

Announcement by the German Federal Environment Agency (Umweltbundesamt)

## Acrylamide and Human Biomonitoring

Opinion of the Human Biomonitoring Commission of the German Federal Environment Agency (Umweltbundesamt)

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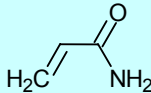
### Introduction

Acrylamide (AA) is produced at higher temperatures when preparing foods containing both proteins and carbohydrates. In addition, tobacco smoke also contains AA. In animal experiments, AA was found to be clearly carcinogenic, producing a large number of different cancer localisations. For reasons of health prophylaxis and as a result of the most recent investigations on human metabolism, it can be assumed that AA is also carcinogenic in humans. These facts make acrylamide one of the most important environmental carcinogens and are the reason for its relevance in environmental medicine. A number of reports provide information on the toxicological effects of acrylamide in animals and humans; these are referred to below [1,2,3,4,5,6]. It is possible to establish the current body burden of humans via various exposure routes by determining the acrylamide-haemoglobin adduct (N-2-carbamoyl-ethylvaline: AAV<sub>al</sub>) in the blood. In this opinion, the Human Biomonitoring

Commission describes the background exposure of the non-smoking general population in Germany based on the AAVal contents in the blood. Compared with these background values, individual, cause-related human biomonitoring results can be evaluated.

## 1 Physico-chemical properties

Acrylamide (AA) is a white, crystalline solid at room temperature. Its melting point is 84°C. The physico-chemical properties of acrylamide are shown in **Table 1**.

<b>Table 1</b>	
<b>Acrylamide data sheet [7]</b>	
<b>Acrylamide</b>	
IUPAC	acrylamide
CAS number	79-06-1
Sum formula	C <sub>3</sub> H <sub>5</sub> NO
Molecular weight [g/mol]	71.078
Properties	white, crystalline solid at RT
Density <sub>30 °C</sub>	1.127 g/cm <sup>3</sup>
Melting point	84 - 84.5 °C
Boiling point	125 °C at 3.3 Pa
Vapour pressure	0.9 Pa at 25 °C
log P <sub>ow</sub>	-0.67 to -1.65
Solubility in water	2.155 g/l at 30 °C
MAK Commission Classification	skin absorption H carcinogen category 2 germ cell mutagen category 2

## 2 Production and use

Nowadays, acrylamide is produced on a major technical scale through catalytic hydration of acrylonitrile. By far the greatest part (99.9%) of the monomeric acrylamide produced in the European Union (EU) is used in the production of polyacrylamides. Among other purposes, polyacrylamides are used as dispersants and flocculants in drinking water treatment. In addition, high-molecular polyacrylamides can be modified chemically by the introduction of non-ionic, anionic or cationic groups for various purposes and subsequently used as ion exchanger, thickener or as processing aid in the paper industry. Besides, acrylamide is also used as a co-polymer in the synthesis of dyestuffs and for various plastics. Other uses for acrylamide polymers are found in the crude oil industry (drill hole cement), the building (additive to hydraulically binders), the paper (to improve tear resistance), the mining

(clarification of circulation water) and the textile industries, in which polyacrylamides are used as colouring aid as well as for binding textile fibres [1].

In research, acrylamide is used in the production of polyacrylamide gels for electrophoresis. The annual production of acrylamide in the EU is estimated at 80,000 to 100,000 tonnes [7]. When using polyacrylamides, their content of monomeric acrylamide must not exceed 0.1 percent by weight, as they otherwise have to be classified as Class 2 carcinogens [8]. In polyacrylamides destined for drinking water treatment, the residual content of monomeric acrylamide may only be 0.025%.

### **3 Emission into the environment**

Acrylamide enters the environment during its production and when using its polymers (residual content of monomers). Due to its high water solubility, acrylamide is mainly released into water and water-containing compartments. The European Risk Assessment Report [7] calculates the total continental release of acrylamide into water from all conceivable sources at a maximum of 280 kg/day. On the other hand, due to the low vapour pressure of acrylamide, its release into the atmosphere is negligibly low at 0.38 kg/day. In the environmental media, particularly water, soil and air, acrylamide is degraded within a few days. This is either via a bacterial process or due to the reaction with hydroxyl radicals. For this reason, no accumulation of acrylamide occurs either in the environment or in the food chain.

### **4 Exposure of humans**

The intake of acrylamide by the non-smoking general population is almost exclusively via food. Acrylamide is produced at elevated temperatures when foods containing both proteins and carbohydrates are prepared. This means that acrylamide is formed during processes such as baking, roasting or frying etc. Compared with this, intake of acrylamide with tobacco smoke is far greater than that with food.

#### **4.1 Intake with food**

In 2002, evidence was produced for the first time by a Swedish working group that acrylamide is produced by the heating of foods [9]. In this case, those foods are involved which – like potatoes – contain both carbohydrates and proteins, and are prepared at elevated temperatures. In further studies, it was possible to show that reducing sugars such as glucose for example, and the amino acid asparagine, play a crucial role in the production of acrylamide [10, 11]. The mechanism of this reaction, during which acrylamide is formed temperature- and time-dependently, is accepted as having been clarified in the meantime [12]. The fact that acrylamide is not formed until reaching temperatures above 120°C is of

great practical importance. A sudden rise in acrylamide formation occurs at temperatures between 170 and 180°C.

Against the background of the carcinogenicity of acrylamide as incontestably confirmed by animal experiments, the realization that the substance occurs in a great number of foods has triggered many activities on a worldwide basis. Already in 2002, the BfR (*Bundesinstitut für Risikobewertung*, [German] Federal Institute for Risk Assessment) published a list with the acrylamide concentrations in various foods (**Table 2**) [13].

<b>Table 2</b>			
<b>Acrylamide concentrations in various food categories in Germany [13]</b>			
<b>Acrylamide (µg/kg)</b>			
<b>Product</b>	<b>Number of investigated samples</b>	<b>Median</b>	<b>Range</b>
Potato chips	221	750	130 – 3680
French fries, cooked	54	250	20 - 3920
Potato sticks	26	1430	630 – 2870
Fried potatoes, cooked	6	240	n.n. – 280
Cracker bread	95	170	n.n. – 2840
Bread	52	< 30	n.n. – 200
Bread rolls	12	< 30	n.n. – 140
Breakfast cereals	39	50	n.n. – 640
Cornflakes	9	170	20 – 640
Butter cookies	8	300	140 – 1090
Gingerbread	17	350	130 – 890
Pretzel sticks	7	250	110 – 360
Powdered coffee	35	280	180 – 290

According to this, the highest acrylamide concentrations were found in potato products such as potato chips, French fries etc. (up to 4000 µg/kg), although bakery products such as bread, gingerbread, butter cookies and also coffee contained appreciable amounts of acrylamide (up to 1000 µg/kg). In Germany, a number of measures have been taken to reduce the acrylamide content in foods. A comparison of the AA contents of foods published by the BfR in 2002 with the corresponding values by the Bavarian State Agency for Health and Food Safety (*Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit; LGL*

*Bavaria*) [14] (**Tables 2 and 3**) show tendencies to a reduction of the AA concentrations in foods. AA is also produced when cooking food at home, which means that the measures taken above naturally have no influence; consequently, preventive health measures can only be undertaken by informing the general public. The BfR has issued relevant information in the internet ([www.bfr.bund.de](http://www.bfr.bund.de)).

<b>Table 3</b>						
<b>Acrylamide concentrations in food samples investigated during the period January 1 to September 19, 2006 (n=264) [14]</b>						
<b>Product group</b>	<b>No. of samples</b>	<b>Acrylamide [µg/kg] Minimum</b>	<b>Acrylamide [µg/kg] Median</b>	<b>Acrylamide [µg/kg] Maximum</b>	<b>Signal value excess</b>	<b>Signal value [µg/kg]</b>
Potato chips	24	168.2	309.9	665.3	-	1000
French fries, cooked	132	19.3	188.5	1532.0	11	530
Potatoes, other products	1	6.1	-	-	-	1000
Gingerbread, products containing gingerbread	14	49.5	614.9	2711.5	6	1000
Children's cookies	6	34.9	120.5	143.4	-	245
Added cereals for babies and infants	10	12.8	39.6	155.0	-	1000
Coffee, roasted	52	45.4	164.7	314.7	-	370
Bakery products containing fats and yeast, also with filling	4	<0.6	28.1	279.2	-	1000
Other samples	21	<0.6	64.4	442.7	-	1000
<b>Total results</b>	<b>264</b>	-	-	-	<b>17</b>	-

Based on the acrylamide concentrations measured in foods and various assumptions on the eating habits of the German population, the mean acrylamide intake with food has been calculated at 0.6 µg/kg body weight. According to such projected values, an acrylamide intake of 3.4 µg/kg body weight and day is found in population groups with a more extensive consumption of foods containing acrylamide such as, for example, deep-fried potatoes, potato chips etc. [15]. Towards the end of 2002, the BfR gathered data on the intake of acrylamide from high-contaminated foods by more than 1000 male and female students aged

15 to 19 at Berlin schools. A mean daily acrylamide intake of 1.1 µg/kg body weight and day was calculated. According to these calculations, 1% of the students consume even more than 6.9 µg acrylamide/kg body weight and day [16].

In an initial approximation, the WHO assumed an average food-based acrylamide intake of between 0.3 and 0.8 µg/kg body weight and day [17].

Another way to determine the amount of acrylamide intake by humans is found in the concentrations of haemoglobin-(Hb) adducts of acrylamide (AA) measured in human blood. Using a linear pharmacokinetic model, it was possible to estimate the daily intake of acrylamide from the concentration of N-2-carbamoylvaline (AAVal) in the blood [18]. This calculation is based on an elimination constant of 0.15 h<sup>-1</sup> for acrylamide. In investigating the Hb-AA adduct content of 25 non-smokers, 0.85 µg/kg body weight and day was calculated as median value for the daily AA intake. The highest AA intake was found to be approx. 2.0 µg/kg body weight and day [19]. In the meantime, more recent investigations on the internal acrylamide exposure of the general population are available, which the LGL Bavaria carried out together with the IASU (*Institut für Arbeits-, Sozial- und Umweltmedizin*, Institute for Occupational, Social and Environmental Medicine) of the University of Erlangen-Nuremberg [20]. Thereby, 1008 persons throughout the entire region of Bavaria were investigated for their AA-Hb adduct level. In the 857 non-smokers, AAVal concentrations in the blood between 3 and 103.4 pmol/g globin were found (median value 26.5 pmol/g globin). From this, a daily AA intake between 0.12 and 4.10 µg/kg body weight and day (median value: 1.07 µg/kg body weight and day) was calculated. This means that the AA intake of individual persons with their food is four times higher than the average for the investigated collective. On the other hand, however, dietary habits were found resulting in an AA intake of only about 1/10 of the average of the investigated collective. The values for AA intake given here agree well with an assessment by the Joint FAO WHO Expert Committee on Food Additives (JECFA) on the AA intake of the general population. Based on data from 17 countries, JECFA calculated an average AA intake of 1 µg/kg body weight and day. For extreme consumers, the estimate was 4 µg/kg body weight and day [21].

In the meantime, a lively discussion has started as to whether acrylamide is also taken up through other sources than food (and tobacco smoke). The possibility of an endogenous formation of AA was also discussed. The reason for this was that a correlation between the dietary habits recorded via questionnaire and the level of internal AA exposure was not recognizable or only recognizable with difficulty [22, 23]. At present, however, this question may be regarded as having been solved. After a fasting period of 2 days, the concentration

of urinary mercapturic acids from AA decreased by 90%. Considering the relatively long half-lives of mercapturic acids from AA, this result means that by far the greatest amount of acrylamide is taken up with the food by non-smokers and that other acrylamide sources in the environment or a possible endogenous formation of AA are quantitatively insignificant.

#### **4.2 Intake with tobacco smoke**

Tobacco smoke is an important source in human exposure to acrylamide. In the filtered mainstream smoke, between 1.1 and 2.34  $\mu\text{g}$  AA/cigarette were found [24]. With a daily consumption of 20 cigarettes, an additional AA intake of up to 47  $\mu\text{g}$  is calculated, which corresponds to a daily dose of up to 0.7  $\mu\text{g}/\text{kg}$  body weight for an adult weighing 70 kg.

Adapting the estimate for acrylamide intake from tobacco smoke to the values from the population study conducted in Bavaria cited above, the following picture is obtained: 148 smokers participated in the study ( $n=1008$ ). Their AA-Hb adduct level was between 8.16 and 331.03 pmol/g globin (median value: 66.99 pmol/g globin). From these adduct levels, a daily AA intake between 0.33 and 13.31  $\mu\text{g}/\text{kg}$  body weight and day (median value: 2.7  $\mu\text{g}/\text{kg}$  body weight and day) can be calculated. When these values are compared with those of non-smokers, this means that smokers take up 2-3 times more AA than non-smokers.

From the same study, it can be seen that the acrylamide intake from passive smoking is probably of subordinate importance. In 126 non-smoking volunteers living together with at least one smoker, acrylamide adduct concentrations between 3 and 103.4 pmol/g globin (median: 27.6 pmol/g globin) were found. The median value of the adduct concentration in the non-smokers without exposure to passive smoking ( $n=723$ ) was only slightly lower (26.20 pmol/g globin).

#### **4.3 Other acrylamide sources**

Compared with the AA intake from foods and tobacco smoke, other sources are quantitatively insignificant. The AA intake via drinking water has been estimated at 0.0036  $\mu\text{g}/\text{kg}$  body weight and day. It is thus lower than that from food by two orders of magnitude. The level of AA intake from cosmetics is estimated to be similar [25].

### **5 Toxicology**

The toxicological effects of acrylamide in animals and humans have been summarized and evaluated by a number of national and international expert committees [1,2,3,4,5,6]. AA is classified as a carcinogenic compound (DFG: Category 2; IARC: Category 2A) [3,6]. This means that AA is a substance which must be viewed as being carcinogenic in humans. In addition, AA is classified as a germ cell mutagen (DFG: Category 2), as such an effect has

been demonstrated by an increased mutation rate in the offspring of exposed mammals. With regard to effects, the most important properties of acrylamide in humans are its potential carcinogenicity and germ cell mutagenicity as well as its demonstrated neurotoxicity.

## **5.1 Chronic toxicity in animal experiments**

### **5.1.1 Carcinogenicity**

In long-term carcinogenicity studies in rats and mice with oral, dermal or intraperitoneal administration, increased incidences of cancer were observed in various localisations. In female rats, these consisted of malignant and benign mammary tumours, tumours of the central nervous system, the thyroid gland, the oral cavity, the clitoris and of the uterus. In the male rats, mesotheliomas of the tunica vaginalis testis and the scrotum, and tumours of the thyroid gland were found. The doses at which increased cancer incidences occurred were in the range between 0.1 and 2 mg AA per kg body weight and day.

With regard to tumour induction, it appeared that mice react more sensitively than rats. This may be attributed to the fact that, in the mouse metabolism, glycidamide (GA) amounts approximately three times higher than those in rats are formed. GA was demonstrated to be the ultimate carcinogenic agent of acrylamide [26].

### **5.1.2 Neurotoxicity**

Acrylamide is a highly effective neurotoxic compound which, in a higher dose range (20 to 50 mg/kg body weight and day), especially affects the central nervous system and produces functional disturbances. Doses in this range (20-50 mg/kg body weight) produced ataxias in rats, dogs and primates [27, 28, 29, 4]. Lower doses produce no clinical effects.

In the range of lower doses (up to 20 mg/kg body weight, the peripheral nervous system is affected in particular, whereby morphological changes are observed. For example, AA produces changes in the microtubule network already at very low concentrations [30]. At doses of 5 or 20 mg/kg body weight, AA is capable of producing an increase in peripheral nerve degeneration which has, however, been found to be reversible [31]. After treatment with 2 mg AA per kg body weight for 18 months, degeneration of the tibial nerve was found in rats [32]. In a long-term study (18 months) at a dose of 2 mg AA per kg body weight, an increased incidence of degeneration of the sciatic nerve was observed histopathologically [33]. The question as to whether these neurotoxic effects are produced by AA itself or its metabolite, glycidamide, is still open.

The NOAEL for systemic neurotoxicity is given as 500 µg/kg body weight and day [34].

## **5.2 Chronic toxicity in humans**

### **5.2.1 Carcinogenicity**

To clarify the question whether acrylamide is also carcinogenic in humans, a number of epidemiological studies were carried out in cohorts exposed to acrylamide at their workplaces. The AA concentrations in the air of the workplace were between 0.1 and 1 mg acrylamide per m<sup>3</sup>. No association between exposure to AA and the occurrence of cancer could be demonstrated [35, 36, 37]. Nevertheless, these studies were assessed as not suitable to answer the question as to a possible carcinogenic effect of AA in humans. Among other factors, the investigated cohorts are too small. There were also considerable deficits in measuring and recording the exposure [6].

A further problem in clarifying the question as to whether AA is carcinogenic also in humans is found in how to determine the exposure. Here, questionnaires are frequently used which were found to be inadequate for determining food intake and tobacco consumption in the field of environmental medicine. It is difficult to obtain a valid record of changing aspects such as e.g. dietary habits, which is highly dependent on memory, age, sex and time of year [23]. In the available environmental medical studies, the statistical power is too low to record internal exposure correctly and relate it with a potential cancer risk due to the small number of investigated persons and other limitations [38, 39, 40].

### **5.2.2 Neurotoxicity**

The central and the peripheral nervous system are the target organs after chronic intake of acrylamide [41]. After acute AA exposure, ataxia, tremor, impaired reflexes, slurred speech and states of mental confusion were observed [42]. Disturbances of the peripheral nervous system make themselves felt by sensations of numbness in hands and feet, loss of foot reflexes, muscular atrophy and ataxia [2]. In a majority of cases, these neurotoxic effects of AA were found to be reversible [43, 44, 45]. In Swedish tunnel workers exposed to a mixture containing acrylamide, reversible deteriorations of the peripheral nervous system were found. Here, the AA-Hb adduct level correlated with the neurological symptoms [46]. 41 Chinese workers exposed to a mixture of acrylamide and acrylonitrile showed significantly more frequent peripheral neurotoxic symptoms than a control group. Here, too, the neurotoxic effects correlated with the internal AA exposure as measured by the urinary AA mercapturic acid elimination and the AA-Hb adduct level in the blood [47].

Various mechanisms for the neurotoxic effects of AA are discussed in the literature. More recently, this discussion has concentrated around two competing hypotheses: (a) inhibition of the kinesin-based fast axonal transport [48], and (b) the direct inhibition of neurotransmission [49]. In either case, however, the binding of the noxious electrophilic AA or GA to nucleophilic

positions on macromolecules, especially SH groups, seem to make up the biochemical cause for the neurotoxic effects of AA. A summary of the discussed mechanisms is found in Friedman [50].

### **5.2.3 Relationship between internal exposure and neurotoxic effects**

In the literature, a number of occupational medical studies are available in which the relationship between internal acrylamide exposure and neurotoxic effects has been investigated.

In 1993, in the People's Republic of China, 41 workers occupied in the catalytic production of AA from acrylnitrite (ACN) were investigated for internal AA exposure. The Hb adducts of acrylamide and acrylonitrile were determined. The AAVal values were between 8.6 and 972.4 µg/l. For the first time, the determination of GA-Val in the blood was also attempted. According to these investigations, the GA-Val values are just as high as or somewhat higher than the AAVal values (54.9-1098 µg/l). From these results, the authors calculated that the intake of AA of the highest exposed workers was about 3 mg/kg body weight and day, and compared this with the dose of 2 mg/kg body weight and day administered to rats, which already produces an increase in tumour rates [51]. An investigation as to whether there is a relationship between Hb adduct levels, mercapturic acid elimination in the urine and neurological effects was carried out with the same workers in 1994. The observed and measured neurological deviations were summarized in a "neurotoxic index" that correlates well with mercapturic acid elimination and the concentration of Hb adducts in the blood. More than 70% of the workers showed symptoms of peripheral neuropathy [47].

In connection with an accident that occurred in 1997 whereby workers building a tunnel in Sweden were exposed to acrylamide, a Swedish working group investigated 210 victims for their internal acrylamide exposure and various neurophysiological effects. AA-Hb adduct levels were found in the blood of 47 workers, that were within the range of background exposure of the general population (0.6 - 2.0 µg/l). The remaining 163 workers were found to have AA-Hb adduct levels between 2.0 and 506.2 µg/l. Clear-cut dose-response associations were found between the AAVal concentration and the symptoms of the peripheral nervous system, such as tingling and numbness in hands and feet. For these effects, the authors derive a NOAEL of 14.6 µg AAVal/l blood [46]. For the neurophysiological investigations, standardised methods in testing motor and sensory nerves of the right extremities were applied.

## **6 Absorption, distribution, kinetics and metabolism**

### **6.1 Absorption and distribution**

AA can be absorbed by inhalation, through the skin, and orally. It is absorbed almost completely, and its solubility in water ensures that it is distributed rapidly throughout the body [52]. After the administration of potato chips containing acrylamide to 6 volunteers (average age 26.6 years) 60.3% of the orally absorbed AA was excreted with the urine after 72 hours in the form of acrylamide and its mercapturic acids, N-acetyl-S-(2-carbamoylethyl)cysteine (AAMA) and N-acetyl-S-(2-hydroxy-2-carbamoyl)cysteine (GAMA). The half-lives were 2.4 h (AA), 17.4 h (AAMA) and 25.1 h (GAMA) [53]. In this context, it is worth noting that the GA, which was demonstrated in animal experiments to be the ultimate genotoxic substance, is eliminated in the form of GAMA more slowly than AAMA. After oral administration of 0.99 mg  $d_3$ -AA to one volunteer, 57 % of the absorbed AA dose was excreted with the urine in the form of both mercapturic acids within 46 hours [54]. Both deuterated mercapturic acids were still demonstrable in the urine even after 46 hours. Whereas the maximum excretion of AAMA is already attained after 11.5 hours, it was 22.5 h in the case of GAMA. It is to be assumed that the amount of acrylamide not excreted with the urine in the form of AA, AAMA or GAMA is bound to nucleophilic structures in the body.

As a water-soluble substance, acrylamide also passes into breast milk. In a Bavarian study conducted in 2005 acrylamide concentrations ranging between 0.1 and 1.3  $\mu\text{g}/\text{kg}$  (median: 0.11  $\mu\text{g}/\text{kg}$ ; 95<sup>th</sup> percentile: 0.33  $\mu\text{g}/\text{kg}$ ) were discovered in 172 breast milk samples [55]. The initial feed of infants investigated in the same study showed comparable results which were, on average, slightly above the mean level of breast milk.

In the breast milk of two mothers, each of whom consumed 100 g potato chips, Sörgel et al. [56] have measured AA concentrations of 3.2 and 18.8  $\mu\text{g}/\text{l}$ . Assuming an intake of 500 ml breast milk, the authors calculated an intake of 2 or 10  $\mu\text{g}$  AA for these children. Compared with the more recent studies on AA exposure of the general population [22, 20], these values appear to be too high. As AA reacts readily with nucleophilic substances, free AA only occurs in body fluids at relatively low concentrations. For this reason, the determination of AA in body fluids is less suited for estimating internal exposure. AA passes the placental barrier. In 11 mother/child pairs it was found that the AA-Hb adduct level in the maternal blood correlates with that in the umbilical cord blood. Although the adduct concentrations in both matrices behave in a 2:1 ratio, it can be assumed that the AA dose ( $\mu\text{g}/\text{kg}$  body weight) is approximately the same in mother and child. Among other evidence, the short life span of the infant erythrocytes resulting in a comparably low AAVal level in the blood argues in favour of this [57].

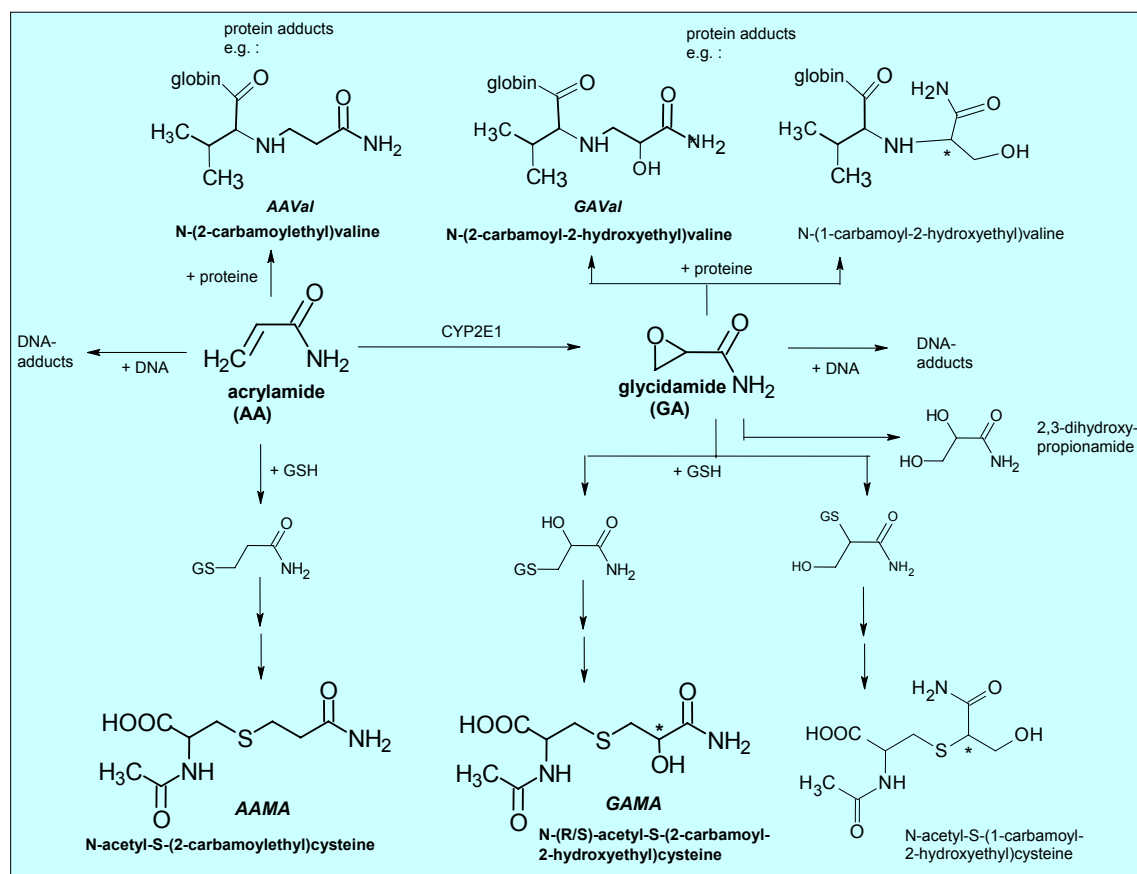
## 6.2 Metabolism

From animal experiments, it is known that a part of the absorbed AA oxidizes to glycidamide (GA) via the cytochrome-P450 enzyme 2E1 [58]. Both the AA and the GA bind to nucleophilic sites of macromolecules in the body, sulfhydryl or amino groups in particular. GA is electrophilic and more reactive than AA, and is thought to be responsible for the mutagenic and carcinogenic effects of AA [59].

Within the phase II metabolism, both AA and GA are bound to glutathione and lastly excreted with the urine in the form of the mercapturic acids AAMA and GAMA. Due to the more pronounced genotoxic and carcinogenic effects of GA versus those of AA, the ratio between the two mercapturic acids AAMA and GAMA is of great importance. This ratio is 5:1 in rats and 2:1 in mice [60]. These results are in agreement with the observation that mice are more sensitive to the carcinogenic effects of AA than rats [61, 62].

Although a number of investigations using animals are available on the metabolism of AA, the human metabolism of this substance has not been investigated until recent years. In the study already mentioned, in which deuterated AA was administered orally, 52% of the dose was excreted in the form of AAMA and 5% as GAMA within 48 hours after administration. This means that the ratio between the less and the more pronounced genotoxic metabolic pathway is approximately 10:1 in humans [54]. A similar ratio between AAMA and GAMA was observed by Fuhr and et al. after administration of potato chips containing acrylamide [53], following which an average 50% of the AA dose was excreted with the urine as AAMA and 5.9% as GAMA within 42 hours after administration. In addition, this working group also found unchanged AA in the urine, although this only made up about 4.4% of the dose. In the investigation involving the general population, the ratio between the two mercapturic acids was somewhat different to that following single AA administration. In the population, the ratio between the reductive and the oxidative metabolic pathway is on average 6:1, and is closer to that observed in rats than after single administration. This is attributed to the fact that, in a larger group of persons, the metabolic steady state is better represented than after single administration of a higher dose [54].

Recently, Fennell and Friedman identified a further metabolite of AA in human urine, i.e. 1,2-dihydroxypropionamide, which is produced by the hydrolysis of GA [63]. Figure 1 shows the human metabolism of acrylamide in simplified form.



**Figure 1** Simplified metabolism of AA in humans

### Hb adducts

Electrophilic, mutagenic chemical substances are able to react with haemoglobin and form covalent bindings. One of the binding sites of haemoglobin is the free amino group of valine located at the end of the haemoglobin molecule (N-terminal valine).

The reaction product of AA with the N-terminal valine of haemoglobin (AAVal) was first demonstrated in persons of the general population in 1997 [64]. The mean value was 31 pmol/g globin. On average, an adduct level higher by three times was measured in tobacco smokers (arithmetic mean = 116 pmol/g globin). It was not possible to demonstrate that the haemoglobin adducts of glycidamide also occur in the blood of the general population until more selective analytical procedures were developed. This knowledge is of great importance because glycidamide, and not unchanged acrylamide, actually represents the carcinogenic agent. In non-smokers, the ratio between N-(R,S)-2-hydroxy-2-carbamoylethylvaline (GAVal) and N-2-carbamoylethylvaline (AAVal) was 0.96 on average (maximum value: 1.7) [65, 66]. The ratio of Hb adduct GAVal to adduct AAVal is consequently five times greater than that of the corresponding mercapturic acids of GA and

AA. This indicates a higher reactivity and efficacy of GA in humans than expressed by the excretion of the two mercapturic acids. The GAVal/AAVal ratio is comparable in humans and rats [54].

In smokers absorbing markedly more AA than non-smokers, the GAVal to AAVal ratio was 0.7. This could mean that the importance of the oxidative metabolism increases as AA exposure decreases in humans.

Both acrylamide and glycidamide form haemoglobin adducts in vivo, whereas DNA adducts are exclusively formed by glycidamide in vivo [3, 67]. The good correlation between the extent of exposure to acrylamide and the haemoglobin adduct levels in rats and mice proves that haemoglobin adducts are suitable as biomarkers for exposure to AA [67].

### **DNA adducts**

In humans, no adducts of AA or GA to DNA could be detected up to now, though this was possible in animal experiments.

After administration of AA to rats and mice, N-7-(2-carbamoyl-2-hydroxyethyl)guanine was found at very similar concentrations in various organs of both species. This indicates that glycidamide (GA) is distributed evenly throughout the body and that only GA shows a mutagenic and carcinogenic potential [68]. Adducts of AA on DNA have not been found up to now. In 2003 it was possible to identify a further DNA adduct of GA. The N-3-(2-carbamoyl-2-hydroxyethyl)adenine level following AA dosage is, however, a hundred times lower than that of the guanine derivative described above [69]. In mice, an increase in DNA adduct concentrations in the liver could already be demonstrated 8 hours after the administration of 0.1 mg/kg body weight [70]. In this context, it is important to note that this dose is only a hundred times higher than that which we generally take up with our food. The fact that we are not yet able to demonstrate GA-DNA adducts in humans is due to the fact that the available analytical methods are not yet sensitive enough.

### **GST polymorphisms**

Interindividual differences in the conjugation of acrylamide with *L*-glutathione by the glutathione-*S* transferases M1 and T1 and a related modulation of a genetic risk measured as chromosome aberrations could not be unequivocally demonstrated [71]. Ex vivo incubations of acrylamide with blood samples of different GSTM1 and GSTT1 activities revealed no modulating effect on the measured haemoglobin adduct levels [72].

## 7 Human biomonitoring

As a basic principle, the Hb adducts and the mercapturic acids from reductive and oxidative metabolism are suitable for the biological monitoring of persons exposed to acrylamide.

These are on the one hand

N-2-carbamoyl-ethylvaline (AAVal) and

N-acetyl-S-(2-carbamoyl)cysteine (AAMA),

which are formed by direct binding of the N-terminal valine of haemoglobin or of glutathione to acrylamide (“Michael” addition).

On the other hand, glycidamide also reacts with haemoglobin and glutathione (oxidative metabolism) to form

N-(R, S)-2-hydroxy-2-carbamoyl-ethylvaline (GAVal) and

N-(R, S)-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA).

As glycidamide represents the ultimate carcinogen of AA, GAVal and GAMA should be the best suited parameters in estimating the exposure to AA. They are closest to the carcinogenic mode of action of AA.

Unfortunately, both these parameters have only been used in a few of the studies investigating groups from the general population up to now. No reliable data on the investigation of persons occupationally exposed to AA are available at present.

By contrast, comprehensive investigations have been carried out in which the Hb adduct of AA in the blood of persons occupationally exposed to AA as well as in persons from the general population was investigated (see 5.2.2, 5.2.3, 8). As there is a close relationship between AAVal and GAVal, AAVal can be used as a suitable parameter for exposure. On average, the ratio between the concentrations of the Hb adducts of AA and GA in humans is approximately 1:1.

As regards excretion of the mercapturic acid of unchanged AA, the data again are insufficient for use in assessing an internal AA exposure of persons occupationally exposed to the substance, or of persons in the general population.

### 7.1 Analytics

To determine AAVal, the erythrocytes are first of all separated from the whole blood. After haemolysis of the erythrocytes the globin is separated from the haemoglobin. In a modified Edman degradation, the terminal valine of the haemoglobin is cleaved and at the same time

derivatised. The acrylamide is bound to this valine. By means of capillary gas chromatography, the analyte is separated from accompanying substances and determined by mass spectrometry. For calibration, a dipeptide, N-2-carbamoyl-ethylvaline-leucine-anilide, is used [66]. Analytical methods such as this one, which are used to determine the Hb adducts of carcinogenic substances, are relatively sophisticated, though they are nowadays applied on a routine basis in appropriately equipped laboratories and carried out with reliable results.

## 7.2 Available data on internal exposure in the general population

In the following, the AAVal values are given in  $\mu\text{g/l}$ . In this way, they can be better compared with references in the literature. To convert  $\text{pmol AAVal/g globin}$  to  $\mu\text{g AAVal/l}$ , a factor of 1:36.90 is used. In other words, 1  $\mu\text{g AAVal/l}$  corresponds to 36.90  $\text{pmol AAVal/g globin}$ .

A number of studies is available in which the AA-Hb adducts are measured in blood samples from the population (**Table 4**).

In 1997, Bergmark described for the first time that the presence of AAVal can be demonstrated in practically all blood samples from the general population [64]). In eight non-smokers, this author observed AAVal concentrations between 0.7 and 1.3  $\mu\text{g/l}$  (arithmetic mean 0.8  $\mu\text{g/l}$ ). By comparison, smokers ( $n=10$ ) were found to have, at an average of 3.1  $\mu\text{g/l}$ , AAVal concentrations which were four times higher. In 2001, Hagmar and et al. measured AAVal values between 0.57 and 2.0  $\mu\text{g/l}$  in ten non-smokers [46]. On investigating 62 persons who had not been exposed to AA occupationally, AAVal concentrations between < 0.3 and 1.4  $\mu\text{g/l}$  (95%: 1.2) were measured in non-smokers. In this collective as well, smokers were found to have AAVal concentrations in the blood which were up to six times higher. The values were between 0.4 and 7.9  $\mu\text{g/l}$  (95<sup>th</sup> percentile: 4.3  $\mu\text{g/l}$ ) [19]. In a more comprehensive study in the German general population ( $n=395$ ) [22], the non-smokers ( $n=296$ ) were found to have mean AAVal-levels of  $0.4 \pm 0.2 \mu\text{g/l}$  (range < 0.3 – 1.2  $\mu\text{g/l}$ ); here too, the corresponding mean value was approximately four times higher in the smokers ( $n=99$ ) at  $1.5 \pm 1.4 \mu\text{g/l}$  (range: < 0.3 – 12  $\mu\text{g/l}$ ).

In a study involving a Bavarian population consisting of 1008 persons in total, a median value of 0.7  $\mu\text{g AAVal/l}$  blood was found in non-smokers ( $n=857$ ). The smokers ( $n=148$ ) showed a median value of 1.8  $\mu\text{g/l}$ . The maximum values for non-smokers and smokers were 2.8 and 9.0  $\mu\text{g/l}$ , the 95<sup>th</sup> percentiles were 1.3 and 5.4  $\mu\text{g/l}$  [20]. In this study, children  $\leq 15$  years were found to have significantly higher AAVal levels than the older non-smoking age groups. This difference is possibly attributable to the higher food intake per kg body weight. Different eating habits may also play a role.

In a study on 70 non-smokers, Swedish researchers found AAl concentrations between 0.5 and 2.7 µg/l [73]. In an American study [74], in which smokers and non-smokers were not evaluated separately, AAl values between 0.7 and 12.3 µg/l were found.

<b>Table 4</b>					
<b>AAI adduct concentration in the blood of the general population (all values in µg/l blood)</b>					
<b>Reference/region/nation</b>	<b>Number/persons</b>	<b>Median</b>	<b>Arithmetic mean</b>	<b>Range</b>	<b>95th percentile</b>
Bergmark 1997 [64] Sweden	8 non-smokers		0.8	0.7 – 1.3	
	10 smokers		3.1	0.7 – 4.0	
Perez et al. 1999 [77] Korea	2 non-smokers		1.1		
	2 smokers		2.2		
Hagmar et al. 2001 [46] Sweden	10 non-smokers			0.57 – 2.0	
Schettgen et. al. 2003 [78]Germany / Bavaria	25 non-smokers	0.57		<0.3 – 1.4	1.2
	47 smokers	1.3		0.4 – 7.9	4.3
Paulsson et al. 2003 [65] / Sweden	5 non-smokers		0.73		
Schettgen et al. 2004b [66] /Germany / Bavaria	13 non-smokers	0.49	0.51	0.2 – 0.84	
	16 smokers	2.2	2.1	0.68 – 5.4	
Bader et al. 2005 [22]/ Germany/Lower Saxony	296 non-smokers		0.4	<0.3 – 1.2	0.8
	99 smokers		1.5	<0.3 – 12	3.8
Hagmar et al. 2005 [73] Sweden	70 non-smokers	0.84		0.5 – 2.7	
	smokers			0.8 – 11.6	
Urban et al. 2006 [79] Germany	60 non-smokers	0.73	0.75	0.5 – 1.4	
	60 smokers	2.1	2.2	0.5 – 5.7	
Vesper et al. 2006 [74] USA	96 smokers and non-smokers	3.5		0.7 – 12.3	8.3
Kütting et al. 2006 [20] Germany / Bavaria	857 non-smokers	0.72	0.76	0.08 – 2.8	1.3
	148 smokers	1.8	2.2	0.2 – 9.0	5.4
	88 children ≤ 15 years	0.91			1.75
	730 adults, non- smokers ≥ 18 years	0.69			1.18

## 8 Background exposure in the population

To derive reference values [76] for AAVal in the blood of the German population, no data from representative population studies are available at present. However, both the data of Bader et al. [22] and the studies by Kütting et al. [20] are considered by the Commission as being, in principle, suitable for describing the background exposure of the general population. Bader et al. [22] determined values between <0.3 and 1.2 µg/l in 296 non-smokers. The mean value was 0.4 µg/l and the 95<sup>th</sup> percentile 0.8 µg/l blood. In 857 non-smokers, the investigations by Kütting et al. [20] showed concentrations between 0.08 and 2.8 µg/l blood, the median being given as 0.72 µg/l, and the 95<sup>th</sup> percentile as 1.3 µg/l blood.

In this study [20] it was found that children ( $\leq 15$  years; n=88) had higher AAVal levels in the blood than adults ( $\geq 18$  years; n=730). Median values and 95<sup>th</sup> percentiles were determined in both groups. These values were 0.91 and 1.75 µg/l in children, and 0.69 and 1.18 µg/l in adults.

From these data recorded in Germany, the following values are given to describe background exposure for non-smokers:

- **1.8 µg AAVal/l blood for non-smoking children,**
- **1.2 µg AAVal/l blood for non-smoking adults.**

On average, 4-5 times higher AAVal values are found in the blood of smokers.

## 9 The question of HBM values

The Commission is at present not in a position to derive toxicologically founded HBM values for acrylamide (AA). The main reason is that AA is a substance for which a carcinogenic effect in humans has to be assumed.

## 10 Measures for reducing body burden of acrylamide

Smoking tobacco is the most important source of AA intake. In non-smokers, intake of AA is almost exclusively via the food. The following foods contain acrylamide in particular: chips, crackers and salted pretzel sticks, cracker bread, French fries, peanut flips, biscuits (cookies) and waffles, fried potatoes, cornflakes and muesli, muesli bars, toast, peanuts and coffee [75]. The AA concentration decreases in these foods in the sequence given, i.e. chips have the highest and coffee the lowest AA content. Especially by reducing the consumption of this type of food containing AA, everybody should be able to reduce his/her health risk from acrylamide. The Federal Institute for Risk Assessment (*Bundesinstitut für Risikobewertung*, BfR) has made a programme available on the internet (www), by means of which everybody can calculate his/her personal AA intake and compare it with the mean AA intake of the

population ([www.bfr.bund.de/cd/8616acrylamidrechner.xlf](http://www.bfr.bund.de/cd/8616acrylamidrechner.xlf)). The BfR gives the mean AA intake of the population as 0.81 µg/kg body weight and day. This corresponds to an acrylamide-haemoglobin adduct level in the blood of 0.54 µg AAVaI/l blood. This mean value is of course markedly lower than the background burden of AAVaI levels in the blood of the general population, as these reflect the 95<sup>th</sup> percentiles and not the average value of the population. In the interest of preventive health measures, attaining a value below the mean value of 0.54 µg AAVaI/l blood should therefore be aimed at, especially as this can be achieved by proper selection of the foods available. This applies all the more, as chips, French fries, peanut flips, etc. are foods which one can go without most easily. In foods cooked at home such as toast or fried potatoes for example, the motto “brown but not black”, should be upheld to reduce the acrylamide content in such foods.

In the light of the above facts, one should immediately start reducing AA adduct levels in the blood exceeding the background exposure values by declining those foods containing AA in particular. As a basic recommendation, tobacco smokers should discontinue their habit under all circumstances, in addition to reducing their AA intake through food.

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