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Research Article

Internal doses of acrylamide and glycidamide in mice fed diets with low acrylamide contents

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The formation of acrylamide during heating of certain foodstuffs constitutes a potential health hazard. The health risk assessment should be based on knowledge about the relation between dietary exposure to acrylamide and internal doses of acrylamide and its genotoxic metabolite glycidamide. The primary aim of this study in mice was to measure these relationships at low levels of acrylamide intake through the diet. A secondary aim was to clarify which extraction method should be used when analyzing acrylamide in food in order to obtain a correct measure of the acrylamide that is available for absorption. In the analysis procedure, alkaline extraction has earlier shown much higher measured acrylamide levels in certain foods compared to water extraction. In this subcronic study the administered diets were composed to give five levels of acrylamide intakes between 3 and 50 μg/kg body weight *per* day (calculated on figures obtained after water extraction). Internal doses of acrylamide and glycidamide were measured through hemoglobin (Hb)-adducts. The results showed linear relationships between the exposure of acrylamide and Hb-adduct levels from both acrylamide and glycidamide at these low exposure levels. The study also showed that the "extra" acrylamide measured with alkaline extraction does not correspond to bioavailable acrylamide.

Keywords: Acrylamide / Diet / Glycidamide / Hemoglobin adducts / Internal dose

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1 Introduction

The finding [1, 2] that acrylamide is formed during heating of food initiated a broad range of research. Acrylamide is a neurotoxic chemical, carcinogenic in laboratory animals and classified as a probable human carcinogen [3, 4]. It is shown to form a genotoxic epoxy metabolite, glycidamide, in laboratory animals and in humans [5 – 7]. Acrylamide was shown to occur in many foods, and in certain products at levels up to mg/kg [2, 8]. In many countries foods have been characterized with regard to acrylamide contents (*e. g.*, IRMM, http://www.irmm.jrc.be/html/activities/acrylamide/database.htm, 2005), and such data together with information from surveys on dietary habits, have been used to estimate the acrylamide intakes in different populations [9 – 11]. For the purpose of health risk estimation, however, the intake of acrylamide has to be related to the internal doses of both acrylamide and glycidamide, which could be inferred from hemoglobin (Hb)-adduct levels [12]. Studies of the relation between estimated dietary intake of acrylamide from questionnaires and the corresponding Hb-adduct levels in human blood samples show that the intake estimates are encumbered with uncertainties [13 – 16].

In studies in rodents nonlinearity between exposure dose of acrylamide and the internal doses of acrylamide and glycidamide has been observed at high exposure doses, administered through i.p. injections [5, 17]. This particularly concerns the formation of glycidamide from administered acrylamide, assumed to be due to saturation of the P450 system involved in the oxygenation [18]. In humans, a nonlinear relationship between the internal doses of acrylamide and glycidamide has been indicated at low levels of exposure to acrylamide [19, 20].

In this subchronic study acrylamide at low daily doses is administered to mice through diet. The primary aim is to determine the relationship between the intake and the internal dose of acrylamide; and between the internal doses of acrylamide and glycidamide. The different administered doses of acrylamide are obtained through heating of

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Abbreviations: bw, body weight; **Hb,** hemoglobin

selected food components, which then are mixed with laboratory animal feed.

A factor with possible influence on the estimated intakes of acrylamide concerns differences in measured acrylamide contents, which depend on the chosen analytical procedure. The content of acrylamide is usually measured as "water-soluble free acrylamide" after extraction of the foodstuff with water (pH 5 – 7 during extraction) followed by liquid chromatographic separation and mass spectrometric detection (*e. g.*, [2, 21]), a methodology which has shown comparable results between different laboratories [22, 23]. In a study of alternative extraction techniques Eriksson and Karlsson [24] found that extraction at alkaline pH (pH $\geq$ 12) could result in up to three times higher levels of acrylamide than the commonly used water extraction, especially in the analysis of fiber-rich foodstuffs. The higher acrylamide content obtained with the alkaline extraction method might correspond to acrylamide that is available for absorption. This would then mean that estimates of the intake based on the commonly used water extraction method would not be correct. Therefore, the present study is also designed to elucidate whether the acrylamide obtained with alkaline extraction contributes to the internal dose.

2 Materials and methods

2.1 Chemicals

Pentafluorophenyl isothiocyanate (PFPITC) (CAS 35923-79-6) (>95%), obtained from Fluka (Buchs, Switzerland) was purified on a Sep-Pak silica cartridge [25]. Myoglobin, from horse skeletal muscle, from Sigma – Aldrich Chemie (Schnelldorf, Germany), was precipitated in acidic acetone solution. Formamide (CAS 75-12-7) from Scharlau Chemie S.A. (Barcelona, Spain) was purified by extraction with *n*-pentane (CAS 109-66-0) prior to use. All other chemicals used were of analytical grade. Standard compounds used for identification and quantification of Hb-adducts were gifts from Dr. E. Bergmark or synthesized according to Paulsson *et al.* [26].

2.2 Instrumentation

The bread products were prepared by using a drying oven (Termaks TS4115) and grounding equipment (Retsch ZM 100, with sieve 0.75 mm). The potato powder was heated in a GC temperature-programmed oven (Hewlett – Packard 5790A). Analysis of the food products and the special diets was performed by a LC-MS/MS (Agilent 1100, Micromass Quattro Premier) according to Eriksson and Karlsson [24]. Hb-adducts were analyzed with GC-MS/MS technique (Varian 3400, Finnigan TSQ700).

2.3 Animal treatment

Thirty male C57BL mice, 5 – 8 wk old with average body weight (bw) of 26 g, supplied from Scanbur-BK (Sollentuna, Sweden) and the Wenner-Gren Institute, Stockholm University were divided into five groups (six mice *per* group) and fed special diets for 40 days. Four groups were given two different diets (A or B) at two acrylamide intake levels ("low-dose" and "high-dose"). A control group was given laboratory animal feed (known to contain a low level of acrylamide). The animals were kept in individual cages (at the animal house at Stockholm University). The mice were weighed every tenth day of the experiment. The study was reviewed and approved by the Ethical Committee on Animal Experiments of Stockholm, Sweden, application nr N/56/02.

2.4 Diet preparation

The diets were composed to give as low exposure doses of acrylamide as possible, yet sufficiently high to give significant difference in the adduct levels from acrylamide and glycidamide between the test groups of mice and compared to the control group. Our hypothesis was that the measured free acrylamide obtained through the alkaline extraction is equally available for absorption as the acrylamide measured after common water extraction. To test this hypothesis, two diets (A and B) were composed so that the calculated acrylamide intake based on alkaline-extraction for both test diets and dose groups should be similar (see Table 1). The compositions also implied equal energy levels for the diets.

All diets were based on grounded laboratory animal feed (RMI (E) FG SQC, from Special Diets Services, Witham, UK). The tests diets were prepared by mixing laboratory animal feed together with the selected food products (Table 2). Diet A contained heated (170°C for 15 min), commercially available mashed-potato powder and French loaf bread dried at 65°C (overnight). Diet B contained soft whole kernel bread, dried at 65°C (overnight). Prior to the diet preparations, the selected food products were analyzed by LC-MS/MS with regard to the acrylamide content obtained by common water extraction or by alkaline (pH = 12) extraction [24]. The contents were with common water extraction and alkaline extraction, respectively: (i) Dried whole kernel bread: 0.3 and 0.9 mg acrylamide/kg. (ii) Heated potato powder: 11 and 14 mg acrylamide/kg. (iii) French loaf bread ~10 μg acrylamide/kg (only analyzed with common water extraction). All diets (including the one for the control group) were prepared by mixing the grounded components with water; the mix was then formed to a cake that was dried at room temperature. The diets were prepared and analyzed four times during the experiment.

Table 1. Acrylamide content in the diets obtained after common water extraction and alkaline extraction, respectively

		Acrylamide content (μg/kg dry weight)[a)]	
		Water extraction	Alkaline extraction
Controls	Standard diet	20 (7)	49 (4)
Low dose	Diet A	120 (10)	166 (12)
	Diet B	70 (10)	164 (22)
High dose	Diet A	334 (20)	401 (13)
	Diet B	157 (17)	375 (23)

a) Mean values from the analyses of the diets prepared four times during the study. SD in parenthesis.

2.5 Hb-adduct analysis

The mice were sacrificed and blood (~1 mL) was collected and prepared for analysis of Hb-adducts according to the *N*-alkyl Edman method [27]. Samples were prepared as described by Paulsson *et al.* [26] for simultaneous analysis of acrylamide and glycidamide adducts, with some modifications due to low amounts of precipitated globin obtained from the mouse blood samples (14 – 30 mg). Myoglobin (5 – 16 mg) was added when necessary to make 30 mg globin samples (myoglobin gives no contribution to the measured adduct levels) [25]. The globin samples were dissolved in formamide (1 mL) and derivatized with PFPITC (5 μL) after addition of 1 M NaOH (30 μL). The detached derivatives, the pentafluorophenylthiohydantoins (PFPTHs) of *N*-(2-carbamoylethyl)-valine from acrylamide, and *N*-(carbamoyl-2-hydroxyethyl)-valine from glycidamide were isolated and purified by extraction. Corresponding deuterium-substituted PFPTH analytes, synthesized from ($^2H_7$)valine, were used as internal standards, and reference globins with known adduct levels from acrylamide and glycidamide to *N*-terminal valine were used for calibration [28]. The calibration curves ($R^2 = 0.998$, for both) included six samples in the range of 0 – 400 pmol/g globin for acrylamide and 0 – 800 pmol/g globin for glycidamide. After evaporation the samples were dissolved in 30 μL toluene and 1 μL was injected on the GC-MS/MS for analysis according to the conditions described in Paulsson *et al*. [26]. The LOQ was estimated to < 1 pmol/g globin for acrylamide and 6 pmol/g globin for glycidamide. The feeding was carried out over the lifetime of erythrocytes (t_{er}) in mice (40 days) to exclude an influence on the measured Hb-adduct levels of the acrylamide intake from the standard diet before start of the experiment [29]. After a continuous exposure for this time the Hb-adduct levels have reached a steady-state level (A_{ss}), which is used for the calculation of daily adduct level increment (a) according to: $a = A_{ss}/(t_{er}/2)$ [30]. The daily adduct level increment is directly proportional to the daily "Area Under the Concentration Curve," that is the internal dose and the rate of formation of the specific adducts from the compound studied [12]. Thus the daily adduct level increment from acrylamide and from glycidamide, respectively, is then calculated as a measure of the internal dose from the respective compound (such calculations also facilitate comparisons with other studies with regard to adduct level increment *per* administered dose).

Table 2. Composition of diets: percentage of the different components in the feeds

		Standard animal feed (%)	Whole kernel bread (%)	Potato powder (heated) (%)	French loaf bread (%)
Standard diet		100	0	0	0
Low dose diet	A	85	0	1	14
	B	85	15	0	0
High dose diet	A	55	0	3	42
	B	55	45	0	0

3 Results

3.1 Diet analyses

The test diets and the laboratory animal feed were analyzed with regard to acrylamide content, with both common water extraction and alkaline extraction [24] (Table 1). As expected Diet B, with a high content of fiber-rich whole kernel bread, gave considerably higher measured acrylamide levels after the alkaline extraction. The calculation of the acrylamide intake (see Table 3) was based on the measured acrylamide content in the diets and an average feed intake for mouse of 4 g *per* day. The average increase in bws in mice during the study was 3.5 g with no significant difference between the groups (not shown).

3.2 Hb-adduct levels

Analysis of blood samples from the mice of both diet groups showed significant increases in both acrylamide- and glycidamide-adduct levels with increasing intake of acrylamide. In Table 3 the calculated intake and the average adduct levels in the samples from each group are presented.

In Fig. 1A and B the daily increase in acrylamide-adduct levels is presented as a function of the daily intake of acrylamide in the diets, calculated from the acrylamide content measured after extraction with both extraction methods. Plots of the daily adduct increment *versus* daily intake, show different slopes for the two diets when the intake was based on the alkaline extraction method (Fig. 1A). The curve for the Diet A showed a higher slope compared to the curve for the Diet B (with fiber-rich bread). The plots for the intakes calculated on acrylamide measured after common water extraction showed nearly equivalent slopes for the two diets (Fig. 1B).

Table 3. Calculated daily intake of acrylamide and measured Hb-adduct levels of acrylamide and glycidamide in each animal group

		Acrylamide intake[a] (μg/kg bw *per* day)		Measured Hb-adduct level[b] (pmol/g globin)	
		Water extraction	Alkaline extraction	Acrylamide	Glycidamide
Controls	Std diet	3	7	12 (1)	42 (6)
Low dose	Diet A	18	26	34 (2)	184 (39)
	Diet B	11	25	20 (1)	85 (12)
High dose	Diet A	51	61	68 (4)	396 (39)
	Diet B	23	58	36 (3)	171 (8)

a) Based on acrylamide contents obtained with the different extraction procedures.
b) Mean values from each group of mice ($n = 6$, except for the group High dose, Diet B, where $n = 5$ as samples from two mice were pooled). SD in parenthesis.

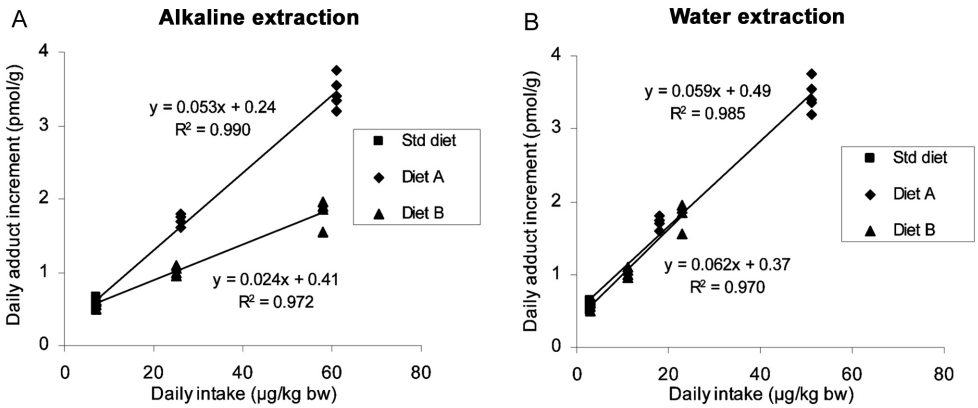

Figure 1. Relationships between estimated daily acrylamide intake and the daily increase in acrylamide-adduct levels in Hb when the intake was inferred from analysis after alkaline extraction (A), and from analysis after common water extraction (B).

Furthermore, a strong correlation ($R^2 = 0.98$, not shown) was found between the acrylamide-adduct levels of all animals and the acrylamide-intakes calculated from the analysis after common water extraction. Analysis of the observed acrylamide-adduct levels with a multiple regression model, simultaneously including the intake calculated after both common and alkaline extraction, showed no significant contribution from the "extra" acrylamide measured after alkaline extraction ($P = 0.93$). These results reject the hypothesis that the increased acrylamide content measured after alkaline extraction is bioavailable.

The primary aim of the study concerned the metabolism of acrylamide to glycidamide at these low exposure levels of acrylamide in the diet. The measurement of Hb-adducts showed that from the lowest dose it was a linear relationship between the glycidamide adduct levels and acrylamide adduct levels as shown in Fig. 2. The ratio of the adduct levels from glycidamide and acrylamide then corresponds to a slope of 6.2.

4 Discussion

This study presents data on Hb-adduct levels from acrylamide and from the metabolite glycidamide, as a measurement of internal doses, in a subchronic feeding experiment in mice at low exposure levels. In the experimental design we considered the earlier finding that extraction at pH ~ 12 in the analysis of certain foodstuffs results in much higher values of measured acrylamide. The results showed that, the extra acrylamide obtained with the alkaline extraction procedure gave no contribution to the internal dose of acrylamide measured as Hb-adduct levels (Table 3 and Fig. 1A and B). This means that for the different diets the common water extraction gives a good measurement of the acrylamide that is available for absorption. Then this also indicates the same bioavailability of acrylamide in the relatively fiber-rich diet, "Diet B, High dose" compared to the other diets.

The extra acrylamide observed by Eriksson and Karlsson [24] has in later studies been suggested to be formed through two different precursors. One study claimed that 3-aminopropionamide is a possible precursor for acrylamide formation under alkaline conditions [31]. Recently it was instead suggested the food heat-generated Amadori product *N*-(1-deoxy-D-fructos-1-yl)-3′-aminopropionamide is converted to acrylamide if exposed to alkaline conditions and that this is the main source for the formed acrylamide [32].

The content of acrylamide in the standard feed was measured to 3 μg/kg (relatively large SD, *cf*. Table 1), thus the

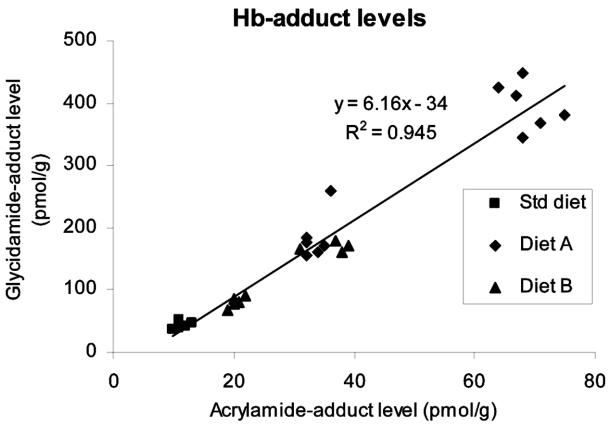

Figure 2. Glycidamide-adduct levels in Hb as a function of acrylamide-adduct levels in pmol/g globin.

control animals correspond to a low-exposure group. The steady-state level of the Hb-adduct from acrylamide in the control animals was 12 pmol/g. Corresponding background acrylamide-adduct levels in mice and rats between 5 and 170 pmol/g have been found in other studies [1, 33–36]. These background levels are expected to mainly originate from acrylamide in the diet. However, in Table 3 and Fig. 1B it is indicated that the adduct increment *per* μg acrylamide intake is relatively higher in the control mice compared to the adduct increment at higher doses. This might be explained by an unknown exposure source, with a measurable impact only on the incremental adduct levels of the control group. Even though acrylamide has not been proved to be formed endogenously, this cannot be excluded. For certain other compounds, like ethylene oxide, background-adduct levels due to endogenous formation of the precursor electrophile has been demonstrated [37–40].

In the present study acrylamide was administered to mice through diet at five dose levels between 3 and 50 μg/kg bw *per* day, which is relatively close to the estimated mean intakes of dietary acrylamide in humans, 0.3–2.0 μg/kg bw *per* day [41]. Hb-adduct levels from acrylamide showed a linear increase with acrylamide intake, and the relationship between the adduct levels from glycidamide and acrylamide was linear as well. The obtained ratio of 6.2 (Fig. 2), could be compared with the corresponding adduct ratio obtained in other studies. Mice treated with i.p. injections with doses from 25 to 100 mg acrylamide/kg bw showed a decrease in the ratio from 3 to 1 [34]. No such obvious deviation from linearity could be observed in the present study. The study, however, confirm that the metabolism to glycidamide is more effective in the mouse at low exposure levels through diet than at the much higher dose rates obtained from i.p. injections at high doses. As follows from the faster metabolism to glycidamide, the acrylamide adduct increment *per* administered dose of acrylamide is lower in the present low-dose oral study (Fig. 1B) compared to our previous study [34] with treatment *via* i.p. (4 and 9–20 nmol acrylamide adducts/g Hb *per* mmol acrylamide/kg bw, respectively).

Tareke *et al*. [42] have administered acrylamide at low levels through diet and gavage (at a single exposure doses of 0.1 mg/kg bw) and drinking water (at about 3 mg/kg bw and day) to mice. The obtained glycidamide to acrylamide adduct ratio was about two times higher (adduct-levels estimated from figures in the paper) in the drinking water experiment than in the low single (diet and gavage) exposure doses. The adduct ratio obtained for the low single exposure doses by Tareke *et al*. [42] is compatible to that of this present study (different analytical set-up and mouse strain). A study in swine with exposure to acrylamide through the drinking water at 0.8 or 8 μg/kg bw during 142 days (in the range of the lowest doses in the present study) showed a direct proportionality between Hb-adduct levels from acrylamide and the intake [43]. Glycidamide adduct-levels were below LOQ in the study.

The dietary acrylamide intake in humans has been estimated to about 0.5 μg/kg bw and day [44]. Rather few studies of "background" acrylamide exposure include analysis of Hb-adducts from both acrylamide and glycidamide [13, 19, 20, 26, 45]. In the most comprehensive study ($n = 96$; smokers and nonsmokers) the results showed acrylamide-adduct levels in the range of 27–453 pmol/g globin. It was indicated that the metabolism of acrylamide to glycidamide is more effective at low doses [20], as earlier indicated in a smaller study ($n = 29$) [19]. With regard to cancer risk estimation of acrylamide it is important that this observation could be rejected or verified in studies of a large number of nonsmoking humans. In the present study in mice no obvious difference in the metabolism of acrylamide at the exposure levels from 50 down to 3 μg acrylamide/kg bw could be verified.

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The authors have declared no conflict of interest.

5 References

[1] Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Törnqvist, M., Acrylamide: A cooking carcinogen? *Chem. Res. Toxicol.* 2000, *13*, 517–522.

[2] Tareke, E., Rydberg, P., Karlsson, P., Eriksson, S., Törnqvist, M., Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* 2002, *50*, 4998–5006.

[3] EC, *European Union Risk Assessment Report*, Acrylamide, Risk Assessment, Office for Official Publications of the European Communities, Luxembourg 2002.

[4] IARC, Acrylamide, in: *Monographs on the Evaluation of Carcinogen Risk to Humans: Some Industrial Chemicals*, no 60, International Agency for Research on Cancer, Lyon 1994, pp. 389 – 443.

[5] Bergmark, E., Calleman, C. J., Costa, L., Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. *Toxicol. Appl. Pharmacol.* 1991, *111*, 352 – 363.

[6] Calleman, C. J., Bergmark, E., Costa, L. G., Acrylamide is metabolized to glycidamide in the rat: Evidence from hemoglobin adduct formation. *Chem. Res. Toxicol.* 1990, *3*, 406 – 412.

[7] Segerbäck, D., Calleman, C. J., Schroeder, J. L., Costa, L. G., Faustman, E. M., Formation of *N*-7-(2-carbamoyl-2-hydroxyethyl) guanine in DNA of the mouse and the rat following intraperitoneal administration of [^{14}C]acrylamide. *Carcinogenesis* 1995, *16*, 1161 – 1165.

[8] Rosén, J., Hällenäs, K.-E., Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. *Analyst* 2002, *127*, 880 – 882.

[9] Dybing, E., Sanner, T., Risk assessment of acrylamide in foods. *Toxicol. Sci.* 2003, *75*, 7 – 15.

[10] Konings, E. J. M., Baars, A. J., van Klaveren, J. D., Spanjer, M. C. *et al.*, Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risk. *Food Chem. Toxicol.* 2003, *41*, 1569 – 1579.

[11] Svensson, K., Abramsson, L., Becker, W., Glynn, A., *et al*., Dietary intake of acrylamide in Sweden. *Food Chem. Toxicol.* 2003, *41*, 1581 – 1586.

[12] Törnqvist, M., Fred, C., Haglund, J., Helleberg, H., *et al.*, Protein adducts: Quantitative and qualitative aspects of their formation, analysis and applications. *J. Chromatogr. B* 2002, *778*, 279 – 308.

[13] Bjellaas, T., Olesen, P. T., Frandsen, H., Haugen, M., *et al.*, Comparison of estimated dietary intake of acrylamide with haemoglobin adducts of acrylamide and glycidamide. *Toxicol. Sci.* 2007, *98*, 110 – 117.

[14] Hagmar, L., Wirfält, E., Paulsson, B., Törnqvist, M., Differences in hemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender. *Mutat. Res.* 2005, *580*, 157 – 165.

[15] Kütting, B., Schettgen, T., Beckmann, M. W., Angerer, J., Drexler, H., Influence of diet on exposure to acrylamide – reflections on the validity of a questionnaire. *Ann. Nutr. Metab.* 2005, *49*, 173 – 177.

[16] Wirfält, E., Paulsson, B., Törnqvist, M., Axmon, A., Hagmar, L., Associations between estimated acrylamide intakes and hemoglobin AA-adducts in a sample from the Malmö Diet and Cancer cohort. *Eur. J. Clin. Nutr.* 2008, *62*, 314 – 323.

[17] Paulsson, B., Kotova, N., Grawé, J., Henderson, A., *et al.*, Induction of micronuclei in mouse and rat by glycidamide, genotoxic metabolite of acrylamide. *Mutat. Res.* 2003, *535*, 15 – 24.

[18] Sumner, S. C. J., Fennell, T. R., Moore, T. A., Chanas, B., *et al.*, Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem. Res. Toxicol.* 1999, *12*, 1110 – 1116.

[19] Schettgen, T., Rossbach, B., Kütting, B., Letzel, S., *et al.*, Determination of haemoglobin adducts of acrylamide and glycidamide in smoking and nonsmoking persons of the general population. *Int. J. Hyg. Environ. Health* 2004, *207*, 531 – 539.

[20] Vesper, H. W., Ospina, M., Meyers, T., Ingham, L., *et al.*, Automated method for measuring globin adducts of acrylamide and glycidamide at optimized Edman reaction conditions. *Rapid Commun. Mass Spectrom.* 2006, *20*, 959 – 964.

[21] Castle, L., Eriksson, S., Analytical methods used to measure acrylamide concentrations in foods. *J. AOAC Int.* 2005, *88*, 274 – 284.

[22] Klaffke, H., Fauhl, C., Mathar, W., Palavinskas, R., *et al.*, Results from two interlaboratory comparison tests organized in Germany and at the EU level for analysis of acrylamide in food. *J. AOAC Int.* 2005, *88*, 292 – 298.

[23] Owen, L. M., Castle, L., Kelly, J., Lloyd, A. S., *et al.*, Acrylamide analysis: Assessment of result from six rounds of food analysis performance assessment scheme (FAPAS) proficiency testing. *J. AOAC Int.* 2005, *88*, 285 – 291.

[24] Eriksson, S., Karlsson, P., Alternative extraction techniques for analysis of acrylamide in food: Influence of pH and digestive enzymes. *LWT – Food Sci. Technol.* 2006, *39*, 393 – 399.

[25] Törnqvist, M., in: Everse, J., Vandegriff, K. D., Winslow, R. W. (Eds.) *Methods in Enzymology*, Academic Press, New York 1994, pp. 650 – 657.

[26] Paulsson, B., Athanassiadis, I., Rydberg, P., Törnqvist, M., Hemoglobin adducts from glycidamide: Acetonization of hydrophilic groups for reproducible gas chromatography/tandem mass spectrometric analysis. *Rapid Commun. Mass Spectrom.* 2003, *17*, 1859 – 1865.

[27] Törnqvist, M., Mowrer, J., Jensen, S., Ehrenberg, L., Monitoring of environmental cancer initiators through hemoglobin adducts by a modified Edman degradation method. *Anal. Biochem.* 1986, *154*, 255 – 266.

[28] Bergmark, E., Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers, and nonsmokers. *Chem. Res. Toxicol.* 1997, *10*, 78 – 84.

[29] Horky, J., Vacha, J., Znojil, V., Comparison of life span of erythrocytes in some inbred strains of mouse using 14C-labelled glycine. *Physiol. Bohemoslov.* 1978, *27*, 209 – 217.

[30] Granath, F., Ehrenberg, L., Törnqvist, M., Degree of alkylation of macromolecules in vivo from variable exposure. *Mutat. Res.* 1992, *284*, 297 – 306.

[31] Goldmann, T., Perisset, A., Bertholet, M.-C., Stadler, R. H., *et al.*, Impact of extraction conditions on the content of acrylamide in model systems and food. *Food Addit. Contam.* 2006, *23*, 437 – 445.

[32] Yaylayan, V. A., *Heat and pH induced generation of acrylamide from N-(1-deoxy-D-fructos-1-yl)-3′-aminopropionamide*, American Chemical Society (ACS) 2007, 234th ACS National Meeting.

[33] Fennell, T. R., Snyder, R. W., Krol, W. L., Sumner, S. C. J., Comparison of the hemoglobin adducts formed by administration of *N*-methylolacrylamide and acrylamide to rats. *Toxicol. Sci.* 2003, *71*, 164 – 175.

[34] Paulsson, B., Grawé, J., Törnqvist, M., Hemoglobin adducts and micronucleus frequencies in mouse and rat after acrylamide or *N*-methylolacrylamide treatment. *Mutat. Res.* 2002, *516*, 101 – 111.

[35] Sumner, S. C. J., Williams, C. C., Snyder, R. W., Krol, W. L. *et al.*, Acrylamide: A comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal, oral, or inhalation exposure. *Toxicol. Sci.* 2003, *75*, 260 – 270.

[36] Twaddle, N. C., Churchwell, M. I., McDaniel, L. P., Doerge, D. R., Autoclave sterilization produces acrylamide in rodent diets: Implications for toxicity testing. *J. Agric. Food Chem.* 2004, *52*, 4344 – 4349.

[37] Törnqvist, M., Gustavsson, B., Kautiainen, A., Harms-Ringdahl, M. *et al.*, Unsaturated lipids and intestinal bacteria as sources of endogenous production of ethene and ethylene oxide. *Carcinogenesis* 1989, *10,* 39 – 41.

[38] Filser, J. G., Denk, B., Törnqvist, M., Kesser, W., Ehrenberg, L., Pharmacokinetics of ethylene in man; body burden with ethylene oxide and hydroxyethylation of hemoglobin to endogenous and environmental ethylene. *Arch. Toxicol.* 1992, *66*, 157 – 163.

[39] Törnqvist, M., Kautiainen, A., Adducted proteins for identification of endogenous electrophiles. *Environ. Health Perspect.* 1993, *99*, 39 – 44.

[40] Törnqvist, M., in: Snyder, R. (Ed.), *Biological Reactive Intermediates V*, Plenum Press, New York 1996, pp. 275 – 283.

[41] WHO Joint FAO (Food and Agriculture Organization of the United Nations)/WHO (World Health Organisation) Expert Committee on Food Additives, JECFA/64/SC, 2005, pp. 7 – 17.

[42] Tareke, E., Twaddle, N. C., McDaniel, L. P., Churchwell, M. I. *et al.*, Relationships between biomarkers of exposure and toxicokinetics in Fischer 344 rats and B6C3F$_1$ mice administered single doses of acrylamide and glycidamide and multiple doses of acrylamide. *Toxicol. Appl. Pharmacol.* 2006, *217*, 63 – 75.

[43] Aureli, F., Di Pasquale, M., Lucchetti, D., Aureli, P., Coni, E., An absorption study of dietary administered acrylamide in swine. *Food Chem. Toxicol.* 2007, *45,* 1202 – 1209.

[44] Dybing, E., Farmer, P. B., Andersen, M., Fennell, T. R., *et al.*, ILSI Report: Human exposure and internal dose assessments of acrylamide in food. *Food Chem. Toxicol.* 2005, *43*, 365 – 410.

[45] Chevolleau, S., Jacques, C., Canlet, C., Tulliez, J., *et al.*, Analysis of hemoglobin adducts of acrylamide and glycidamide by liquid chromatography-elecrospray ionization tandem mass spectrometry, as exposure biomarkers in French population. *J. Chromatogr. A* 2007, *1167*, 125 – 134.

