REVIEW

Toxicology and risk assessment of acrolein in food

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Acrolein is an α,β-unsaturated aldehyde formed by thermal treatment of animal and vegetable fats, carbohydrates and amino acids. In addition it is generated endogenously. As an electrophile, acrolein forms adducts with gluthathione and other cellular components and is therefore cytotoxic. Mutagenicity was shown in some in vitro tests. Acrolein forms different DNA adducts in vivo, but mutagenic and cancerogenous effects have not been demonstrated for oral exposure. In subchronic oral studies, local lesions were detected in the stomach of rats. Systemic effects have not been reported from basic studies. A WHO working group established a tolerable oral acrolein intake of 7.5 μg/kg body weight/day. Acrolein exposure via food cannot be assessed due to analytical difficulties and the lack of reliable content measurements. Human biomonitoring of an acrolein urinary metabolite allows rough estimates of acrolein exposure in the range of a few μg/kg body weight/day. High exposure could be ten times higher after the consumption of certain foods. Although the estimation of the dietary acrolein exposure is associated with uncertainties, it is concluded that a health risk seems to be unlikely.

Keywords:
Acrolein / Exposure / Genotoxicity / Risk assessment / Toxicity

1 Introduction

Acrolein (propenal, acrylaldehyde, CAS No. 107-02-8) belongs to the α,β-unsaturated aldehydes. The substance is a highly volatile (boiling point 52.5°C) colourless liquid with a melting point of −88°C, which dissolves very well in water (log Kow = −1.1 to –1.02).

Acrolein is formed from amino acids, fats or carbohydrates during thermal food processing. During the preparation of carbohydrate-containing foods, acrolein can be formed in the course of the Maillard reaction. It occurs in fruits, for instance raspberries, grapes, strawberries and blackberries as well as in vegetables, tomatoes, fish and cheese. Furthermore, it can be detected in spirits and wines. Consequently, humans are exposed to acrolein through consumption of many foods [1]. For a total exposure assessment, inhalation exposure (e.g. smoking) and endogenous formation, e.g. through lipid peroxidation and polyamine metabolism, must also be taken into account.

Knowledge on toxicology and evidence of acrolein in numerous food, including possibly high levels of acrolein in fried potato chips [2], prompted us to conduct a risk assessment in order to answer the question whether oral intake of acrolein via foods could lead to health risks. The toxicology data in the literature are summarised. Furthermore, we assessed dietary exposure to acrolein based on the available food content data. Our assessments are approximations due to analytical problems with current methods for acrolein determination in foods and due to limited availability of food content data. A better estimate of exposure seems to be possible with data from human biomonitoring of the main metabolites of acrolein in urine. Finally, we evaluated whether dietary intake of acrolein in food poses health risks.

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Abbreviations: 3-HPMA, *S*-(hydroxypropyl)-mercapturic acid; **AAMA,** *N*-acetyl-*S*-(2-carbamoylethyl)-cysteine; **BfR,** Federal Institute for Risk Assessment; **bw,** body weight; **DNPH,** 2,4-dinitrophenylhydrazine; **GAMA,** *N*-acetyl-*S*-(2-hydroxy-2-carbamoylethyl)-cysteine; **GSH,** glutathione; **MRL,** minimum risk level; **NOAEL,** no observed adverse effect level; **NTP,** National Toxicology Program; **SPME,** Solid phase micro extraction

2 Toxicity of acrolein

2.1 Toxicokinetics and metabolism

Seven days after the administration of a single dose of 2.5 mg radioactively labeled acrolein/kg body weight (bw) to

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fasting rats, a total of 52–63% of radioactivity was excreted in the urine, 12–15% with the faeces and approximately 30% as CO_2 in respiratory air. Approximately 90% of the dose was excreted within 24 h. Even with daily dosing (14 days unlabeled acrolein, followed by a single dose of radioactively labeled acrolein), the excretion profile was similar. The tissue contents (measured as radioactivity) were minimal (<1.2%). They were higher after intravenous administration [3].

After oral administration of a single or repeated dose of 2.5 mg/kg bw or a single high dose of 15 mg/kg bw acrolein to rats, the following metabolites were detected in urine: oxalic acid, malonic acid, *N*-acetyl-*S*-2-carboxy-2-hydroxyethylcysteine, *N*-acetyl-*S*-3-hydroxypropylcysteine, *S*-(hydroxypropyl)-mercapturic acid (3-HPMA), *N*-acetyl-*S*-2-carboxyethylcysteine and 3-hydroxy-propionic acid. After oral or intravenous administration of acrolein, 3-HPMA was identified in urine as the main metabolite [4]. This corresponds to findings that 78.5% of an oral dose of 13 mg acrolein/kg bw in rats was excreted as 3-HPMA. Other metabolites were not analysed [5]. Oxalic acid was only detected after oral administration. The metabolite pattern was otherwise comparable for the two routes of administration. In faeces, an inert homopolymer of acrolein was identified following oral administration (15% of a dose of 2.5 mg acrolein/kg bw and 30% of a 15 mg/kg bw dose). The authors assumed that the polymer was formed in the intestinal tract [4]. Other metabolites that have been identified in vitro include acrylic acid, glycidaldehyde and glyceraldehyde [6, 7].

According to Parent et al. [4] the metabolism routes of acrolein consist of (i) epoxidation of the double bond followed by a conjugation with glutathione (GSH), (ii) Michael-type addition of water followed by oxidative degradation and (iii) GSH addition to the double bond and an oxidative or reductive change of the aldehyde function. The GSH adducts are further metabolised to corresponding mercapturic acids and excreted in the urine (Fig. 1).

The main metabolism route of acrolein occurs through the formation of GSH adducts. The latter is formed during the direct reaction of acrolein with GSH, but can also be catalysed by glutathione-*S*-transferases. In different cell lines, a complete GSH depletion was observed from 10 μM acrolein [8–11]. After intraperitoneal administration of 5 mg acrolein/kg bw the GSH content in the liver of rats was reduced by approximately 50% [12].

In several studies, the release of acrolein was held responsible for cytotoxicity of the aldehydic mercapturic acid. The results of Hashmi et al. [13] on LLC-PK1 cells suggest that the mercapturic acids themselves could be cytotoxic. So far a noteworthy release of acrolein was only observed from mercapturic acids that have not been detected in vivo. It is, however, unclear whether these could have been detected under the conditions of the analysis. Whether acrolein can also be released under physiological conditions is unclear, since the pH of the urine is lower than in the

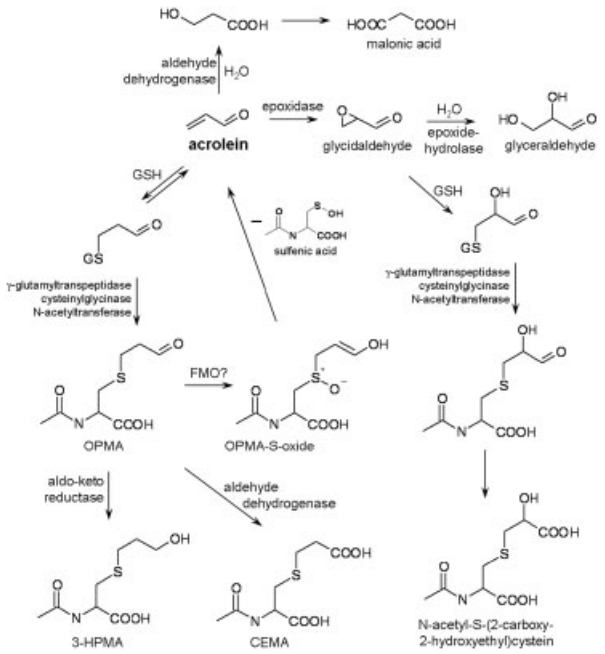

Figure 1. Metabolism of acrolein. Modified from Stevens and Maier [1].

cited in vitro studies. 3-HPMA found in the urine is relatively stable according to the studies conducted so far and is not bladder-toxic. Furthermore, *N*-acetyl-*S*-2-carboxyethylcysteine is not cytotoxic either.

Overall, so far there is no evidence suggesting that oral intake of acrolein would reach in significant amounts in organs such as the bladder, kidney or brain that could trigger toxic effects [5, 14].

2.2 Generally toxic effects

Acrolein reacts with SH groups as well as primary and secondary amines. Acrolein exposure may lead to GSH depletion and formation of adducts with thiol groups of cysteine residues and amino groups of proteins, nucleic acids and other cellular components [15–17]. In addition to this adduct formation, acrolein may also cause cross-linking between proteins as well as between peptides/proteins and DNA [17–19].

Consequently, acrolein is highly cytotoxic in vitro and in vivo. In cell culture studies, acrolein (≥10 μM) reduces the viability of numerous cell lines such as bronchial endothelial and epithelial cells, bronchial and cardial fibroblasts, retinal pigment epithelial cells as well as neuronal cells [16, 20–26]. Acrolein causes cell death via apoptotic processes, e.g. in human lung cells [27]. Furthermore, acrolein modulates several signalling pathways including those that involve the transcription factors, nuclear factor-κB and activator protein-1 [1, 16].

Acrolein may cause oxidative stress through the depletion of GSH, for instance in neuronal cells, bronchial

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epithelial cells as well as in endothelial cells of the umbilical cord vein [23, 24, 28]. Oxidative stress could subsequently lead to DNA damage [29]. GSH protects from damaging influences through conjugate formation with acrolein [21, 22]. Although acrolein reduces the GSH level in the cell through this conjugate formation and hence promotes oxidative stress, normal levels can be restored through a new synthesis of GSH [30, 31]. A proteomic analysis of acrolein-exposed bronchial epithelial cells revealed that acrolein especially modifies stress proteins, cytoskeletal proteins and proteins involved in redox signalling [32].

Target organs of toxicity of acrolein are primarily the local tissues affected. After inhalation intake of acrolein, irritations of the entire respiratory tract may result in inflammation, hyperplasias as well as metaplasias in the respiratory epithelium [33, 34]. Oral exposure leads to gastro-intestinal symptoms, gastric ulcers and/or gastric bleeding, whereby the severity rises with an increase in dose [34, 35].

In summary, the mechanism of acrolein toxicity includes increased levels of proteins/DNA adducts, decreased GSH levels and interference with cell signalling pathways such as oxidative stress signalling, nuclear factor-κB and other nuclear factors.

2.3 Acute, subchronic and chronic toxicity

The LD50 of acrolein (single oral dose) has been determined to be between 7 and 46 mg/kg bw for rats, mice and hamsters [36]. In a subchronic National Toxicology Program (NTP) study [37], hyperplasias of the squamous epithelium were observed in the forestomach of rodents but no tumours. Rats were exposed to 0, 0.75, 1.25, 2.5, 5 and 10 mg acrolein/kg bw/day and mice to 0, 1.25, 2.5, 5, 10 and 20 mg acrolein/kg bw/day for 14 wk by gavage (application 5 days/wk, dissolved in methylcellulose). All mice with the highest acrolein dose died within the first week after the start of the study. Increased mortality rates were also observed in rats and mice in the other higher acrolein dose groups. The incidence of the hyperplasias in the forestomach was increased in male rats in the 5 and 10 mg/kg bw acrolein group as well as in female rats and mice from the 2.5 mg/kg bw dose group [35, 37]. From the occurrence of hyperplasias in the forestomach, a NOAEL (no observed adverse effect level) of 1.25 mg/kg bw/day for female mice and male rats and a NOAEL of 0.75 mg/kg bw/day for female rats can be derived. For male mice, no NOAEL can be derived since the lowest acrolein dose of 1.25 mg/kg bw already resulted in a slightly higher hyperplasia rate.

Moreover, Beagle dogs were exposed daily to 0.1, 0.5 and 1.5 mg acrolein/kg bw/day for 1 year (in gelatine capsules) whereby the highest dose was increased after 4 wk to 2 mg/kg bw. The main effect of acrolein in this study was frequent vomiting of the animals after the treatment whereby the frequency decreased after some time, possibly due to an adaptive process. Changes of biochemical parameters such as reduction of calcium, albumin and total protein levels in the serum were observed. The authors stated that the cause could be related to the vomiting or a functional disorder of the kidney or liver, although there were no pathological indications for the latter [38].

In studies of chronic acrolein toxicity, rats were administered orally (gavage) doses of 0.05, 0.5 and 2.5 mg acrolein (dissolved in water)/kg bw/day over a period of 2 years. In all treated rats, reduced levels of creatinine phosphokinase were observed in serum but the underlying cause could not be explained. No increased incidence of microscopically detectable lesions was reported. However, increased mortality rates were observed, which were dose-dependent [39].

2.4 Reproduction toxicity

The reproduction and developmental toxicity of acrolein was examined in rats and rabbits [40, 41]. Rabbits were administered daily 0, 0.1, 0.75 and 2 mg acrolein/kg bw in water by gavage between days 7 and 19 of gestation. In the highest dose group, an increase in the resorption rates of the foetuses was observed which was, however, not statistically significant. Foetal malformations were not observed. At higher doses of 4 and 6 mg/kg bw/day there were reports of high maternal mortality, spontaneous miscarriages, increased resorption rates of the foetuses and gastric ulcers. Acrolein neither induced developmental effects nor did it exhibit any teratogenic effects [40]. Furthermore, rats of the parental generation (F0 Generation) were exposed to 0, 1, 3 and 6 mg acrolein/kg bw initially for 70 days, and the females were subsequently treated with acrolein up to and including the lactation period. A similar treatment was carried out for rats in the F1 Generation to examine their offspring (F2 Generation). Reproduction parameters, such as fertility and viability, and morphology of the offspring remained largely unchanged after acrolein treatment. A reduced weight of the offspring in the F1 Generation was, however, observed at the highest acrolein dose. Gastric lesions were established especially in the high-dose group and in some animals of the F0 and F1 Generations receiving the medium dose. In the highest dose group, an increased mortality rate was observed for the F0 and F1 Generations. Acrolein was not classified as a reproduction toxin. However, it showed toxic effects at a dose of 3 mg/kg bw/day and higher [41].

2.5 Genotoxicity

2.5.1 Genotoxicity in vitro

The results on the mutagenicity of acrolein in bacterial test systems with *Salmonella typhimurium* strains do not show a

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uniform picture, since both positive and negative results were observed [18, 37, 42, 43].

In somatic cells, acrolein caused damage to DNA. DNA strand breaks were observed in fibroblasts of the skin, bronchial epithelial cells and leukaemic cells treated with acrolein [18]. Sister chromatid exchange and chromosomal anomalies were reported in Chinese hamster ovary cells and other cell types [18, 42, 43]. Acrolein showed highly mutagenic effects in human xeroderma pigmentosum cells that were deficient in the nucleotide excision repair systems but not in normal fibroblasts [42, 44]. Further in vitro tests in V79 cells confirmed the mutagenic potential of acrolein (1–2 μM) [45]. Other mutagenicity analyses with or without activation did not show any mutagenic effects in mammalian cells [37, 46].

2.5.2 Genotoxicity in vivo

In vivo studies on genotoxicity resulting from acrolein exposure are very limited and show negative results [36]. After oral administration of up to 10 mg/kg bw acrolein for 14 wk, no effects of acrolein on the formation of micronuclei in normal chromatic erythrocytes in peripheral blood of male and female mice were observed [37].

2.5.3 Adducts with DNA in vitro and in vivo and their genotoxicity

Acrolein forms adducts with all DNA bases in vitro [47]. In cell culture, DNA adducts with desoxyguanosine were detected after acrolein exposure in human lung cells [48] and in Chinese hamster ovary cells [49]. In addition, acrolein adducts with desoxyadenosine have been detected in rat liver epithelial cells [50].

DNA adducts with desoxyguanosine were detected ex vivo in many human tissues as well as in tissues of untreated rodents. Background levels of acrolein DNA adducts were found in the liver of rats and mice [51], whereby a more recent study conducted by the same researchers also detected adducts in the skin of the mouse as well as in the lung, kidney, brain, colon, prostate gland and mammary gland of the rat [52]. After inhalation exposure to acrolein (1 and 10 ppm over 6 h) higher acrolein-guanine adduct levels were detected in the abdominal aorta of cockerels. Ten days after the single acrolein exposure, the DNA adduct levels had been repaired and returned to normal values [53]. Data on DNA adduct levels after an oral acrolein exposure in animal tests are not available.

Background levels of acrolein DNA adducts were also detected in the liver and in the mammary gland and leucocytes of humans [51, 52]. Acrolein-DNA adducts were also detected in brain [54] as well as in other human tissues, such as the lung and the colon [55]. In oral tissue (mostly gingival tissue) higher levels of acrolein-guanine adducts were reported in smokers compared to non-smokers [56]. With guanosine, acrolein forms two isomers, i.e. α-OH-Acr-dGuo and γ-OH-Acr-dGuo. Background levels of the two isomers were detected in the human placenta and leucocytes. Significantly lower levels of the α-OH-Acr-dGuo adduct were reported compared to γ-OH-Acr-dGuo [57]. Zhang et al. [58] also detected primarily γ-OH-Acr-dGuo and hardly any α-OH-Acr-dGuo adducts in leucocytes of smokers and non-smokers but found no differences in the adduct levels of γ-OH-Acr-dGuo between smokers and non-smokers. Furthermore, human lung tissue of smokers or former smokers (1 wk to 26 years since last smoking) was examined for α- and γ-OH-Acr-dGuo adducts whereby both isomers were detected. In lung tissue, similar levels of α-OH-Acr-dGuo and γ-OH-Acr-dGuo adducts were observed, whereby no differences were observed between smokers and former smokers. The levels of acrolein DNA adducts of approximately 1 per 10^7 nucleotides found in this study in lung tissue are higher than the levels of DNA adducts of some carcinogenic substances such as the polycyclic aromatic hydrocarbons with approximately 0.3 adducts per 10^7 nucleotides and benzo[a]pyrene-diol-epoxide with non-detectable values up to 0.1–0.5 adducts per 10^7 nucleotides [59].

Although mainly γ-OH-Acr-dGuo is formed in most tissues except for the lung, this adduct seems to have weak mutagenic potential since it can be rapidly repaired by the cell [60–62]. Although acrolein-DNA adducts were detected in *cII* transgene of embryonal fibroblasts of the mouse, in particular with guanosine, no mutagenicity was found [42].

Other studies have demonstrated mutagenic effects of γ-OH-Acr-dGuo. This controversy regarding the mutagenicity of γ-OH-Acr-dGuo is discussed in detail by Liu et al. [43]. The ring structure of γ-OH-Acr-dGuo seems to play a role as far as mutagenicity is concerned. In double-strand DNA and paired with desoxycytosine, γ-OH-Acr-dGuo occurs with an open ring structure [63]. These adducts can be easily repaired and hence show very low mutagenic potential. However, if γ-OH-Acr-dGuo occurs in a closed ring structure, as is the case with free nucleoside in solution or in single-strand DNA, it possesses a rather strong mutagenic potential [19, 43]. γ-OH-Acr-dGuo can also cause cross-linking between peptides/proteins and DNA, which can lead to DNA damage and mutations [19, 60, 64].

As opposed to the rather weak mutagenic potential of γ-OH-Acr-dGuo, α-OH-Acr-dGuo was detected as a potent mutagen since it can hardly be repaired in the cell [60, 62]. Although α-OH-Acr-dGuo is formed after acrolein exposure in different organs in smaller amounts compared to γ-OH-Acr-dGuo [57, 58], there could be an accumulation of α-OH-Acr-dGuo in certain tissues such as the lung, so that similar levels of the two acrolein-guanosine adduct isomers develop [59].

2.6 Carcinogenicity

Given the cytotoxicity of acrolein, its possible carcinogenicity is difficult to test because of the necessity to conduct long-term exposure studies [43].

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In female rats that were exposed to 625 ppm acrolein in drinking water (5 days/wk) for approximately 2 years, a slightly higher incidence of adenomas of the adrenal cortex was observed. In five out of 20 rats, these tumours were observed whereas in the control group only one case was identified [65].

Parent et al. [39] did not observe any influence of acrolein on tumour development in rats that were treated with 0.05, 0.5 and 2.5 mg acrolein (dissolved in water)/kg bw/day for 2 years by gavage. The increased tumour incidence in the adrenal cortex observed in the first study [65] could not be confirmed. The study by Lijinsky and Reuber [65] seems to have problems with the stability of acrolein so that the actual acrolein exposure cannot be estimated. This is supported by the fact that the acrolein dose calculated by Parent et al. [39] of the Lijinsky and Reuber study is approximately 50 mg/kg bw/day and therefore above the lethal dosage of rats [34, 39].

A further study in mice did likewise not show any influence of acrolein on tumour formation. Animals were exposed daily to 0.5, 2.0 and 4.5 mg acrolein/kg bw (dissolved in water) over 18 months by gavage. The treated animals showed a reduced bw increase and a higher mortality in the higher dose group [66].

In another study, neonatal mice received 75 or 150 nmol acrolein intraperitoneally on days 8 and 15 after birth. After 12 months, the animals were examined for liver tumours. Acrolein did not lead to an induction of tumours [67].

Sakata et al. [68] reported on hyperplasias in the urinary bladder after intraperitoneal injection of acrolein. Acrolein (25 mg/kg bw) was administered to male rats intraperitoneally or intragastrically. Many animals died because of the high toxicity of acrolein. Three out of 12 animals that were still alive 2 days after intraperitoneal treatment developed hyperplasias in the bladder. These hyperplasias were not observed after intragastric acrolein treatment. In a subsequent experiment, the intraperitoneal acrolein injection of 6 mg/kg bw resulted in a significant increase in proliferation in the mucosa of the bladder. Lower doses did not have any effect.

Cohen et al. [69] reported a tumorigenic effect of acrolein on the bladder. Acrolein (2 mg/kg bw) was administered to male rats intraperitoneally twice a wk for 6 wk. Then they were treated for 20 wk with 3% uracil and another 6 wk with the control diet. The majority of the animals (60%) treated with acrolein and uracil showed papillomas (benign tumours of the epithelium) in the bladder whereas uracil alone caused papillomas in 27% of the male rats. No tumours were observed in the control animals. This study suggests a possible tumour-initiating activity of acrolein in the urinary bladder if it is applied intraperitoneally.

2.7 Epidemiology

The possible carcinogenicity of acrolein was examined in an epidemiological study on workers in chemical plants. A relationship between the probable inhalation exposure with acrolein and an increased risk of non-Hodgkin's lymphoma (odds ratio 2.6; two cases), multiple myelomas (odds ratio 1.7; one case) and non-lymphatic leukaemia (odds ratio 2.6; three cases) was observed. However, no statistical significance was determined [70]. This study presents several deficiencies such as the small number of cases, insufficient evaluation of statistical analysis and a restricted characterisation of the exposure so that the validity of this study is limited [36].

In epidemiological studies, an increased lung cancer incidence was reported for non-smoking Chinese women cooking in a traditional wok style. The international Agency for Research on Cancer (IARC) classified emissions from ''high-temperature frying'' as ''probably carcinogenic to humans'' (2A) [71]. Compared to the corresponding controls, these women excreted a significantly higher level of mercapturic acids in urine (referred in each case to creatinine), which were derived from reactions of acrolein, crotonaldehyde and benzene with GSH [72].

2.8 Further aspects

Metabolites such as acrylic acid, glycidaldehyde and glyceraldehyde are also formed from acrolein. It is speculated that they could play a role in the toxicological effects of acrolein [34, 73]. For glycidaldehyde, carcinogenic effects were observed in laboratory rodents after dermal application [34, 74].

In many more recent publications, acrolein and also other lipid peroxidation products, such as 4-hydroxy-2-*trans*-nonenal, have been associated with neurological diseases. The endogenous level in the brain or medulla is increased in patients suffering from Alzheimer, Parkinson, amyotrophic lateral sclerosis and other neurological diseases (e.g. [14, 75–78]). Increased DNA-acrolein adduct levels were also determined in the brain of Alzheimer patients [54]. Whether the oral intake of acrolein via foods is relevant in this connection is rather unlikely due to its toxicokinetic properties. This aspect should, however, continue to be investigated and clarified.

3 Summary and conclusions of the hazard assessment

Concerning the dosage in the longer-term experiments administered to the animals in drinking water, the earlier studies are not representative (volatility and instability of acrolein) in order to be able to use them for a characterisation of the dose–effect relationship (e.g. [65]). In chronic studies in which acrolein was administered dissolved in water to rats and mice, in doses of up to 2.5 mg/kg bw/day (concentration in water: 0.25 mg/mL) for rats and 4.5 mg/kg bw/day (concentration 0.45 mg/mL)

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for mice, increased mortality was reported as a primary endpoint. The underlying cause was not discussed or was designated as unclear [39, 66]. In a reproduction toxicity study with rats, an erosion of the glandular stomach as well as hyperplasias/hyperkeratosis of the forestomach were reported at a mean dosage of 3.0 mg/kg bw/day (concentration in water 0.6 mg/mL) [41]. In a subchronic study with Beagle dogs, acrolein was administered in gelatin capsules. This resulted in changes of biochemical parameters in the serum and temporary clinical-toxic symptoms. The dose was 2 mg/kg bw/day [38]. A possible cause for these differences in the observed effects could be the differences in oral application in each case (vehicle, concentration).

In subchronic studies in rats and mice, acrolein was administered by gavage using a solution in methylcellulose [37]. Lesions of the stomach were detected, including hyperplasias of the forestomach as well as necroses, inflammations, haemorrhagia of the glandular stomach and forestomach. The findings indicate that these effects are due to the irritation caused by acrolein at the site of contact with the tissues. No systemic effects have been reported that were associated with oral intake of acrolein.

Taken together, the most sensitive and most appropriate study for risk assessment for oral intake of acrolein is the above-mentioned NTP study with rats and mice [37]. The LOAEL (low observed adverse effect level) for the described effects amounted to 1.25 mg acrolein/kg bw/day (the applied concentration was 0.25 mg/mL for rats and 0.125 mg/mL for mice). No effects were identified in rats (NOAEL) at 0.15 mg/mL [37]. Based on the administered amount, this value corresponds to a dose of 0.75 mg/kg bw/day. After application of the usual safety factor of 100 – which must be seen as very conservative for an irritant in this case and actually applies to systemically effective substances – a tolerable intake of 7.5 μg/kg bw/day would result. Nonetheless the Federal Institute for Risk Assessment (BfR) would refer in a first approach as a basis for the risk assessment to be conducted here to this tolerable acrolein dose which was derived by a working group of the World Health Organisation (WHO) in 2002 [36].

Acrolein forms different DNA adducts in numerous tissues of humans and animals. It is not clear whether only the endogenous formation is relevant or whether also an exogenous exposure, e.g. orally via foods, can generate these adducts. Concerning inhalation intake it was shown by animal experiment that premutagenic DNA adducts develop in the aorta at levels that were five times higher than for the controls. After 10 days the adduct levels returned to normal by DNA repair [53]. The detection of in vivo adducts means, however, that a certain systemic distribution should be expected, at least after inhalation intake.

In vitro mutagenicity of acrolein and acrolein-DNA adducts were demonstrated in some but not all tests. Evidence for in vivo formation of acrolein adducts from ingested acrolein is still lacking. Therefore, the question whether acrolein is mutagenic after oral exposure should be clarified in further investigations. It is interesting to note that evidence for a carcinogenic effect of acrolein after oral exposure cannot be derived from the available studies. Although one of the studies shows possibly an initiating effect on the urinary bladder epithelium, this is only evidenced after intraperitoneal application. A subsequent promotion with uracil resulted in papillomas. The findings that acrolein is able to form DNA adducts without carcinogenic potential require further investigation.

The International Agency for Research on Cancer [79] classified acrolein concerning its carcinogenic potential in group 3 (''There is inadequate evidence in humans for the carcinogenicity of acrolein. There is inadequate evidence in experimental animals for the carcinogenicity of acrolein'').

The German MAK (''maximale Arbeitsplatz-Korzentration'': maximum Workplace concentration) Commission [80] classified acrolein in group to 3B, which is defined as follows: ''Indications for a carcinogenic effect exist based on in vitro or animal tests which are, however, not sufficient for classification into another category. For a final decision, further investigations are required. If the substance or its metabolites do not show any genotoxic effects, an MAK or BAT value can be defined.''

The Agency for Toxic Substances & Disease Registry (ATSDR) laid down an ''Oral Minimal Risk Level'' (MRL) for acrolein of 4 μg/kg bw/day for an ''intermediate'' oral exposure (15–364 days) based on a benchmark dose lower confidence limit 10% (BMDL10) of 0.36 mg/kg/day for epithelial hyperplasias of the forestomach in mice and taking into account a safety factor of 100. MRL values are based on non-carcinogenic effects; carcinogenic effects are not taken into account with this value. A chronic MRL value was not derived due to lack of data [34].

4 Exposure

4.1 Endogenous formation

2-Alkenals such as acrolein can also be formed endogenously as a result of lipid peroxidation so that a permanent endogenous background exposure of 2-alkenals can be assumed [81, 82]. For instance, acrolein was formed during oxidation of low density lipoprotein [83]. Lipid peroxidation is probably not the only endogenous source of formation. Other sources have to be considered as well, such as myeloperoxidase-related degradation of threonine and amino-oxidase-related degradation of spermine and spermidine. As far as the exact endogenous acrolein formation is concerned, there are currently no data available [1, 84]. The extent of endogenous formation of acrolein and its biological relevance should therefore be clarified.

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4.2 Formation and analysis of acrolein in food

Acrolein can be formed during different steps of food processing from amino acids, fats and carbohydrates. It is formed, inter alia, by heating and/or overheating of vegetable and animal fats, e.g. during frying and deep frying. During this reaction, triglycerides are hydrolytically cleaved in the presence of residual water and the generated glycerine continues to be dehydrated. Due to its high reactivity, acrolein has a polymerisation tendency in water and can hence not be analytically detected as free acrolein. Furthermore, acrolein is formed through the conversion of amino acids (Strecker degradation within the framework of the Maillard reaction) and through the oxidative deamination of polyamines [1, 85].

Due to the volatility of acrolein, special attention has to be given to sampling and sample storage to avoid loss of acrolein. The analytics of acrolein in foods are extremely complicated and require great effort. Early methods in the 1960s were based on paper and thin layer chromatography and/or spectroscopic techniques using UV-VIS or fluorescent spectroscopy after derivatisation of acrolein with hydroxylamine, morpholine, *N*-methylhydrazine or sodium bisulphate [85].

Since the 1980s, HPLC and UV detection of the 2,4-dinitrophenylhydrazine (DNPH) derivatives or GC-MS after derivatisation of acrolein with pentafluorobenzylhydroxylamine (PFBHA) or pentafluorophenylhydrazine (PFPH) to the corresponding oxime derivatives became the preferred analytical tools for quantification. The use of SPME-GC-MS has also been described, in particular for the determination of acrolein formed during heat processing of foods and released into the ambient air [85].

In a study by Osório and de Lourdes [86] who used SPME-GC/MS, acrolein contents in French fries of 1–5 μg/kg were reported. During repetition of the measurements, concentration differences of up to 100% were obtained. If foods are heated in oil, the emission of acrolein is reduced to approximately 20 mg/kg/h for soya bean oil and to approximately 10 mg/kg/h for rape seed oil. It can be derived from these data that during the heating of 100 g French fries approximately 150 μg of acrolein are released in 10 min [87].

During the last years, the analytical methods were extended to LC-MS/MS after derivatisation to the corresponding DNPH derivatives whereby the accuracy and precision were improved through the use of labelled internal standards (d_4-acrolein, $^{13}C_3$-acrolein) within the framework of an isotope dilution assay [88]. All analytical methods listed so far have not yet been standardised for foods and there are so far no studies on comparison of methods through inter-laboratory tests. Only one method validation study with air samples and standardised solutions using an HPLC-DNPH method was carried out by Hafkenscheid and van Oosten [89].

4.3 Contents of acrolein in food

Due to missing data on measurements of food in Germany a literature search has been performed (Table 1). These data can only give a rough impression of the acrolein content of some food groups and may, therefore, neither be representative nor transferable to the situation in Germany. The preparation processes and hence the contents in the foods can differ widely between countries. Moreover, the (few) analysed foods in the different food groups are not necessarily representative for the consumption habits in Germany.

Data and especially current data on acrolein contents in foods are only available to a very limited extent (Table 1). Therefore, they do not allow a representative exposure assessment. The contents vary very strongly depending on the preparation/processing and composition of the foods. For that reason, measurements should be made particularly in processed foods in a ready-to-eat condition. According to the existing published exposure data, the highest contents were measured in alcoholic beverages such as wines and

Table 1. Acrolein contents and intakes of different food groups

Food	Acrolein content (mg/kg or L)	Source of data [ref.]	Mean consumption (g/day)	Intake (μg/day)
Domiati (Feta)	0.3–1.3 (Depending on maturity)	[107]	42 (cheese)	13-55
Donuts	0.1–0.9	[108]	Approx. 60 g/unit	6–54/unit
Wine	Up to 3.8	[105]	43 (wine/sparkling wine)	Up to 163
Fruits	<0.01–0.05	[90] in [80]	337 (without juice and nuts)	<3–17
Vegetables	Up to 0.59	[90] in [80]	263 (without potatoes); 341 (with potatoes)	Up to 155; up to 201
Lager beer	0.001–0.002	[109] in [90]	142 (beer total)	0.1–0.3
Brandy/cognac	1.4–1.5	[110]	3 (spirits total)	2–33
Whisky (scotch, bourbon)	0.7–11.1	[111]		
Coating of fried fish	0.1	[108]		

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spirits. Some published results only show the qualitative detection of acrolein, e.g. in caviar and lamb [90], in salted pig meat [91], in poultry meat [92, 93], in cocoa beans and chocolate liquor [94], and in non-alcoholic beverages such as coffee and tea [90].

4.4 Exposure assessment: intake via food (Germany)

The assessments of consumption are based on data of the ''Dietary History'' interviews of the National Nutrition Survey II (NVS II), which was collected using the programme ''DISHES 05'' [95]. With this method, 15 371 individuals were interviewed and their consumption habits of the last 4 wk (starting from the date of the interview) were recorded retrospectively. It provides good estimates for long-term intakes of substances if foods are summed up in general categories or if foods are considered, which are regularly consumed. The consumption data (apart from donuts) were evaluated within the framework of a project carried out by the BfR under the title ''Dietary Exposure due to Environmental Contaminants'' (LExUKon [96]). For the calculation of consumption amounts, recipes (dishes) and almost all composited foods like cakes and bread were broken down into their unprocessed ingredients. Corresponding processing factors such as drying were taken into account. The recipes are (largely) based on standard recipes and hence ignore variation in preparation/production and the ensuing consumption amounts.

A rough intake assessment of individual foods/food groups was carried out with summarised content data of different international publications from 1981 to 2001 (Table 1).

A calculation of the overall intake cannot be made from the determined values since there were only individual minimum and maximum values/intakes in the food groups, which involve major uncertainties. A summing, therefore, does not reflect a realistic intake. Moreover, not all sources could be included due to missing content data. Therefore, possible non-quantifiable additional exposures should be considered.

Due to the inclusion of all consumption amounts from composite foods and recipes regardless of their way of preparation, it has to be assumed that the food groups shown underlie an over-estimation of acrolein intake (does not refer to the contents). Assumptions of consumption had to be calculated, since no reliable data for individual foods were available. In most cases, conservative assumptions were made (e.g. for cheese, oil or French fries), which probably lead to an over-estimate of the acrolein intake of the respective food groups. Since the available contents are not representative, an under-estimate or an over-estimate of the calculated intakes cannot be excluded.

4.5 High acrolein contents in fried potatoes?

Acrolein and acrylamide react in the organism in considerable amounts with GSH and, after further degradation, they are excreted as corresponding mercapturic acids in urine (see Section 2.1). Acrolein is mainly converted to 3-HPMA whereas acrylamide, another heat-generated contaminant in food, leads to *N*-acetyl-*S*-(2-carbamoylethyl)-cysteine (AAMA) and *N*-acetyl-*S*-(2-hydroxy-2-carbamoylethyl)-cysteine (GAMA). These mercapturic acid derivatives were measured as biomarkers in urine within the framework of a human study with 13 test subjects (non-smokers) who had eaten 150 g potato chips [2]. From the amounts of 3-HPMA, AAMA and GAMA excreted in urine over 72 h, average elimination half-lives of 5, 12 and 38 h were calculated, respectively. Based on the calculated ''Area under the Curve'' (AUC), the total excretion of 3-HPMA within 72 h was higher by a factor of 15 compared to the sum of AAMA and GAMA. The authors concluded from the excretion kinetics that the exposure to acrolein is attributable to the intake of the test meal. The deep-frozen urine samples examined during these analyses originated from a study conducted earlier [97, 98]. Test subjects consumed self-made potato chips with relatively high acrylamide content (acrylamide dose 1 mg). Assuming that acrolein and acrylamide are metabolised in equal proportions into the corresponding mercapturic acid derivatives, an acrolein exposure from the consumed 150 g potato chips in the range of 15 mg and an acrolein concentration of 100 mg/kg food can be calculated for these chips.

A subsequent examination of the potato chips used in the study for their acrolein content was not possible, because non-consumed chips had not been stored under the necessary conditions (personal communication by Prof. Eisenbrand). The chips were produced with the goal of reaching the highest possible acrylamide content (frying at 190°C for 5 min [98]). This may also have resulted in a very high acrolein content. Publications about direct measurements of acrolein in fried potatoes are not available, except for a Brazilian study [86]. After the frying of potato slices (170°C for 12–15 min) in different oils, the Brazilian research group detected much lower acrolein contents of up to 5 μg/kg.

In a more recent paper, the acrolein contents were measured with two newly developed methods in frying oils [88]. There were major differences in the examined oils. After heating to 140°C for 24 h coconut oil contained 6.7 mg/kg, whereas linseed oil contained 242.3 mg/kg. In different oils after the deep frying of potato chips (180°C for 2.5 min/cycle) acrolein contents between approximately 0.40 mg/kg (coconut oil) and 2.75 mg/kg (linseed oil) were measured after the deep frying of potato chips (180°C for 2.5 min/cycle) after nine cycles. Interestingly, control oils that were heated under comparable conditions but without potato slices showed significantly higher contents (up to a maximum of approximately fivefold). The authors hypothesised that the presence of water and air during deep frying might lead to oxidative

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and hydrolytic processes. Contents in frying oils in a single-digit mg/kg range would suggest significantly higher acrolein contents than the maximum value of 5 μg/kg measured by Osório and de Lourdes [86].

Overall, the data presented here suggests that fried potato chips or French fries might possibly present significant contents of acrolein, which could make a major contribution to the overall exposure. For clarification purposes, the development of reliable analytical methods will be necessary to determine acrolein content in fried potato chips and French fries.

4.6 Acrolein exposure assessment by determination of the mercapturic acid 3-HPMA in urine

A possibly promising approach to determine the total exposure to acrolein is the one used in the study by Watzek et al. [2] who measured the excretion of the acrolein metabolite 3-HPMA in urine. This is particularly of importance considering the analytical difficulties for the quantitative determination of acrolein in foods and the many foods that possibly could contribute to acrolein exposure. Such an approach also covers inhalation exposure as well as an endogenous formation (see Section 4.1). During the last years, methods for the determination of mercapturic acid derivatives in urine were developed not only for acrolein but also for other volatile, small molecular compounds such as acrylamide, 1,3-butadiene, ethylene oxide, propylene oxide or glycidol [88, 99]. These studies focused on environmental and occupational exposures.

In spot urine of 14 non-smokers, Schettgen et al. [99] were able to determine 3-HPMA in a concentration of 155 μg/L (median, range 37–730 μg/L). Eckert et al. [100] found a 3-HPMA concentration of 179 μg/L (median, range 32.6–2325 μg/L) in 54 non-smokers. Assuming that the urine production was 1.5 L/day and 50% of the acrolein taken up was excreted as 3-HPMA, the recorded data of the two studies would lead to an acrolein exposure of 124 μg/day (median, range 30–585 μg/day) and 143 μg/day (range 26–1862 μg/day) respectively. Assuming a bw of 60 kg, a median exposure of 2.1 or 2.4 μg/kg bw/day would be calculated, while peak concentrations might possibly reach 30 μg/kg bw.

These estimates could come close to the oral acrolein exposure. An uncertainty lies in the lack of knowledge as to the acrolein percentage that is excreted as 3-HPMA in urine. Furthermore, the estimates from morning urines may be low due to the rapid excretion as 3-HPMA (elimination half-life approximately 5 h [2]). Morning urine would contain low amounts of acrolein metabolites if the exposure took place around noon of the previous day.

On the other hand, oral intake of acrolein can also be over-estimated from urinary metabolite concentrations because both endogenous formation and inhalation exposure (in particular kitchen vapours, road traffic, passive smoking) could be relevant. Numerous studies document that tobacco smoke has high acrolein contents and smokers have a significantly higher acrolein exposure than non-smokers (e.g. 3-HPMA median 1219 μg/L for smokers compared to 179 μg/L for non-smokers [100]). The average acrolein concentration in the air of two US cities (14.3 μg/m^3) given in a toxicological review of acrolein evaluated by the US-EPA (2003, available online: http://www.epa.gov/iris/toxreviews/0364tr.pdf) would lead to a comparatively high inhalation intake of 286 μg/day for an average adult with breathing rate of 20 m^3/day. Compared to the data of 3-HPMA excretion in urine of non-smokers, this appears to be very high. The acrolein exposure via air was reviewed by Stevens and Maier [1] and reported to amount to approximately 154 μg/day. Other reports estimated the daily acrolein exposure via inhalation to be only approximately 26 μg acrolein/day for adult non-smokers [101].

The approach of exposure estimation through urine excretion of mercapturic acid derivatives also permits a direct comparison of acrylamide and acrolein exposure. In the study by Schettgen et al. [99] not only 3-HPMA (median of 155.0 μg/L) but also AAMA (median of 52.6 μg/L) was measured in spot urine of 14 non-smokers. Converted to the amounts of the respective original substances, this corresponds to a ratio of 2.56 to 1 for the exposure, if it is assumed that acrolein and acrylamide are converted in equal proportions to the corresponding mercapturic acid derivatives (for acrolein not known, acrylamide is converted up to approximately 50% into AAMA [98]). For acrylamide, the results of Schettgen et al. [99] would allow for an estimate of exposure with a median of 0.8 μg/kg bw/day (assumptions: 1.5 L urine, 50% metabolism rate, 60 kg bw). A similar result (0.75 μg/kg bw/day on average, for an assumed bw of 60 kg) was reached by Urban et al. [102] in a study with 60 non-smokers who collected urine for 24 h (mean AAMA excretion of 73.1 μg/day); the result corresponded to the average estimate from the nutrition protocol. Lower AAMA concentrations in spot urine (median 29 μg/L, range 3–83 μg/L) were found by Boettcher et al. [103] for 16 non-smokers, which would lead to a correspondingly lower estimate of the acrylamide assumption (median approximately 0.45 μg/kg bw/day). EFSA (European Food Safety Authority) recently published intake estimates for Germany presenting mean values of 0.31–0.34 μg/kg bw and 95th percentiles of 0.79–0.83 μg/kg bw/day [104]. Overall, the data on acrylamide show that a reliable exposure estimate is possible through the AAMA excretion in urine.

4.7 Conclusions on the exposure data

A valid estimate of the oral acrolein exposure from content and consumption data is currently not feasible. The reason is primarily the limited availability of analytical techniques for the detection of acrolein in foods. Consequently, foods were sporadically examined for acrolein with different methods so far. The excretion of the mercapturic acid

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metabolite 3-HPMA in urine is probably more appropriate for a rough estimate. The few results available so far suggest that the mean acrolein exposure is roughly higher by factor 3 compared to acrylamide and is on average in a range of a few μg/kg bw/day. Following consumption of certain foods, a significantly higher exposure can be expected. For instance, the consumption of two glasses of wine (0.4 L) with the highest measured content (3.8 mg/L [105]) would lead to an intake of approximately 25 μg/kg bw. A higher acrolein exposure can also be expected from the consumption of potato chips.

For the improvement of exposure data, the following would be necessary:

(i) Development of valid analytical methods for foods as well as the examination of a broad range of foods in ready-to-eat form with a focus on the foods with high acrolein contents (e.g. potato chips, wine).
(ii) Validation of 3-HPMA excretion in urine as an exposure marker for acrolein; with this approach the extent of endogenous acrolein formation and inhalation exposure for non-smokers (in particular kitchen vapours, road traffic, passive smoking) could likewise be clarified.

5 Risk characterisation and discussion

The toxic hazard potential of acrolein is primarily marked by its reactivity as an α,β-unsaturated carbonyl compound, which leads to cytotoxic effects such as local lesions of the mucous membrane when acrolein comes into direct contact with tissue, e.g. during oral intake in the oral/buccal cavity and the gastro-intestinal tract.

Due to these local effects, there is a threshold below which acrolein effects would no longer have to be expected according to the current findings. Systemic effects in organs have not been found in animal. Based on the results of subchronic animal experiment studies on local effects (in the stomach/forestomach of the rat), a WHO working group derived a tolerable oral acrolein intake of 7.5 μg/kg bw/day [37].

A reliable estimate of the acrolein exposure via diet is not possible. Food analytics of acrolein are methodologically problematic so that there are only study results to a limited extent for certain foods.

The more recently used approach to record an integral acrolein exposure via the excretion of its metabolite 3-HPMA in urine (human biomonitoring) appears, however, to be promising; based on the few data available so far for the quantified 3-HPMA in urine of non-smokers it can be roughly estimated that the mean acrolein exposure could be in the range of a few μg/kg bw/day and that exposure could be higher by approximately a factor of 10 after the consumption of certain foods with high acrolein levels.

Due to this only inaccurately quantifiable exposure, the assessment of the risk of oral intake of acrolein via foods is currently only insufficiently possible. For acrolein exposures in the range of a few μg/kg bw/day, health risks would be negligible considering the mentioned value of the WHO working group of 7.5 μg/kg bw/day. After consumption of certain foods, a significantly higher daily intake might have to be expected under certain circumstances, which would exceed the mentioned WHO value. Whether such elevated exposures pose health risks is not clear. It has to be taken into consideration that for acrolein, no systemic effects were reported from studies with oral administration and that for the derivation of the WHO value the toxicological end point of a local, direct cell damaging effect was used as a basis for which the applied overall safety factor of 100 has to be considered as highly conservative.

Since acrolein is also formed endogenously and has been associated in more recent articles with the pathophysiology of neurodegenerative diseases in humans [15, 76–79] and in animal tests with atherosclerotic changes [106], the question arises whether acrolein taken up orally through foods is relevant in this connection. At present this question cannot be answered and should therefore be further investigated. For toxicokinetic reasons it appears very unlikely that for the usual food contents relevant amounts of acrolein reach the corresponding target organs and/or tissues.

Acrolein forms different DNA adducts in numerous tissues. It is not clear whether only the endogenous formation or also the exogenous exposure through foods is relevant here. For inhalation intake it was shown in animal experiments that pre-mutagenic DNA adducts were formed in the aorta which were however completely repaired [54]. This result also means that a certain systemic distribution of acrolein would have to be expected, at least after inhalation intake.

In vitro the mutagenicity of acrolein and its DNA adducts have been shown in some but not all tests. In vivo evidence has not been demonstrated so far. Therefore, the question whether acrolein is mutagenic after oral exposure should continue to be investigated.

The finding that acrolein is able to form DNA adducts without a carcinogenic effect having been evidenced so far after oral exposure, is not yet understood from the current point of view. For that reason a possible biological relevance and/or toxicological relevance of these adducts should be examined and clarified.

The authors have declared no conflict of interest.

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