

Toxicokinetics of Acrylamide in Humans after Ingestion of a Defined Dose in a Test Meal to Improve Risk Assessment for Acrylamide Carcinogenicity

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Abstract

High amounts of acrylamide in some foods result in an estimated daily mean intake of 50 µg for a western style diet. Animal studies have shown the carcinogenicity of acrylamide upon oral exposure. However, only sparse human toxicokinetic data is available for acrylamide, which is needed for the extrapolation of human cancer risk from animal data. We evaluated the toxicokinetics of acrylamide in six young healthy volunteers after the consumption of a meal containing 0.94 mg of acrylamide. Urine was collected up to 72 hours thereafter. Unchanged acrylamide, its mercapturic acid metabolite *N*-acetyl-*S*-(2-carbamoyl-ethyl)-cysteine (AAMA), its epoxy derivative glycidamide, and the respective metabolite of glycidamide, *N*-acetyl-*S*-(2-hydroxy-2-carbamoyl-ethyl)-cysteine (GAMA), were quantified in the urine by liquid chromatography-mass spectrometry. Toxicokinetic variables were obtained by noncompartmental

methods. Overall, 60.3 ± 11.2% of the dose was recovered in the urine. Although no glycidamide was found, unchanged acrylamide, AAMA, and GAMA accounted for urinary excretion of (mean ± SD) 4.4 ± 1.5%, 50.0 ± 9.4%, and 5.9 ± 1.2% of the dose, respectively. Apparent terminal elimination half-lives for the substances were 2.4 ± 0.4, 17.4 ± 3.9, and 25.1 ± 6.4 hours. The ratio of GAMA/AAMA amounts excreted was 0.12 ± 0.02. In conclusion, most of the acrylamide ingested with food is absorbed in humans. Conjugation with glutathione exceeds the formation of the reactive metabolite glycidamide. The data suggests an at least 2-fold and 4-fold lower relative internal exposure for glycidamide from dietary acrylamide in humans compared with rats or mice, respectively. This should be considered for quantitative cancer risk assessment. (Cancer Epidemiol Biomarkers Prev 2006;15(2):266–71)

Introduction

Acrylamide has recently been detected at high amounts in everyday foodstuff, mainly in fried and baked starch-enriched food (1). It is formed mainly by a reaction of reducing sugars with asparagine when heating the food to >120°C (2, 3). Food composition and processing conditions have a major effect on acrylamide formation (4, 5). Based on food contents, the average daily intake of acrylamide for adults in western countries was estimated to be in the range of 0.2 to 1.4 µg/kg body weight, with 0.5 µg/kg body weight probably as the best guess. However, depending on the different diets in younger age groups, a higher exposure is assumed in children and adolescents, reaching up to 3.4 µg/kg body weight daily (95th percentile) in a Berlin cohort (reviewed in ref. 6).

This finding was troublesome because acrylamide has previously been recognized as a carcinogen in rodents (7, 8), and is classified as probably carcinogenic in humans (ref. 9; reviewed in ref. 10). The mutagenicity of acrylamide was also confirmed at low concentrations in different mammalian cell

lines (11). Based on conventional risk assessment calculations of limited data from two rodent studies, the additional population cancer risk estimated to result from a daily lifetime uptake of 70 µg acrylamide with the food was in the order of magnitude of 1×10^{-3} (10, 12). These extrapolations, however, must be considered as very preliminary. Epidemiologic studies failed to confirm an increased cancer risk caused by acrylamide intake with food in humans, but these studies have relevant methodologic limitations (13–16).

One major uncertainty about these risk extrapolations are possible differences in the toxicokinetics of acrylamide between rodents and humans (6). The fate of acrylamide seems to be qualitatively similar in the mammalian species examined more extensively to date, including mice, rats, and humans (Fig. 1). Acrylamide was rapidly absorbed following oral administration in all species (6). A first exploratory study in healthy volunteers confirmed this finding for humans following oral administration of acrylamide-containing food (17). Moreover, it was shown that acrylamide could cross the blood/placenta barrier in a human placenta *in vitro* model as well as the blood/breast milk barrier *in vivo* of lactating mothers (17). These observations suggest that orally ingested acrylamide is able to reach any human tissue. A fraction of the dose is metabolized to form the epoxy derivative glycidamide (6). In mice, it has been shown that this step is mediated by the cytochrome P450 enzyme, CYP2E1 (18). Both acrylamide and glycidamide have the capability to bind covalently to nucleophilic sites of biological macromolecules. The major targets of these compounds seem to be the –SH and –NH₂ groups of proteins and nucleic acid nitrogens. Although both

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acrylamide and glycidamide DNA adducts are formed *in vitro*, only glycidamide adducts have been found after the administration of acrylamide or glycidamide *in vivo* (19). This supports the importance of glycidamide rather than the parent compound acrylamide as the mediator of the genotoxic effects of acrylamide (6, 19). Acrylamide and glycidamide adducts to the NH₂-terminal valine of human hemoglobin are used as convenient biomarkers for external acrylamide and/or internal glycidamide exposure (6). Acrylamide and glycidamide are also able to form glutathione conjugates, possibly mediated by glutathione S-transferases, which thereafter, are converted to the respective mercapturic acid metabolites. Both *N*-acetyl-S-(2-carbamoyl-ethyl)cysteine (AAMA) and *N*-acetyl-S-(2-hydroxy-2-carbamoyl-ethyl)cysteine (GAMA) have been found in rodent (20, 21) and human urine (22, 23). These metabolites have also been proposed as biomarkers for acrylamide and glycidamide exposure (23).

The available data on urinary excretion of acrylamide metabolites in humans originate from a study in which a small collective of the general population was continuously exposed to acrylamide through diet and tobacco smoke (23) and from two studies with experimental administration of acrylamide. Fennel and coworkers (24) determined acrylamide and its urinary metabolites in the urine 24 hours after p.o. administration of a high 3 mg/kg bodyweight dose, applying an analytic method with rather low sensitivity. Only very recently, Boettcher et al. (22) measured AAMA and GAMA in one individual up to 2 days after administration of a single oral acrylamide dose of 13 µg/kg body weight dissolved in water. More extensive toxicokinetic data for acrylamide, also addressing interindividual variation and uptake out of drinking water versus complex food matrices, are essential for acrylamide risk assessment. Thus, in the present clinical study, a defined dose of acrylamide was administered in acrylamide-containing food to six healthy volunteers, and the urinary excretion of acrylamide and its metabolites glycidamide, AAMA, and GAMA were followed for 3 days.

Materials and Methods

The study was conducted according to the pertinent version of the Declaration of Helsinki (Washington, 2002) and was

approved by the Ethics Committee of the University of Cologne. Each volunteer provided written informed consent. Six young Caucasian nonsmokers (three women and three men) were included in the study: mean ± SD for age was 26.6 ± 5.6 years, body height was 179 ± 10 cm; their body weight of 75.4 ± 14.4 kg corresponded to a body mass index of 23.3 ± 2.2 kg/m². Participants were required to be healthy as determined by medical history, physical examination including ECG, body temperature and blood pressure measurements, and standard clinical chemistry evaluation. Main exclusion criteria included pregnancy or lactation, environmental or occupational exposure to acrylamide, therapeutic or illicit drug intake, and alcohol intake of 30 g daily or more.

Acrylamide-containing food was prepared by frying 150 g batches of self-prepared potato chips ("Princess" potatoes) at 190°C for 5 minutes. Frying was done on the day prior to administration using a conventional household fryer (4). The chips were of uniform surface to volume ratio and were mixed repeatedly during frying to achieve a uniform degree of browning. After preparation of the entire amount required for the study, all chips were mixed thoroughly, and six representative samples were drawn by chance. These samples were crunched in liquid nitrogen, and two fractions of 1 g each of the powder obtained were used for further analysis. Acrylamide was quantified in these samples as described (4). The concentration found in these samples was 6.23 ± 0.99 µg/g of potato chips. The chips were maintained overnight at +4°C. In the morning after preparation, each participant in the study obtained a meal consisting of 150 g of the potato chips, corresponding to an acrylamide dose of 938 µg (13.3 µmol), equivalent to a mean dose of 12.4 µg/kg body weight. This is ~20-fold more than the estimated average daily intake of acrylamide in the diet (see above).

Subjects arrived at the clinical ward the evening prior to acrylamide administration and were discharged 24 hours thereafter. The acrylamide-containing meal was administered within 30 minutes after 11 hours of fasting, further food intake was allowed 6 hours after this meal. Food known or suspected to contain high amounts of acrylamide were excluded from the prestudy examination until the last urine sampling. Fluid intake was restricted to 240 mL of water 1 hour prior to the acrylamide dose with the meal, and 2, 4, and 6 hours thereafter. After this period, drinking limitations were

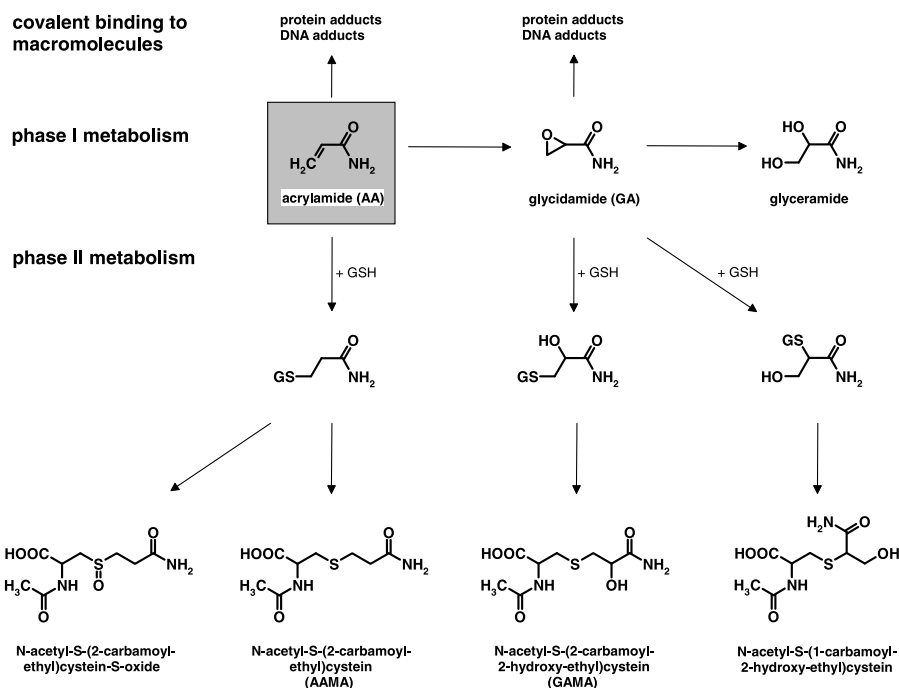


Figure 1. Presumed metabolic scheme of acrylamide. The scheme was partly adopted from Boettcher et al. (22), Dybing et al. (6), and Fennel et al. (24). Not all of the metabolites shown have been confirmed unequivocally in humans. In the present study, only acrylamide, glycidamide, AAMA, and GAMA have been quantified, with glycidamide concentrations being lower than the lower limit of quantification (2.5 ng/mL) in all samples.

suspended. Urine was collected just prior to the acrylamide dose and 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 16, 16 to 24, 24 to 36, 36 to 48, and 48 to 72 hours postdose. Urine was kept at +4°C until completion of the respective collection period and frozen at -80°C thereafter. Adverse events were monitored throughout the study.

Unchanged acrylamide and the epoxy metabolite glycidamide in urine were quantified using an Applied Biosystems (Foster City, CA) API3000 liquid chromatography-mass spectrometry device as previously described (17). The lower limits of quantification were 0.5 and 2.5 ng/mL for acrylamide and glycidamide, respectively. The concentrations of the mercapturic acid metabolites, AAMA and GAMA, were quantified using an Applied Biosystems API2000 liquid chromatography-mass spectrometry device as previously described (25). The lower limit of quantification for AAMA and GAMA was 5 ng/mL.

The molar amounts excreted were calculated by multiplying the respective concentrations with the corresponding urine volumes which were measured by weighing the urine containers prior to and after the end of a collection interval. The amounts excreted were transformed to excretion rates by division of the individual time intervals. The terminal elimination rate was obtained by linear correlation of mean postdose time of the sampling interval and log excretion rates using the WinNonlin Professional pharmacokinetic software version 4.01 (Pharsight Corp., Mountain View, CA). Extrapolation of excreted amounts to infinity was done by division of the estimated excretion rate at the end of the collection period with the last quantifiable concentration by the elimination rate constant and adding this value to the amount excreted until this point of time. No baseline corrections were made for AAMA and GAMA as concentrations prior to acrylamide administration were >6-fold lower than those found at the end of the 72-hour sampling period.

Results

In the predose samples, neither acrylamide nor glycidamide concentrations were detected above the lower limit of quantification. Baseline AAMA concentrations reached 252 ± 142 nmol/L (mean \pm SD), GAMA concentrations were 42 ± 40 nmol/L.

After the meal, a rapid onset of acrylamide excretion was seen in the first sampling period, whereas an increased excretion of AAMA and GAMA was delayed until the 2 to 4 hours sampling period (Figs. 2 and 3). No concentrations of glycidamide exceeding the limit of quantification (2.5 ng/mL) were detected during the entire sampling period. Maximal urinary excretion rates of acrylamide, AAMA, and GAMA were usually observed during the 2 to 4, 6 to 8, and 12 to 16 hours sampling intervals, respectively (Table 1). Corresponding concentrations measured in the respective intervals were 533 ± 131 nmol/L for acrylamide, $5,986 \pm 5,693$ nmol/L for AAMA, and 384 ± 276 nmol/L for GAMA; these concentrations decreased to <7, 437 ± 177 , and 94 ± 67 nmol/L, respectively, for the last sampling interval 48 to 72 hours postdose. The apparent terminal elimination half-lives for the substances were much shorter for acrylamide, 2.4 ± 0.4 hours compared with 17.4 ± 3.9 and 25.1 ± 6.4 hours for AAMA and GAMA, respectively.

Unchanged acrylamide, AAMA, and GAMA accounted for urinary excretion of (mean \pm SD) $4.4 \pm 1.5\%$, $50.0 \pm 9.4\%$, and $5.9 \pm 1.2\%$ of the dose, respectively (Table 1; Fig. 3). As the sum of acrylamide, AAMA, and GAMA, $60.3 \pm 11.2\%$ (median, 63.9%; range, 38.2-69.1%) of the overall acrylamide dose was recovered in urine. One subject (no. 3) showed consistently lower excreted amounts for acrylamide, AAMA, and GAMA (Fig. 2), suggesting a decreased absorption of

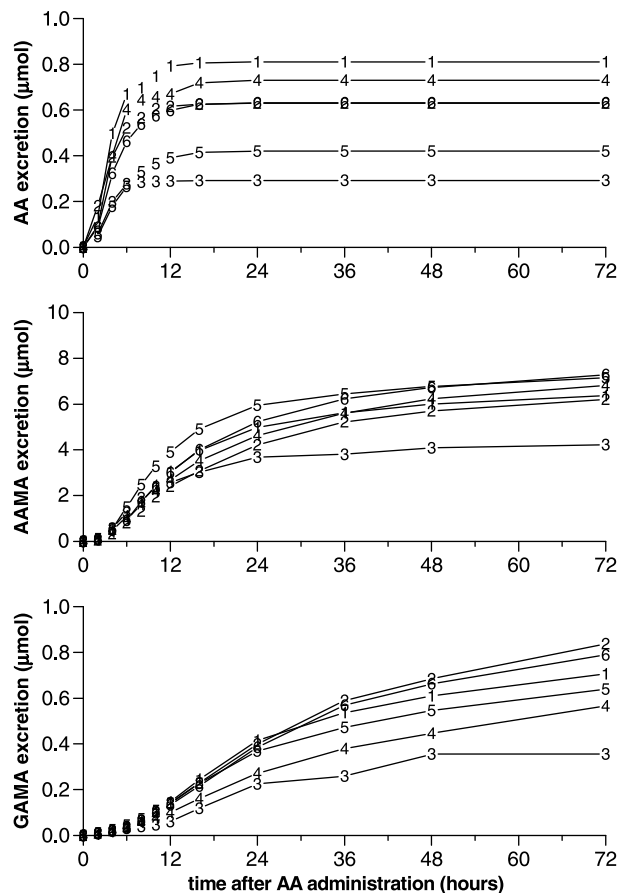


Figure 2. Individual cumulative excretion of acrylamide and its metabolites AAMA and GAMA (μmol) following a single dose of 13.3 μmol (0.94 mg) of acrylamide administered as a potato chips test meal. Numbers indicate individual subjects (women, nos. 1-3; men, nos. 4-6). Note the different y-axis scales used.

acrylamide rather than an anomaly in metabolism. Excretion of acrylamide was virtually completed within 1 day, extrapolation of excreted amounts to infinity added amounts of <0.2% on average. In contrast, mean excretion of AAMA and GAMA reached only 96.2% and 88.7% of the respective total excreted amount within the 72-hour sampling interval excretion.

The mean \pm SD ratio of overall GAMA/AAMA amounts excreted was 0.118 ± 0.017 (median, 0.115; range, 0.100-0.150). However, because of the different excretion versus time profiles for these two mercapturic acid metabolites, this ratio, which had a predose value of 0.204 ± 0.062 (mean \pm SD), changed with the postdose time and varied from 0.027 ± 0.007 in the 4 to 6 hours sampling interval to 0.248 ± 0.036 in the 48 to 72 hours interval. There were no adverse events from the prestudy until the final examination of the study participants.

Discussion

In the present study, the metabolic fate of a 0.94 mg (13.3 μmol) acrylamide dose administered in potato chips was investigated in humans. The data shows that most of the dose is absorbed, and that detoxification of acrylamide is more efficient than formation of the more reactive epoxy metabolite glycidamide in humans.

Absorption and Recovery of Acrylamide. There are only two studies in which a relatively low defined dose has been given to laboratory animals to assess toxicokinetic data. The

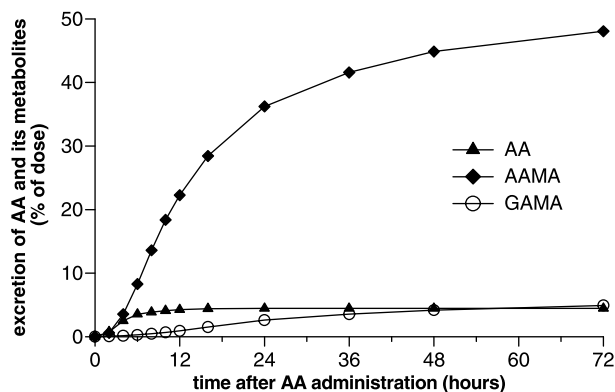


Figure 3. Mean cumulative recovery ($n = 6$) of acrylamide and its metabolites AAMA and GAMA (% of dose) following a single dose of 13.3 μmol (0.94 mg) of acrylamide administered as a potato chips test meal.

lowest dose given to rodents was 0.1 mg/kg (26, 27), whereas typical doses tested were in the 1 to 50 mg/kg range (6). Despite these higher relative doses in the animals, the 60.3% mean fraction of the dose recovered in the urine in this study was similar to that reported in rodents. Miller et al. (28) recovered 71% of the dose in urine after gavage to F344 rats. Also, in male rats, 53% of a 50 mg/kg dose appeared in urine within 24 hours after gavage (21). In B6C3F1 mice, the absorbed fraction of a 0.1 to 0.26 mg/kg, as calculated from the sum of normalized areas under the curve for glycidamide and acrylamide in plasma relative to the areas under the curve following i.v. administration was roughly 100% when given in aqueous solution and had reached $\sim 40\%$ when given with the diet (26). In a very similar study in Fischer 344 rats, the calculated values of the absorbed fraction of a 0.09 to 0.14 mg/kg dose were in the range of 80% to $>100\%$ when given as a solution, and the absorbed fraction was reduced to $\sim 60\%$ to 80% when given with the food (27). These values, however, are derived from experiments with three mice or three to seven rats per group and/or data points, and show pronounced variability, and therefore, provide only a very preliminary estimate. Data on the toxicokinetics of acrylamide in humans are scarce. The first direct evidence in humans that acrylamide is absorbed from acrylamide-containing food was reported by

Sörgel et al. (17). Two studies in humans with experimental administration of a defined acrylamide dose conducted has thus far suggested extensive absorption of acrylamide: after oral acrylamide administration in drinking water, Fennell et al. (24) reported that (mean) 34% of the dose (3 mg/kg body weight) was excreted within the first day of administration, whereas Boettcher et al. (22) found $\sim 50\%$ after 1 day, and altogether, 57% of the dose after 2 days in one individual. This is close to our results, taking the ongoing elimination of AAMA and GAMA after 24 and/or 48 hours into account (in the present study, the corresponding mean values were 43.3% and 53.5% of the dose excreted after 24 and 48 hours, respectively; see Fig. 3). Further studies with radiolabeled acrylamide in dogs and miniature pigs (29) confirm that independent of the dose, intestinal acrylamide absorption is virtually complete in all mammalian species tested, including humans (6).

Assuming complete absorption, it is tempting to speculate on the fate of the dose fraction which is not recovered in urine in all these studies. This fraction is either transformed to metabolites not quantified in the respective studies, excreted via the bile, or may also remain in the body by formation of adducts to macromolecules. Further studies are needed to clarify this question.

Conversion of Acrylamide to the More Reactive Glycidamide. The major metabolic steps of acrylamide involve the formation of the reactive epoxide glycidamide and subsequent conjugation to glutathione in all species tested (6). In all studies in humans, only minor amounts of unchanged acrylamide and of glycidamide, if any, were detected in the urine. Thus, for an assessment of the extent of glycidamide formation from acrylamide based on urinary recovery, the ratio of the respective phase II metabolites must be considered. In the present study, the mean ratio of GAMA/AAMA amounts excreted was 0.12. In the 3 mg/kg study reported by Fennell et al. (24), glycidamide conjugates were below the limit of quantification, whereas acrylamide conjugate excretion accounted for 26.2% of the dose. Assuming a limit of quantification (which was not given explicitly) of 0.06 mmol/L for both glycidamide conjugates, the mean ratio of glycidamide/acrylamide conjugates was <0.3 . For dietary exposure in humans, Boettcher et al. (23) reported a median ratio of 0.16; the corresponding value for the participant in the experimental study reported by Boettcher et al. (22) was 0.09. In summary, it seems that the conversion of acrylamide to glycidamide in humans is usually slower than that of

Table 1. Toxicokinetic variables of acrylamide, AAMA, and GAMA

Variable (unit)	Time of maximal excretion rate (h)	Maximal excretion rate (nmol/h)	Terminal elimination half-life (h)	Amount excreted up to 72 hours (μmol)	Amount excreted extrapolated to infinity, μmol (% of dose)
Acrylamide					
Mean	3.0	113	2.4	0.59	0.59
SD	0.0	45	0.4	0.19	0.19
Median	3.0	109	2.3	0.63	0.63
Min	2.9	66	2.1	0.29	0.29
Max	3.0	179	3.1	0.81	0.81
AAMA					
Mean	6.9	378	17.4	6.34	6.60
SD	1.8	69	3.9	1.12	1.24
Median	7.0	368	16.8	6.59	6.88
Min	4.9	314	11.4	4.22	4.26
Max	9.0	502	22.9	7.28	7.61
GAMA					
Mean	14.9	20	25.1	0.65	0.77
SD	2.5	4	6.4	0.17	0.16
Median	13.9	21	22.3	0.67	0.78
Min	13.8	14	20.2	0.36	0.48
Max	20.0	24	37.2	0.84	0.96

acrylamide to glutathione conjugates, and that the acrylamide dose has no pronounced effect on the fraction converted to glycidamide.

It is important to compare the extent of glycidamide formation in humans to that in rodents because glycidamide is clearly the more reactive moiety (19), and current cancer risk extrapolation is based on rodent studies. Distinct species differences have been reported between rats and mice concerning the extent of oxidative acrylamide metabolism. In mice, urinary excretion of glycidamide and its metabolites suggests that ~50% to 70% of a 50 mg/kg acrylamide dose given orally as a solution is converted to glycidamide (20, 21). At the same dose in rats, only ~30% to 40% is excreted as glycidamide and glycidamide-derived metabolites, with no major effect of the i.p. or p.o. route of administration (20, 21). Thus, based on urinary excretion, the extent of glycidamide formation in humans is ~2-fold lower than in rats and 4-fold lower than in mice. However, the effects of dose and mode of administration on toxicokinetics of acrylamide need to be taken into account for this comparison. This information may be derived from other studies reporting the formation of hemoglobin adducts or plasma concentrations, although a direct comparison to urinary metabolite excretion is not possible. In a study in rats using 5 to 100 mg/kg acrylamide i.p., based on hemoglobin adduct formation, it was estimated that 51% of a dose was converted to glycidamide, compared with 13% for a 100 mg/kg dose. This dose dependency has been explained by a saturable formation of glycidamide at higher doses in rodents (30). Plasma concentrations were measured by Doerge et al. (26, 27) following administration of a low 0.1 mg/kg dose of acrylamide or glycidamide to rats and mice. In mice, the glycidamide/acrylamide area under the curve ratios were (mean of male and female animals) 0.76, 2.7, and 2.2 following i.v. administration, oral gavage as a solution, or administration with food, respectively (26), whereas the corresponding values in rats were 0.14, 0.77, and 1.0 (27). These data show clearly that (a) lower doses do not result in a decreased formation of glycidamide (and probably are related to an increased glycidamide formation), (b) conversion of acrylamide to glycidamide is more extensive in rats compared with mice, and (c) conversion of acrylamide to glycidamide is more extensive on oral administration than on i.v. administration, caused by first-pass metabolism. Thus, as the design of the present study maximizes glycidamide formation, the assumption (based on urinary metabolite excretion) that average internal exposure towards the epoxide glycidamide in humans is 2-fold lower than in rats and 4-fold lower than in mice when given at the same relative dose and route seems to be a conservative estimate.

Interindividual Variation in Acrylamide Toxicokinetics. In the present study, the interindividual coefficient of variation of acrylamide toxicokinetic variables was ~20% to 30%, with no apparent outliers (Fig. 2). The GAMA/AAMA ratio for overall amounts excreted was very uniform (median, 0.115; range, 0.100-0.150), which is in contrast to that reported for spot urine samples in individuals without the administration of experimental doses of acrylamide (median, 0.16; range, 0.03-0.53; ref. 23). This may be the result of a standardized design in the present study including prohibition of alcohol and smoking, an 11-hour fasting period before dosing, and a diet without acrylamide-rich food in the prestudy period, but probably also depends on the different routes of acrylamide intake from cigarette smoke and food in the study by Boettcher et al. (23). Furthermore, in our study, we observed >20-fold changes of the average GAMA/AAMA ratio depending on the postdose sampling time, indicating that the variable and undefined time lag between exposure and spot urine sampling in the former study may have a major effect on variability of the GAMA/AAMA ratio. Whether diversity in the activity of

CYP2E1 (18, 31) or of glutathione S-transferases in humans have a major effect on the metabolism of acrylamide and thus may put individuals at increased risk for acrylamide carcinogenicity remains to be determined.

Conclusion

In conclusion, most of a 0.94 mg dose of acrylamide ingested with food is absorbed in humans. The predominant metabolite excreted is AAMA, suggesting that detoxification, together with elimination of unchanged acrylamide, is more efficient than formation of the more reactive epoxy metabolite glycidamide. The metabolite pattern is similar to that observed for lower dietary exposure and to experimental high-dose (3 mg/kg) intake in humans. Formation of the glycidamide-derived metabolite, GAMA, is probably at least 2-fold lower than in rats and at least 4-fold lower than in mice at the same relative doses, suggesting that extrapolations of the cancer risk in humans caused by oral acrylamide exposure from studies in rodents should be corrected by these factors.

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