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Thermally induced/process-related contaminants: The example of acrolein and the comparison with acrylamide

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α,β-Unsaturated aliphatic carbonyl compounds are naturally widespread in food, but are also formed during the thermal treatment of food. This applies, for example to the genotoxic carcinogen acrylamide, but also to acrolein, the simplest α,β-unsaturated aldehyde. First observations indicate that human exposure to acrolein (AC) may be higher than the exposure to acrylamide (AA). The DFG Senate Commission on Food Safety (SKLM) therefore compared data on AC and AA available in the scientific literature, evaluating current knowledge on formation, occurrence, exposure, metabolism, biological effects, toxicity, and carcinogenicity and defined knowledge gaps as well as research needs in an opinion on November 19th 2012 in German. The English version was agreed on April 17th 2013.

Thermally induced/process-related contaminants: The example of acrolein and the comparison with acrylamide

1. Introduction

A previous SKLM evaluation on health effects of α,β-unsaturated aliphatic carbonyl compounds in food was issued 2002 [1]. Such substances are naturally occurring in food, but also added as flavourings. Examples are 2-hexenal or 2,4-nonadienal. In addition, they may be generated during the thermal treatment of food. As has been shown for example for the genotoxic carcinogen acrylamide (2-propenamide, CAS Nr. 79-06-1). Another example is acrolein (2-propenal, CAS Nr. 107-02-8), the simplest α,β-unsaturated carbonyl compound.

Pilot studies using exposure biomarkers suggest that human exposure to acrolein (AC) may be higher than that to acrylamide (AA). Mercapturic acids (MA), resulting from the coupling of the α,β-unsaturated aldehydes to glutathione, are excreted in urine and can be utilised as biomarker of exposure over the last 48 hours. In a pilot study monitoring the mercapturic acid content of spot urine samples from occupationally non-exposed nonsmokers (n = 14, [2]) the excretion of *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine (3-HPMA), a biomarker of exposure to AC, was found to be at least three times that of *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine (AAMA), a biomarker of exposure to AA.

Similar results were obtained in a further pilot human study on 13 volunteers. In this case, a defined test meal of potato crisps/chips experimentally heat treated to achieve a high AA intake (1 mg per person), the excretion of HPMA was found substantially higher than that of the AA-related mercapturic acids. Based on the AUC-values in urine („area under the curve“, AUC), the AC associated mercapturic acid

excretion after 72 hours was about 15 times that of the AA- plus glycidamide-associated mercapturic acids. Glycidamide (2,3-epoxypropionamide, GA) is the genotoxic metabolite of AA [3, 4].

The exposure level is an essential criterion to assess the potential health risks derived from the intake of such process-related substances. Occurrence and thermal formation of AA as well as its dietary intake are well known. In contrast, for AC, occurrence in food are not sufficient for a reliable exposure assessment. It is expected that thermal food treatment processes entailing the formation of AA, will also induce the formation of AC, even though different precursor molecules and mechanisms of formation are involved. The present opinion summarizes the knowledge on AC and AA, highlighting knowledge gaps and research needs.

2. Summary of data on AC and AA

2.1. Formation and occurrence

2.1.1. Breathing air

Acrolein

AC is formed during combustion processes, particularly in the course of incomplete combustion of fuels, wood or plastics. In the exhaust fumes of combustion engines 0.05-27.7 mg AC/m^3 was measured [5]. An appreciable source for inhalative AC exposure are workplaces in commercial kitchens, in which edible oil is heated up to temperatures above 180 °C (roasting/deep-frying). Depending on the conditions (e.g. oil grade, temperature, time) 5 to 250 mg AC/kg of used oil may be released in the ambient air [6-8]. In the ambient air of kitchens concentrations of up to 0.55 mg AC/m^3 air were detected while heating deep-frying fat [9]. The traffic-related burden of the breathing air in Hong Kong is estimated to be in the order of about 1.8 tons AC per year, whereas the burden due to commercial kitchens may amount to 7.7 tons/year [10].

Cigarette smoke can also contribute to the inhalative uptake of AC. The AC content in the smoke of cigarettes lies within the range of about 3 to 220 µg/cigarette [5] whereas in cigarette main stream smoke 56-118 µg AC per cigarette were detected. The formation of AC increases with rising glycerol and sugar contents in tobacco [11-13].

US EPA estimates the average AC concentration in the atmosphere at about 14.3 µg per m^3 [14]. However, due to the high reactivity of AC its persistence in the environment and the exchange between different environmental compartments is low [15].

Table 1 (Annex I) summarizes data on AC concentrations in the environment.

Acrylamide

The occurrence of AA in the environment has only sporadically been analyzed so far. Normally no significant entry of AA from anthropogenic sources into the environment occurs. Up to the present time natural sources of AA have not been identified. In 1997 an AA-containing chemical grout was used during a tunnel construction in Sweden. Due to an incorrect utilization high amounts of AA were released into the environment [16, 17].

According to the European drinking water directive the limit value for AA in drinking water is 0.1 µg/l. This value has been calculated based on specifications for the maximum monomer release from the corresponding water treatment polymer in contact with water. If this limit value is not exceeded, AA exposure through drinking water is assumed negligible.

In the main stream smoke of a cigarette 1-2 µg AA have been measured [18, 19]. Hence, cigarette smoke also is a source of exposure.

2.1.2. Food

Acrolein

AC can be formed from fats, amino acids and carbohydrates when heating food [13, 20]. Heat-induced formation of AC from glycerides/glycerol in the fat phase of food [21], thermal decomposition of amino acids such as methionine [22] and threonine [23] or the heating of carbohydrate-rich foods [24, 25] can also lead to the formation of AC.

Concerning the occurrence of AC in fresh, untreated foods almost no data are available. The few results available point out that AC can be present in low amounts in fruits and vegetables [26], as well as in foods of animal origin including fish [27] and cheese [28]. AC has also been detected in processed foods, in products

containing heated animal fats and plant oils as well as in the volatile components of certain foods such as fish, bread, poultry and beef [15]. However, data on the AC content in heated foods are largely missing. Nevertheless it can be presumed that thermally treated foods, depending on the type of thermal treatment, may contain significant amounts of AC.

The formation of AC during the heating of oil depends on the fatty acid composition, the heating time and the temperature [29]. In oils and fats not subject to heating processes during/after refining, AC contents were reported in the lower trace range, up to 20 µg/kg [30]. In contrast, used deep-frying fats, showed markedly increased AC contents (0.2-1.4 mg/kg = ppm) [9, 30, 31].

AC can be formed during production of spirits under conditions favouring its formation, e.g. by dehydration of glycerol. In addition, certain microorganisms such as heterofermentative lactobacilli und enterobacteriaceae can metabolically form 3-hydroxypropionaldehyde (3-HPA), from which AC can be formed by water elimination during distillation [32].

Other alcoholic beverages, e.g. red wine and lager beer, may contain AC, mostly in low concentrations [5, 26, 30, 33].

Reliable analytical methods for the determination of AC are only available for a few food groups at present. Gas chromatographic determination mostly makes use of head space analysis, sampling the volatilized AC fraction in the headspace for determination [4, 34, 35]. However, biomarker study results suggest that a considerable proportion of AC may escape such head space analysis, e.g. by forming reversible non-volatile associates with food constituents, presumably not accessible to headspace gas chromatography, but still liberating AC in the gastrointestinal tract.

Data on the occurrence of AC in drinking water are very limited, but indicate no significant human exposure [15].

Table 2 (Annex II) lists data on acrolein contents in foods.

Acrylamide

Various mechanisms of AA formation in food have been discussed [22, 36, 37]. The reaction of reducing carbohydrates with amino acids, in particular asparagine, during nonenzymatic browning (Maillard reaction) appears to represents the most important mechanism of formation of AA in foods.

AA is mainly formed in carbohydrate-rich, strongly heated foods, such as for example French fries, potato chips/crisps and coffee [38, 39].

A compilation of AA contents of foods is given in Tables 3 and 4 (Annex I).

2.1.3. Endogenous formation

2-Alkenals can be formed endogenously during lipid metabolism as a consequence of lipid peroxidation. Endogenous background exposure of the organism to 2-alkenals may be influenced by nutrition and by physiological states (acute or chronic infections, inflammation) [40-42].

Acrolein

No data are available up to now regarding the endogenous exposure of the organism to AC. However, it has been described that AC can be formed as a by-product of certain metabolic pathways such as lipid metabolism, glycolysis, the amino acid turnover or the oxidative deamination of polyamines [13]. For example, the amino acid threonine may be oxidized to 2-hydroxypropanal under oxidative stress (infections, inflammatory processes) or enzymatically by myeloperoxidase. AC may subsequently be formed from 2-hydroxypropanal by water elimination. Furthermore, the degradation of polyamines such as spermine and spermidine by copper-dependent amine oxidases can lead to 3-aminopropanal, which in turn can release AC by elimination of ammonia.

Acrylamide

The possible contribution of the endogenous formation of AA to overall AA exposure has not been studied so far. However, endogenous formation of AA may be presumed to be rather low in comparison with the exogenous exposure through food, since after a 48-hour fasting period a 90% decrease in the AA mercapturic acid excretion was observed [43].

However, in rats on AA-free chow, a clearly higher urinary mercapturic acid output was observed compared to the estimated (very low) AA intake. This observation has been interpreted as evidence for some endogenous formation of AA in rats (in the range of about 0.5 µg/kg b.w./day) [44]. Results from other studies presented evidence for a possible endogenous formation of AA also in humans [45, 46].

2.2 Exposure

Acrolein

Because of the limited database regarding acrolein levels in foods, a reliable assessment of the AC exposure through foods is not possible at present. Based on the assumption that all foods contain maximal reported levels of AC an exposure of around 1 mg/person/day (17 µg/kg b.w./day) may be estimated (see Annex I, Table 5).

A more reliable exposure estimate may be possible by making use of exposure biomarkers, e.g. through the quantitation of AC-associated mercapturic acids such as 3-HPMA and 2-carboxyethylmercapturic acid (CEMA) in urine. To achieve this, the proportion of an oral AC dose excreted as mercapturic acids in urine has to be known. Since data in humans are missing so far, the conversion rate into mercapturic acids may be estimated on the basis of results from animal studies.

After the oral administration of ^{14}C-labelled AC to rats (single dose of 2.5 mg/kg b.w.) 59% of the administered radioactivity was excreted in the 24 h-urine. After pre-treatment of the animals with 2.5 mg AC/kg b.w. for 14 days, followed by a single dose of 2.5 mg ^{14}C-AC/kg b.w., the 24 h-urine excretion remained almost unchanged (53%). Thus, about 60% of the administered radioactivity was excreted via the urine, about 30% of it as 3-HPMA [47, 48]. In conclusion, about 20% of the administered total dose was found excreted as 3-HPMA in rats.

The median 3-HPMA concentration in spot urine samples of 14 and 54 nonsmokers was 155 and 179 µg/l [2, 49], respectively. On the assumption of a daily urine volume of 1.5 l and of about 50% of oral AC being excreted as 3-HPMA, the estimated AC exposure is 124-143 µg/day or 2.1-2.4 µg/kg b.w./day. Based on maximal instead of median values the estimated exposure would be about 30 µg/kg b.w./day [50].

Literature data on 3-HPMA excretion in 24 h-urine of nonsmokers, respectively former smokers on abstention for a longer time vary considerably, from 200-300 µg

to 800-1000 µg 3-HPMA/24 h-urine (Table 6, Annex I). Assuming that about 20% of the AC intake is excreted as 3-HPMA in analogy to data in rats, an exposure of about 300-1400 µg/day or 5-24 µg/kg b.w./day can be estimated.

Acrylamide

The AA exposure can strongly vary, depending on individual consumption habits and different AA contents of single food groups. AA exposure in adults mainly results from consuming heated potato products (not cooked), bread and roasted coffee [38]. At present, the average daily uptake of AA by adults is estimated to be 1 µg/kg b.w./day [51, 52] or 0.31-1.1 µg/kg b.w./day [38]. The maximal exposure through foods is estimated to reach up to 4 µg/kg b.w./day [51, 52] or 0.58-2.3 µg/kg b.w./day (95^{th} percentile; [38]).

2.2.1. Further sources of exposure

Acrolein

The environmental AC exposure of humans mainly occurs through inhalation. Based on an average AC concentration in the atmosphere of about 14.3 µg/m^3 (6.2 ppb) and a mean respiratory volume in humans of about 20 m^3/24 h [53], an AC exposure via the atmosphere of 286 µg/day can be estimated.

In the case of smokers (about 20 cigarettes/day) an additional exposure (50-100 µg/cigarette) of up to 2 mg, corresponding to 0.03 µg/kg b.w./day, has to be taken into account. Smokers have been reported to show two times higher levels of the main AC metabolite 3-HPMA in urine than nonsmokers. After cessation of smoking for 4 weeks the 3-HPMA levels in urine decrease by about 78% (median value; [54]).

Acrylamide

Tobacco smoke is also a source of exposure for AA. Heavy smokers (about 20 cigarettes/day) may inhale up to 40 µg acrylamide/day through tobacco smoke. Fourfold higher amounts of AA metabolites are found in the urine of smokers as compared to nonsmokers [55].

The tobacco specific contribution to AC or AA exposure may be estimated by concomitantly monitoring exposure towards acrylonitrile (ACN). ACN leads to the formation of specific Michael addition products, such as the hemoglobin adduct at the N-terminal valine [56].

2.3. Metabolism

Acrolein

The metabolism of acrolein is not yet fully understood. The current state of knowledge is summarized in Annex II. A main metabolic pathway presumably proceeds through the formation of a glutathione adduct, followed by reductive /oxidative degradation of the mercapturic acid to the urinary metabolites 3-HPMA/CEMA. However, oxidative metabolism into acrylic acid may also occur as indicated by the detection of excreted CEMA [54, 57, 58]. Epoxidation of AC to the unstable metabolite glycidaldehyde may also be inferred, but has not been described up to now.

Acrylamide

In comparison, the metabolism of AA has been fairly well analyzed. A detailed description of the metabolic pathways is found in Annex II. The epoxidation of AA to glycidamide, mainly catalyzed by cytochrome P450 2E1, is considered to be essential for the genotoxicity and carcinogenicity of AA [59, 60]. Glycidamide forms DNA adducts, with the N^7-guanine adduct representing by far the most prevalent adduct formed [59].

In analogy to AC, AA and glycidamide are bound to glutathione to become excreted after further phase II metabolism as AA mercapturic acid (AAMA) and glycidamide mercapturic acid (GAMA) [61].

2.4. Reactivity and biological effect

α,β-unsaturated carbonyl compounds and acrylic acid derivatives possess a conjugated structure with electrophilic character, which can react with nucleophiles such as thiol groups or primary and secondary amine structures in amino acids and proteins in a Michael addition reaction. They predominantly form adducts with thiol groups of cysteine residues [62-65]. Free thiol groups can be present as thiol or thiolate. The highly nucleophilic thiolate is considered to be the preferred target structure for α,β-unsaturated carbonyl compounds [64, 65].

An adduct formation with lysine and histidine residues, representing harder nucleophiles, seems to occur predominantly at higher dose range or in the final stage of chronic diseases, when the cysteine-thiolate-pool is saturated [66].

Quantum mechanical calculations suggest that the relative „softness“ or „hardness“, the electrophilicity index and the energy level of the lowest unoccupied molecular orbital (LUMO) correlate with the reactivity towards cysteine, but also with the biologic effect, such as for example the extent of the synaptosomal neurotoxicity [64, 66]. According to these calculations [65] AC is significantly more reactive than AA. AC, which has more Michael reactivity than AA, reacts faster than AA via covalent binding to macromolecules and glutathione. This could mean that AC largely becomes scavenged before it might reach DNA or other critical cellular targets. The lower reactivity of AA probably explains why the epoxidation to the genotoxic glycidamide - at least in the liver - occurs to a significant degree.

The cellular toxicity of AC, but also that of AA at high concentrations, is at least in part due to the depletion of cellular glutathione [67]. Moreover, the irreversible formation of 1,4-Michael adducts with thiolate groups of cysteine residues can lead to modulation of various signal transduction pathways. For example, interaction of AC with „antioxidant response elements/electrophile response elements“ [68, 69] or with the mitogen-activated protein kinase in the MAP kinase signal transduction pathway has been described. Furthermore, interactions with transcription factors such as NF-κB [70, 71] or Nrf-2 [72, 73], with the tumour suppressor gene *p53* [74], with certain DNA repair enzymes [74, 75] and with the thioredoxin reductase/thioredoxin system have also been reported [76]. Triggering of apoptosis [77, 78], at higher concentrations (in-vitro > 10-25 µM) also of necrosis [14, 79] *in vitro* has been reported as well.

2.4.1. Neurotoxicity

Neurotoxic effects have been described in laboratory animals and in humans exposed to AA and/or ACN [80-82]. The acute exposure to AA leads to ataxia, tremor, amentia as well as speech and reflex disturbance in humans [83]. Swedish tunnel workers, which were highly exposed to an AA-containing mixture, showed reversible peripheral nerve dysfunctions [84]. Neurotoxic effects were also observed in rats, mice, cats, dogs and monkeys after repeated AA uptake [85, 86]. The animals developed neuropathies, tremor, impaired coordination, motor dysfunctions, neuromuscular weakness and reduced nerve conduction velocity [85, 86]. A „non observed adverse effect level“ (NOAEL) of 0.2 mg/kg AA/kg b.w./day was deduced for neurotoxicity, based on morphological changes in the nervous system as endpint [52]. While in animal tests higher AA doses (20-50 mg/kg b.w./day) mainly impair the central nervous system [87-90] lower doses (2-20 mg/kg b.w./day) appear to affect the peripheral nervous system [91-93].

Different mechanisms regarding the neurotoxic effect of AA are being discussed [94]. There is evidence that AA and glycidamide react with proteins that are involved in the axonal transport such as the microtubuli-associated proteins and the motor proteins dynein and kinesin [94-96]. This entails the inhibition of axonal transport [97, 98]. Inhibition of neurotransmitter uptake through the membrane and of their storage in vesicles also seems to play a role [99, 100]. In addition, time-dependent depletion of glutathione in the neurons is discussed to contribute to the neurotoxic effect of AA [101].

In vitro-structure-activity studies indicate that other conjugated α,β-unsaturated aldehydes can also lead to a concentration-dependent inhibition of the neurotransmitter (dopamine) release, uptake and storage in vesicles [64, 102]. This effect correlates with a decrease in free sulfhydryl groups. For AA, an IC_{50} value within the milimolar range was reported for inhibition of dopamine transport into rat synaptosomes (438 mM), whereas for AC it lies within the micromolar range (53 µM). Therefore, the neurotoxicity of α,β-unsaturated carbonyl compounds *in vitro* seems to mainly correlate with the formation of Michael adducts with sulfhydryl groups of presynaptic proteins [62, 64, 102, 103].

Current studies link AC and other lipid peroxidation products with various different neurodegenerative diseases such as e.g. Alzheimer disease, Parkinson disease and

amyotrophic lateral sclerosis (ALS) [104-107]. Endogenous AC levels appear to be increased in the brain and in the medulla of patients. It is considered unlikely, however, that orally ingested AC may reach the brain. After uptake, AC is quickly metabolized in the organism and/or rapidly scavenged by its reaction with macromolecules [50].

2.5. Toxicity

Acrolein

The effects of AC after an inhalative exposure have been well studied, whereas there are only a few reports on the effects of AC after oral exposure.

Inhalative exposure to AC mainly results in irritation and inflammation of exposed mucosae and necroses of the pulmonary tissue in rats [14]. Oral exposure causes inflammation and necrosis in the forestomach of rats. In some animal studies a depletion of glutathione was observed which was reversible after 24 hours [108-111]. Oral LD_{50} values of 7 – 46 mg/kg b.w./day after a single application are reported for rats, mice and hamsters [15].

In a 14-week oral subchronic toxicity study (90 days), rats were treated with 0, 0.75, 1.25, 2.5, 5 and 10 mg AC/kg b.w./day (dissolved in 0.5 % methylcellulose; 5 days/week) by gavage. Mice were treated with 0, 1.25, 2.5, 5, 10 and 20 mg AC/kg b.w./day (dissolved in 0.5 % methylcellulose; 5 days/week) by gavage [112]. In the higher dosage groups (10 mg/kg b.w. in the case of male and female rats and 20 mg/kg b.w. in the case of female mice) lesions in the forestomach and glandular stomach were observed. The occurrence of hyperplastic lesions in the epithelium of the forestomach was the most sensitive parameter. The incidence was significantly increased in male rats at doses of 5 and 10 mg/kg b.w., in female rats at doses ≥ 2.5 mg/kg b.w. as well as in male and female mice at doses of 2.5, 5, and 10 mg/kg b.w. Based on the occurrence of hyperplastic lesions in the forestomach, NOAEL values of 1.25 mg/kg b.w./day for female mice and male rats and of 0.75 mg/kg b.w./day for female rats were deduced [15, 50]. In male mice an increase of the number of epithelial hyperplastic lesions already occurred at the lowest dose tested, so that a dose without effect could not be determined.

In a two-generation-study in rats hyperplastic lesions in the forestomach and the glandular stomach, ulcers and haemorrhages in the glandular stomach were observed at a dose of 3 mg/kg b.w./day [113].

Nausea and vomiting was observed in dogs after repeated administration of 0.1, 0.5 and 1.5 mg AC/kg b.w./day in gelatine capsules. After 4 weeks the dosage of 1.5 mg/kg b.w./day was increased to 2 mg/kg b.w./day. At this dose level biochemical parameters in serum were altered [114].

Acrylamide

Data on the acute and chronic toxicity of AA were summarized by JECFA [115] and MAK [96].

After a single administration of AA at higher concentrations, tremor, ataxia, convulsion, muscle weakness, circulation collapse as well as body weight loss were observed in rats, mice, guinea pigs, rabbits and cats [86]. For rats, guinea pigs and rabbits oral LD_{50} values of 150-200 mg/kg b.w. have been reported [96]. Subchronic oral exposure lead to neurotoxic effects, such as those described in chapter 2.4.1 (Neurotoxicity).

Studies on carcinogenicity are described in chapter 2.6.

New approaches in molecular epidemiology aim at correlating biomarkers of exposure with effects in humans. In this context, in a human pilot study an increased formation of reactive oxygen species in leucocytes together with an increased plasma level of C-reactive protein was reported after a four-week consumption of potato crisps/chips (157 µg AA/person) [116]. An association between AA-related hemoglobin adduct levels in the umbilical cord blood, used as biomarker of exposure of the mother during the last trimester of pregnancy, and of developmental effects such as reduced birth weight of infants was reported [117]. Further studies are needed to substantiate these preliminary findings.

2.6. Genotoxicity and mutagenicity

Acrolein

For AC the formation of DNA adducts (*in-vitro* and *in-vivo*), mainly with guanine residues, has been described [118-120]. The main DNA adduct *in vitro* is γ-

hydroxypropanodeoxyguanosine (γ-OH-PdG), the minor adduct α-hydroxypropanodeoxyguanosine (α-OH-PdG). Both adducts induce a base substitution (G→T, G→A) [121-123]. The mutagenicity of α-OH-PdG is well documented, the relevance of γ-OH-PdG is still being discussed at the present time [122, 124-126]. AC-DNA adducts were also detected in untreated rats and mice as well as in human samples (blood, liver, mammary gland) [119, 127]. In human samples the N^7-adduct 7-(2´-carboxyethyl)guanine was present in substantially higher concentrations than the cyclic adducts. Whether this observation is directly related to an AC exposure, remains open [128]. This adduct could for example also be the result of the formation of acrylic acid from AC and its reaction with the N^7-position of guanine in DNA.

Data on the mutagenicity/genotoxicity of AC are not clear. Genotoxic/mutagenic effects have been reported at non-cytotoxic concentrations in *in-vitro* systems [14]. Depending on the strain and test conditions, bacterial test systems showed positive as well as negative results [112, 129].

In mammalian cells the results are also inconsistent. Depending on the experimental parameters, HPRT mutations have been observed in a few cases [129]. In V79 cells AC was mutagenic in the mM concentration range [130]. In HepG2 cells AC at lower concentrations (12.5 und 25 µM) led to DNA strand breaks and at higher concentrations (50-100 µM) to DNA-protein crosslinks [131]. Sister chromatid exchange and chromosomal anomalies were observed in the micromolar concentration range, among others, in CHO cells [129]. In contrast, AC was not mutagenic in human and murine fibroblasts, independently from their DNA repair capacity [132]. In further studies in various mammalian cell types, no mutagenic effect was observed with and without activation system [112, 133].

The evidence from *in vivo* studies regarding the genotoxic/mutagenic effect of AC is limited, but is negative so far [15, 50, 112].

Acrylamide

While a direct reaction of AC with DNA has been demonstrated a DNA damaging effect of AA is only expected to occur after its metabolic activation to glycidamide [134, 135]. The main DNA adduct *in vivo* is glycidamide-N^7-guanine (N7-GA-Gua), the minor adduct (2 orders of magnitude lower level) glycidamide-N^3-adenosine (N3-

GA-Ade) [59, 136]. In the lower dose range (0.1-100 µg/kg b.w.), which is more relevant for food based human exposure, no dose dependency for the formation of the N7-GA-Gua adduct in rats was observed, with adduct frequencies invariably < 2 adducts per 10^{-8} nucleotides. At higher doses (>100 µg/kg b.w.) the N7-GA-Gua-adduct was dose-dependently detected in different rat organs at a frequency of > 2 adducts per 10^{8} DNA bases. This is at the lower bound of DNA adduct background levels found in the liver of nonsmokers, formed as a consequence of exposure to DNA interacting electrophilic/ genotoxic compounds of different origin (exogenous or endogenous) [44].

Evidence for a possible interaction with chromatin-associated proteins such as protamine in germ cells (spermatids) as well as kinesin-dependent proteins was described. Such reactions may also contribute to the genotoxic effect of AA [96].

The mutagenic potential of glycidamide has been shown to be relatively low [137-140]. For example, in the HPRT mutagenicity assay in mammalian cells, GA induced a mutagenic effect only at concentrations higher by several orders of magnitude than effected by activated forms of *N*-nitroso compounds or polycyclic aromatic hydrocarbons [138].

2.7. Carcinogencity

Acrolein

Up to now, chronic toxicity/carcinogenicity studies for AC administered p.o. have been performed in rats [141], mice [142] and Beagle dogs [114]. Moreover, there exists a non-representative study in male rats, in which only the mortality and histopathological changes in selected tissues were analyzed [143]. In more comprehensive studies the highest doses of 2.5 mg/kg b.w. in rats and 4.5 mg/kg b.w. in mice did not lead to substance-induced lesions, and there was no evidence for increased tumour incidences. However, for as yet unknown reasons the mortality in rats and mice was enhanced [141, 142]. In these studies AC dissolved in water was administered.

Different bodies/commissions classify the carcinogenic potential of AC as follows:

- IARC category 3 (not classifiable as to its carcinogenicity to humans, based on inadequate evidence in humans and in experimental animals for the carcinogenicity of acrolein) [5]
- MAK Commission (Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area) of the German Research Foundation (DFG): Category 3B (Substances that cause concern that they could be carcinogenic for man but cannot be assessed conclusively because of lack of data) [129]
- US EPA: „data are inadequate for an assessment of human carcinogenic potential by either the inhalation or oral routes of exposure.” [14]

Acrylamide

In long-term studies in rats, a carcinogenic potential of AA was demonstrated after administration via drinking water at doses of 0.5 to 2 mg/kg b.w./day in male animals or at doses of up to 3 mg/kg b.w./day in female animals [92, 93]. Enhanced occurrence of certain tumours such as mesotheliomas of the tunica vaginalis testis, mammary fibroadenomas and thyroid tumours (follicular adenomas) has been reported [96, 115]. The carcinogenic potential of AA was confirmed in a two-year NTP study [144]. Following the administration of AA at doses of 0.33, 0.66, 1.32 and 2.71 mg /kg b.w. to male rats, at doses of 0.44, 0.88, 1.84 and 4.02 mg/kg b.w. to female rats, at doses of 1.04, 2.20, 4.11 and 8.93 mg/kg b.w. to male mice and at doses of 1.10, 2.23, 4.65 and 9.96 mg/kg b.w. to female mice, a reduction of the body weight in rats as well as a reduction of the survival rate in mice was observed. In both species tumours were found in different organs, some of them already at the lowest AA dose, so that no threshold could be deduced in this study [144, 145]. Overall, mice seem to be more sensitive than rats towards tumour induction by AA, female rats developed a greater diversity of tumour types than male rats [144, 146].

The carcinogenicity of AA is classified as follows:

- IARC: Category 2A (probably carcinogenic to humans) [146]
- MAK Commission of the DFG: Category 2 (to be regarded as carcinogenic for humans) [96]
- US EPA: "likely to be carcinogenic to humans." [147]

2.8. Risk assessment of other bodies

Acrolein

The most comprehensive study for the evaluation of the effects of AC administered p.o. is a subchronic NTP study in rats, in which hyperplasia in the epithelium of the forestomach was the most sensitive parameter [112]. Based on a NOAEL value of 0.75 mg/kg b.w./day and by applying a safety factor of 100, a „tolerable daily intake“ (TDI) of 7.5 µg/kg b.w./day was deduced [15, 50].

On the basis of the subchronic NTP study the US Agency for Toxic Substances and Disease Registry (ATSDR) derived an oral „Minimal Risk Level“ (MRL) for AC of 4 µg/kg b.w./day for an average duration of exposure (15-364 days) [148]. The derivation was performed starting from the lower 95% confidence interval of the Benchmark-Dose for a 10% additional risk (BMDL10) for epithelial hyperplastic lesions to develop in the forestomach of mice and by taking into account a safety factor of 100 [148]. The MRL is based on non-carcinogenic effects.

Acrylamide

The NOAEL value for morphological alterations in the nervous system was reported to be 0.2 mg/kg b.w./day. Based on this NOAEL value a „Margin of Exposure“ (MOE) of 200 in the case of an average consumption of 1 µg/kg b.w./day and a MOE of 50 in the case of a maximal consumption of 4 µg/kg b.w./day was calculated [51, 52]. According to an estimation of JECFA, neurotoxic effects at the estimated average intake level are unlikely. In contrast, for highly exposed individuals morphological alterations of nerves were not excluded [51, 52].

If the exposure is compared with the $BMDL_{10}$ (benchmark dose lower confidence limit for a 10% additional tumour risk), the MOE values are in a similar order of magnitude. Based on a $BMDL_{10}$ of 0.31 mg/kg b.w./day for the induction of mammary tumours in rats, the calculated MOE values are 310 for average and 78 for high exposure. For tumours in the Harderian glands of mice the $BMDL_{10}$ is 0.18 mg/kg b.w./day and the MOE values 180 and 45 for average and high exposure, respectively [51, 52].

On the basis of $BMDL_{10}$ values of 0.30 mg/kg b.w./day (mammary tumours in rats) or 0.16 mg/kg b.w./day (tumours in the Harderian glands of mice) and an average uptake for adults of 0.34 µg/kg b.w./day or a 95^{th} percentile uptake of 0.83 µg/kg b.w./day (in each case „upper bound“, [38]), the German Federal Institute for Risk

Assessment (BfR) calculated MOE values of 471 and 882 (average) or 193 and 361 (95th percentile), respectively [145].

3. Conclusions and recommendations

The SKLM published a first opinion on α,β-unsaturated carbonyl compounds in 2002 [1]. According to the Commission the basic statements of this opinion still apply:

„2-Alkenals, like other α,β-unsaturated carbonyl compounds, are highly reactive compounds. They react on the one hand they easily with proteins and DNA, causing cytotoxic and genotoxic effects, on the other hand they are rapidly detoxified by oxidation or reduction as well as by glutathione conjugation. In this respect they are comparable to many other naturally occurring substances, to which man has already always been exposed and for which efficient detoxification mechanisms exist in many cases. The presently available data are inadequate for a comprehensive risk assessment. They indicate, however, that toxicity and genotoxicity will become apparent only at high doses, when such detoxification mechanisms have become overloaded. It must be assumed however, that doses which lead to such overloading vary not only from substance to substance but also depend on the cell type and tissue exposed."

More recent study results suggest that the diet-related exposure to AC or its precursors in foods, measured by determining the excretion of mercapturic acids in urine, may be higher than that of AA. There is also evidence for an endogenous formation of AC and AA in the intermediate metabolism. To what extent the endogenous formation contributes to the total exposure, is not known. The question, how to assess an exogenous exposure to toxicologically relevant compounds against the background of a possibly substantial endogenous formation of these substances, remains open at the moment.

For a health evaluation of process-related α,β-unsaturated carbonyl compounds formed in foods, the assessment of the exposure to these compounds is of particular importance. In this context, in the case of AC it cannot be ruled out that the existing analytical methods only detect a fraction of the AC „exposure equivalents".

The extent of the exposure to AC (or AC exposure equivalents) and its sources have insufficiently been analyzed up to now. Regarding the AC levels in heated foods only

a few results are available so far. However, it can be assumed that thermally treated foods could substantially contribute to the AC intake. Due to the limited data on the AC content of foods, an estimation of the exposure through foods is not possible at the moment. A rough calculation of the daily AC intake based on the highest contamination levels measured would indicate for the most unfavourable case (all food samples consumed are contaminated) a value of 17 µg AC/kg b.w./day (see Annex I).

The assessment of an oral AC exposure through the excretion of the AC metabolite 3-hydroxypropylmercapturic acid (3-HPMA) in urine suffers from some uncertainties as well. The extent of the metabolization of AC to 3-HPMA and/or CEMA and of their urinary excretion in humans is currently not known. A possible contribution of an endogenous formation of AC or AA in the organism as well potential inhalative exposure through kitchen vapour, traffic or passive smoking cannot be reliably estimated at the moment.

Based on spot urine samples from nonsmokers, the average AC exposure was estimated to be 2.1-2.4 µg/kg b.w./day and the maximal AC exposure 30 µg/kg b.w./day [50]. An assessment on the basis of the 24-hour 3-HPMA excretion in urine led to similar values, the lower exposure level being about 5-7 µg/kg b.w./day and the higher one being about 24 µg/kg b.w./day.

The above estimate of 2 – 30 µg/kg b.w. suggest an AC exposure through foods at or even above the TDI value of 7.5 µg/kg b.w./day (which refers to a lifetime exposure). More research is required for a reliable assessment of the food-related exposure to be put into correlation with the excretion of exposure biomarkers in urine.

4. Research needs

For the assessment of the AC intake through foods, reliable data covering occurrence in unprocessed and ready-to-eat foods are required, as well as elucidation of the influence of processing and preparation procedures (such as roasting or frying). Major AC exposure sources are not known at present.

In addition to the development of a reliable analytical method for the assessment of AC in foods, the SKLM recommends to determine the AC exposure in comparison to the AA exposure. This could be done by using validated exposure biomarkers such

as the corresponding mercapturic acids in urine or hemoglobin adducts in blood. It has to be clarified to which extent exposure from endogenous sources also contribute to the total exposure. Moreover, the causes for the observed discrepancy between the analytical values detected so far and the excretion of exposure biomarkers in urine have to be clarified.

In analogy to the biomarker for a long-term AA exposure, namely the formation of hemoglobin adducts during the lifespan of the erythrocyte, it is also advisable to develop corresponding long-term biomarkers for the AC exposure.

Further research is needed concerning the mechanisms of AC formation in foods, particularly during thermal processing or bacterial fermentation. In this respect the comparison with AA formation and an analysis of the possible similarities and differences regarding the formation pathways of AC are of scientific interest. From an epidemiological point of view no convincing relationship between AA exposure and tumour formation has been established. Of note, epidemiological evidence has not taken into consideration the presumably substantially higher exposure to AC. Concomitant determination of biomarkers for AA and AC exposure may be useful for instance in order to study associations with further non-transmissible diseases (such as diabetes and neurological diseases) or other presumed effects (such as reduced weight at birth).

For an adequate safety assessment of AC, extended genotoxicity and mutagenicity studies (*in vitro* and *in vivo*) for AC are required in addition to the exposure assessment. Moreover, studies on the biokinetics of AC after oral exposure should be performed. This should clarify whether orally administered AC is systemically bioavailable to an extent that leads to the formation of DNA adducts in different organs. A dose-effect study on the formation of DNA adducts may provide further significant information for risk assessment. In addition, *in vitro* studies in primary hepatocytes could give mechanistic information, e.g. regarding the rate of the glutathione adduct formation in comparison to DNA adduct formation. Moreover, the dose-dependent formation of DNA adducts measured in experimental systems should be compared with the background level of DNA damage measured in human samples. Finally, the question whether the combined exposure to AA and AC influences the biological effect of the respective individual compounds should be analyzed.

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Annex I

Table 1. Acrolein concentrations in the environment

Source		Concentration	Reference
water	surface water (irrigation canal)	20-200 µg/l	[1]
cigarettes	mainstream smoke	10-140 µg/cigarette	[2]
	sidestream smoke	100-1700 µg/cigarette	[2]
		3-220 µg/cigarette	[3]
air/atmosphere	city air	0.002-0.035 mg/m^3 8.2-24.6 µg/m^3 (on average 14.3 mg/m^3)	[4]
	exhaust gases petrol engines diesel engines	 0.05-27.7 mg/m^3 0.12-0.21 mg/m^3	 [3] [3]
	smoky indoor air	2.3-275 µg/m^3	[3]
	heating of deep-frying fat in commercial kitchens	0.55 mg/m^3	[5]

Table 2. Acrolein contents in foods

Food	Concentration	Reference
fruits	10-50 µg/kg	[3]
vegetables	10-590 µg/kg	[3]
donuts	100-900 µg/kg	[6]
cod filet	100 µg/kg	[6]
	100-900 µg/kg	[7]
cheese	290-1300 µg/kg	[8]
red wine	up to 3800 µg/kg	[7]
	7.0-8.8 µg/l	[9]
lager beer	1.11-2 µg/l	[10]
beer	1.37 µg/l	[11]
heated lard	109 µg/l	[12]
sunflower oil	163 µg/l	[12]
heated canola and soy oil	ca. 390-440 µg/l	[5]
heated vegetable oil	62-520 µg/l	[13]
deep-frying fat after use	0.2-1.4 mg/kg	[9]
vegetable oils, not heated	1-20 µg/kg	[9]
French fries	1-5 µg/kg	[14]

Note to Table 2:

Concerning the occurrence of acrolein in **raw/unprocessed foods** only few data are available. Acrolein was detected in fruits, e. g. raspberries, grapes, strawberries and blackberries (0.01-0.05 mg/kg), in vegetables such as cabbage, carrots and tomatoes (≤ 0.59 mg/kg) as well as in foods of animal origin such as fish (0.1-0.9 mg/kg) and cheese (0.29-1.3 mg/kg) [7, 8]. Moreover, acrolein is utilized as an herbicide, which according to the literature could explain the detection of acrolein traces in raw turkey [15], lettuce [16] and tomatoes [12].

Acrolein has also been detected in **processed foods** such as sugarcane molasses, soured and salted pork as well as products containing heated fats of animal origin and vegetable oils, likewise in the volatile components of cooked fish (Carangidae), white bread, chicken breast, ripe arctic blackberries and beef [10].

Acrolein is formed during the **heating of vegetable oils**. Acrolein contents in fats/oils are highly variable with amounts of about 1 µg/kg up to 1.4 mg acrolein/kg [9]. Oils and fats, which have not undergone any further heating process than refining, showed lower concentrations (1-20 µg/kg). However, very high amounts of acrolein (0.2-1.4 mg/kg) were detected in deep-frying fats especially after use [9]. After heating up to 240-280°C, 391.8 µg acrolein/l were measured in canola oil and 442.7 µg acrolein/l in soy oil [5]. After heating corn oil, sunflower oil, groundnut oil and olive oil up to 145ºC for 2 hours, acrolein concentrations of 1.1-9.3 µM (62-520 µg/l) were reported [13]. Yet, in the absence of heat treatment, no acrolein was detected. The formation of acrolein during the heating of oil depends on high temperatures. After a rise in temperature from 150 to 400°C an increase of the acrolein content by about 2 orders of magnitude was observed [13].

A recent study showed that acrolein formation during the heating of oils depends on fatty acid composition, heating time and temperature [17]. Oils with a high amount of unsaturated fatty acids, particularly linolenic acid (e.g. canola oil and linseed oil), generated higher concentrations of acrolein during heating than oils with a high saturated or monounsaturated fatty acid content (e.g. coconut oil, olive oil). Maximal acrolein concentrations were formed in most oils in the range of 140-180 ºC, whereas a further temperature increase up to 220-260ºC resulted in lower acrolein concentrations. The authors explain this observation with an increased reaction of acrolein with other degradation products of acrolein at high temperatures [17]. Heating at 140ºC for 24 h led to acrolein contents of up to 240 mg/kg (linseed oil), 160 mg/kg (canola oil), 15 mg/kg (olive oil), 40 mg/kg (thistle oil), 70 mg/kg (deep-frying fat) and 7 mg/kg (coconut oil) [17].

In the course of the production of spirits, acrolein can be formed during the distillation step by dehydration of glycerol in the presence of acids on hot metallic surfaces. Furthermore, acrolein might be formed as a result of the metabolic activity of certain microorganisms such as heterofermentative lactobacilli and enterobacteria. These microorganisms form metabolically 3-hydroxypropionaldehyde (3-HPA), an acrolein

precursor. During distillation acrolein is formed as a consequence of water elimination. Contamination sources are, among others, dust, dirt and soil, which adhere to the raw material during the mash production or are present in the washing water, an insufficient cleaning of the fermentation tanks, pipelines, pumps etc., the natural accompanying microorganisms of compressed and dried yeast as well as human beings with their natural bacterial flora [18].

In a study on 516 spirit samples [19] contents of 0.002 to > 0.05 mg/ml (2 to > 50 mg/l) and 0.0003 to 0.002 mg/ml (0.3 to 2 mg/l) were measured in 4 and 55% of the samples, respectively. In potato spirits 0.003 to > 0.05 mg/ml (3 to > 50 mg/l) [19] and in whisky 0.67 to 11.1 µg/l were detected. Current data confirm this order of magnitude range. Twenty-eight analyzed spirits showed acrolein contents between <14.4 µg/l and 0.74 mg/l, in the case of a fruit spirit 5 mg/l were measured [9].

An earlier study reports acrolein contents of up to 3.8 mg/kg in red wine [6]. According to current studies acrolein was measurable only in 9% of the samples, with significant lower contents (7.0-8.8 µg/l) [9].

Acrolein concentration in beer was in the range of 1.37 µg/l [11] and in lager beer between 1.11 and 2 µg/l, on average 1.6 µg/l [10].

In **drinking water** acrolein has not been found so far. According to US EPA [2] acrolein has a relatively short half life in surface water. The volatilization of the substance on the surface of the water is considered as a key event.

Table 3: Acrylamide content in foods [20]

Commodity group	Signal value, 8th calculation 18.11.2010, acrylamide [µg/kg]	Number of samples	Minimum acrylamide [µg/kg]	Median acrylamide [µg/kg]	Maximum acrylamide [µg/kg]
Cereal products					
crispbread	480	144	5	**250**	950
breakfast cereals	260	69	10	**68**	780
viennoiseries from shortcrust pastry	260	292	5	**90**	678
dry baked goods or diabetics	450	106	10	**136**	1832
„Zwieback“ or biscuits for babies and small children	160	84	5	**35**	196
gingerbread and gingerbread-containing pastry	1000	567	5	**278**	4200
speculoos	300	176	15	**134**	1042
Potato products					
potato chips/crisps	790	363	8	**275**	3453
French fries, freshly prepared	530	590	5	**194**	2030
hash browns	870	34	111	**502**	3025
Coffee products					
coffee, roasted	280	243	19	**187**	861
instant coffee	900	63	200	**728**	1373
coffee substitute	1000	58	77	**639**	1798

Table 4: Acrylamide contents in foods in the EU in the years 2007-2009 [21]

Food group	2007		2008		2009	
	Sample number	**Median µg/kg**	Sample number	**Median µg/kg**	Sample number	**Median µg/kg**
bread:						
with crust	155	**116**	92	**107**	130	**186**
soft	127	**30**	211	**30**	110	**27**
not specified	54	**58**	17	**19**	84	**49**
breakfast cereals	134	**100**	136	**75**	153	**87**
coffee:						
instant	51	**188**	63	**482**	46	**584**
not specified	41	**183**	11	**210**	14	**237**
roasted	153	**197**	267	**164**	172	**193**
French fries	647	**246**	536	**220**	469	**247**
potato chips/crisps	280	**413**	458	**436**	388	**394**

Table 5: Current available data regarding the daily acrolein intake through foods

Food	Acrolein content	Estimated daily intake	
		Consumption	Acrolein intake
cheese	1.3 mg/kg	**50 g**	**65 µg**
donuts	0.9 mg/kg	**400 g**	**360 µg**
cod filet	0.9 mg/kg	**200 g**	**180 µg**
red wine	3.8 mg/l 7-8 µg/l	**400 ml** **400 ml**	**1520 µg** **3 µg**
spirits	max. 5 mg/l	**20 ml**	**100 µg**
fruits	0.05 mg/kg	**300 g**	**15 µg**
vegetables	0.6 mg/kg	**400 g**	**240 µg**
oil, heated	0.200 mg/kg	**50 g**	**10 µg**

Note to Table 5:

Because of the difficulties in quantifying acrolein in foods, merely not representative results regarding acrolein contents in a few foods are known. Therefore, no reliable estimation of the acrolein exposure through foods is possible at present. A very rough exposure estimation based on the provisional and limited content data available (without the earlier maximal value for red wine), assuming high consumption, maximal contents of the respective food item and occurrence of acrolein in all samples would lead to a value of about 1 mg/day, respectively 17 µg/kg b.w./day. If the maximal value for red wine is taken into consideration, the exposure level rises to about 2.5 mg/day, respectively 42 µg/kg b.w./day. The acrolein intake through beer and unheated oils seems to be relatively low and was estimated to be negligible. The analytically measurable acrolein intake through French fries and potato chips/crisps also appears to be low, although the mercapturic acid excretion data yield another picture [22, 23].

Table 6: 3-Hydroxypropylmercapturic acid (3-HPMA) excretion in urine of nonsmokers or smokers after quitting smoking

Reference	N (nonsmoker)	3-HPMA in urine (µg/24 h; mean ± standard deviation)	Remarks
[24]	41	812 ± 123	
[25]	100	337 ± 383	
[26]	49	983 (879-1088)	unexpected high level, large variations → probably acrolein sources other than tobacco smoke involved
[27]	50	214 (196-232)	special diet: no smoked, roasted and grilled foods, no alcohol and coffee
[28]	17 smokers, 56 days after quitting smoking	1500 ± 1005 (nmol/24h)	
[29]	21	683 pmol/mg creatinine (median) 1900 ± 3000 pmol/mg creatinine	
[30]	20 smokers, 8 days after quitting smoking	250-300	81% decrease if compared to the baseline performed in a clinic, standardized meal to minimize dietetic confounders
		3-HPMA, spot urine (µg/l) median (range)	
[31]	14	155 (37-730 µg/l)	
[32]	54	179 (32.6-2325 µg/l)	

Note to Table 6:

In order to be able to perform an exposure estimation based on the 24 h excretion in urine, an approximate lower level of 3-HPMA excretion/24 h urine of about 200-300 µg und an approximate upper level of about 800-1000 µg was deduced from the table.

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Annex II: Metabolization of acrylamide and acrolein

Metabolism of acrolein

An overview of the acrolein metabolization pathways is shown in Fig. 1. The main metabolization pathway of acrolein involves the formation of a glutathione (GSH) adduct. The glutathione adduct can be formed by direct reaction of acrolein with GSH, a reaction that is also catalyzed by different glutathione-*S*-transferases (mainly GST A4-4) [1, 2]. The amino acids glutamate and glycine are removed from the primary adduct and an acetylation reaction leads to the formation of *S*-(3-oxopropyl)-N-acetylcysteine (OPMA). The aldehyde OPMA can be oxidized by the aldehyde dehydrogenase to carboxyethylmercapturic acid (CEMA). However, the predominant reaction is the reduction of OPMA catalysed by the aldo-keto-reductase resulting in the main acrolein metabolite 3-hydroxypropylmercapturic acid (3-HPMA), which is then excreted via urine [3-5]. In the case of OPMA it has been suggested that flavin-containing monooxygenases or cytochrome P450-dependent monooxygenases may catalyze the formation of an OPMA-*S*-oxide, which is not stable and decomposes to sulfenic acid and acrolein [6].

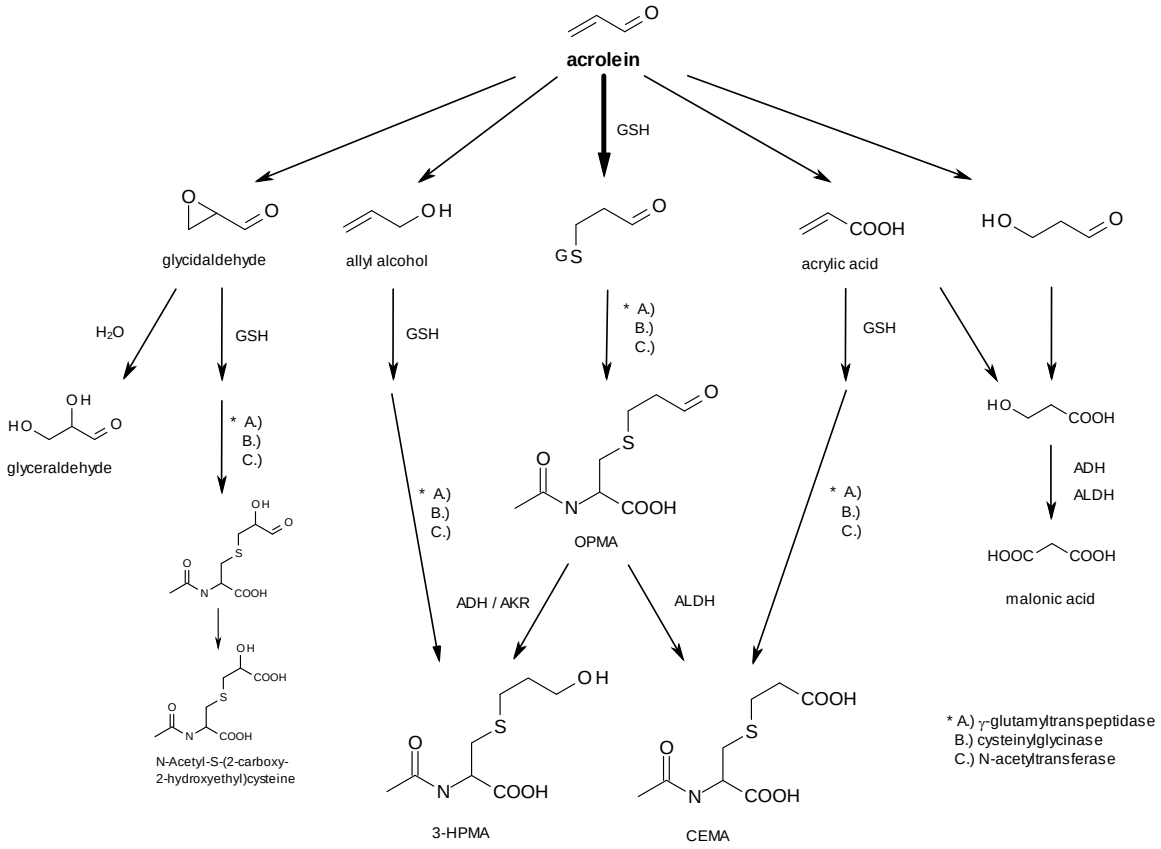

Fig. 1: Overview of the metabolization pathways of acrolein. (ADH: alcohol dehydrogenase, ALDH: aldehyde dehydrogenase, AKR: aldo-keto-reductase, CEMA: carboxyethylmercapturic acid, 3-HPMA: S-(3-hydroxypropyl)-mercapturic acid, OPMA: S-(3-oxopropyl)-mercapturic acid, GSH: glutathione)

Another potential secondary pathway is the oxidation of acrolein to acrylic acid, which can be converted to 3-hydroxypropionic acid and further oxidized to malonic acid. An epoxidation of acrolein leads to the unstable metabolite glycidaldehyde, which can be hydratized to glyceraldehyde or coupled to glutathione and subsequently degraded to the mercapturic acid *N*-acetyl-*S*-(2-carboxy-2-hydroxyethyl)cysteine. After administering ^{14}C-labeled acrolein (2.5 mg/kg b.w.) to rats, Parent et al. [7] identified 3-HPMA, oxalic acid, CEMA, 3-hydroxypropionic acid, *N*-acetyl-*S*-(2-carboxy-2-hydroxyethyl)cysteine and traces of malonic acid as metabolites. Part of the radioactivity was found in faeces, but no metabolites could be identified. After i.v. administration of ^{14}C-labeled acrolein, no radioactivity was identified in form of oxalic acid. However, after oral administration 16-36% of the radioactivity was shown to correspond to oxalic acid, which suggests that the gut flora is involved in the

metabolization of acrolein [7]. As cumulative 24-hour excretion after oral administration of radioisotope labelled acrolein (2.5 mg/kg b.w.) to rats, about 59% of the administered radioactivity was found in urine, 12% in faeces, about 27% as CO_2 and less than 0.7% (after i.v. administration <1.2%) in tissues [8]. Twenty-four hours after i.v. administration of the same dose (2.5 mg/kg b.w.), 54% of the administered radioactivity was found in urine, 0.6% in faeces and 22% as CO_2. A comparison group, which was pretreated for 14 days with 2.5 mg/kg b.w. acrolein p.o. and thereafter received 2.5 mg/kg b.w. ^{14}C-labeled acrolein p.o., showed only a slightly altered pattern when compared to the animals having received a single dose. Fifty-three percent of the administered radioactivity was recovered in urine, 10% in faeces and 27% as CO_2 [8].

Metabolism of acrylamide

An overview of the metabolization pathways of acrylamide is shown in Fig. 2. Of substantial relevance for the carcinogenicity of acrylamide is the cytochrome P450 2E1-catalyzed epoxidation to glycidamide (GA), thus leading to genotoxicity by forming DNA-adducts [9, 10]. The formation rate of GA decreases in the sequence mouse > rat > human [11]. Because of their electrophilic properties, acrylamide as well as glycidamide easily react with nucleophilic centers in macromolecules such as e.g. sulfhydryl and amino groups in hemoglobin and serum albumin [12]. In addition to the reaction with glutathione, irreversible binding to the blood proteins albumin and hemoglobin plays an important role in the detoxification of acrylamide and glycidamide. In analogy to acrolein, acrylamide and glycidamide are bound to glutathione (direct reaction with GSH or GST-mediated) and finally excreted as phase II metabolites acrylamide mercapturic acid (AAMA) and glycidamide mercapturic acid (GAMA) [13]. These mercapturic acids can be regarded as biomarkers of short-term exposure to acrylamide. In addition, humans (but not rodents) also form an AAMA-sulfoxide [14]. The metabolization pathway via glutathione is of particular importance for the detoxification of acrylamide and glycidamide in mice, rats and humans, since up to 60% of an administered acrylamide dose can be excreted as mercapturic acids [15-17].

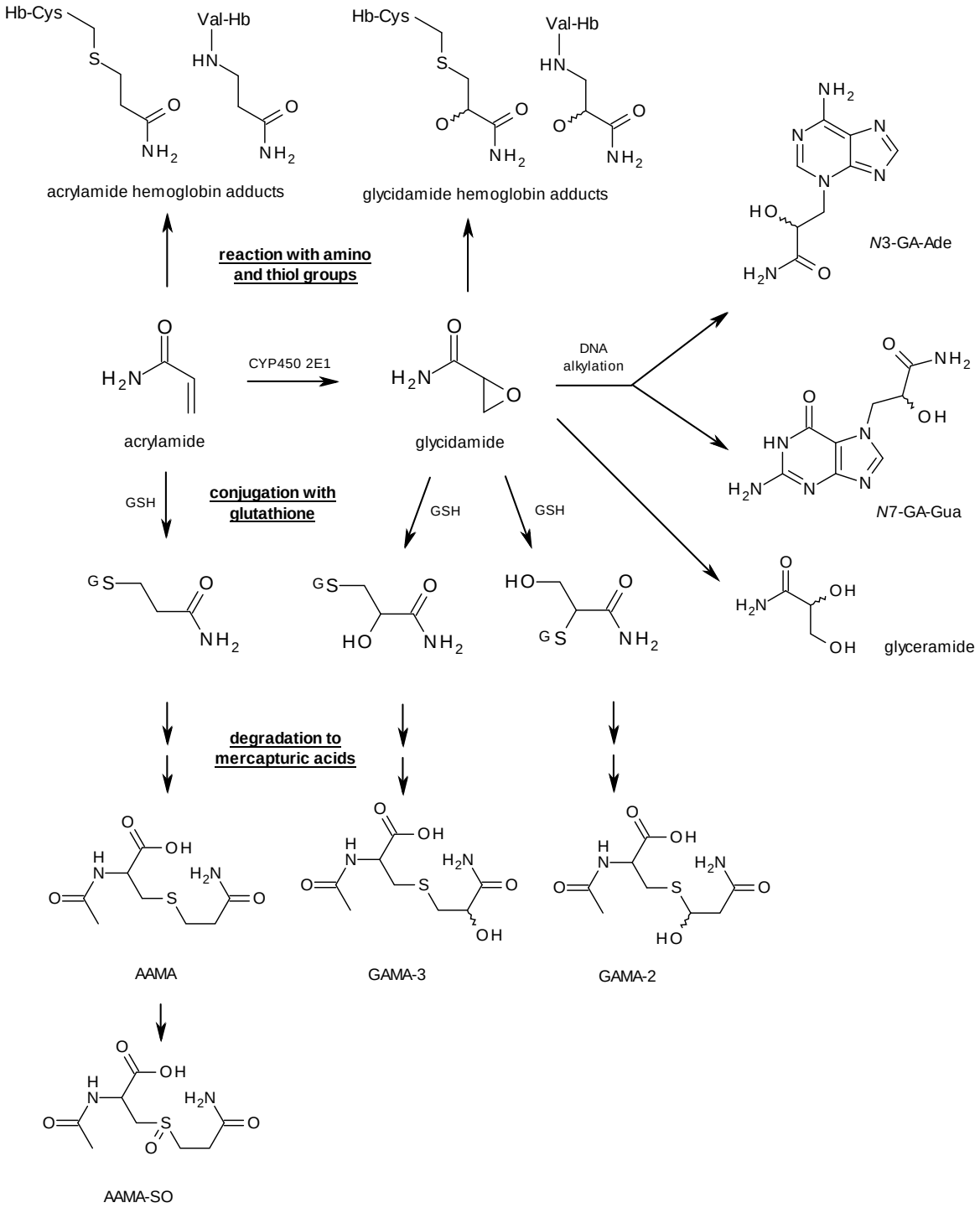

Fig. 2: Metabolization pathways of acrylamide. (AAMA: acrylamide mercapturic acid; AAMA-SO: AAMA-sulfoxide; GAMA: glycidamide mercapturic acid; Hb: hemoglobin; N3-GA-Ade: N^3-glycidamide-adenine adduct; N7-GA-Gua: N^7-glycidamide-guanine adduct)

Another detoxification pathway for glycidamide is the epoxide hydrolase-mediated formation of glyceramide (2,3-dihydroxypropionamide). Of note, administration of a dose of 3 mg $^{13}C_3$-acrylamide/kg b.w. to humans, glyceramide made up 11% of the urinary metabolites, while after treating rats with the corresponding dose no glyceramide was detected [15]. After the oral administration of 50 mg $^{13}C_3$-acrylamide/kg b.w. to Fischer 344 rats and B6C3F1 mice, glyceramide made up 2 and 5% of the total urinary metabolites respectively, which suggests that this metabolite is of little importance in the two above-mentioned species [18].

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