily cigarette consumption, and other urinary biomarkers of smoke exposure (such as nicotine exposure) [31].

A clear correlation between the number of cigarettes per day and the urinary levels of CEMA has been shown [7, 31]. A recent study in which smokers consuming 20 cigarettes per day (cpd) or more were forced to smoke exactly 20, 15, 5 or 0 cpd during a 5-day confinement period showed a dose-relationship between the number of cigarettes and the levels of urinary CEMA (i.e. 218.0, 168.0, 93.2 and 38.3 µg/24h, respectively) [36].

Smoking cessation studies of 5 to 8 days showed significantly levels of CEMA in urine by about 7- to 10-fold [24, 36, 37] (Table 2).

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Table 1 Representative urinary CEMA levels in smokers and non-smokers

Author (year)	Levels in non-smokers (or ex-smokers)	Levels in smokers	Units; variability
Schettgen et al. (2009) [7]	2 (<1-21.3)	240 (2-1382)	ng/mL; median (range)
Schettgen et al. (2012) [20]	1.5 (5.9)	148 (393)	µg/g creat or ng/mg creat; median creat [(95 percentile)
Minet et al. (2011) [31]	1.3 ± 0.7 (1.1)	96.9 ± 81.8 (75.4) ISO tar yield 1 mg	µg/24h; mean ± SD (median)
Minet et al. (2011) [31]	1.3 ± 0.7 (1.1)	139.3 ± 72.1 (140.2) ISO tar yield 4 mg	µg/24h; mean ± SD (median)
Minet et al. (2011) [31]	1.3 ± 0.7 (1.1)	214.8 ± 113.8 (186.6) ISO tar yield 10 mg	µg/24h; mean ± SD (median)
Ding et al. (2009) [33]	ND-158	27-744	µg/g creat or ng/mg creat
Xiaotao et al. (2014) [32]	0.641 (0 to 7.02)	26.34 (0.551–162)	ng/mL; mean (range)
Jacob et al. (2013) [35]	NM	70.9 (45.4–110.9)	µg/24h; geometric mean (95% CI)
Alwis et al. (2012) [34]	4.60 ± 35	187 ± 181	ng/mL; mean ± SD
Pluym et al. (2015) [38]	0.46 (0.23-8.6)	72.5 (28.6-140.4)	µg/g creat or ng/mg creat; median (min-max)

Table 2 Representative urinary CEMA levels after smoking cessation

Author (year)	Levels in ex-smokers or switchers	Levels in smokers	Units; variability
Scherer et al. (2014) [24]	1.3 ± 0.9	197 ± 101.3	µg/24h; mean ± SD
Scherer et al. (2010) [37]	25.7 ± 13.4 (14.9-75.5) [a]	259.2 ± 117.6 (128.2–608.0)	µg/24h; mean ± SD
Theophilus et al (2015) [36]	38.3 ± 15.3 [b]	263 ± 83.4	µg/24h; mean ± SD

[a] After 8 days of smoking cessation

[b] After 5 days of smoking cesstion

List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette [39]
ACToR	Aggregated Computational Toxicology Resource
AD	Addictive
ADME	Absorption and Distribution, Metabolism, and Excretion
CA	Carcinogen

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CAS number	Chemical Abstracts Service number
Creat	Creatinine
CEMA	N-acetyl-S-(2-cyanoethyl)cysteine or 2-cyanoethylmercapturic acid,
CEO	Cyanoethylen-epoxide
CEVal	2-cyanoethylvaline
CHEMA	N-acetyl-S-(1-cyano)-2-hydroxethyl-cysteine
CI	Confidence interval
CT	Cardiovascular toxicant
FDA	Food and Drug Administration
GSTM1	Glutathione S-transferase mu 1
GSTT1	Glutathione S-transferase theta-1
HC	Health Canada
HCI	Health Canada Intense regime [40]
HEMA	2-hydroxyethyl methacrylate
HPHC	Harmful and Potentially Harmful Constituents
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime [41]
IUPAC	International Union of Pure and Applied Chemistry
MW	Molecular weight
ND	Not detectable
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant
RT	Respiratory toxicant
SD	Standard deviation
WHO	World Health Organization

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