APPENDIX A6.1.3.24 ACRYLAMIDE - FACTSHEET

	HPHC	BoExp assessed by PMI
IUPAC name	Prop-2-enamide	1) N-acetyl-S-(2-carbamoylethyl)-L-cysteine 2) N-(R/S)-acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine
Usual abbreviation	AA; AM	1) AAMA, MA-AA, APC, NASPC 2) GAMA, MA-GA
Other names	Acrylamide, 2-propenamide, propenamide, acrylic amide, ethylenecarboxamide	1) N-acetyl-S-(3-amino-3-oxopropyl)-L-cysteine, (2S)-2-acetamido-3-(3-amino-3-oxopropyl)sulfanylpropanoic acid (IUPAC), N-acetyl-S-(3-amino-3-oxopropyl)cysteine, N-(R/S)-acetyl-S-(2-carbamoyl-hydroxyethyl-L-cysteine 2) N-Acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine; N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)cysteine, N-Acetyl-S-(2-hydroxy-3-propionamide)-L-cysteine
CAS number	79-06-1	1) 81690-92-8 2) 137698-08-9
Formula and molecular weight	C_3H_5NO MW: 71.08	1) $C_8H_{14}N_2O_4S$ MW: 234.27 2) $C_8H_{14}N_2O_5S$ MW: 250.28

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Main characteristics

Chemical class	Aliphatic nitrogen, acid derivative, carboxylic acid amide
HPHC lists	FDA-93 [1], PMI-58
Source in cigarettes	Amount in main stream smoke (3R4F): 1.37 µg/cigarette (ISO); not reported (HCI) [2] Smoke phase: mainly particulate phase (gas/vapor phase < 1%) [3] Presence in tobacco / pyrosynthesis (T°): present in uncombusted tobacco [4] / Acrylamide can be formed during heating of biological materials (containing sugars) at temperatures below 100°C, but at least in food, seems to peak between 120-200°C [5]. Information specific to tobacco could not be found, but acrylamide is likely to also occur at relatively low temperatures. In tobacco, acrylamide may also be formed form amino acids in the presence of nitrates [6].
Other sources [7]	Acrylamide is formed in foods that are rich in carbohydrates (particularly potatoes) when they are fried, grilled, or baked at high cooking temperatures [8, 9]. Levels of acrylamide in these foods increase with higher temperatures and longer cooking times. Protein-based foods (such as meats) probably contain low amounts of acrylamide. Ingestion of foods that contain acrylamide is a primary source of exposure for the general public. Average estimated intake of acrylamide from food sources ranged from 0.3 to 0.8 µg/kg bw/day. Acrylamide may be released to the environment during production and use of polyacrylamide resins, which are used as clarifiers in water treatment. Acrylamide can be released to the environment during formulation of cosmetics or other consumer products and in laboratories. Due to its low vapor pressure and high water solubility, acrylamide is rarely identified in atmospheric samples and is seldom considered to be an air pollutant. Inhalation exposure is likely limited to tobacco smoke exposure. Acrylamide is sometimes present in drinking water due to leaching of monomer during treatment processes, as well as release from grouting agents in wells and dams and releases from plastics and dye industries. It is rarely found in soil samples.
Toxicity class (FDA) [1]	CA

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Toxicity – CA [7, 10]	IARC: group 2A: probably carcinogenic to humans [10] Supporting evidence from human studies on cancer risk from acrylamide in food, which include case-control studies and reports form ongoing prospective cohort studies, found no significant associations between dietary acrylamide and the risk of cancers; however, risks for some cancers (pancreas and Hodgkin's disease [10]) were slightly elevated in a few instances [7]. Studies in rats and mice provide clear evidences of acrylamide-induced carcinogenicity.
Toxicity – RT	-
Toxicity – CT	-
Toxicity – RDT	-
Toxicity – AD	-
Toxicity - others	Information in humans is available from numerous case reports in which acrylamide exposure has been associated with signs of impaired neurological performance in central and peripheral nervous systems that include impaired motor function and muscle weakness [7]. All available toxicity data can be found on the Website Aggregated Computational Toxicology Resource (ACToR) [11].

ADME properties

Absorption [7] - Available data in rats and mice indicate that acrylamide is readily and rapidly absorbed following inhalation and oral exposure, and somewhat less readily absorbed following dermal exposure. Formation of hemoglobin adducts of acrylamide following inhalation exposure also provide some information regarding the extent of absorption via this exposure route. No studies were located regarding mechanisms for acrylamide absorption across lung, gut, or skin; processes may include passive and/or facilitative mechanisms.

Metabolism [7] - Available data in rats and mice indicate species differences in acrylamide metabolism. Additional comparative toxicokinetic studies may be warranted pending the development of new PBPK models that would be adequate for humans.

Acrylamide metabolism is relatively rapid. Metabolism is assumed to take place primarily in the liver, but may occur in the brain and the skin. Acrylamide reacts readily with glutathione to form a glutathione conjugate, which is further metabolized to N-acetyl-S-(2-carbamoylethyl)-cysteine (AAMA) (Figure 1, left-hand pathway) [7, 12]. Another initial step, likely catalyzed by CYP2E1, involves oxidation of acrylamide to the epoxide derivative, glycidamide. Studies of CYP2E1 null and wild-type mice have demonstrated the importance of CYP2E1 in catalyzing this step, suggesting that CYP2E1 may be involved in metabolism of acrylamide in man [13]. Glycidamide can react with glutathione to form conjugates that are further metabolized to two isomeric mercapturic

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acids, namely, N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-cysteine (GAMA) and N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)-cysteine (GAMA-2) (Figure 1, right-hand pathway). Glycidamide may also undergo hydrolysis, perhaps catalyzed by epoxide hydrolases, leading to the formation of 2,3-dihydroxypropionamide and 2,3-dihydroxypropionic acid (not shown).

Both acrylamide and glycidamide react with nucleophilic sites in macromolecules (including hemoglobin and DNA) in Michael-type additions [7], producing adducts that can be measured (e.g., AAVal, GAVal [14]). In rats, the binding index of acrylamide to cysteine in hemoglobin was higher than glycidamide. The lower binding index for glycidamide may be the result of lower reactivity toward hemoglobin-cysteine and shorter half-life in blood.

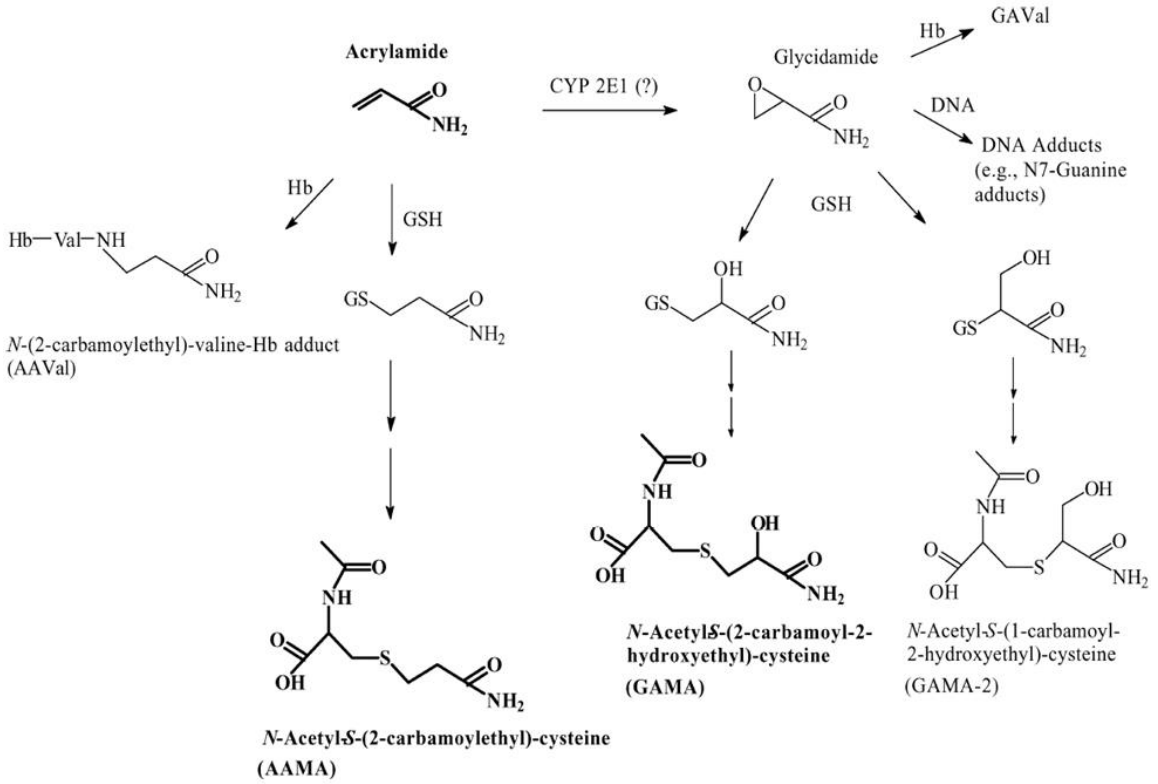


Figure 1 Metabolic scheme for acrylamide and its metabolite glycidamide [12]

Figure by Urban M, Kavvadias D, Riedel K, Scherer G, Tricker AR. Urinary mercapturic acids and a hemoglobin adduct for the dosimetry of acrylamide exposure in smokers and nonsmokers. Inhal Toxicol. 2006; 18(10):831-9. Reprinted with permission of the publisher Informa Healthcare. Copyright © Informa Healthcare USA, Inc.

Excretion [7] - Urinary excretion of conjugated acrylamide derivatives represents the major excretory pathway for acrylamide. Following oral administration of single 50 mg/kg doses of radiolabeled acrylamide to wild-type mice, considerable amounts of metabolites derived from glycidamide were found in the 24-hour urine [15]. Approximately 22% of the excreted metabolites were derived from glutathione conjugation with acrylamide and glycidamide (AAMA and GAMA, respectively) and

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28% were derived from glycidamide and its hydrolysis products (2,3-dihydroxypropionamide and 2,3-dihydroxypropionic acid). In contrast, no evidence was found for the formation of glycidamide-related metabolites in the 24-hour urine of CYP2E1 null mice or wild-type mice treated with the CYP2E1 inhibitor, aminobenzotriazole, thus indicating the importance of CYP2E1, at least in mice [15]. In another study, Sumner et al. [16] exposed male rats and mice nose-only to a mixture of radiolabeled acrylamide vapors for 6 hours. During a 24-hour post-exposure period, the distribution of the inhaled dose in the rats was: 31% in the urine, 56% retained in the body, 3% in the feces, and 2% as exhaled organic volatiles and $^{14}CO_2$. The distribution of the inhaled dose in the mice was: 27% in the urine, 46% in tissues, 5% in feces, 2% as organic volatiles, and 1% as $^{14}CO_2$.

Most human data have been generated in the frame of dietary exposure. Fuhr et al. [17] evaluated the toxicokinetics of acrylamide in six young healthy volunteers after the consumption of a meal containing 0.94 mg of acrylamide. Urine was collected up to 72 hours thereafter. Unchanged acrylamide; its mercapturic acid metabolite (AAMA), its epoxy derivative, glycidamide, and its mercapturic acid metabolite (GAMA) were quantified in the urine by LC-MS. Toxicokinetic variables were obtained by non-compartmental methods. Overall, approximately 60% of the dose was recovered in the urine. Although no glycidamide was found, unchanged acrylamide, AAMA, and GAMA accounted for urinary excretion of approximately 4.5, 50, and 6% of the dose, respectively. These results indicate that most of the acrylamide ingested with food is absorbed in humans.

Boettcher et al. [18] investigated the human metabolism of acrylamide to AAMA and GAMA in a healthy male volunteer who received a single dose of about 1 mg deuterium-labelled [d_3]-acrylamide, representing 13 µg/kg bw, in drinking water. Urine samples before dosing and within 46 hours after the dosing were analyzed for [d_3]-AAMA and [d_3]-GAMA. Total recovery of [d_3]-AAMA and [d_3]-GAMA in the 24-hour urine was about 51% of the administered dose, which provides additional demonstration of the rapid and extensive absorption of ingested acrylamide. This sum of AAMA and GAMA recovery was similar to that found in rats (53–66%) given a single dose of 0.1 mg/kg bw by gavage [19]. After 2 days, AAMA accounted for 52% of the total administered acrylamide dose. GAMA accounted for 5%, and appeared as a minor metabolite of acrylamide. A urinary ratio of 0.1 was observed for GAMA/AAMA compared to previously reported values of 0.2 for rats and 0.5 for mice [20, 21]. The authors concluded that the metabolic fate of acrylamide in humans was more similar to that in rats than in mice as previously demonstrated in terms of hemoglobin adducts. In a study by Kopp et al. after single oral administration (drinking water) to humans, the major metabolite AAMA accounting for approximately 71% of all relevant metabolites excreted with urine, while 20% were excreted as AAMA-sulfoxide [22]. While assessing urinary levels of AAMA and GAMA in humans following controlled administration of acrylamide [17, 23], it should be noted that GAMA and AAMA sulfoxide metabolites are isomeric and may thus not separate well during their measurement, which means that the formation of GAMA may be overestimated since the sulfoxide can account for a major portion of the metabolism of acrylamide [24].

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After exposure to acrylamide, a first phase of increase in urinary concentration lasts about 18 h with a broad plateau between 8 and 18 h for AAMA, and 22 h for GAMA [23]. Elimination half-lives of both AAMA and GAMA were estimated to be approximately 3.5 h for the first phase and more than 10 h up to few days for the second phase.

Exposure levels in smokers and non-smokers

Ever since researchers at the Swedish National Food Administration and Stockholm University reported the presence of the chemical acrylamide in a variety of fried and oven-baked foods in the early 2000 [8, 9], several studies have looked at the exposure of acrylamide following dietary exposure. Gas chromatography–mass spectrometry methods have been used to determine the adducts of acrylamide and its metabolites (such as glycidamide) with hemoglobin in blood samples [8]. These methods are sensitive enough to measure adducts at blood levels relevant to potential dietary acrylamide exposure and to determine a time-averaged estimate. The Fourth National Report on Human Exposures to Environmental Chemicals reported the levels of acrylamide and glycidamide hemoglobin adducts from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 [25]. These levels were generally similar to those seen in several previous studies of non-occupationally exposed subjects, although different analytic methods can affect results. Acrylamide adducts are detected in every individual, but smokers have a significantly increased adduct level compared to non-smokers [12, 14, 26, 27]. Smokers have adduct levels that are three to four-fold higher than non-smokers.

Only a few studies assessing AAMA and or GAMA metabolites in the urine of smokers and non-smokers are available Table 1 and Table 2). In a study by Urban et al. [12], smokers had significantly higher levels of AAMA and GAMA than non-smokers. The GAMA/AAMA ratio was significantly lower in smokers compared to non-smokers (Table 3). The GAMA/AAMA ratio is often reported, since glycidamide is thought to be the ultimate carcinogenic metabolite of acrylamide [28]. In smokers, urinary excretion rates were significantly correlated with markers of smoking dose, such as daily cigarette consumption, nicotine equivalents in urine, cotinine in saliva, and CO in exhaled breath [12]. AAVal levels were three-fold higher in smokers than in non-smokers and were also significantly correlated with those markers of smoking dose. From these data, Urban et al. estimated that cigarette smoking explains up to 70% of the variation in acrylamide exposure in the absence of occupational exposure to this toxicant [12]. 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 AAMA (i.e. 44.2, 45.0, 31.4and 32.7 µg/24h, respectively), or GAMA (i.e. 254, 234, 177 and 166 µg/24h, respectively) [29].

In two 7-days smoking abstinence studies, AAMA levels were very moderately decreased (1.3- to 2-fold), and GAMA was decreased by similar magnitudes [30, 31]. A 6-day study in confinement showed a decrease of about 2-fold of AAMA levels after smoking abstinence [32]. Finally, in a recent smoking cessation study of 5 days, AAMA levels were decreased by 1.3-fold, and GAMA by 1.7-fold [29]. No human data were located regarding age-related differences in susceptibility to acrylamide toxicity in humans, while

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conflicting reports are available from animal studies [7]. Genetic polymorphisms in the acrylamide metabolizing P-450 enzyme CYP2E1 have been identified in humans [27, 33]. Polymorphisms in CYP2E1 could conceivably confer a differential risk to acrylamide toxicity and carcinogenicity.

Table 1 Representative urinary AAMA levels in smokers, non-smokers or quitters

Author (year)	Levels in non-smokers (or ex-smokers)	Levels in smokers	Units; variability
Urban (2006) [12]	73.1 ± 46.6 [0.0731 ± 0.0466]	185.7 ± 107.8 [0.1857 ± 0.1078]	µg/24h [mg/24h]; mean ± SD
Boettcher (2005) [34]	29 (3–83)	127 (17–338)	µg/L; median (range)
Li (2005) [35]	113 ± 22	159 ± 43	µg/L; mean ± SD
Li (2005) [35]	76 ± 30	135 ± 88	µg/g creat; mean ± SD
Tricker (2012) [30]	147.77 ± 27.38 [0.14777 ± 0.02738] [a]	188.01 ± 51.90 [0.18801 ± 0.05190]	µg/24h [mg/24h]; mean ± SD
Tricker (2012) [31]	61.6 ± 22.2 [0.0616 ± 0.0222] [a]	122.2 ± 69.9 [0.1222 ± 0.0699]	µg/24h [mg/24h]; mean ± SD
Alwis (2012) [36]	82 ± 128	196 ± 180	ng/mL; mean ± SD
Theophilus (2015) [29]	166 ± 78.3 [0.166 ± 0.078] [b]	289 ± 83.9 [0.289 ± 0.084]	µg/24h [mg/24h]; mean ± SD
Pluym (2015) [37]	11.1 (5.4-84.3)	64.8 (37.1-123)	µg/g creat; median (range)

[a] After 8 days of smoking abstinence

[b] After 5 days of smoking abstinence

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Table 2 Representative urinary GAMA levels in smokers, non-smokers or quitters

Author (year)	Levels in non-smokers or quitters	Levels in smokers	Units; variability
Urban (2006) [12]	15.9 ± 13.0 [0.0159 ± 0.013]	27.6 ± 20.1 [0.0276 ± 0.0201]	µg/24h [mg/24h]; mean ± SD
Boettcher (2005) [34]	5 (<LOD-14)	19 (3-45)	µg/L; median (range)
Tricker (2012) [30]	11.66 ± 2.44 [0.01166 ± 0.00244] [a]	14.98 ± 5.58 [0.01498 ± 0.00558]	µg/24h [mg/24h]; mean ± SD
Alwis (2012) [36]	28 ± 38	57 ± 57	ng/mL; mean ± SD
Theophilus (2015) [29]	32.7 ± 13.0 [0.0327 ± 0.013] [b]	44.2 ± 16.0 [0.0442 ± 0.0160]	µg/24h [mg/24h]; mean ± SD
Pluym (2015) [37]	3.9 (1.6-16.5)	12.7 (6.4-19.6)	µg/g creat; median (min-max)

[a] After 8 days of smoking abstinence

[b] After 5 days of smoking abstinence

Table 3 Representative urinary ratios of GAMA/AAMA found in the urine of smokers or non-smokers

Author (year)	Levels in non-smokers	Levels in smokers	Units; variability
Urban (2006) [12]	0.23 ± 0.29	0.15 ± 0.08	mean ± SD
Boettcher (2005) [34]	0.22 (0.10–0.53)	0.15 (0.03–0.30)	median (range)

List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette [38]
AAMA	N-acetyl-S-(2-carbamoylethyl)-L-cysteine
ACToR	Aggregated Computational Toxicology Resource
AD	Addictive
ADME	Absorption and Distribution, Metabolism, and Excretion
CA	Carcinogen
CAS number	Chemical Abstracts Service number
CPD	Cigarettes per day
CT	Cardiovascular toxicant
FDA	Food and Drug Administration
GAMA	N-(R/S)-acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine
HC	Health Canada
HCI	Health Canada Intense regime [39]

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HPHC	Harmful and Potentially Harmful Constituents
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime [40]
IUPAC	International Union of Pure and Applied Chemistry
LC-MS	Liquid chromatography–mass spectrometry
MW	Molecular weight
NHANES	National Health and Nutrition Examination Survey
PBPK	Physiologically based Pharmacokinetic
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant
RT	Respiratory toxicant
SD	Standard deviation
WHO	World Health Organization

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