

Chemistry, Biochemistry, and Safety of Acrylamide. A Review

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Acrylamide ($\text{CH}_2=\text{CH}-\text{CONH}_2$), an industrially produced α,β -unsaturated (conjugated) reactive molecule, is used worldwide to synthesize polyacrylamide. Polyacrylamide has found numerous applications as a soil conditioner, in wastewater treatment, in the cosmetic, paper, and textile industries, and in the laboratory as a solid support for the separation of proteins by electrophoresis. Because of the potential of exposure to acrylamide, effects of acrylamide in cells, tissues, animals, and humans have been extensively studied. Reports that acrylamide is present in foods formed during their processing under conditions that also induce the formation of Maillard browning products heightened interest in the chemistry, biochemistry, and safety of this vinyl compound. Because exposure of humans to acrylamide can come from both external sources and the diet, a need exists to develop a better understanding of its formation and distribution in food and its role in human health. To contribute to this effort, this integrated review presents data on the chemistry, analysis, metabolism, pharmacology, and toxicology of acrylamide. Specifically covered are the following aspects: nonfood and food sources; exposure from the environment and the diet; mechanism of formation in food from asparagine and glucose; asparagine–asparaginase relationships; Maillard browning–acrylamide relationships; quenching of protein fluorescence; biological alkylation of amino acids, peptides, proteins, and DNA by acrylamide and its epoxide metabolite glycidamide; risk assessment; neurotoxicity, reproductive toxicity, and carcinogenicity; protection against adverse effects; and possible approaches to reducing levels in food. Further research needs in each of these areas are suggested. Neurotoxicity appears to be the only documented effect of acrylamide in human epidemiological studies; reproductive toxicity, genotoxicity/clastogenicity, and carcinogenicity are potential human health risks on the basis of only animal studies. A better understanding of the chemistry and biology of pure acrylamide in general and its impact in a food matrix in particular can lead to the development of improved food processes to decrease the acrylamide content of the diet.

Keywords: Acrylamide; glycidamide; polyacrylamide; asparagine; food chemistry; food processing; food safety; Maillard reactions; carcinogenicity; developmental toxicity; genotoxicity; hemoglobin adducts; DNA adducts; neurotoxicity; risk assessment

INTRODUCTION

Beginning in 1964 we published a series of studies on the kinetic, synthetic, and mechanistic aspects of Michael-type nucleophilic addition reactions of amino (NH_2) and sulfhydryl (SH) groups of amino acids, peptides, and proteins to the double bond of acrylamide and related conjugated vinyl compounds (1–22). **Tables 1–3** summarize some of our findings. The summary of reaction rates of the NH_2 groups of the model compounds glycine and diglycine with acrylamide and several other vinyl compounds indicates that the rates with acrylamide are much lower than those observed with the other vinyl compounds. **Table 3** and **Figure 1** show that under the cited conditions, acrylamide can selectively modify the SH groups of bovine serum albumin and wheat gluten. Our observations suggested that acrylamide could exert its biological effects

Table 1. Second-Order Rate Constants ($k_2 \times 10^4$ in L/mol/s) for Reaction of the NH_2 Groups of Diglycine ($\text{NH}_2-\text{CH}_2\text{CONHCH}_2\text{COOH}$) and Glycine ($\text{NH}_2-\text{CH}_2\text{COOH}$) with Conjugated Vinyl Compounds at pH 8.4 at 30 °C^a

vinyl compound	diglycine	glycine
$\text{CH}_2=\text{CH}-\text{CONH}_2$ (acrylamide)	1.3	0.49
$\text{CH}_2=\text{CH}-\text{CON}(\text{CH}_3)_2$ (<i>N,N</i> -dimethylacrylamide)	0.21	0.072
$\text{CH}_2=\text{CH}-\text{CN}$ (acrylonitrile)	9.5	3.9
$\text{CH}_2=\text{CH}-\text{SO}_2\text{CH}_3$ (methyl vinyl sulfone)	62.7	

^a Adapted from refs 5 and 6.

by analogous modifications of amino acids, peptides, and proteins in vivo.

At about the same time, studies began appearing on the biological manifestations of acrylamide in cells, tissues, and animals (23). These studies stimulated interest in understanding the chemical basis for these biological effects. Not surprisingly,

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Table 2. Second-Order Anion Rate Constants (k_A^-) for the Reaction of the NH_2 Groups of Glycine with Conjugated Vinyl Compounds at pH 8.4 at 30 °C^a

vinyl compound	$k_A^- \times 10^4$, L/mol/s
$\text{CH}_2=\text{CH}-\text{CONH}_2$ (acrylamide)	6.3
$\text{CH}_2=\text{CH}-\text{PO}(\text{OCH}_2\text{CH}_2\text{Cl})_2$ [bis(2-chloroethyl) vinylphosphonate]	20.9
$\text{CH}_2=\text{CH}-\text{CN}$ (acrylonitrile)	50.0
$\text{CH}_2=\text{CH}-\text{SO}_2\text{CH}_3$ (methyl vinyl sulfone)	306.0
$\text{CH}_2=\text{CH}-\text{COCH}_3$ (methyl vinyl ketone)	4000.0

^a See refs 1 and 3–6 for the derivation and significance of the pH-independent anion rate constants.

Table 3. Amino Acid Composition of Untreated and Alkylated BSA and Wheat Gluten at pH 7 and 30 °C^a

amino acid	native BSA	BSA + acrylamide, 90 min	native wheat gluten	gluten + acrylamide, 90 min
lysine	1.36	1.36	0.27	0.28
histidine	0.34	0.32	0.49	0.50
arginine	0.47	0.48	0.66	0.67
aspartic acid	1.19	1.18	0.72	0.79
threonine	0.73	0.74	0.87	0.87
serine	0.63	0.63	2.07	2.16
glutamic acid	1.77	1.79	12.1	12.5
proline	0.61	0.68	5.42	5.36
glycine	0.34	0.35	1.71	1.72
alanine	1.00	1.00	1.00	1.00
cysteine	0.44	0.0	0.64	0.0
valine	0.73	0.78	1.35	1.37
isoleucine	0.27	0.28	1.12	1.14
leucine	1.26	1.32	2.21	2.19
tyrosine	0.36	0.34	0.74	0.77
phenylalanine	0.54	0.56	1.24	1.25

^a Values are ratios to alanine. Adapted from refs 5 and 6.

it turned out that the reactions we observed in vitro were also occurring in vivo. These include alkylation of nonprotein SH groups such as that of reduced glutathione (GSH) and protein SH groups as well as modification of NH_2 groups of proteins and nucleic acids.

To cross-fertilize information among several disciplines wherein an interest in acrylamide has developed (including soil science, environmental science, plant science, food science, microbiology, nutrition, pharmacology, toxicology, and medicine), this review attempts to integrate and correlate the widely scattered literature on the formation, analysis, and reactions of acrylamide in relation to its biological properties. Specifically covered are the following relevant aspects: mechanisms of formation and distribution in food; role of asparagine in the plant and in acrylamide formation; other sources of acrylamide; risk assessment; reactions of both acrylamide and its epoxide metabolite glycidamide with amino acids, peptides, proteins, and nucleic acids; quenching of protein fluorescence; preventing formation; metabolism; neurotoxicity; genotoxicity; and carcinogenicity. Suggestions for future research to better define fundamental and applied aspects of acrylamide chemistry, biochemistry, and safety and to catalyze progress in minimizing possible adverse effects are also offered. Understanding the chemistry of formation of acrylamide during food processing and its reactions both in vitro and in vivo may make it possible to design effective means to prevent or arrest undesirable consequences of acrylamide in the diet.

ACRYLAMIDE AND POLYACRYLAMIDE IN THE WORKPLACE

Acrylamide (2-propenamide) is a colorless and odorless crystalline solid with a melting point of 84.5 °C and is formed from the hydration of acrylonitrile. The compound is soluble in water, acetone, and ethanol, has a high mobility in soil and groundwater, and is biodegradable (24, 25). It is used as a cement binder and in the synthesis of polymers and gels. Polyacrylamide polymers and copolymers are used in the paper and textile industries, as flocculants in the treatment of wastewater, as soil conditioners, in ore processing, and in cosmetics.

Acrylamide is also widely used in scientific research to selectively modify SH groups in structural and functional proteins and as a quencher of tryptophan fluorescence in studies designed to elucidate the structure and function of proteins. Polyacrylamide gels are used in the research laboratory to separate proteins and other compounds by electrophoresis. Other sources of acrylamide include acrylamide entrapped in polyacrylamide, depolymerized polyacrylamide in the soil and in food packaging, microbial enzyme-catalyzed transformation of acrylonitrile to acrylamide, and tobacco smoke. Some of these will be briefly examined below.

Degradation of Polyacrylamide to Acrylamide. As mentioned, acrylamide polymers and copolymers are reported to have numerous applications, especially as soil conditioners to reduce soil erosion and in wastewater treatment as flocculants to improve the process of sludge thickening and dewatering. Other uses include mixing with pesticides as a thickening agent and as a medium for hydroponically grown crops, in sugar refining (26, 27), and as a binder of bone cement (28). In the United States, such polymers are estimated to comprise 0.5–1% of the sludge solid mass, amounting to ~25–50 million kilograms of polymer annually (29). These investigators describe an NMR method to measure the amount of the cationic polyelectrolyte Percol 787, a copolymer of acrylamide, in biosolids. This method may be useful to assess to what extent, if any, the copolymer depolymerizes to monomeric acrylamide during storage of the sludge and in the soil (30). Such depolymerization is an undesirable event because the free acrylamide could then be released into the aqueous environment.

Studies by Smith et al. (24) showed that heat, light, and outdoor environmental conditions, but not pH, promoted depolymerization of polyacrylamide to acrylamide. Wallace et al. (31) studied the effects of 1% polyacrylamide added to the soil on the mineral nutrition of tomatoes and wheat. They concluded that the added soil conditioner is unlikely to pollute the soil with sufficient acrylamide monomer arising from depolymerization to constitute a potential hazard. Analysis of residual acrylamide in beans, corn, potatoes, and sugar beets grown in soil treated with polyacrylamide to reduce erosion showed levels of <10 ppb (26). The acrylamide absorbed by the field crops was largely degraded after 18 h. Related studies showed that acrylamide does not bioaccumulate in mushrooms or tomatoes (32, 33). The mechanism of destruction of acrylamide after it enters the plant is not known.

Microbial Production and Biodegradation of Acrylamide. Microbes produce enzymes that catalyze both the synthesis and the biodegradation of acrylamide, as indicated by the following selected examples from the literature. Microbial enzymes involved in the production of acrylamide by *Rhodococcus rhodochromus* include nitrile hydratase, amidase, glutamine synthetase, and dehydrogenase (34, 35). Nitrile hydratase is used industrially to produce acrylamide (36, 37). The enzyme nitrile

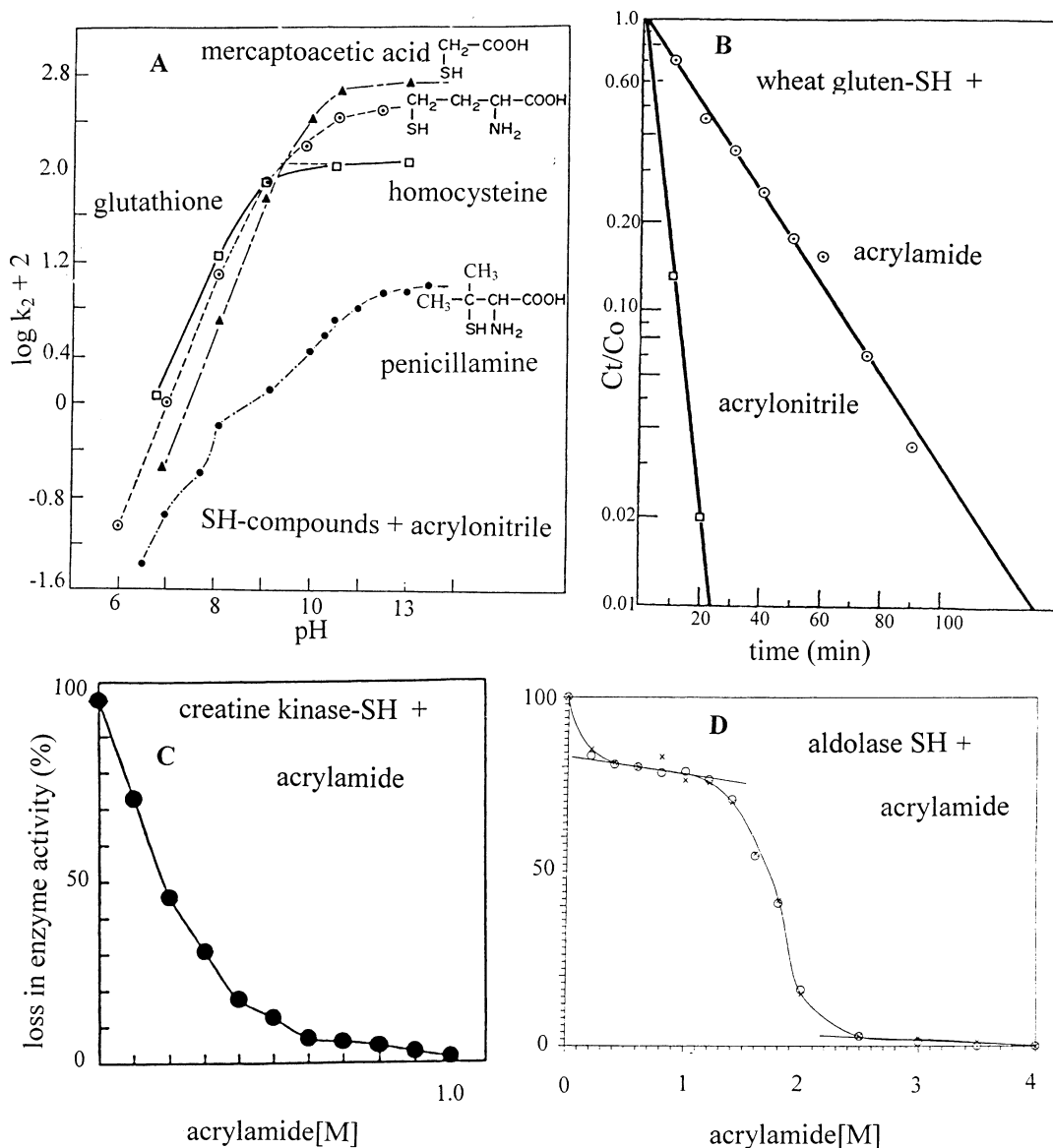


Figure 1. Reactions of amino acid and protein SH groups with acrylonitrile and acrylamide: (A) pH rate profile for the reaction of the SH groups of mercaptoacetic acid, homocysteine, glutathione, and penicillamine with acrylonitrile (3); (B) time-dependent alkylation of wheat gluten SH groups by acrylamide and acrylonitrile, where C_t = concentration at time t and C_0 = initial concentration (6); (C) inhibition of enzyme activity of creatine kinase by alkylation of its SH groups with acrylamide (adapted from ref 89); (D) inhibition of enzyme activity of aldolase by alkylation of its SH groups with acrylamide (adapted from ref 90).

hydratase produced by *Nocardia* cells catalyzes the hydrolysis of acrylonitrile to acrylamide (38). Encapsulated immobilized *Rhodococcus* bacteria produce an acrylamide-degrading amidase. An amidase capable of degrading acrylamide has also been isolated from *Klebsiella pneumoniae*. The microorganism *Pseudomonas stutzeri* metabolized acrylamide released from a copolymer at a concentration <440 mg/L under aerobic conditions (39, 40). The denitrifying bacteria used the resulting acrylic acid and ammonia as sources of carbon and nitrogen (41). Acrylamide is also a good substrate for an amidase produced by the human gastric pathogen *Helicobacter pylori* (42). These observations suggest that it may be possible to reduce acrylamide levels with the aid of acrylamide-degrading enzymes both in foods and in the digestive tract after consumption.

ACRYLAMIDE IN PROCESSED FOOD

The observation that acrylamide used as a sealing adjuvant in tunnel construction in Sweden was responsible for adverse

health effects in exposed humans eventually led Tareke et al. (43) to an association of acrylamide with food. They found that rats fed a fried chow diet had significantly higher levels of the hemoglobin (Hb) adduct of acrylamide, measured as *N*-(2-carbamoyl-ethyl)valine, than those fed a control diet. Analysis of the heat-treated feed revealed the presence of acrylamide in amounts that paralleled those of the Hb adducts. The authors suggested that heat-treated food is probably a major source of acrylamide for humans, which could account for the high background levels of Hb adducts (40 pmol/g of globin) in nonsmokers. This observation in the year 2000 was largely ignored. However, 2 years later, Tareke et al. (44) demonstrated relatively high levels of acrylamide in heat-processed commercial foods and in foods cooked at high temperatures, especially in carbohydrate-rich foods. These widely publicized findings stimulated worldwide studies on determining acrylamide levels in food and on the nature of the acrylamide

Table 4. Acrylamide Levels in Processed Foods Listed Alphabetically

food	acrylamide ^a ($\mu\text{g}/\text{kg}$ = ppb)
almonds, roasted	260
asparagus, roasted	143
baked products: bagels, breads, cakes, cookies, pretzels	70–430
beer, malt, and whey drinks	30–70
biscuits, crackers	30–3200
cereals, breakfast	30–1346
chocolate powder	15–90
coffee powder	170–351
corn chips, crisps	34–416
crispbread	800–1200
fish products	30–39
gingerbread	90–1660
meat and poultry products	30–64
onion soup and dip mix	1184
nuts and nut butter	64–457
peanuts, coated	140
potato, boiled	48
potato chips, crisps	170–3700
potato, French-fried	200–12000
potato, puffs, deep-fried	1270
snacks, other than potato	30–1915
soybeans, roasted	25
sunflower seeds, roasted	66
taco shells, cooked	559

^a Values were selected from the following references and websites on acrylamide: 44, 49, 78–82, 84; (a) CFSN/FDA Exploratory Survey: <http://www.cfsan.fda.gov/~dms/acrydata.html> and <http://www.cfsan.fda.gov/~dms/acrydat2.html>; (b) Acrylamide Infonet: <http://www.acrylamide-food.org/>; (c) WHO/FAO Acrylamide: http://www.who.int/fst/Acrylamide/Acrylamide_index.html; and (d) JIFSAN/NCFT Acrylamide in Food Workshop: http://www.jifsan.umd.edu/Acrylamide/acrylamide_workshop.html.

precursors in unprocessed food. The acrylamide contents of several food categories are listed in **Table 4**.

Mechanism of Formation of Acrylamide in Food. Heating equimolar amounts of asparagine and glucose at 180 °C for 30 min resulted in the formation of 368 μmol of acrylamide/mol of asparagine (45). Adding water to the reaction mixture resulted in an increase in acrylamide to 960 $\mu\text{mol}/\text{mol}$. A temperature-dependence study showed that acrylamide formation also increases with temperature from about 120 to 170 °C and then decreases. Under similar conditions, methionine formed about one-sixth the amount of acrylamide. A similar temperature dependence was observed by Tareke et al. (44) in laboratory-heated foods. Moderate amounts (5–50 $\mu\text{g}/\text{kg}$) were detected in heated protein-rich foods and higher levels (150–4000 $\mu\text{g}/\text{kg}$) in carbohydrate-rich foods such as beetroot and potatoes. No acrylamide was found in unheated or boiled foods. However, Ezejiet al. (46) found that acrylamide is formed during the boiling/autoclaving of starch. Other amino acids producing low amounts of acrylamide include alanine, arginine, aspartic acid, cysteine, glutamine, methionine, threonine, and valine (see references in footnote to **Table 4**).

Mass spectral studies showed that the three C atoms and the N atom of acrylamide were all derived from asparagine (47). Stadler et al. (45), Zyzak (47), Mottram et al. (48), Becalski et al. (49), Sanders et al. (50), and Yaylayan et al. (51) also found that reducing sugars containing an aldehyde group such as glucose react with asparagine above 100 °C to form an *N*-glycoside, which is then cleaved at the C–N bond to an intermediate that can be transformed to acrylamide, possibly as shown in **Figure 2**. The yield of acrylamide from the model studies was ~0.1%. A pathway from the *N*-glycoside to acrylamide has been proposed by Yaylayan et al. (51a).

Because 2-deoxyglucose, glyoxal, and glycerol, which do not participate in the classical Maillard reactions, also combined with asparagine to form acrylamide, other pathways or mechanisms leading to acrylamide may also be operative (45). Both mechanisms, one requiring the participation of a dicarbonyl moiety and the second a monocarbonyl aldehyde or ketone, therefore predict that acrylamide may result from the general reaction of asparagine with any aldehyde or ketone. This suggestion is supported by the observation that heating the aldehyde octanal or the ketone 2-octanone with asparagine in a sealed tube at 175 °C produced measurable amounts of acrylamide (49).

In the presence of asparagine, the mechanism of formation of acrylamide from methionine (and possibly other amino acids) probably involves first a decarboxylation and deamination of methionine to methional, $\text{CH}_3\text{SCH}_2\text{CHO}$, which then behaves like any other aldehyde by reacting with the $\alpha\text{-NH}_2$ group of asparagine to form the Schiff base ($\text{Asn-NH}=\text{CHCH}_2\text{SCH}_3$). The latter is then transformed to an *N*-glycoside, which can then undergo decarboxylative deamination to form acrylamide by a mechanism analogous to that shown in **Figure 2** for the *N*-glycoside derived from asparagine and glucose. Note that this mechanism postulates that methionine is not transformed directly to acrylamide but, rather, is the source of a carbonyl compound analogous to that of glucose. In the absence of asparagine, methional may be transformed to acrolein by elimination of H_2S . Acrolein could then be transformed to acrylamide as outlined below.

The fatty acid oxidation product acrolein ($\text{CH}_2=\text{CH}-\text{CHO}$) could, in principle, be directly transformed to acrylamide by reaction with NH_3 to form $\text{CH}_2=\text{CH}-\text{CHOH}(\text{NH}_2)$ followed by oxidation to acrylamide or could react with asparagine to form an *N*-glycoside, which is then transformed to acrylamide (51b). Plant lipoxigenases also catalyze the formation of other reactive aldehydes in food, which could react with asparagine to form acrylamide. The three-carbon dehydroalanine $\text{CH}_2=\text{CH}(\text{NH}_2)\text{COOH}$ (derived from alkali-induced dehydration of serine and desulfurization of cysteine/cystine) (52, 53) could, in principle, also be an acrylamide precursor. This possibility is supported by the observations that lye (alkali)-treated, but not untreated, olives contain acrylamide (Lauren Jackson, private communication).

Asparagine appears to be a key participant in acrylamide formation, so there is a need to know the amounts of this free amino acid in various food categories as well as its chemistry and biochemistry. **Table 5** lists the asparagine content of selected foods.

Acrylamide in Processed Food. Acrylamide in food is largely derived from heat-induced reactions between the amino group of the free amino acid asparagine and the carbonyl group of reducing sugars such as glucose during baking and frying. Foods rich in both of these precursors are largely derived from plant sources such as potatoes and cereals (barley, rice, wheat) but apparently not animal foods such as poultry, meat, and fish (**Table 5**). Widely consumed processed foods with high levels of acrylamide include French fries, potato chips, tortilla chips, bread crust, crispbread, and various baked goods and cereal formulations. However, the observed wide variations in levels of acrylamide in different food categories as well as in different brands of the same food category (e.g., French fries; potato chips) appear to result not only from the amounts of the precursors present but also from variations in processing conditions (e.g., temperature; time; nature of frying oil; nature of food matrix).

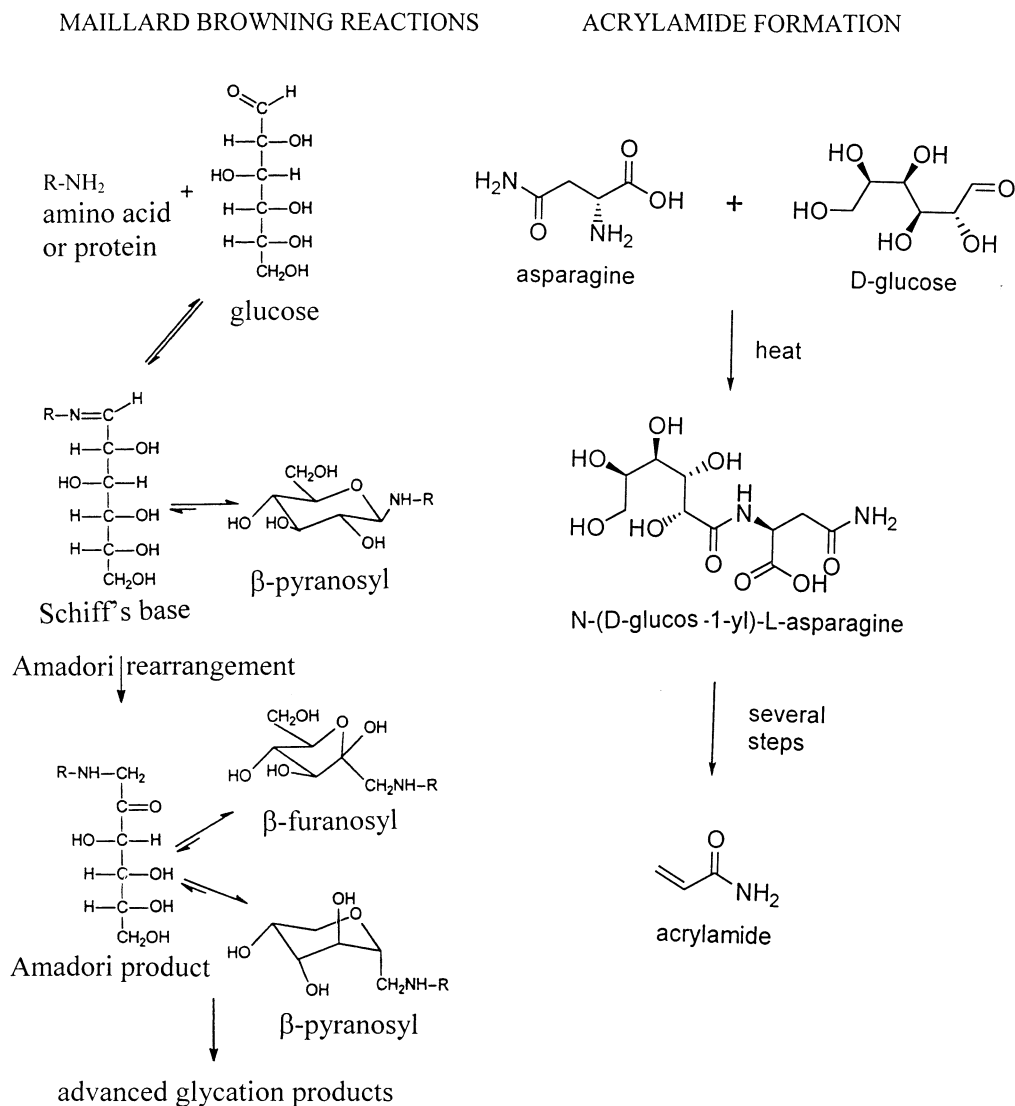


Figure 2. Acrylamide formation from asparagine and glucose. The α -NH₂ group of asparagine participates in a nucleophilic addition reaction with the aldehyde group of glucose to form a Schiff base, which then undergoes an Amadori rearrangement to the shown glucose–asparagine derivative (*N*-glycoside). The latter can then undergo decarboxylative deamination, losing the COOH and α -NH₂ groups associated with asparagine to form acrylamide (right side), or can proceed via the Maillard reactions to form browning products (left side).

Heat and high pH also induce the formation of dehydroalanine and cross-linked amino acids such as lanthionine, lysinoalanine, and histidinoalanine, as well as *D*-amino acids (52, 53). Dehydroalanine could, in principle, act as a biological alkylating agent analogous to acrylamide (see below). Browning products, lysinoalanine, and some *D*-amino acids are reported to significantly impact the nutritional quality and possibly the safety of food. These considerations suggest the need to define the dietary significance of both pure acrylamide and acrylamide plus some of the other compounds formed during food processing. These could act synergistically or antagonistically in animals and humans. As noted below, although biological and toxicological effects of pure acrylamide have been widely studied, deeper insights into possible adverse effects of acrylamide in food have not.

Maillard Browning Reactions and Acrylamide Formation. Because a major objective of this review is to develop a better understanding of the dietary significance of acrylamide and because its formation cannot be separated from the broader aspects of the reactions leading to food browning, the nature of the browning process that induces the formation of Maillard products including acrylamide will be briefly mentioned here.

For a more detailed discussion of both nonenzymatic and enzymatic browning reaction pathways, see refs 54 and 55.

Amino–carbonyl and related reactions of food constituents involve those changes commonly termed browning reactions (**Figure 2**). Specifically, heat-induced reactions of amines, amino acids, peptides, and proteins with reducing sugars and vitamin C (nonenzymatic browning, often called Maillard reactions) and quinones (enzymatic browning catalyzed by polyphenol oxidase) cause deterioration of food during storage and processing. The production of toxic compounds may further reduce the nutritional value and safety of foods. These compounds include rat kidney-damaging metal chelators, mutagens, carcinogens, anti-mutagens, antioxidants, antibiotics, and anti-allergens. Maillard reactions may also result in the formation of desirable flavors and antimicrobial compounds active against human pathogens.

Because some individuals are sensitive to the antibrowning compound sodium sulfite, we explored the potential of sulfur amino acids to prevent browning. The antioxidant and antitoxic effects of SH-containing amino acids such as cysteine, cysteine methyl ester, cysteine ethyl ester, *N*-acetylcysteine, cysteinylglycine, and the tripeptide reduced glutathione are due to a number of mechanisms including their ability to act as (a)

Table 5. Asparagine Content of Foods Listed Alphabetically

food	asparagine ^a (mg/kg)	refs
almonds, 19 cultivars	980–6410	252
apples, fresh pulp, 5 cultivars	315–588	63
apple juice	323	253
asparagus, dry	11000–94000	57, 58
beans, green pods, dry	3840	254
broccoli, whole, dry	1920	255
broccoli, florets, fresh	578	256
broccoli, stems, fresh	189	256
cassava, processed, dry	10	257
cauliflower, fresh	54–1060	254
cocoa powder, unroasted	309	258
cocoa, roasted (125°C, 3 min)	145	258
cocoa, roasted (135°C, 3 min)	94	258
grape juice	4	253
lentils, dry	1900–6200	60
meat, bovine	0.4	259
meat, pork	11	260
pineapple juice	247	253
potatoes, fresh, 4 varieties	2500–3500	261
potatoes, fresh	1703–2581	262
potatoes, dry	580–3300	254
potatoes, dry	7700	263
rice, milled	29	264
rice, bran	282	264
rice, germ	236	264
spinach, dry	460–1470	254
wheat grain	1540	265
wines	0.67–27	266

^a Various reported concentration units were normalized to mg/kg (ppm) for solids and to mg/L for liquids.

reducing agents, (b) scavengers of reactive oxygen (free radical traps), (c) destroyers of fatty acid hydroperoxides, (d) strong nucleophiles that can trap electrophiles and their intermediates, and (e) inducers of cellular detoxification. Our results demonstrated that SH-containing amino acids were nearly as effective as sodium sulfite in preventing browning in apples, potatoes, fruit juices, and protein-containing foods such as nonfat dry milk and barley and soy flours. On the basis of reported reactions of these compounds with acrylamide (described below), it is likely that the sulfur amino acids simultaneously reduced levels of acrylamide in the heated foods.

ASPARAGINE AS THE MAJOR PRECURSOR OF ACRYLAMIDE IN FOOD

The free amino acid asparagine, a genetically coded non-essential amino acid first isolated from asparagus juice in 1806 (56–58), probably is a major precursor of acrylamide. Selecting cultivars for food use that contain low levels of asparagine and/or devising conditions to hydrolyze asparagine to aspartic acid chemically or enzymatically with asparaginase or other amidases prior to food processing may result in low-acrylamide foods. The following aspects of asparagine chemistry and biochemistry are examined in this section: function in the plant; analysis and concentration in foods; deamidation; and its role in leukemia and antibiotic resistance.

Occurrence and Function in Plant Foods. Because the amino acid asparagine is a major precursor for the heat-induced formation of acrylamide, suppression of the biosynthesis of free asparagine could turn out to be a useful approach to reduce acrylamide formation. Here we briefly examine the biosynthesis and function of asparagine in some plant foods. Martin and Ames (59) found that asparagine is the free amino acid present in the highest amount in potatoes (93.9 mg/100 g). Rozan et al.

(60) found that asparagine was quantitatively by far the most important amino acid present in five varieties of lentil seeds, ranging from 18 to 62 mg/g of dry weight. These and related studies summarized in **Table 5** show that asparagine levels in foods vary widely.

Asparagine seems to play a key role in the regulation of nitrogen metabolism for the soybean plant and probably also for many other plants (61). The amino acid can act as a shunt for the long-distance transport as well as storage of nitrogen in the plant. In the soybean plant, highest levels (up to 2.4 mg/g of fresh weight) of asparagine occur in stems and lower level roots and leaves. Application of a herbicide resulted in a 3–6-fold increase in asparagine levels. Changes in asparagine content appear to be a good indicator of changes in nitrogen metabolism of plants induced by pesticides and environmental factors. Possible consequences of suppressing genes that govern the formation of enzymes involved in asparagine biosynthesis are not known.

Analysis of Asparagine. After extraction from the plant matrix, analysis of free asparagine and other free amino acids is a challenging problem because they often coelute with other protein and nonprotein amino acids present in the extracts (62). To overcome this problem, Vasinitis et al. (63) developed protocols for the analysis of free amino acids in apples by HPLC. Jia et al. (61) describe a two-step ion chromatographic procedure for the analysis of asparagine. The first analysis yields data for free aspartic acid, and the second step involves hydrolysis of asparagine to aspartic acid followed by analysis of the asparagine-derived aspartic acid. Martin and Ames (59) used capillary electrophoresis to measure the content of asparagine in fresh and fried potato slices. Rozan et al. (60) analyzed asparagine and other free amino acids in lentils. Tomatoes also have a high content of free amino acids measured by ion exchange chromatography (64).

Table 5 shows the reported asparagine contents of a variety of foods. Note the wide range of values in the different food categories. Discussion of cited changes in asparagine levels resulting from storage at various temperatures as well as exposure to heat and other processing conditions is beyond the scope of this paper. Further improvement in the analysis of free asparagine in plant materials and in processed foods will facilitate studies on its stability to food processing and on defining its role in the formation of Maillard browning products and as a precursor of acrylamide. The development of an immunoassay (ELISA) and the use of asparaginase or other amidases for the analysis of asparagine merit study.

Deamidation of Asparagine. In principle, simple acid or base- or enzyme (asparaginase, amidase)-catalyzed hydrolysis of asparagine to aspartic acid and ammonia in food could be a useful approach to reduce the extent of heat-induced acrylamide formation. In vivo deamidation serves as a molecular timer of biological events including the aging process and the progression of disease and is involved in the mechanism of postsynthetic formation of proteins of biological significance (65, 66). Factors (pH, buffer ions, ionic strength, temperature) that influence the deamidation of peptide- and protein-bound asparagine residues have been extensively studied (67). However, this does not seem to be the case for free asparagine.

Factors that were found to be optimal for the deamidation of protein-bound asparagine should be tested on free asparagine in food.

Therapy of Leukemia with Asparaginases. Because asparaginase may be used to hydrolyze asparagine in food in an effort to reduce acrylamide formation, it is relevant to mention

previous uses of the enzyme. The enzyme L-asparaginase is widely used in medicine in the treatment of childhood acute lymphoblastic leukemia (68, 69). The enzyme hydrolyzes L-asparagine to L-aspartic acid and ammonia and L-glutamine to L-glutamic acid and ammonia, thus depleting free asparagine and glutamine in blood. The molecular basis of the therapeutic effects is due to the fact that the growth of malignant cells is more dependent on an exogenous source of asparagine and glutamine than the growth of normal cells.

Pritsa and Kyriakidis (70) describe the isolation of L-asparaginase EC 3.5.1.1, molecular mass 33 kDa, from *Thermus thermophilus*. Currently, there are three asparaginase preparations available: an enzyme derived from *Escherichia coli* (ASP, Elspar), an *E. coli* enzyme modified by covalent attachment to poly(ethylene glycol) (PEG, Oncospar), and an enzyme derived from *Erwinia chrysanthemum* (ERW, Erwinase). The nature of the enzyme administered to patients significantly affected the pharmacological characteristics in terms of clearance of enzyme activity, ability to deplete serum asparagine, and development of anti-asparaginase antibodies (71). These considerations suggest that all available enzyme preparations should be evaluated for their ability to hydrolyze free asparagine in food. Such evaluation should include assessment of the safety of asparaginase-treated food.

Asparagine and Bacterial Resistance. Asparagine can adversely affect food safety in another way; its presence in the growth medium enhanced the resistance of human pathogens to inactivation at low pH (72). Will exposure of asparagine-rich foods such as apples (Table 5) to *E. coli* or *Salmonella* also induce antibiotic resistance (73, 74)?

ANALYSIS OF ACRYLAMIDE AND METABOLIC PRODUCTS

Acrylamide is absorbed by animals and humans via ingestion or inhalation or through the skin. Extensive efforts have been made to assess human exposure to acrylamide by monitoring several metabolites excreted in the urine as well as products resulting from biological alkylations by acrylamide. Analytical methods for acrylamide include those based on gas chromatography (GC), high-performance liquid chromatography (HPLC), mass spectrometry (MS), and combinations of these. This section offers capsule summaries of analytical methods that have been used to measure acrylamide in food and in vivo as well as metabolic transformation products found in urine, plasma, and tissues.

The use of a bromo derivative of acrylamide is illustrated with the following examples. Polyacrylamide is used to flocculate lime in the production of sugar at high temperatures, so acrylamide may be present in sugar as a result of depolymerization. Cutie and Kallos (75) attempted to optimize the analysis of acrylamide in sugar based on derivatization to the dibromo derivative, separation by HPLC, and detection by MS through a thermospray interface. The method has a limit of detection of ~200 parts per trillion. This and related studies by Schultzova and Tekel (76) showed that the amount of acrylamide in sugar (<5 $\mu\text{g}/\text{kg}$) represents a negligible risk to the consumer.

An HPLC method with UV detection at 200 nm for the direct and simultaneous determination of acrylamide and its epoxide glycidamide in rat plasma is described by Barber et al. (77). The limit of detection for acrylamide was 0.05 $\mu\text{g}/\text{mL}$ and that for glycidamide 0.25 $\mu\text{g}/\text{mL}$. The half-life of acrylamide in plasma following a single injection of 50 mg/kg was ~2.8 h. These authors also used a GC method to determine the hemoglobin adduct of acrylamide in rats.

As illustrated with the following examples, acrylamide-containing processed food samples seem to require more complex analytical methods, presumably because of the need for extensive manipulation to remove interfering compounds and impurities during the extraction step. Rosen and Hellenäs (78) used liquid chromatography–tandem mass spectrometry (LC-MS-MS) for the analysis of acrylamide. The method is based on the addition of [$^2\text{H}_3$]acrylamide as an internal standard, extraction with water, mixed mode solid phase extraction, ultrafiltration, and use of a graphitized carbon column for chromatography. Comparative results were obtained for a range of foods analyzed independently by a GC-MS method. Similar methods were used by Tareke et al. (44), Becalski et al. (49), and Clarke et al. (79) to measure the acrylamide content of a large number of foods (Table 4). The development of “improved” methods for analyzing acrylamide in processed food is currently an active area of research (80–86). Pedersen and Olsson (87) developed an improved extraction method for acrylamide from potato chips.

Due to the complexity of these methods, development of simpler HPLC, immunoassay (ELISA), and acrylamide-specific enzyme assays for the analysis of acrylamide, metabolites, amino acid, and protein derivatives in processed food and in vivo merits study. It should be emphasized that assessment of human exposure to acrylamide discussed below is complicated by the fact that it can be taken up by several routes (through the skin, by inhalation, from drinking water, and from food). It may therefore be preferable to measure hemoglobin or DNA adducts in vivo as biomarkers of exposure irrespective of route.

CHEMISTRY AND BIOCHEMISTRY OF ACRYLAMIDE

As mentioned earlier, we carried out extensive studies on the reactions of conjugated vinyl compounds including acrylamide, acrylonitrile, methyl acrylate, methyl vinyl sulfone, and vinylpyridine with wheat gluten, soy proteins, and keratin (wool, human hair) proteins designed to prepare derivatives of potential industrial use and to assess the role of cysteine/cystine and lysine residues in protein structure, function, and nutrition. These studies revealed that SH groups of cysteine residues as well as the $\epsilon\text{-NH}_2$ group of lysine side chains have a strong avidity for the double bond of conjugated vinyl compounds. Our seminal studies in this area provide a chemical basis for the biological effects of acrylamide and its reactive epoxide metabolite glycidamide in vivo. The in vivo reactions involve biological alkylation reactions of proteins such as hemoglobin, enzymes, and DNA.

Protein Acrylamide Reactions and Interactions. *General Aspects.* Acrylamide has two reactive sites, the conjugated double bond and the amide group. The electrophilic double bond can participate in nucleophilic reactions with active-hydrogen-bearing functional groups both in vitro and in vivo. These include the SH of cysteine, homocysteine, and glutathione (GSH), $\alpha\text{-NH}_2$ groups of free amino acids and N-terminal amino acid residues of proteins, the $\epsilon\text{-NH}_2$ of lysine, and the ring NH group of histidine (Figure 3). Exposing acrylamide to pH extremes results in its hydrolysis to acrylic acid and ammonia. As mentioned earlier, various kinetic, mechanistic, and synthetic studies have been carried out to define the course and mechanisms of the reactions of acrylamide and numerous other vinyl compounds. As one practical result, several vinyl compounds have been shown to be useful specific blocking agents for SH groups in proteins. Another result is the apparent relationship between the levels of Hb adducts of acrylamide in the plasma of animals and humans and the extent of exposure to the toxin. Selected findings on this subject are reviewed below.

PROTEIN ALKYLATIONS BY ACRYLAMIDE

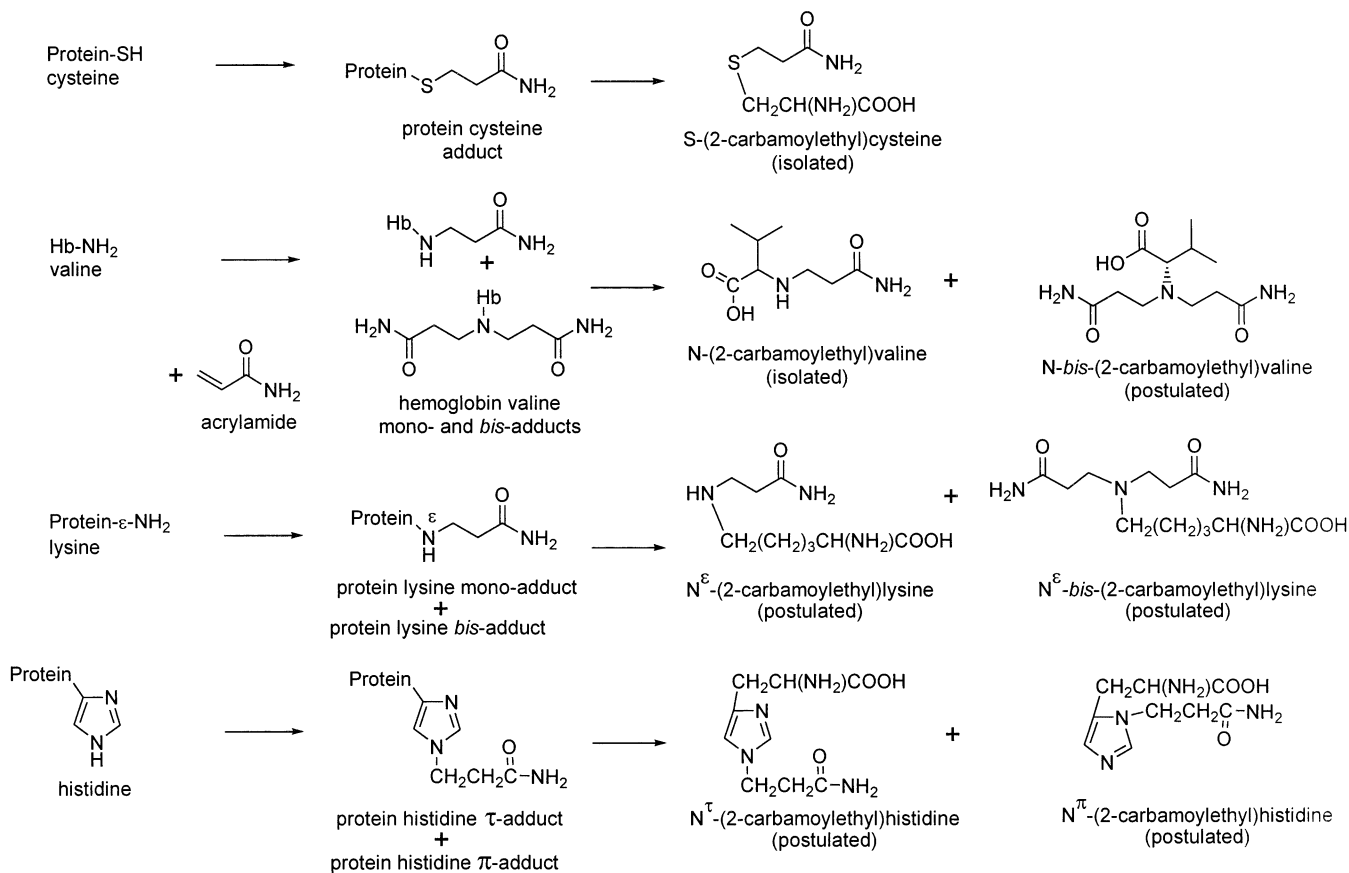


Figure 3. Known and theoretically possible amino acid derivatives that can result from reactions of proteins with acrylamide. Enzymatic hydrolysis or Edman degradation of the carbamoylethyl proteins produces carbamoylethyl amino acid derivatives (shown). Acid hydrolysis forms the corresponding carboxyethyl ($-\text{CH}_2\text{CH}_2\text{COOH}$) substituted amino acids + NH_4Cl (not shown). One or two acrylamide molecules can alkylate the $\epsilon\text{-NH}_2$ group of lysine to form mono- and disubstituted lysine derivatives, respectively. The monosubstituted protein-bound lysine derivative would form N^ϵ -(2-carbamoylethyl)lysine on enzymatic hydrolysis (shown) and N^ϵ -(2-carboxylethyl)lysine [$\text{HOOCCH}_2\text{CH}_2\text{NH}(\text{CH}_2)_4\text{CH}(\text{NH}_2)\text{COOH}$] on acid hydrolysis (not shown). The disubstituted derivative would form N^ϵ -bis(2-carbamoylethyl)lysine (shown) on enzymatic hydrolysis and N^ϵ -bis(2-carboxylethyl)lysine [$(\text{HOOCCH}_2\text{CH}_2)_2\text{N}(\text{CH}_2)_4\text{CH}(\text{NH}_2)\text{COOH}$] on acid hydrolysis (not shown). Acrylamide can alkylate histidine on one or the other imidazole ring nitrogens to form the N^τ - and N^π -(2-carbamoylethyl)histidine isomers, respectively. Enzymatic hydrolysis of the modified protein-bound histidines forms two carbamoylethyl histidine isomers (shown). On acid hydrolysis these form the corresponding N^τ - and N^π -(2-carboxylethyl)histidines (not shown). See ref 16 for a detailed discussion of the stereochemistry of related transformations.

Studies by Druckrey et al. (88) showed that acrylamide can chemically modify proteins. The specific functional groups involved were, however, not defined. Our studies on reactions of protein functional groups with vinyl compounds included such reactions of bovine serum albumin (BSA) and wheat gluten with acrylamide. Amino acid analysis of the modified proteins showed that under the cited conditions, alkylation by acrylamide was limited to cysteine SH groups, which were transformed to cysteinyl- S - β -propanamide derivatives (Tables 1–3). Our kinetic studies revealed that SH groups were 100–300 times more reactive with conjugated vinyl compounds than were amino groups.

This discovery stimulated efforts to use acrylamide as a specific alkylating agent for protein SH groups, as evidenced by its application to the following selected proteins: creatine kinase (89) (Figure 1C), aldolase (90) (Figure 1D), β -lactoglobulin (91), bovine serum albumin (92), fucosidase from peas (93), and glyceraldehyde-3-phosphate dehydrogenase (94). Such studies facilitated the determination of amino acid sequences as well as the location of disulfide bonds, as discussed in detail for related reactions of SH groups with vinylpyridines (22).

We found that acrylamide can also alkylate NH_2 groups, as shown with the model compounds glycine and diglycine (Tables 1 and 2). Acrylamide was much less reactive than the related vinyl compounds acrylonitrile and methyl vinyl sulfone. Note that N,N -dimethylacrylamide reacted 6 times more slowly than did acrylamide. Danileviciute et al. (95) also studied reaction rates of protein (casein, insulin) NH_2 groups with acrylamide.

Evidence for the involvement of the CONH_2 group of acrylamide in hydrogen-bonding interactions derives from our observation that dimethyl sulfoxide (DMSO), which can participate in such bonding, altered the rates of acrylamide with both glycine and diglycine. The mechanism for this effect may involve changes in the electron density of the double bond of acrylamide (4). These considerations imply that, depending on conditions, acrylamide can alkylate both protein SH and/or NH_2 groups. Extensive studies with hemoglobin adducts of acrylamide relevant to the theme of this paper demonstrate this possibility. Some of these are outlined below.

Reactions of Amino Acids and Proteins with Acrylamide in Polyacrylamide Gels. Polyacrylamide gels prepared by free

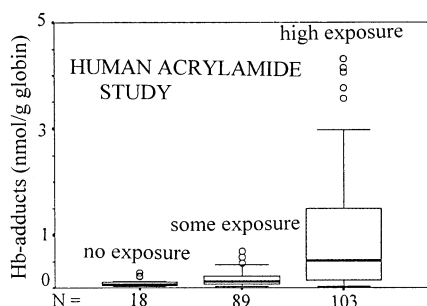


Figure 4. Relationship between N-terminal valine-acrylamide adduct levels of hemoglobin and exposure of humans to acrylamide. Modified from ref 108.

radical-catalyzed polymerization of acrylamide are widely used to separate and purify mixtures of proteins and other biomolecules. Specialized applications include those combining zymography with a gradient of polyacrylamide (96) and immobilized copolymers of acrylamide (97). Significant amounts of acrylamide monomer are present in such gels, so it is not surprising that acrylamide in the gel can alkylate SH groups of cysteine and ϵ -NH₂ lysine residues of proteins during electrophoresis. Such in situ alkylation can be adapted to facilitate the characterization of proteins (98). Caution should be exercised during the preparation and use of the polymeric gels to avoid exposure to acrylamide.

Acrylamide also reacts with primary, secondary, and tertiary amine buffer components used in polyacrylamide gel electrophoresis and with ϵ -NH₂ groups of lysine residues of bovine serum albumin and cytochrome *c* (99). These reactions induced a downward shift in the pH of the buffers and changes in the isoelectric points and electrophoretic mobilities of the proteins. Acrylamide lowered the pH of milk following heating at 140 °C for 30 min, possibly by hydrolyzing to acrylic acid or reacting with the basic casein ϵ -NH₂ groups of lysine (100).

These results are not unexpected, in view of our earlier finding that the p*K* value of the α -NH₂ group of phenylalanine [C₆H₅-CH(NH₂)COOH] is shifted downward from 9.0 to 8.1 after modification with acrylamide to C₆H₅CH₂CH(NHCH₂CH₂-CONH₂)COOH. The decrease in the basic nature of the amino groups of phenylalanine and of tyrosine modified with acrylonitrile was greater, as evidenced by a shift in p*K* values from 9.0 to 6.6 (7, 101). The reduction in basicity is due to the inductive, electron-withdrawing action of the carbamoyloethyl and cyanoethyl groups. An analogous downward shift probably occurs in the p*K* value of the α -NH₂ group of the N-terminal valine residue of hemoglobin modified by acrylamide (see below).

Hemoglobin Acrylamide and Glycidamide Adducts. Adducts formed as a result of reaction between the α -NH₂ group of N-terminal valine of Hb both with acrylamide [*N*-(2-carbamoyl-ethyl)-L-valine] and with glycidamide [*N*-(2-carbamoyl-2-hydroxyethyl)-*RS*-valine] seem to be useful biomarkers of human exposure to acrylamide (102–104). For example, Bergmark (105) detected the following amounts of (carbamoyl-ethyl)valine adducts in the blood of humans: nonsmokers, 31 pmol/g; laboratory personnel working with acrylamide, 54 pmol/g; and smokers, 116 pmol/g. Hb adducts in nonsmokers may originate from the diet. Similar observations were made by Schettgen et al. (106, 107) and Hagmar et al. (108) (Figures 4 and 5).

Compared to rats, mice exhibited higher *in vivo* levels of the glycidamide than of the acrylamide adduct of valine. The epoxide group of glycidamide appears generally more reactive

than the double bond of acrylamide with hemoglobin (103) (Figure 5). Because glycidamide is formed by the cytochrome P450 2E1-catalyzed epoxidation of acrylamide, the epoxide may only be present *in vivo* (109, 110).

Can glycidamide also form in food as result of epoxidation of acrylamide by cytochromes from animal and plant sources?

The ability of acrylamide to modify the α -NH₂ group of the sterically hindered terminal amino acid valine of hemoglobin is surprising, in view of the facts that (a) NH₂ groups are much less reactive with acrylamide than are SH groups and (b) steric factors associated with valine would be expected to hinder the accessibility of acrylamide. A possible explanation is that the valine residue protrudes from the surface of the globin protein, allowing rapid access to acrylamide. Hydrophilic and hydrophobic effects proximate to the microenvironment of valine may also be involved in enhancing the p*K* value and hence the nucleophilic reactivity of its amino group.

Does alkylation of the SH group of cysteine, the α -NH₂ group of the N-terminal valine, and possibly also the ϵ -NH₂ of lysine and the NH group of histidine by acrylamide (Figure 3) affect the affinity of hemoglobin for oxygen, as is the case for hemoglobin following deamination of its N-terminal valine and other residues by ninhydrin (111)?

Quenching of Protein Fluorescence by Acrylamide. Non-covalent interactions of acrylamide with proteins and DNA could also impact nutrition and food safety and so will be briefly mentioned here. Fluorescence occurs when the absorption of a photon by a molecule is followed by emission of light of longer wavelength (112). The ratio of the number of emitted to absorbed photons is known as the quantum yield, *Q*. The fluorescence of the tryptophan residue of proteins is widely used to obtain information about the structure and function of proteins. Acrylamide can reduce the quantum yield by a process known as quenching involving non-covalent interactions with the fluorophore. If quenching occurs, tryptophan must be on the surface of the protein. Otherwise, the amino acid is probably located internally.

Acrylamide induces quenching of tryptophan (113) and tyrosine (114) fluorescence. The quenching reaction involves physical contact between acrylamide and the excited indole ring of tryptophan. Because acrylamide is a neutral, highly polar molecule, it readily diffuses to and senses the microenvironment of fluorophores by nonpolar charge-transfer complex formation (115). Tryptophan quenching by acrylamide is therefore widely used in studies of protein structure and folding.

The following are some examples from the extensive literature on this subject. Acrylamide quenching studies of tryptophan fluorescence facilitated studies of interactions between tropinin-C and tropinin-I (116), the microenvironment of a buried tryptophan residue of cytochrome *c* (117), a conformational change in phytochrome A (118), the tryptophan environment in carbonic anhydrase (119), conformational changes of myosin during ATP hydrolysis (120), the structure of a major coat protein of a bacteriophage (121), unfolding of lipoxygenase (122), the structure of lactalbumins (123), and catalytic sites of ATPase (124).

Quenching of tryptophan in aldolase (90) is a time-dependent process. High concentrations of acrylamide irreversibly inactivate the enzyme (Figure 1D). In this case, acrylamide interacts non-covalently with tryptophan and covalently with cysteine residues. Quenching of fluorescence by acrylamide has also been used to study DNA-binding domains (115, 125). It is not known whether the charge-transfer effects associated with protein and DNA quenching are involved in the biological effects of

HEMOGLOBIN ALKYLATION BY GLYCIDAMIDE

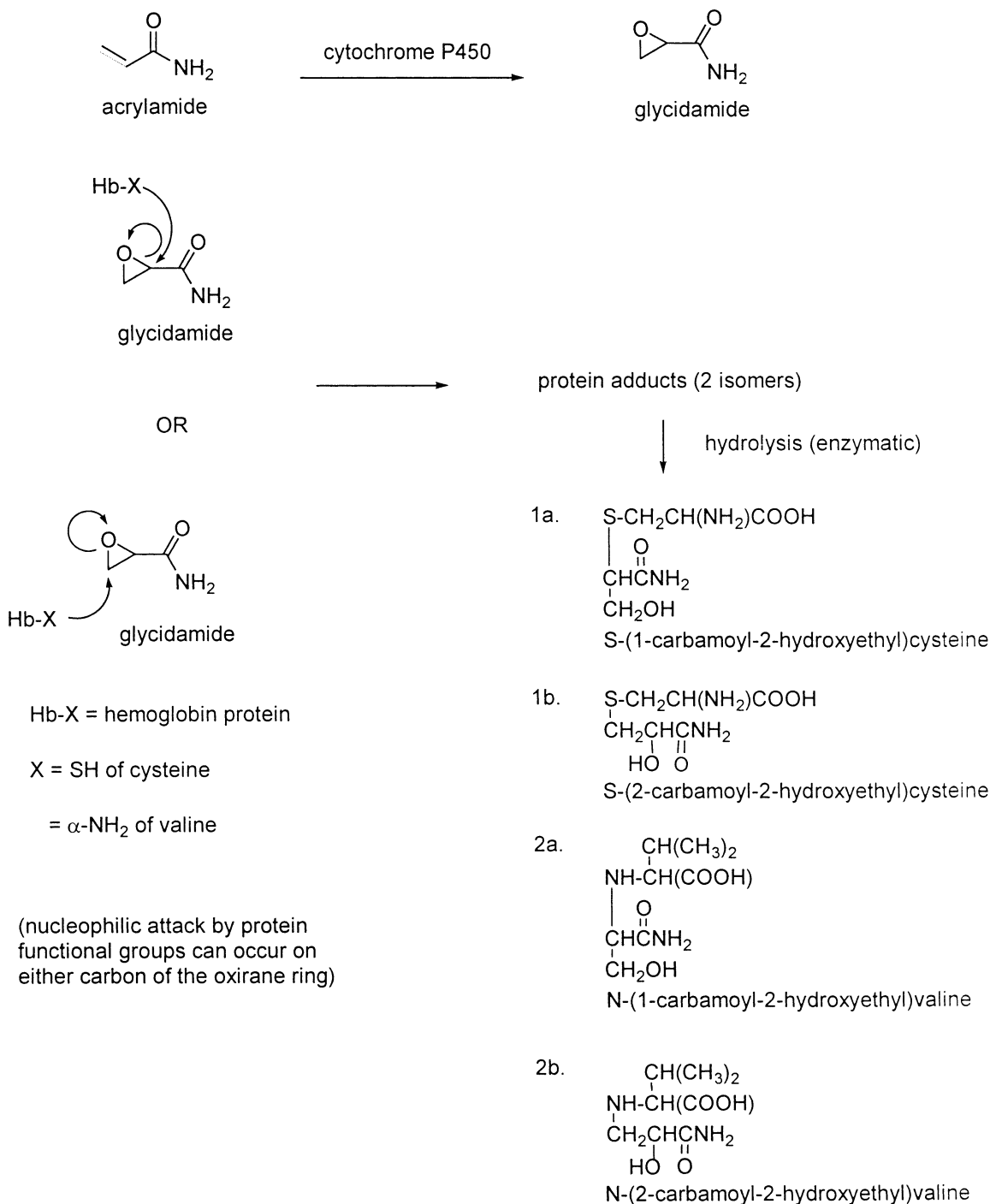


Figure 5. Alkylation of the SH group of cysteine and of the α -NH₂ group of valine residues of hemoglobin by glycidamide (acrylamide epoxide).

acrylamide and whether such interactions will affect the digestibility and nutritional utilization of tryptophan in food proteins (126).

Absorption of UV Light by Acrylamide. In addition to the perturbation of the fluorescence of aromatic acids, acrylamide can also interact with UV light. Thus, filtration of sunlight through acrylamide significantly reduced the incidence of UV-light-induced malformations of frog limbs (127). This beneficial effect of acrylamide may be due to its ability to absorb some of the high-energy UV light, thus reducing light-induced damage.

Will UV light induce the polymerization of processing-generated acrylamide in a food matrix such as potato chips (128–130)?

HUMAN EXPOSURE TO ACRYLAMIDE AND RISK ASSESSMENT

Risk can be defined as the probability that an individual contracts a disease or the number of cases in a population. It is estimated that ~100000 people may come in contact with acrylamide in the United States (131, 132). Pantusa et al. (133) measured the exposure of laboratory workers to airborne

acrylamide derived from either crystalline acrylamide or commercial solutions used to make polyacrylamide gels. Mean air concentrations for a 15-min exposure during the sampling from the original containers were 7.20 and 5.81 $\mu\text{g}/\text{m}^3$ for users of crystalline and solution acrylamide, respectively. Although the extent of exposure increased with time, the calculated 8-h time-weighted average exposures were below current occupational exposure limits.

Bailey et al. (134) describe a method for monitoring the exposure of acrylamide in rats down to levels of 0.5 mg/kg based on GC-MS analysis after hydrolysis of the cysteine residue of Hb as *S*-(2-carboxyethyl)-*L*-cysteine. Previously, we described the synthesis, mass spectra, and ion exchange chromatography of this and related cysteine derivatives (2, 14, 135). Studies by Calleman et al. (136, 137) showed that glycidamide also alkylates the same SH group, forming after protein hydrolysis *S*-(2-carboxy-2-hydroxyethyl)-(*S*)-cysteine. Moreover, in their efforts to characterize Hb-acrylamide adducts, Springer et al. (138) discovered two new unknown adducts, possibly lysine or histidine derivatives of acrylamide (Figure 3). As already mentioned, direct determination of adducts to N-terminal valines in hemoglobin is an important tool for risk assessment. It is especially useful to monitor the *in vivo* human exposure to acrylamide from dermal contact, the diet, drinking water, smoking, and the workplace.

The following consumption levels of acrylamide-containing food categories per user are estimated for the U.S. diet (139) ($\mu\text{g}/\text{day}$, % of total acrylamide in diet): potato chips (23, 17); French fries (15, 17); breads (3, 12); cereals (6, 12); biscuits/cookies (4, 8); home fries (27, 5); fried pastries (12, 5); other salty snacks (13, 4); battered/fried foods (3, 4); and popcorn (10, 4). A 2-day Swiss dietary study with 27 participants (13 women and 14 men aged 16–67) showed a mean daily intake of acrylamide of 0.277 $\mu\text{g}/\text{kg}$ of body weight (140). The percentage exposure to acrylamide derived from different meals during the daily intake was as follows: breakfast, 8; lunch, 21; dinner, 22; snacks, 13; and coffee, 36. The high contribution of coffee to the total is noteworthy. Gingerbread (lebkuchen) contained 7 times the amounts of acrylamide found in fried potatoes. On the basis of the calorie content of the U.S. diet, the risk assessment by Petersen (139) suggests that up to 40% of all foods contain acrylamide. On the basis of the amount consumed per kilogram of body weight, the exposure data also suggest that children may be more at risk than are adults.

Soergel et al. (141) found that from 10 to 50% of dietary acrylamide in pregnant women is transferred via blood through the placenta to the fetus. Breast milk was found to contain up to 18.8 $\mu\text{g}/\text{L}$ of acrylamide. Because water soluble acrylamide can pass both placental and blood-brain barriers, the authors suggest that to protect fetuses pregnant women should not consume high-acrylamide food.

From combined animal test results and human exposure data, Dearfield et al. (142) calculated an estimated heritable genetic risk to humans exposed to acrylamide of 7.3×10^{-5} – 3.1×10^{-2} new dominant diseases due to gene mutation/ 10^6 offspring at an uptake of 1.3×10^{-5} μg of acrylamide/kg of body weight/day. Whether the predicted rate will be confirmed by epidemiological studies is currently an active area of research, especially in view of the fact that ingestion of acrylamide with food may be much higher than with water.

Efforts have been made to relate exposure to nondietary acrylamide to the incidence of human cancers. Thus, Marsh et al. (143) updated the mortality experienced by 8508 workers with potential exposure to acrylamide at three plants in the

United States from 1984 to 1994 (144). They found little evidence of a causal relationship between exposure to acrylamide and mortality from cancer sites. However, among cancer organ sites examined in an exploratory analysis of the data, they found increases in the standard mortality ratio (SMR) for some individuals exposed to acrylamide. The authors therefore recommend additional follow-up of the cohort to establish whether the observed excess of thyroid cancer and the association found between the exposure to acrylamide and pancreatic cancer warrant further study. A regrouping of the data from the two human studies (145) revealed a monotonic dose-response pattern in deaths due to pancreatic cancer, with the SMRs increasing up to 2.26 of expected deaths. It is not known to what extent, if any, the amount of acrylamide present in cigarette smoke and/or food contributes to the etiology of human cancers (104, 146, 147).

Mucci et al. (148) found a lack of an excess risk of cancer of the bowel, bladder, or kidney in Swedish consumers of foods containing moderate (30–299 $\mu\text{g}/\text{kg}$) or high (300–1200 $\mu\text{g}/\text{kg}$) levels of acrylamide. Pelucci et al. (149a) and Dybing and Sanner (149b) reported similar results. Although the absence of an association in a population-based study seems reassuring, there is a need to extend the epidemiological evaluation to other cancer sites (e.g., lung, pancreas, testis), in view of the fact that smoking significantly increases the body burden of acrylamide (106, 107). Moreover, these studies were not designed to detect an estimated stochastic (random) small increase in the incidence of cancer.

TOXICOLOGY OF ACRYLAMIDE AND GLYCIDAMIDE

Metabolism and Detoxification. With the above-cited chemical, biochemical, and risk-associated aspects as a background, this section will discuss the metabolism and the different toxicological manifestations of acrylamide and glycidamide.

Figure 6 illustrates metabolic pathways for acrylamide and glycidamide. Conjugation to GSH catalyzed by glutathione-*S*-transferase (GST) and excretion as mercapturic acid is a major pathway for the metabolism and detoxification of acrylamide (150, 151). The mercapturic acid [*N*-acetyl-*S*-(2-carbamoyl-ethyl)-cysteine] is excreted in the human urine. The mercapturic acid isolated from the urine of workers exposed to acrylamide can be measured by an HPLC procedure with a detection limit of 1 pmol (152). The urine of mice and rats exposed to acrylamide also contained several other cysteine metabolites (110).

The GSH concentration in the human liver is high, ranging from 3 to 5 $\mu\text{mol}/\text{g}$ of liver wet weight. Conditions that can decrease GSH levels and hence increase the toxicity of acrylamide at much lower exposure include (a) malnutrition associated with consumption of diets low in the sulfur amino acids cysteine and methionine, which are needed for the synthesis of GSH (153, 154); (b) oxidative stress, which may result in oxidation of GSH to GSSG; and (c) liver damage associated with alcoholic hepatitis, cirrhosis, and other malignant disorders (155). The rate of protein synthesis as well as GSH levels of neuroblastoma cells decreased on exposure to acrylamide (150, 156). The resulting depletion in GSH could result in reduced protection of cell membranes against oxidative stress.

Risk-based decision for the multisite carcinogenicity and neurotoxicity of acrylamide and its epoxide metabolite, glycidamide, may well be facilitated by a physiologically based pharmacokinetic (PBPK) model for the kinetics of distribution within five compartments of the rat: arterial blood, venous blood, liver, lung, and all other tissues (157). On the basis of the available data on the proportion of various metabolites in

METABOLISM OF ACRYLAMIDE AND GLYCIDAMIDE

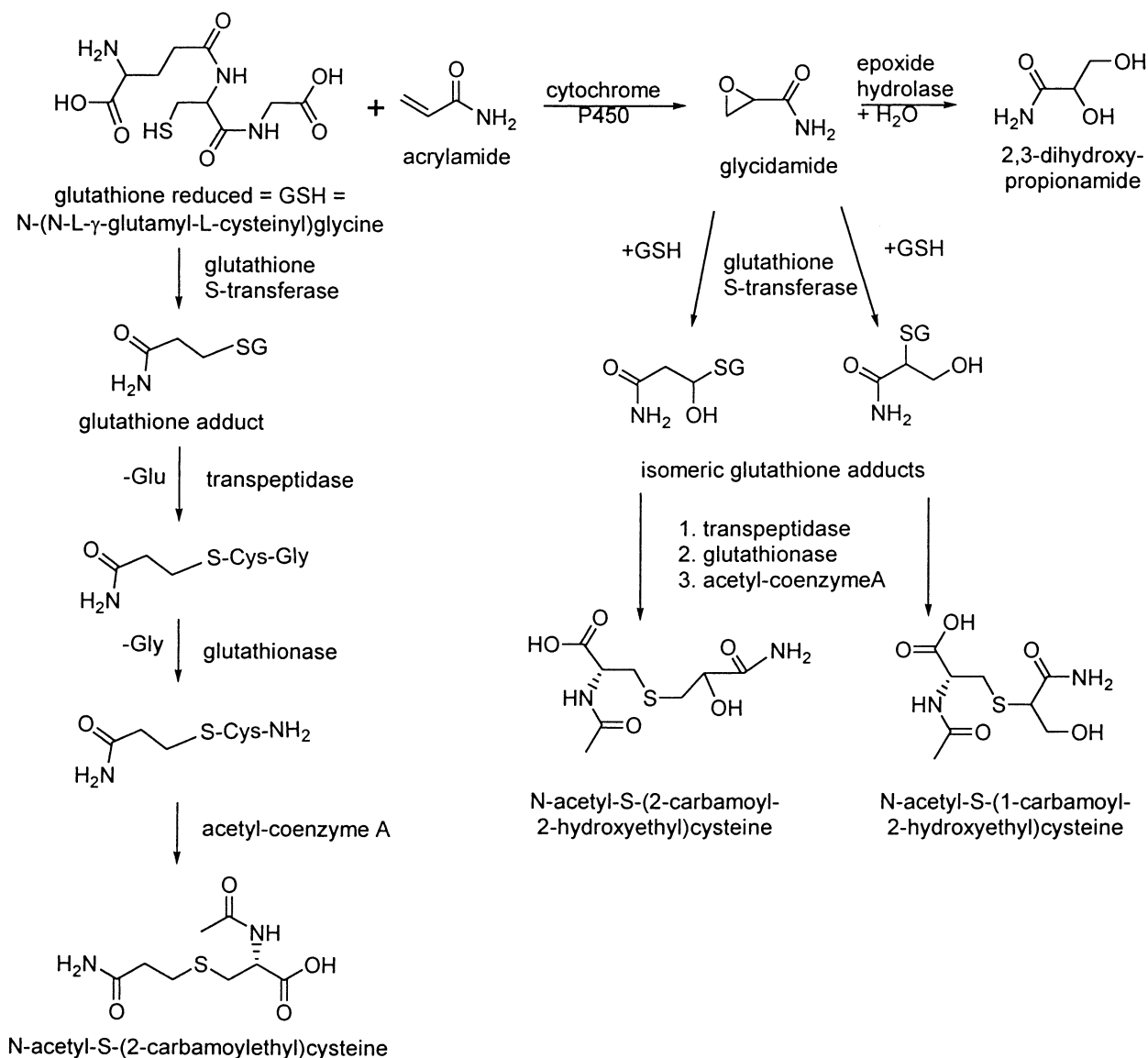


Figure 6. Metabolic pathways of acrylamide and glycidamide in mice. The final products are all eliminated from the kidneys into the urine.

the urine of the rat, the metabolism of acrylamide via cytochrome P-450 is described by a V_{max} of 1.6 mg/h/kg and a K_m of 10 mg/L and via glutathione-S-transferase (GST)-catalyzed conjugation to GSH. The kinetic analysis suggests that among the potential modes of action considered, reaction with SH groups appears to be the most biologically relevant (77, 158, 159). A parallel metabolic pathway involves epoxide hydrolase-catalyzed hydration of glycidamide to 2,3-dihydroxypropionamide (157).

Does acrylamide induce the synthesis of phase 2 detoxifying enzymes as do related vinyl compounds (160)?

Consistent with these conclusions is the suggestion that rapid alkylation of cysteine SH groups by acrylamide presents a detoxification pathway unless alkylation of protein SH groups of neurologically associated protamines leads to genetic damage through chromosome breakage (142, 161). Only reduced levels of acrylamide and glycidamide that survive the so-called "thiol barrier" would be available to react with DNA. The clastogenic (genotoxic) action of acrylamide appears to be largely due to

alkylation of DNA by glycidamide (162, 163). This model can be further tested by establishing whether externally introduced thiol compounds such as *N*-acetyl-L-cysteine and GSH can compete for acrylamide and thus reduce its toxicity, in view of the observation that acrylamide-induced morphological changes and reduction in GSH levels in Syrian hamster embryo cells were ameliorated by co-addition of *N*-acetyl-L-cysteine (164). We found that *N*-acetyl-L-cysteine is well utilized by mice as a nutritional source of cysteine (165).

Neurotoxicity. Human Studies. Workers exposed to acrylamide exhibited symptoms of peripheral neuropathy, suggesting that the compound is a human neurotoxin (137, 166). Occupational exposure of Swedish tunnel workers to a grouting agent containing acrylamide and *N*-methylolacrylamide resulted in mild and reversible peripheral nervous system symptoms (108). Hb-acrylamide adduct levels shown in Figure 4 were correlated with neurologic symptoms. Subjects with levels > 1 nmol/g of globin experienced tingling or numbness in the hands or feet. Acrylamide and *N*-methylolacrylamide ($\text{CH}_2=\text{CH}_2-$

CONHCH₂OH) differ in their ability to form Hb adducts in rats (167). We found that the latter can react with proteins (protein–NH₂) to form cross-linked proteins (protein–NH–CH₂CH₂–CONHCH₂–NH–protein) that resist degradation by ruminal bacteria (17). Such transformations benefit ruminant nutrition (168).

Short-term occupational exposure of 71 workers to acrylamide in small factories producing acrylamide in China induced the following symptoms: weak legs, loss of toe reflexes and sensations, and numb hands and feet preceded by skin peeling from the hands (169). Longer exposure resulted in more severe symptoms including cerebellar dysfunction followed by neuropathy. The authors emphasize the need to prevent dermal exposure to acrylamide.

Mechanisms of Neurotoxicity. Early studies showed that acrylamide induced neuropathological changes (peripheral distal axonopathy) in laboratory animals (170), whereas more recent studies show that nerve terminals are the initial sites for lesions with axonopathy as a conditional effect related to long-term, low-dose intoxication (171). Currently, there are two main competing mechanistic hypotheses of acrylamide neurotoxicity: inhibition of kinesin-based fast axonal transport (172) and direct inhibition of neurotransmission (171).

A single intraperitoneal injection of acrylamide (100 mg/kg) caused an increase in the expression of mRNA for neurofilament proteins in the rat brain (173), suggesting that the mechanism of adverse effects may include alteration of the expression of genes governing the synthesis of brain proteins. In vitro and in vivo studies with animals also indicate that acrylamide–sulfhydryl interactions may be paramount in the pathogenesis of neurotoxicity including impairment of regeneration of nerve function, impairment in regeneration of axons following physical injury, impairment of axonal nerve transport, skeletal weakness, and hind limb paralysis and gait impairment in animals (174–176). The biochemical basis for the neuropathy may also involve modification of amino acids and proteins present in neurons and suppression of amino acid incorporation into proteins of the nervous system (177, 178).

Seven beef cattle accidentally exposed to acrylamide while grazing exhibited clinical signs of impaired nerve function in the hindlegs, irritability, and sensitivity to touch (179). The severity of the neurological symptoms correlated with levels of Hb–acrylamide adducts.

Although measurement of Hb–acrylamide adducts appears to be an accurate biomarker for acrylamide-induced neurotoxicity, the nature of the cellular SH components of nerve tissues involved have not been elucidated. These could include both nonprotein SH groups (e.g., cysteine, homocysteine, GSH) as well as protein-bound SH groups (e.g., kinesin, dynein), paramount for maintaining native structure and function. It is also relevant that exposure of chick dorsal root ganglion to acrylamide resulted in dose-dependent morphological changes distinct from effects of sulfhydryl alkylation (159).

Srivistava et al. (180) showed that acrylamide inhibited the action of brain GST and reduced the levels of brain GSH. These changes were accompanied by increased brain dopamine receptors in a concentration-dependent manner. Related studies by Gupta and Abou-Donia (181) suggest that acrylamide interacts with tubulin and other cytoskeleton proteins, resulting in accumulation of microfilaments as well as in increases of brain and spinal cord neurofilament proteins. Studies by Ho et al. (182) indicate that acrylamide may down-regulate the microtubular system and neurofilaments, thus blocking intracellular transport in the receptor of the major neurotransmitter γ -amino-

butyric acid (GABA) in the central nervous system of chicken embryos. The authors suggest that acrylamide pathogenesis is due both to its effect on neurofilaments and to changes in the expression of neurotransmitter receptors. Studies by Tandrup and Jakobsen (183) suggest that a primary structural event in acute acrylamide intoxication is damage to the dorsal root ganglion.

LoPachin and colleagues (171, 184–190) proposed that the nerve terminal is the primary site of acrylamide action leading to inhibition of neurotransmission and the resulting neurotoxicological consequences. Their proposed mechanism of the inhibition of neurotransmission at central and peripheral synapses is based on adduct formation between acrylamide and cysteine-rich terminal proteins that mediate fusion of membranes during exocytosis. Acrylamide is a weak thiol-alkylating compound. It can, therefore, be administered on a subchronic daily basis at relatively high dose rates. Recent studies show that acrylamide is not unique among sulfhydryl reagents because *N*-ethylmaleimide and iodoacetic acid also produced concentration-dependent decreases in neurotransmitter release (R. M. LoPachin, private communication).

Since short-term oral feeding of high amounts of acrylamide to rats (21 mg/kg/day at 7, 14, 28, and 38 days) results in degeneration of the brainstem, cerebellum, and spinal cord (186–188), may long-term consumption of low levels of acrylamide contribute to the causes of Alzheimer's or other degenerative diseases of the human brain?

A possible explanation for the neurotoxicity of acrylamide is that it is a bipolar molecule, wherein the CH₂=CH part can undergo hydrophobic interactions and the CONH₂ part, hydrogen-bonding interactions with cell components. This property may enhance its ability to alter cell membrane structures and accelerate its diffusion and penetration to nerve terminal sites associated with normal function of the nervous system. Among these interactions are hydrogen bonding with H₂O, –CO–NH– (peptide bonds), –COO[–] of aspartic and glutamic acid residues, and positively charged molecules (e.g., acetylcholine) forming charged, non-covalent intermediates. Charge-transfer interactions with tryptophan and nucleic acids mentioned earlier may also influence this process.

It is also widely recognized that SH groups within the same protein and in different proteins exhibit various degrees of reactivity toward the same alkylating agent. Elsewhere, we describe such slow and fast reactions of protein SH groups with alkylating agents (9) and those of ϵ -NH₂ groups of BSA with ethyl vinyl sulfone (191). A more recent example is the application of SH chemistry to map the topology of a human plasma cell surface membrane protein (192).

We suggest that SH groups associated with components of the peripheral nervous system fall into the highly reactive category. This would explain the apparent high affinity of these groups for acrylamide. One factor that would be expected to influence rates is the extent of ionization of the SH groups in tissues. Reaction rates with acrylamide are a direct function of the p*K* values governing the equilibrium, –SH \rightleftharpoons –S[–] + H⁺. Thus, **Figure 1A** shows that rates increase rapidly as the pH approaches the p*K* value of the SH groups and, with further increase in pH, approach an asymptotic value. The dependence of rates on pH can be ascribed to the effect of pH on the concentration of the ionized RS[–] forms and their relative reactivities. It is possible that the microenvironment in the vicinity of SH sites in the nerve terminals favors such ionization, thus enhancing the nucleophilic character and hence reactivity of the SH groups with acrylamide. It would therefore be

worthwhile to ascertain reaction rates of SH groups of proteins associated with both the peripheral and central nervous systems, comparing them to rates of the low molecular weight compounds L-cysteine and GSH.

Direct evidence for hydrogen-bonding interactions of acrylamide comes from measurements of interaction energies of dimer formation between the amide group of acrylamide and 9-methyladenine (-52.0 kJ/mol) and 1-methylcytosine (-57.0 kJ/mol) (193). The enthalpies of dimer formation between the nucleic acids and acrylamide are similar to the corresponding energies for dimer formation with the amide group of asparagine.

To facilitate future studies, it is also instructive to examine electronic and charge effects, which may influence reaction rates of SH and NH_2 groups with acrylamide. The mechanism of formation of the respective transition states differs in several features that energetically favor reaction of the ionized SH group (i.e., S^- , mercaptide ion, sulfur anion) more than that of the NH_2 group. Model studies show that both groups have to approach the double bond of acrylamide almost at right angles to the plane of the molecule but that the NH_2 group has to assume a more restricted orientation than the sulfur atom to form the transition state. Unlike the NH_2 group, S^- has two lone pairs of electrons left after bonding is initiated, and the sulfur atom has empty 3d orbitals that may overlap and stabilize the double bond in forming the transition state.

Transition states for the reaction of S^- and NH_2 groups differ in another respect. When S^- goes from the negatively charged ground state as it approaches the double bond of acrylamide to its transition state, the charge becomes uniformly dispersed, whereas in the case of the NH_2 group, energetically less favorable charge separation occurs. Again, this difference in charge rearrangement during alkylation by acrylamide favors the transition state and hence the reaction rate of S^- compared to NH_2 groups (2).

The cited observations suggest that SH groups are involved in neurotransmission. However, the mechanism of the involvement is not clear. Do they interact in SH-SS redox cycles, possibly also involving nitric oxide (NO) (194) and GSH? Does modification of SH groups by acrylamide change the redox potential and internal pH of the nerve endings?

Protective Effects. The following compounds have been shown to protect against or to accelerate recovery from acrylamide-induced neuropathy: vitamin B₆ (195), thioctic acid (196), sodium pyruvate (197), 4-methylcatechol (198), and α - and β -asarones present in an ethanol-water extract of rhizomes of the *Acorus calamus* plant, which is used in India to treat epilepsy and other diseases (199). Another positive observation is that acrylamide is reported to inhibit infection of human glia cells by the poliovirus (200).

Because SH-containing *N*-acetyl-L-cysteine and GSH protected against acrylamide-induced morphological transformations of Syrian hamster embryo (SHE) cells (164), presumably by preferential reaction with the acrylamide, can sulfhydryl compounds mitigate adverse effects of acrylamide in vivo? Moreover, in view of our observation that homocysteine reacts with acrylonitrile, and presumably also with acrylamide, at the same rate as GSH over the pH range of 6–9 (Figure 1A), it is quite likely that acrylamide also alkylates plasma homocysteine, a cardiovascular disease risk factor (201).

Is alkylation of plasma homocysteine by acrylamide in vivo, if it occurs, of physiological or pathological significance?

Reproductive Toxicity. *Genotoxicity.* Acrylamide is reported to induce dominant lethal mutations in spermatids (clastogenic or chromosome damaging effects) of mice and rats and is thus

considered to be a mammalian germ cell mutagen (202, 203). Although not active in the in vitro Ames test, acrylamide does elicit mutagenic effects in stem cell spermatogonia (142). A cytometer-based dose-response micronucleus assay showed that very low doses of acrylamide can damage chromosomes (204). The linearity of the dose-response suggests that acrylamide and glycidamide are DNA-reactive clastogens and represent a health risk to mouse spermatids and other organs. Studies by Paulsson et al. (162) support the view that in the mouse, glycidamide is the predominant genotoxic factor in acrylamide exposure.

Developmental and Reproductive Effects. Feeding water solutions of acrylamide (50–200 ppm) to female and male rats prior to breeding and through the gestation and lactation period (up to 10 weeks) produced disruptions in mating, interference with sperm ejaculation, depression in body weight gain and food intake, and depression in pup body weight at birth and weight gain during lactation (205). Feeding rats a water solution of acrylamide by gavage during organogenesis produced maternal and developmental toxicity in mice at 45 mg/kg/day and only maternal toxicity in rats at >7.5 mg/kg/day (206). Feeding acrylamide to rats at levels of 5–20 mg/kg/day induced neurological manifestations lasting for 30–90 days. At neurotoxic doses, acrylamide also acts as a reproductive toxicant, as evidenced by reduced fertility rates, increased resorptions of fetuses, and reduced litter size in pregnant females, and by formation of abnormal sperm and decreased sperm count in males (207–209). Dose-related chromosomal damage in rat germ cells was observed following dermal exposure to acrylamide (210). The molecular mechanisms of reproductive toxicity could be the result of alkylation of SH groups in the sperm nucleus and tail, depletion of GSH, and/or DNA damage in the testis (142).

To separate neurotoxic from reproductive effects, Tyl et al. (211, 212) designed a two-generation reproductive study with neurotoxic endpoints. The results show that in rats, neurotoxicity and reproductive toxicity were affected by different doses of acrylamide in the drinking water. The no observable effect level (NOEL) for the prenatal dominant lethality was 2.0 mg/kg/day, whereas the NOEL for adult toxicity was <0.5 mg/kg/day. Evidently, neurotoxicity appears to be the cause or is a major contributor to reproductive toxicity. The available evidence is consistent with similar mechanisms leading to both fertility and neurotoxic endpoints. Dominant lethality effects, with different dose-response profiles, appear to operate by a mechanism involving chromosomal damage during spermatogenesis in the testis.

Because male-mediated genotoxicity may affect the survival and health of offspring, Holland et al. (213) investigated the formation of acrylamide-induced chromatin adducts of male cells of mice. Such adducts and the observed dose-dependent morphologic abnormalities in preimplantation embryos indicate that acrylamide can reach sperm cell nuclei.

Although acrylamide caused toxicity in the pregnant mother, there was no evidence for acrylamide-induced neurotoxicity in the offspring of F1 males (214). Rats suffering from protein malnutrition were more susceptible to acrylamide toxicity than those on control diets (154). Otherwise, the roles of nutrients (proteins, carbohydrates, fats, vitamins, minerals) on the severity of acrylamide toxicity as well as possible additive and synergistic effects of heat-induced food mutagens and carcinogens and dietary acrylamide are largely unknown.

Does long-term exposure to acrylamide adversely affect human fertility?

REACTION PRODUCTS FROM DNA PLUS ACRYLAMIDE AND GLYCIDAMIDE

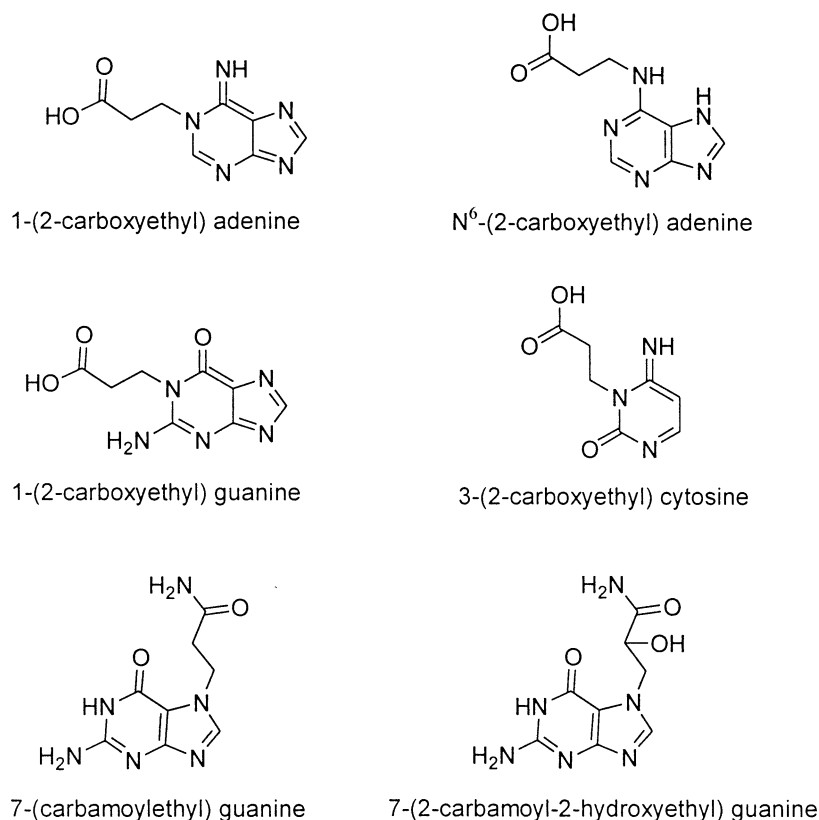


Figure 7. Structures of adenine, cytosine, and guanine derivatives resulting from hydrolysis of DNA treated with acrylamide *in vitro* and of the 7-(2-carbamoyl)-2-hydroxyethyl)guanine adduct derived from alkylation of DNA by glycidamide *in vivo* (142, 221, 222).

Carcinogenicity. Animal Studies. On the basis of numerous studies, the International Agency for Research on Cancer has classified acrylamide as “probably carcinogenic to humans” (132), as is apparently also *N*-methylolacrylamide (215). Some of the studies published before as well as after the classification will be mentioned here. Experimental animal studies showed that acrylamide could induce an increased incidence of cancers of the brain and central nervous system, the thyroid and other endocrine glands, and reproductive organs of mice (216). A lifetime oncogenicity study in rats administered acrylamide in drinking water at 0–2 mg/kg/day to males and at 0–3 mg/kg/day to females showed increases in the incidence of tumors in several organs, especially at the higher doses (217). The question arises whether exposure of humans to acrylamide derived from environmental sources as well as from ingested foods constitutes a human health hazard, in view of the conclusion by Granath et al. (218) that risks associated with genotoxic compounds are indicated to be independent of species and that relative cancer risks observed in animals also apply to humans. An application of a multiplicative model for cancer risk assessment shows that mice were ~10 times more sensitive than rats to acrylamide (219). The acrylamide metabolite glycidamide appears to be the major carcinogen in rodents; hemoglobin adduct levels from glycidamide were 3–10 times higher in mice than in rats. The authors also suggest that to facilitate risk assessment it is essential to know hemoglobin–glycidamide adduct levels in humans associated with exposure to acrylamide.

Possible carcinogenic manifestations of acrylamide could be species-dependent, so to better relate animal data to human risk, it may be worthwhile to define possible carcinogenicity of acrylamide in nonhuman primates. In this regard, it is worth

noting that our studies showed that the lysinoalanine-induced kidney damage in rats was not observed in baboons (52). See also the above section on Risk Assessment.

Mechanisms of Carcinogenesis. Whole-body autoradiography showed that the distribution of injected [¹⁴C]acrylamide in fish was highest in the kidney, urinary bladder, blood, gallbladder, intestine, and eye lens (220). Acrylamide and glycidamide are reported to modify DNA both *in vitro* and *in vivo* (142, 221, 222). Binding of [¹⁴C]acrylamide to DNA of mice was significantly greater after topical (dermal) than after oral administration (223). Glycidamide, but not acrylamide itself, induced *in vitro* mutations in *S. typhimurium* (142, 224). However, acrylamide per se does react with DNA *in vitro* (221, 222), resulting in the formation of adenine and cytosine derivatives after hydrolysis as shown in **Figure 7**. Segerback et al. (225) measured DNA adducts of acrylamide-treated rats and mice and found only the glycidamide derivative, *N*-7-(2-carbamoyl-2-hydroxyethyl)guanine. Glycidamide was 100–1000 times more reactive with DNA than was acrylamide.

Acrylamide also induced morphological changes and reduction in GSH levels in Syrian hamster embryo cells. These changes were ameliorated by co-addition of *N*-acetyl-L-cysteine (164). These observations suggest that acrylamide and glycidamide can both act as biological alkylating agents inducing base-substituted mutations in DNA, which may lead to the initiation of the carcinogenic process. Respective rates of acrylamide and glycidamide with DNA need to be clarified via quantitation of DNA adducts.

A reviewer pointed out that a fundamental question is whether acrylamide or glycidamide causes changes in DNA that result in inheritable effects in offspring cells; such changes also

comprise effects on chromosome structure (clastogenic effects) revealed by micronuclei. If inheritable genotoxic effects are shown to occur, the probability (risk) of cancer is expected to increase linearly with dose, without any safe threshold dose below which the risk is zero (226). If, instead, cancer is due to promotion, for example, by hormonal interactions or other epigenetic effects, a threshold dose-response is expected.

Can the guanine DNA adduct formed *in vivo* serve as a biomarker of exposure to acrylamide as is the case for the corresponding adduct of hemoglobin mentioned earlier?

It is relevant to note that the mechanism of carcinogenesis by glycidamide may be analogous to that described by us for the liver carcinogen aflatoxin B₁ (227). The double bonds of acrylamide and of the furan ring of aflatoxin B₁ are both transformed to reactive epoxides, which then alkylate DNA. As is the case with acrylamide, sulfhydryl compounds such as *N*-acetyl-L-cysteine and GSH prevented the alkylation. Both acrylamide and aflatoxin B₁ are also spermatotoxic (228). Our *in vitro* observations on the reduction of aflatoxin B₁ mutagenicity were confirmed by *in vivo* studies on the reduction of aflatoxin-induced tumors in rodents and poultry by *N*-acetyl-L-cysteine (229, 230). We also found that sulfhydryl compounds inactivated a potent tetrachloroimide mutagen produced in simulated processed water (231). Whether this approach will also be effective in preventing acrylamide-induced carcinogenesis merits study.

Generally, assessments of possible health risks should include the following reported criteria unique for acrylamide: (a) Humans can be exposed to acrylamide through both dietary and external work environment sources (as well as smoking) and with intake by different routes. (b) It is a biological alkylating agent that binds to DNA as well as to essential proteins and enzymes, causing genotoxicity (mutations), clastogenicity (chromosomal damage), and gene mutations in somatic and germ (sperm) cells. (c) It increases the incidence of cancer in rats at a dose of 1–2 mg/kg of body weight/day. (d) It exerts at least *three* major adverse effects in animals, neurotoxicity, developmental toxicity, and carcinogenicity. (e) It is reported to be a *cumulative* neurotoxin. (f) Reported studies with pure acrylamide may not be directly relevant to acrylamide in processed food, which may contain other potentially toxic compounds (aflatoxin B₁; furfuraldehyde; browning mutagens; mutagenic and carcinogenic heterocyclic amines; embryotoxic glycoalkaloids) or protective compounds (antioxidative sulfur amino acids, flavonoid and phenolic antioxidants, plant amidases that can hydrolyze acrylamide). These could have additive, synergistic, or antagonistic effects on the biological actions of acrylamide.

The mechanism of potato glycoalkaloid-induced developmental toxicity in frog embryos involves disruption of cell membranes (232–234) and that induced by acrylamide arises from alkylation of essential sites; what would be the effect of concurrent consumption of potato diets containing both glycoalkaloids and acrylamide? Unlike acrylamide, glycoalkaloids are not genotoxic at the DNA level (235). Moreover, will liver glycosidase-inhibiting calystegine alkaloids also present in fresh potatoes (236) affect the biological activities of acrylamide in processed potato products?

IODOACETAMIDE-INDUCED COLITIS AND ACRYLAMIDE

Intracolonic or intrarectal administration of the sulfhydryl reagent iodoacetamide (237) induces reversible mucosal erosion and ulcerations in the colon of rats (colitis, gastroenteritis, jejunitis). This results in impairment of amino acid absorption.

Compared to infection by *Helicobacter pylori*, iodoacetamide-induced gastritis in rats was associated with more severe histological changes including vascular engorgement and mucus thinning (238). The molecular basis for this effect appears to be chemical modification of sulfhydryl groups that are essential in maintaining the mucosal integrity of the colon. Because alkylation of SH groups by iodoacetamide results in the formation of adducts (R–S–CH₂–CONH₂) analogous to those formed with acrylamide (R–S–CH₂–CH₂–CONH₂), the question arises whether analogous modification by acrylamide can also contribute to the pathogenesis of colonic injury. Both acrylamide and iodoacetamide can induce allergic contact dermatitis (239).

Are the reported acrylamide-induced disruptions of the cell polarity of human colon epithelial cells (240) and of the cytoskeleton that modulates a sodium ion current in human jejunal smooth muscle cells (241) related to the etiology of colitis? Sulfhydryl compounds present in probiotic bacteria ameliorated the iodoacetamide-induced colitis (242).

STRATEGIES TO REDUCE THE ACRYLAMIDE CONTENT OF FOOD

A number of research needs related to biological effects of acrylamide have been mentioned in the text. The following additional research approaches are designed to facilitate reducing acrylamide levels in processed food. These complement those recommended at the Joint Institute for Food Safety and Applied Nutrition/National Center for Food Safety and Technology (JIFSAN/NCFST/FDA) Acrylamide in Food Workshop (see footnote to **Table 4** for website):

1. Establish databases of free asparagine and glucose content in different food categories, preferably on a dry basis.

2. Determine the relationship between asparagine levels in unprocessed foods and levels of acrylamide after processing.

3. Define the kinetics of acrylamide formation as influenced by processing conditions such as time, temperature, pH, water activity, surface area, and food composition. From the available evidence, it appears that the initial rate-determining step in heat-induced formation of acrylamide involves a second-order reaction between the α-NH₂ of asparagine and the carbonyl group of glucose to form an *N*-glycoside. Thus, the rate of formation will be proportional to the concentration of each of the two precursors. Therefore, lowering either the asparagine or glucose content can be expected to result in reduced acrylamide formation. Decreases in asparagine content may be achieved as follows:

- a. Selecting from available cultivars (e.g., potatoes, cereal grain) those that contain low levels of asparagine for food use.

- b. Breeding and/or suppressing genes that encode enzymes which govern the biosynthesis of asparagine.

- c. Acid- and/or asparaginase/amidase-catalyzed hydrolysis of asparagine to aspartic acid and ammonia.

- d. Acetylating asparagine to *N*-acetylasparagine, thus preventing formation of *N*-glycoside intermediates that form acrylamide. This approach was effectively used by us to prevent lysinoalanine formation in soy proteins. The acetylated proteins were nutritionally utilized by rats to the same extent as were the native proteins (243,244).

4. Destroy and trap acrylamide after it is formed via the following:

- a. Acid- or enzyme-catalyzed hydrolysis of the amide group of acrylamide to acrylic acid plus ammonia.

- b. UV light-, radiation-, and/or free radical-induced polymerization of monomeric acrylamide to polyacrylamide in pro-

cessed food. Free radicals in food that may catalyze polymerization of acrylamide include one-electron oxidation intermediates of phenolic compounds and flavonoids (e.g., catechin, chlorogenic acid, tyrosine) (55, 245), Maillard browning products (246), tryptophan (247, 248), and fatty acids (249).

c. Reaction of acrylamide with SH-containing amino acids, esters, peptides, and proteins. We successfully used this approach to reduce nonenzymatic and enzymatic food browning (21, 54, 250) and the lysinoalanine content of processed food proteins. The mechanism of inhibition of lysinoalanine formation involves trapping a dehydroalanine intermediate, analogous to that proposed for acrylamide (12, 18, 251). It is important to show that added reagents do not affect the quality and safety of final products.

d. Prevent formation by lowering pH of baking and frying formulations with citric acid (267).

In conclusion, acrylamide in foods is largely derived from heat-induced reactions between the α -amino group of the free amino acid asparagine and carbonyl group(s) of reducing sugars such as glucose. Secondary sources include analogous reactions in which several other free amino acids and carbonyl compounds participate. Reduction of asparagine and/or glucose content in unheated foods is expected to result in low-acrylamide foods. The available information on adverse manifestations of acrylamide and its major metabolite glycidamide indicates that neurotoxicity is a documented effect in human epidemiological studies; reproductive toxicity, genotoxicity, and carcinogenicity are potential human health risks only on the basis of animal studies. The cited conclusions and interpretations will undoubtedly be modified in the future as more information becomes available about what nature intends for acrylamide (268, 269).

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LITERATURE CITED

- Friedman, M.; Wall, J. S. Application of a Hammett-Taft relation to kinetics of alkylation of amino acid and peptide model compounds with acrylonitrile. *J. Am. Chem. Soc.* **1964**, *86*, 3735–3741.
- Friedman, M.; Cavins, J. F.; Wall, J. S. Relative nucleophilic reactivities of amino groups and mercaptide ions in addition to reactions with α,β -unsaturated compounds. *J. Am. Chem. Soc.* **1965**, *87*, 3572–3582.
- Friedman, M.; Wall, J. S. Additive linear free energy relationships in reaction kinetics of amino groups with α,β -unsaturated compounds. *J. Org. Chem.* **1966**, *31*, 2888–2894.
- Friedman, M. Solvent effects in reactions of amino groups in amino acids, peptides, and proteins with α,β -unsaturated compounds. *J. Am. Chem. Soc.* **1967**, *89*, 4709–4713.
- Cavins, J. F.; Friedman, M. New amino acids derived from reactions of ϵ -amino groups in proteins with α,β -unsaturated compounds. *Biochemistry* **1967**, *6*, 3766–3770.
- Cavins, J. F.; Friedman, M. Specific modification of protein sulfhydryl groups with α,β -unsaturated compounds. *J. Biol. Chem.* **1968**, *243*, 3357–3360.
- Friedman, M.; Romersberger, J. A. Relative influences of electron-withdrawing functional groups on basicities of amino acid derivatives. *J. Org. Chem.* **1968**, *33*, 154–157.
- Friedman, M.; Krull, L. H.; Cavins, J. F. The chromatographic determination of cystine and cysteine and the half-cysteine residues in proteins as *S*- β -(4-pyridylethyl)-L-cysteine. *J. Biol. Chem.* **1970**, *245*, 3868–3871.
- Friedman, M. *The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides, and Proteins*; Pergamon Press: Oxford, U.K., 1973; 485 pp.
- Friedman, M. Reactions of cereal proteins with vinyl compounds. In *Industrial Uses of Cereal Grains*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: Minneapolis, MN, 1973; pp 237–251.
- Finley, J. W.; Friedman, M. Chemical methods for available lysine. *Cereal Chem.* **1973**, *50*, 101–105.
- Snow, J. T.; Finley, J. W.; Friedman, M. Relative reactivities of sulfhydryl groups with *N*-acetyl dehydroalanine and *N*-acetyl dehydroalanine methyl ester. *Int. J. Pept. Protein Res.* **1976**, *8*, 57–64.
- Millard, M. M.; Friedman, M. X-ray photoelectron spectroscopy of BSA and ethyl vinyl sulfone modified BSA. *Biochem. Biophys. Res. Commun.* **1976**, *70*, 445–451.
- Friedman, M. Mass spectra of cysteine derivatives. *Adv. Exp. Med. Biol.* **1977**, *86A*, 713–725.
- Friedman, M.; Finley, J. W.; Yeh, L. S. Reactions of proteins with dehydroalanines. *Adv. Exp. Med. Biol.* **1977**, *86B*, 213–224.
- Friedman, M. Crosslinking amino acids—stereochemistry and nomenclature. *Adv. Exp. Med. Biol.* **1977**, *86B*, 1–27.
- Friedman, M.; Broderick, G. A. Protected proteins in ruminant nutrition. *Adv. Exp. Med. Biol.* **1977**, *68B*, 545–558.
- Masri, M. S.; Friedman, M. Transformation of dehydroalanine in proteins to *S*- β -(2-pyridylethyl)-cysteine side chains. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 321–325.
- Friedman, M.; Levin, C. E.; Noma, A. T. Factors governing lysinoalanine formation in soy proteins. *J. Food Sci.* **1984**, *49*, 1282–1288.
- Masri, M. S.; Friedman, M. Protein reactions with methyl and ethyl vinyl sulfones. *J. Protein Chem.* **1988**, *7*, 49–54.
- Friedman, M. Improvement in the safety of foods by SH-containing amino acids and peptides. A review. *J. Agric. Food Chem.* **1994**, *42*, 3–20.
- Friedman, M. Application of the S-pyridylethylation reaction to the elucidation of the structures and functions of proteins. *J. Protein Chem.* **2001**, *20*, 431–453.
- McCollister, D.; Oyen, F.; Rowe, V. Toxicology of acrylamide. *Toxicol. Appl. Pharmacol.* **1964**, *6*, 172–181.
- Smith, E. A.; Pruen, S. L.; Oehme, F. W. Environmental degradation of polyacrylamides. 1. Effects of artificial environmental conditions: temperature, light and pH. *Ecotoxicol. Environ. Saf.* **1996**, *35*, 121–135.
- Smith, E. A.; Prues, S. L.; Oehme, F. W. Environmental degradation of acrylamides II. Effects of environmental (outdoor) exposure. *Ecotoxicol. Environ. Saf.* **1997**, *37*, 76–91.
- Bologna, L. S.; Andrawes, F. F.; Barwenik, F. W.; Lentz, R. D.; Sojka, R. E. Analysis of residual acrylamide in field crops. *J. Chromatogr. Sci.* **1999**, *37*, 240–244.
- Pogorelova, S. P.; Bourenko, T.; Kharitonov, A. B.; Willner, I. Selective sensing of triazine herbicides in imprinted membranes using ion-sensitive field effect transistors and microgravimetric quartz crystal microbalance measurements. *Analyst* **2002**, *127*, 1484–1491.
- Dos Santos, L. A.; Carrodegua, R. G.; Boschi, A. O.; De Arruda, A. C. Dual-setting calcium phosphate cement modified with ammonium polyacrylate. *Artif. Organs* **2003**, *27*, 412–418.
- Chang, L. L.; Bruch, M. D.; Griskowitz, N. J.; Dentel, S. K. NMR spectroscopy for determination of cationic polymer concentrations. *Water Res.* **2002**, *36*, 2555–2564.
- Lande, S. S.; Bosch, S. J.; Haward, P. H. Degradation and leaching of acrylamide in soil. *J. Environ. Qual.* **1997**, *8*, 133–137.
- Wallace, A.; Wallace, G. A.; Abouzam, A. M. Effect of excess level of a polymer as a soil conditioner on yield and mineral nutrition of plants. *Soil Sci.* **1986**, *141*, 377–379.
- Castle, L. Determination of acrylamide in mushrooms grown on polyacrylamide gel. *J. Agric. Food Chem.* **1993**, *41*, 1261–1263.

- (33) Castle, L.; Campos, M. J.; Gilbert, J. Determination of acrylamide monomer in hydroponically grown tomato fruits by capillary gas chromatography—mass spectrometry. *J. Sci. Food Agric.* **1991**, *54*, 549–555.
- (34) Austarova, O. B.; Leonova, T. E.; Poliakova, I. N.; Sineokaia, I. V.; Gordeev, V. K. Adaptation of acrylamide producer *Rhodococcus rhodochrous* M8 to changes in ammonium concentration in medium (in Russian). *Prikl. Biokhim. Mikrobiol.* **2000**, *36*, 21–25.
- (35) Stevens, J. M.; Rao Saroja, N.; Jaouen, M.; Belghazi, M.; Schmitter, J. M.; Mansuy, D.; Artaud, I.; Sari, M. A. Chaperone-assisted expression, purification, and characterization of recombinant nitrile hydratase NII from *Comamonas testosteroni*. *Protein. Express. Purif.* **2003**, *29*, 70–76.
- (36) Cowan, D.; Cramp, R.; Pereira, R.; Graham, D.; Almatawah, Q. Biochemistry and biotechnology of mesophilic and thermophilic nitrile metabolizing enzymes. *Extremophiles* **1998**, *2*, 207–216.
- (37) Kobayashi, M.; Nagasawa, T.; Yamada, H. Enzymic synthesis of acrylamide: a success story not yet over. *Trends Biotechnol.* **1992**, *10*, 402–408.
- (38) Chen, Z.; Sun, X.; Shi, Y.; Shen, Z.; Zhao, J.; Sun, X. Study on production of acrylamide by microbial method (II)—enzyme catalytic kinetics and de-active dynamics of nitrile hydratase. *Shengwu Gongcheng Xuebao* **2002**, *18*, 225–230.
- (39) Nawaz, M. S.; Khan, A. A.; Seng, J. E.; Leakey, J. E.; Siitonen, P. H.; Cerniglia, C. E. Purification and characterization of an amidase from an acrylamide-degrading *Rhodococcus* sp. *Appl. Environ. Microbiol.* **1994**, *60*, 3343–3348.
- (40) Nawaz, M. S.; Billedeau, S. M.; Cerniglia, C. E. Influence of selected physical parameters on the biodegradation of acrylamide by immobilized cells of *Rhodococcus* sp. *Biodegradation* **1998**, *9*, 381–387.
- (41) Wang, C. C.; Lee, C. M. Denitrification with acrylamide in pure culture of bacteria isolated from acrylonitrile-butadiene-styrene resin manufactured for wastewater treatment. *Chemosphere* **2001**, *44*, 1047–1053.
- (42) Van Vliet, A. H.; Stoof, J.; Poppelaars, S. W.; Bereswill, S.; Homuth, G.; Kist, M.; Kuipers, E. J.; Kusters, J. G. Differential regulation of amidase- and formamidase-mediated ammonia production by the *Helicobacter pylori* fur repressor. *J. Biol. Chem.* **2003**, *278*, 9052–9057.
- (43) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Toernqvist, M. Acrylamide: a cooking carcinogen? *Chem. Res. Toxicol.* **2000**, *13*, 517–522.
- (44) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Toernqvist, M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J. Agric. Food Chem.* **2002**, *50*, 4998–5006.
- (45) Stadler, R. H.; Blank, I.; Varga, N.; Robert, F.; Hau, J.; Guy, P. A.; Robert, M. C.; Riediker, S. Acrylamide from Maillard reaction products. *Nature* **2002**, *419*, 449–450.
- (46) Ezeji, T.; Groberg, M.; Qureshi, N.; Blaschek, H. Continuous production of butanol from starch-based packing peanuts. *Appl. Biochem. Biotechnol.* **2003**, *106*, 375–382.
- (47) Zyzak, D. V. Acrylamide formation mechanism in heated food. JIFSAN/NCFST Acrylamide in Food Workshop. http://www.jifsan.umd.edu/Acrylamide/acrylamide_workshop.html, 2002.
- (48) Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448–449.
- (49) Becalski, A.; Lau, B. P. Y.; Lewis, D.; Seaman, S. W. Acrylamide in foods: occurrence, sources, and modeling. *J. Agric. Food Chem.* **2003**, *51*, 802–808.
- (50) Sanders, R. A.; Zyzak, D. V.; Stojanovic, M.; Tallmadge, D. H.; Eberhart, B. L.; Ewald, D. K. An LC/MS acrylamide method and its use in investigating the role of asparagines (abstract). Presented at the Acrylamide Symposium, Annual AOAC International Meeting, Los Angeles, CA.
- (51) (a) Yaylayan, V. A.; Wnorowski, A.; Perez Locas, C. Why asparagine needs carbohydrates to generate acrylamide. *J. Agric. Food Chem.* **2003**, *51*, 1753–1757. (b) Yasahura, A.; Tanaka, Y.; Hengel, M.; Shibamoto, T. Gas chromatographic investigation of acrylamide formation in browning model system. *J. Agric. Food Chem.* **2003**, *51*, 3999–4003.
- (52) Friedman, M. Chemistry, biochemistry, nutrition, and microbiology of lysinoalanine, lanthionine, and histidinoalanine in food and other proteins. *J. Agric. Food Chem.* **1999**, *47*, 1295–1319.
- (53) Friedman, M. Chemistry, nutrition, and microbiology of D-amino acids. *J. Agric. Food Chem.* **1999**, *47*, 3457–3479.
- (54) Friedman, M. Food browning and its prevention: an overview. *J. Agric. Food Chem.* **1996**, *44*, 631–653.
- (55) Friedman, M. Chemistry, biochemistry, and dietary role of potato polyphenols. A review. *J. Agric. Food Chem.* **1997**, *45*, 1523–1540.
- (56) Kotecha, P. M.; Kadam, S. S. Asparagus. In *Handbook of Vegetable Science and Technology*; Salunkhe, D. K., Kadam, S. S., Eds.; Dekker: New York, 1998; pp 511–521.
- (57) Hurst, P. A.; Clark, C. J. Postharvest changes in ammonium, amino-acids and enzymes of amino-acid metabolism in asparagus spear tips. *J. Sci. Food Agric.* **1993**, *63*, 465–471.
- (58) Hurst, P. L.; Boulton, G.; Lill, R. E. Towards a freshness test for asparagus: spear tip asparagine content is strongly related to post-harvest accumulated heat-units. *Food Chem.* **1998**, *61*, 381–384.
- (59) Martin, F. L.; Ames, J. M. Formation of Strecker aldehydes and pyrazines in a fried potato model system. *J. Agric. Food Chem.* **2001**, *49*, 3885–3892.
- (60) Rozan, P.; Kuo, Y. H.; Lambein, F. Amino acids in seeds and seedlings of the genus *Lens*. *Phytochemistry* **2001**, *58*, 281–289.
- (61) Jia, M.; Keutgen, N.; Matsushashi, S.; Mitzuniwa, C.; Ito, T.; Fujimura, T.; Hashimoto, S. Ion chromatographic analysis of selected free amino acids and cations to investigate the change of nitrogen metabolism by herbicide stress in soybean (*Glycine max*). *J. Agric. Food Chem.* **2001**, *49*, 276–280.
- (62) Eggum, B. O.; Sorensen, H. Chemistry and analysis of amino acids. In *Absorption and Utilization of Amino Acids*; Friedman, M., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. 3, pp 265–295.
- (63) Vasanits, A.; Kutlan, D.; Sass, P.; Molnar-Perl, I. Retention/quantitation properties of the *o*-phthalaldehyde-3-mercaptopropionic acid and the *o*-phthalaldehyde-*N*-acetyl-L-cysteine amino acid derivatives in reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* **2000**, *870*, 271–287.
- (64) Friedman, M.; Fitch, T. E.; Levin, C. E.; Yokoyama, W. H. Feeding of tomatoes to hamsters reduces their plasma low-density lipoprotein cholesterol and triglycerides. *J. Food Sci.* **2000**, *65*, 897–900.
- (65) Robinson, N. E. Protein deamidation. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5283–5288.
- (66) Riha, W. E., 3rd; Izzo, H. V.; Zhang, J.; Ho, C. T. Nonenzymatic deamidation of food proteins. *Crit. Rev. Food Sci. Nutr.* **1996**, *36*, 225–255.
- (67) Carlson, A. D.; Riggan, R. M. Development of improved high-performance liquid chromatography conditions for nonisotopic detection of isoaspartic acid to determine the extent of protein deamidation. *Anal. Biochem.* **2000**, *278*, 150–155.
- (68) Bushman, J. E.; Palmieri, D.; Whinna, H. C.; Church, F. C. Insight into the mechanism of asparaginase-induced depletion of antithrombin III in treatment of childhood acute lymphoblastic leukemia. *Leuk. Res.* **2000**, *24*, 559–565.
- (69) Lanvers, C.; Vieira Pinheiro, J. P.; Hempel, G.; Wuerthwein, G.; Boos, J. Analytical validation of a microplate reader-based method for the therapeutic drug monitoring of L-asparaginase. *Anal. Biochem.* **2002**, *309*, 117–126.
- (70) Pritsa, A. A.; Kyriakidis, D. A. L-asparaginase of *Thermus thermophilus*: purification, properties and identification of essential amino acids for its catalytic activity. *Mol. Cell. Biochem.* **2001**, *216*, 93–101.

- (71) Asselin, B. L. The three asparaginases. Comparative pharmacology and optimal use in childhood leukemia. *Adv. Exp. Med. Biol.* **1999**, *457*, 621–629.
- (72) Jonge, R.; Ritmeester, W. S.; Leusden, F. M. Adaptive responses of *Salmonella enterica* serovar *Typhimurium* DT104 and *S. typhimurium* strains and *Escherichia coli* O157 to low pH environments. *J. Appl. Microbiol.* **2003**, *94*, 625–632.
- (73) Deliganis, C. V. Death by apple juice: the problem of foodborne illness, the regulatory response, and further suggestions for reform. *Food Drug Law J.* **1998**, *54*, 681–728.
- (74) Friedman, M.; Henika, P. R.; Mandrell, R. E. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food Prot.* **2002**, *65*, 1545–1560.
- (75) Cutie, S. S.; Kallos, G. J. Determination of acrylamide in sugar by thermospray liquid chromatography/mass spectrometry. *Anal. Chem.* **1986**, *58*, 2425–2428.
- (76) Schultzova, J.; Tekel, J. Acrylamide monomer occurrence in sugar. *Dtsch. Lebensm.-Rundsch.* **1996**, *92*, 281–282.
- (77) Barber, D. S.; Hunt, J. R.; Ehrich, M. F.; Lehning, E. J.; LoPachin, R. M. Metabolism, toxicokinetics and hemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing. *Neurotoxicology* **2001**, *22*, 341–353.
- (78) Rosen, J.; Hellenas, K. E. Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. *Analyst* **2002**, *127*, 880–882.
- (79) Clarke, D. B.; Kelly, J.; Wilson, L. A. Assessment of performance of laboratories in determining acrylamide in crispbread. *J. AOAC Int.* **2002**, *85*, 1370–1373.
- (80) Ahn, J. S.; Castle, L.; Clark, D. B.; Lloyd, A. S.; Philo, M. R.; Speck, D. R. Verification of the findings of acrylamide in heated foods. *Food Addit. Contam.* **2002**, *19*, 1116–1124.
- (81) Gertz, C.; Klostermann, S. Analysis of acrylamide and mechanisms of its formation in deep-fried products. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 762–771.
- (82) Hofler, F.; Maurer, R.; Cavalli, S. Rapid analysis of acrylamide in foods with ASE and LC/MS. *GIT Labor-Fachz.* **2002**, *46*, 968–970.
- (83) Nemoto, S.; Takatsuki, S.; Sasaki, K.; Maitani, T. Determination of acrylamide in foods by GC/MS using ¹³C labeled acrylamide as an internal standard. *Shokuhin Eisegaku Zasshi* **2002**, *43*, 371–376.
- (84) Ono, H.; Chuda, Y.; Ohnishi-Kameyama, M.; Yada, H.; Ishizaka, M.; Kobaya, H.; Yoshida, M. Analysis of acrylamide by LC-MS/MS and GC-MS in processed Japanese foods. *Food Addit. Contam.* **2003**, *20*, 215–220.
- (85) Ahn, J. S.; Castle, L.; Clarke, D. B.; Lloyd, A. S.; Philo, M. R.; Speck, D. R. Verification of the findings of acrylamide in heated foods. *Food Addit. Contam.* **2002**, *19*, 1116–1124.
- (86) Yoshida, M.; Ono, H.; Ohnishi-Kameyama, M.; Chuda, Y.; Yada, H.; Kobayashi, H.; Ishizaka, M. Analysis of acrylamide level in processed foodstuffs in Japan. *Nippon Shokuhin Kougaku Kaishi* **2002**, *49*, 822–825.
- (87) Pedersen, J. R.; Olsson, J. O. Soxhlet extraction of acrylamide from potato chips. *Analyst* **2003**, *128*, 332–334.
- (88) Druckrey, H.; Conbruch, U.; Schmahl, D. Effect of monomeric acrylamide on proteins (in German). *Z. Naturforsch. B* **1953**, *86*, 145–150.
- (89) Meng, F. G.; Zhou, H. W.; Zhou, H. M. Effects of acrylamide on creatine kinase from rabbit muscle. *Int. J. Biochem. Cell Biol.* **2001**, *33*, 1064–1070.
- (90) Dobryszyccki, P.; Rymarczuk, M.; Gapinski, J.; Kochman, M. Effect of acrylamide on aldolase structure. II. Characterization of aldolase unfolding intermediates. *Biochim. Biophys. Acta* **1999**, *1431*, 351–362.
- (91) Bordini, E.; Hamdan, M. Investigation of some covalent and noncovalent complexes by matrix-assisted laser desorption/ionization time-of-flight and electrospray mass spectrometry. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1143–1151.
- (92) Levi, V.; Gonzalez Flecha, F. L. Reversible fast dimerization of bovine serum albumin detected by fluorescence resonance energy transfer. *Biochim. Biophys. Acta* **2002**, *1599*, 141–148.
- (93) Codina, A.; Vilaseca, M.; Tarrago, T.; Fernandez, I.; Ludevid, D.; Giralt, E. Location of disulfide bonds in mature α -L-fucosidase from pea. *J. Pept. Sci.* **2001**, *7*, 305–315.
- (94) Vyas, I.; Lowndes, H. E.; Howland, R. D. Inhibition of glyceraldehyde-3-phosphate dehydrogenase in tissues of the rat by acrylamide and related compounds. *Neurotoxicology* **1985**, *6*, 123–132.
- (95) Danileviciute, M.; Adomeniene, O.; Dienys, G. Kinetics of interaction of acrylamide with amino groups of some proteins. *Org. React. (Tartu)* **1981**, *18*, 217–224.
- (96) Choi, N.-S.; Kim, B.-Y.; Lee, J.-Y.; Yoon, K.-S.; Han, K.-Y.; Kim, S.-H. Relationship between acrylamide concentration and enzymatic activity in an improved single fibrin zymogram gel system. *J. Biochem. Mol. Biol.* **2002**, *35*, 236–238.
- (97) Petersen, D. W.; Kleinow, K. M.; Kraska, R. C.; Lech, J. J. Uptake, disposition, and elimination of acrylamide in rainbow trout. *Toxicol. Appl. Pharmacol.* **1985**, *80*, 58–65.
- (98) Mineki, R.; Taka, H.; Fujimura, T.; Kikkawa, M.; Shindo, N.; Murayama, K. In situ alkylation with acrylamide for identification of cysteinyl residues in proteins during one- and two-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis. *Proteomics* **2002**, *2*, 1672–1681.
- (99) Geisthardt, D.; Kruppa, J. Polyacrylamide gel electrophoresis: reaction of acrylamide at alkaline pH with buffer components and proteins. *Anal. Biochem.* **1987**, *160*, 184–191.
- (100) Fox, P. F.; Nash, B. M.; Horan, T. J.; O'Brien, J.; Morissey, P. A. Effect of selected amides on heat-induced changes in milk. *J. Dairy Sci.* **1980**, *47*, 211–219.
- (101) Friedman, M. A novel differential titration to determine pK values of phenolic groups in tyrosine and related aminophenols. *Biochem. Biophys. Res. Commun.* **1966**, *23*, 626–632.
- (102) Perez, H. L.; Cheong, H. K.; Yang, J. S.; Osterman-Golkar, S. Simultaneous analysis of hemoglobin adducts of acrylamide and glycidamide by gas chromatography–mass spectrometry. *Anal. Biochem.* **1999**, *274*, 59–68.
- (103) Paulsson, B.; Grawe, J.; Tornqvist, M. Hemoglobin adducts and micronucleus frequencies in mouse and rat after acrylamide or *N*-methylolacrylamide treatment. *Mutat. Res.* **2002**, *516*, 101–111.
- (104) Tornqvist, M.; Ehrenberg, L. Estimation of cancer risk caused by environmental chemicals based on in vivo dose measurement. *J. Environ. Pathol. Toxicol. Oncol.* **2001**, *20*, 263–271.
- (105) Bergmark, E. Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. *Chem. Res. Toxicol.* **1997**, *10*, 78–84.
- (106) Schettgen, T.; Weiss, T.; Drexler, H.; Angerer, J. A first approach to estimate the internal exposure to acrylamide in smoking and non-smoking adults from Germany. *Int. J. Hyg. Environ. Health* **2003**, *206*, 9–14.
- (107) Schettgen, T.; Broding, H. C.; Angerer, J.; Drexler, H. Hemoglobin adducts of ethylene oxide, propylene oxide, acrylonitrile and acrylamide—biomarkers in occupational and environmental medicine. *Toxicol. Lett.* **2002**, *134*, 65–70.
- (108) Hagmar, L.; Tornqvist, M.; Nordander, C.; Rosen, I.; Bruze, M.; Kautiainen, A.; Magnusson, A. L.; Malmberg, B.; Aprea, P.; Granath, F.; Axmon, A. Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. *Scand. J. Work Environ. Health* **2001**, *27*, 219–226.
- (109) Sumner, S. C. J.; MacNeela, J. P.; Fennell, T. R. Characterization and quantitation of urinary metabolites of [1,2,3-¹³C]acrylamide in rats and mice using carbon-13 nuclear magnetic resonance spectroscopy. *Chem. Res. Toxicol.* **1992**, *5*, 81–89.
- (110) Sumner, S. C. J.; Selvaraj, L.; Nauhaus, S. K.; Fennell, T. R. Urinary metabolites from F344 rats and B6C3F1 mice coadministered acrylamide and acrylonitrile for 1 or 5 days. *Chem. Res. Toxicol.* **1997**, *10*, 1152–1160.

- (111) Kokkini, G.; Stevens, V. J.; Peterson, C. M.; Cerami, A. Modification of hemoglobin by ninhydrin. *Blood* **1980**, *56*, 701–705.
- (112) Freifelder, D. *Physical Biochemistry: Applications to Biochemistry and Molecular Biology*, 2nd ed.; Freeman: San Francisco, 1982; 761 pp.
- (113) Eftink, M. R.; Jameson, D. M. Acrylamide and oxygen fluorescence quenching studies with liver alcohol dehydrogenase using steady-state and phase fluorometry. *Biochemistry* **1982**, *21*, 4443–4449.
- (114) Follenius, A.; Gerard, D. Acrylamide fluorescence quenching applied to tyrosyl residues in proteins. *Photochem. Photobiol.* **1983**, *38*, 373–376.
- (115) Bousquet, J. A.; Ettner, N. A possible tertiary structure change induced by acrylamide in the DNA-binding domain of the Tn10-encoded Tet repressor. A fluorescence study. *J. Protein Chem.* **1996**, *15*, 205–218.
- (116) Wang, Z. Y.; Sarkar, S.; Gergely, J.; Tao, T. Ca²⁺(+)-dependent interactions between the C-helix of troponin-C and troponin-I. Photocross-linking and fluorescence studies using a recombinant troponin-C. *J. Biol. Chem.* **1990**, *265*, 4953–4957.
- (117) Fox, T.; Ferreira-Rajabi, L.; Hill, B. C.; English, A. M. Quenching of intrinsic fluorescence of yeast cytochrome *c* peroxidase by covalently- and noncovalently-bound quenchers. *Biochemistry* **1993**, *32*, 6938–6943.
- (118) Wells, T. A.; Nakazawa, M.; Manabe, K.; Song, P. S. A conformational change associated with phototransformation of *Pisum* phytochrome A as probed by fluorescence quenching. *Biochemistry* **1994**, *33*, 708–712.
- (119) Martensson, L. G.; Jonasson, P.; Freskgard, P. O.; Svensson, M.; Carlsson, U.; Jonsson, B. H. Contribution of individual tryptophan residues to the fluorescence spectrum of native and denatured forms of human carbonic anhydrase II. *Biochemistry* **1995**, *34*, 1011–1021.
- (120) Park, S.; Ajtai, K.; Burghardt, T. P. Cleft containing reactive thiol of myosin closes during ATP hydrolysis. *Biochim. Biophys. Acta* **1996**, *1296*, 1–4.
- (121) Spruijt, R. B.; Wolfs, C. J.; Verver, J. W.; Hemminga, M. A. Accessibility and environment probing using cysteine residues introduced along the putative transmembrane domain of the major coat protein of bacteriophage M13. *Biochemistry* **1996**, *35*, 10383–10391.
- (122) Srinivasulu, S.; Rao, A. G. The detection of kinetic intermediates during the unfolding of lipoygenase-1 by urea or guanidine hydrochloride. *Biochim. Biophys. Acta* **1996**, *1294*, 115–120.
- (123) France, R. M.; Grossman, S. H. Acrylamide quenching of apo- and holo- α -lactalbumin in guanidine hydrochloride. *Biochem. Biophys. Res. Commun.* **2000**, *269*, 709–712.
- (124) Weber, J.; Senior, A. E. Features of F1-ATPase catalytic and noncatalytic sites revealed by fluorescence lifetimes and acrylamide quenching of specifically inserted tryptophan residues. *Biochemistry* **2000**, *39*, 5287–5294.
- (125) Espinosa, V.; Kettlun, A. M.; Zanocco, A.; Cardemil, E.; Valenzuela, M. A. Differences in nucleotide-binding site of isoapyrases deduced from tryptophan fluorescence. *Phytochemistry* **2003**, *63*, 7–14.
- (126) Friedman, M.; Cuq, J. L. Chemistry, analysis, nutritional value, and toxicology of tryptophan. *J. Agric. Food Chem.* **1988**, *36*, 1079–1093.
- (127) Ankley, G. T.; Diamond, S. A.; Tietge, J. E.; Holcombe, G. W.; Jensen, K. M.; Defoe, D. L.; Peterson, R. Assessment of the risk of solar ultraviolet radiation to amphibians. I. Dose-dependent induction of hindlimb malformations in the northern leopard frog (*Rana pipiens*). *Environ. Sci. Technol.* **2002**, *36*, 2853–2858.
- (128) Krull, L. H.; Friedman, M. Anionic graft polymerization of methyl acrylate to protein functional groups. *J. Polym. Sci. A-1* **1967**, *5*, 2535–2546.
- (129) Eskins, K.; Friedman, M. Graft photopolymerization of styrene to wheat gluten proteins. *Photochem. Photobiol.* **1970**, *12*, 245–247.
- (130) Eskins, K.; Dintzis, F.; Friedman, M. Photopolymerization of methyl acrylate in dimethyl sulfoxide. *J. Macromol. Sci.* **1971**, *A5*, 543–548.
- (131) EPA. *Preliminary Assessment of Health Risks from Exposure to Acrylamide*; Office of Toxic Substances, U.S. Environmental Protection Agency: Washington, DC, 1988.
- (132) IARC. *Some Industrial Chemicals*; International Agency for Research on Cancer: Lyon, France, 1994.
- (133) Pantusa, V. P.; Stock, T. H.; Morandi, M. T.; Harrist, R. B.; Afshar, M. Inhalation exposures to acrylamide in biomedical laboratories. *Aihaj* **2002**, *63*, 468–473.
- (134) Bailey, E.; Farmer, P. B.; Shuker, D. E. Estimation of exposure to alkylating carcinogens by the GC-MS determination of adducts to hemoglobin and nucleic acid bases in urine. *Arch. Toxicol.* **1987**, *60*, 187–191.
- (135) Friedman, M.; Noma, A. T.; Wagner, J. Ion-exchange chromatography of sulfur amino acids on a single column amino acid analyzer. *Anal. Biochem.* **1979**, *98*, 293–304.
- (136) 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.
- (137) Calleman, C. J.; Bergmark, E.; Stern, L. G.; Costa, L. G. A nonlinear dosimetric model for hemoglobin adduct formation by the neurotoxic agent acrylamide and its genotoxic metabolite glycidamide. *Environ. Health Perspect.* **1993**, *99*, 221–223.
- (138) Springer, D. L.; Bull, R. J.; Goheen, S. C.; Sylvester, D. M.; Edmonds, C. G. Electrospray ionization mass spectrometric characterization of acrylamide adducts to hemoglobin. *J. Toxicol. Environ. Health* **1993**, *40*, 161–176.
- (139) Petersen, B. Exposure and biomarkers. JIFSAN/NCFST Acrylamide in Food Workshop. http://www.jifsan.umd.edu/Acrylamide/acrylamide_workshop.html, 2002.
- (140) *Assessment of Acrylamide Intake by Duplicate Diet Study*; Swiss Office of Public Health: Berne, Switzerland, 2002.
- (141) Sorgel, F.; Weissenbacher, R.; Kinzig-Schippers, M.; Hofmann, A.; Illauer, M.; Skott, A.; Landersdorfer, C. Acrylamide: increased concentrations in homemade food and first evidence of its variable absorption from food, variable metabolism and placental and breast milk transfer in humans. *Chemotherapy* **2002**, *48*, 267–74.
- (142) Dearfield, K. L.; Douglas, G. R.; Ehling, U. H.; Moore, M. M.; Sega, G. A.; Brusick, D. J. Acrylamide: a review of its genotoxicity and an assessment of heritable genetic risk. *Mutat. Res.* **1995**, *330*, 71–99.
- (143) Marsh, G. M.; Lucas, L. J.; Youk, A. O.; Schall, L. C. Mortality patterns among workers exposed to acrylamide. *Occup. Environ. Med.* **1999**, *56*, 181–190.
- (144) Collins, J. J.; Swaen, G. M.; Marsh, G. M.; Utidjian, H. M.; Caporosi, J. C.; Lucas, L. J. Mortality patterns among workers exposed to acrylamide. *J. Occup. Med.* **1989**, *31*, 614–617.
- (145) Schultz, M. R.; Hetz-Piccio, I.; van Wijngaarden, E.; Hernandez, J. C.; Ball, L. M. Dose-response relation between acrylamide and pancreatic cancer. *Occup. Environ. Med.* **2001**, *58*, 609–616.
- (146) Smith, C. J.; Perfetti, T. A.; Rumpel, M. A.; Rdogman, A.; Doolittle, D. J. "IARC Group 2a Carcinogens" reported in cigarette mainstream smoke. *Food Chem. Toxicol.* **2000**, *38*, 371–383.
- (147) Schettgen, T.; Weiss, T.; Drexler, H.; Angerer, J. A first approach to estimate the internal exposure to acrylamide in smoking and non-smoking adults from Germany. *Int. J. Hyg. Environ. Health* **2003**, *206*, 9–14.
- (148) Mucci, L. A.; Dickman, P. W.; Steinek, G.; Adami, H.-O.; Augustsson, K. Dietary acrylamide and cancer of the large bowel, kidney, and bladder. Absence of an association in a population-based study in Sweden. *Br. J. Cancer* **2003**, *88*, 84–89.
- (149) (a) Pelucchi, C.; Franceschi, S.; Levi, F.; Trichopoulos, D.; Bosetti, C.; Negri, E.; La Vecchia, C. Fried potatoes and human cancer. *Int. J. Cancer* **2003**, *105*, 558–560. (b) Dybing, E.; Sanner, T. Risk assessment of acrylamide in foods. *Toxicol. Sci.* **2003**, in press.

- (150) Odland, I.; Romert, L.; Clemenson, C.; Walum, E. Glutathione content, glutathione transferase activity and lipid peroxidation in acrylamide-treated neuroblastoma NIE 115 cells. *Toxicol. in Vitro* **1994**, *8*, 263–267.
- (151) Sumner, S. C.; Fennell, T. R.; Moore, T. A.; Chanas, B.; Gonzalez, F.; Ghanayem, B. Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem. Res. Toxicol.* **1999**, *12*, 1110–1116.
- (152) Wu, Y. Q.; Yu, A. R.; Tang, X. Y.; Zhang, J.; Cui, T. Determination of acrylamide metabolite, mercapturic acid by high performance liquid chromatography. *Biomed. Environ. Sci.* **1993**, *6*, 273–280.
- (153) Khanna, V. K.; Husain, R.; Seth, P. K. Low protein diet modifies acrylamide neurotoxicity. *Toxicology* **1988**, *49*, 395–401.
- (154) Khanna, V. K.; Husain, R.; Seth, P. K. Protein malnourishment: a predisposing factor in acrylamide toxicity in pregnant rats. *J. Toxicol. Environ. Health* **1992**, *36*, 293–305.
- (155) Mulders, T. M.; Breiner, D. D.; Mulder, G. J. Glutathione conjugates in man. In *Human Drug Metabolism*; Jeffery, E. H., Ed.; CRC Press: Boca Raton, FL, 1992; pp 133–142.
- (156) Hashimoto, K.; Ivanov, V. V.; Inomata, K.; Kawai, T.; Mizunuma, K.; Klimatskaya, L. G.; Fefelova, Y. A. Biological monitoring of exposure to alkylating xenobiotics through their determination in the compounds having hemoglobin, plasma proteins, and urinary mercapturic acids, by using a new analytical approach. II. Acrylamide. *Vopr. Med. Khim.* **1995**, *41*, 22–25.
- (157) Kirman, C.; Gargas, M.; Deskin, R.; Tonner-Navarro, L.; Andersen, M. A physiologically based pharmacokinetic model for acrylamide and its metabolite, glycidamide, in the rat. *J. Toxicol. Environ. Health, Part A* **2003**, *66*, 253–274.
- (158) Miller, M. J.; Carter, D. E.; Sipes, I. G. Pharmacokinetics of acrylamide in Fisher-334 rats. *Toxicol. Appl. Pharmacol.* **1982**, *63*, 36–44.
- (159) Martenson, C. H.; Odom, A.; Sheetz, M. P.; Graham, D. G. The effect of acrylamide and other sulfhydryl alkylators on the ability of dynein and kinesin to translocate microtubules in vitro. *Toxicol. Appl. Pharmacol.* **1995**, *133*, 73–81.
- (160) Dinkova-Kostova, A. T.; Massiah, M. A.; Bozak, R. E.; Hicks, R. J.; Talalay, P. Potency of Michael reaction acceptors as inducers of enzymes that protect against carcinogenesis depends on their reactivity with sulfhydryl groups. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3404–3409.
- (161) Paulsson, B.; Kotova, N.; Grawe, J.; Henderson, A.; Granath, F.; Golding, B.; Tornqvist, M. Induction of micronuclei in mouse and rat by glycidamide, genotoxic metabolite of acrylamide. *Mutat. Res* **2003**, *535*, 15–24.
- (162) Granath, F.; Ehrenberg, L.; Paulsson, B.; Tornqvist, M. Cancer risk from exposure to occupational acrylamide. *Occup. Environ. Med.* **2001**, *58*, 608–609.
- (163) Abramsson-Zetterberg, L. The dose–response relationship at very low doses of acrylamide linear in the flow cytometer-base mouse micronucleus assay. *Mutat. Res* **2003**, *535*, 215–222.
- (164) Park, J.; Kamendulis, L. M.; Friedman, M. A.; Klaunig, J. E. Acrylamide-induced cellular transformation. *Toxicol. Sci.* **2002**, *65*, 177–183.
- (165) Gumbmann, M. R.; Friedman, M. Effect of sulfur amino acid supplementation of soy flour on the growth and pancreatic weights of rats. *J. Nutr.* **1987**, *117*, 1018–1023.
- (166) Costa, L. G. Biomarker research in neurotoxicology: the role of mechanistic studies to bridge the gap between the laboratory and epidemiological investigations. *Environ. Health Perspect.* **1996**, *104* (Suppl. 1), 55–67.
- (167) Fennell, T. R.; Snyder, R. W.; Krol, W. L.; Sumner, S. C. Comparison of the hemoglobin adducts formed by administration of *N*-methylolacrylamide to rats. *Toxicol. Sci.* **2003**, *71*, 164–175.
- (168) Friedman, M.; Diamond, M. J.; Broderick, G. L. Dimethylolurea as a tyrosine reagent and protein protectant against ruminal degradation. *J. Agric. Food Chem.* **1982**, *30*, 72–77.
- (169) He, F. S.; Zhang, S. L.; Wang, H. L.; Li, G.; Zhang, Z. M.; Li, F. L.; Dong, X. M.; Hu, F. Neurological and electroneuro-myographic assessment of the adverse effects of acrylamide on occupationally exposed workers. *Scand. J. Work Environ. Health* **1989**, *15*, 125–129.
- (170) Miller, M. S.; Spencer, P. S. The mechanisms of acrylamide axonopathy. *Annu. Rev. Pharmacol. Toxicol.* **1985**, *25*, 643–666.
- (171) LoPachin, R. M. The role of fast axonal transport in acrylamide pathophysiology: Mechanism or epiphenomenon? *Neurotoxicology* **2002**, *23*, 253–257.
- (172) Sickles, D. W.; Stone, J. D.; Friedman, M. A. Fast axonal transport: A site of acrylamide neurotoxicity? *Neurotoxicology* **2002**, *23*, 223–251.
- (173) Endo, H.; Kittur, S.; Sabri, M. I. Acrylamide alters neurofilament protein gene expression in rat brain. *Neurochem. Res.* **1994**, *19*, 815–820.
- (174) Kemplay, S.; Cavanagh, J. B. Effects of acrylamide and other sulfhydryl compounds in vivo and in vitro on staining of motor nerve terminals by the zinc iodide-osmium technique. *Muscle Nerve* **1984**, *7*, 94–100.
- (175) Martenson, C. H.; Sheetz, M. P.; Graham, D. G. In vitro acrylamide exposure alters growth cone morphology. *Toxicol. Appl. Pharmacol.* **1995**, *131*, 119–129.
- (176) Ko, M. H.; Chen, W. P.; Hsieh, S. T. Neuropathology of skin denervation in acrylamide-induced neuropathy. *Neurobiol. Dis.* **2002**, *11*, 155–165.
- (177) Hashimoto, K.; Ando, K. Alteration of amino acid incorporation into proteins of the nervous system in vitro after administration of acrylamide to rats. *Biochem. Pharmacol.* **1973**, *22*, 1057–1066.
- (178) Hashimoto, K.; Ando, K. Alteration of amino acid incorporation into nervous protein as an indicator of chemically induced neuropathy. *Adverse Eff. Environ. Chem. Psychotropic Drugs* **1975**, *1*, 185–188.
- (179) Godin, A.-C.; Bengtsson, B.; Niskanen, R.; Tareke, E.; Tornqvist, M.; Forslund, K. Acrylamide and *N*-methylolacrylamide poisoning in a herd of Charolais crossbreed cattle. *Vet. Rec.* **2002**, *151*, 724–728.
- (180) Srivastava, S. P.; Sabri, M. I.; Agrawal, A. K.; Seth, P. K. Effect of single and repeated doses of acrylamide and bis-acrylamide on glutathione-*S*-transferase and dopamine receptors in rat brain. *Brain Res.* **1986**, *37*, 319–323.
- (181) Gupta, R. P.; Abou-Donia, M. B. Alterations in the neutral proteinase activities of central and peripheral nervous systems of acrylamide-, carbon disulfide-, or 2,5-hexanedione-treated rats. *Mol. Chem. Neuropathol.* **1996**, *29*, 53–66.
- (182) Ho, W.-H.; Wang, S.-M.; Yin, H.-S. Acrylamide disturbs the subcellular distribution of GABA receptor in brain neurons. *J. Cell. Biochem.* **2002**, *85*, 561–571.
- (183) Tandrup, T.; Jakobsen, J. Long-term acrylamide intoxication induces atrophy of dorsal root ganglion A-cells and of myelinated sensory axons. *J. Neurocytol.* **2002**, *31*, 79–87.
- (184) LoPachin, R. M.; Ross, J. F.; Lehning, E. J. Nerve terminals as the primary site of acrylamide action: A hypothesis. *Neurotoxicology* **2002**, *23*, 43–59.
- (185) LoPachin, R. M.; Ross, J. F.; Reid, M. L.; Das, S.; Mansukhani, S.; Lehning, E. J. Neurological evaluation of toxic axonopathies in rats: Acrylamide and 2,5-hexanedione. *Neurotoxicology* **2002**, *23*, 95–110.
- (186) Lehning, E. J.; Balaban, C. D.; Ross, J. F.; Reid, M. A.; LoPachin, R. M. Acrylamide neuropathy—I. Spatiotemporal characteristics of nerve cell damage in rat cerebellum. *Neurotoxicology* **2002**, *23*, 397–414.
- (187) Lehning, E. J.; Balaban, C. D.; Ross, J. F.; LoPachin, R. M. Acrylamide neuropathy—II. Spatiotemporal characteristics of nerve cell damage in brainstem and spinal cord. *Neurotoxicology* **2002**, *23*, 415–429.
- (188) Lehning, E. J.; Balaban, C. D.; Ross, J. F.; LoPachin, R. M. Acrylamide neuropathy—III. Spatiotemporal characteristics of nerve cell damage in forebrain. *Neurotoxicology* **2003**, *24*, 125–136.

- (189) Lehning, E. J.; LoPachin, R. M.; Mathew, J.; Eichberg, J. Changes in Na-K ATPase and protein kinase C activities in peripheral nerve of acrylamide-treated rats. *J. Toxicol. Environ. Health* **1994**, *42*, 331–342.
- (190) LoPachin, R. M.; Balaban, C. D.; Ross, J. F. Acrylamide axonopathy revisited. *Toxicol. Appl. Pharmacol.* **2003**, *188*, 135–153.
- (191) Friedman, M.; Williams, L. D. A mathematical analysis of kinetics of consecutive, competitive reactions of protein amino groups. *Adv. Exp. Med. Biol.* **1977**, *86B*, 299–319.
- (192) Zhu, Q.; Lee, D. W.; Casey, J. R. Novel topology in C-terminal region of the human plasma membrane anion exchanger, AE1. *J. Biol. Chem.* **2003**, *278*, 3112–3120.
- (193) Galetich, I.; Stepanian, S. G. Combined mass spectrometric and *ab initio* study of the point contacts between 9-methyladenine and the amide group. *J. Phys. Chem. A* **2000**, *104*, 8965–8971.
- (194) Meffert, M. K.; Calakos, N. C.; Scheller, R. H.; Schulman, H. Nitric oxide modulates synaptic vesicle docking/fusion reactions. *Neuron* **1996**, *16*, 1229–1236.
- (195) Loeb, A. L.; Anderson, R. J. Antagonism of acrylamide neurotoxicity by supplementation with vitamin B₆. *Neurotoxicology* **1981**, *2*, 625–633.
- (196) Kemplay, S.; Martin, P.; Wilson, S. The effects of thioctic acid on motor nerve terminals in acrylamide poisoned rats. *Neuropathol. Appl. Neurobiol.* **1988**, *14*, 275–285.
- (197) Sabri, M. I.; Dairman, W.; Fenton, M.; Juhasz, L.; Ng, T.; Spencer, P. S. Effect of exogenous pyruvate on acrylamide neuropathy in rats. *Brain Res.* **1989**, *483*, 1–11.
- (198) Saita, K.; Hanaoka, Y.; Furukawa, S.; Hayashi, K.; Matsukara, S. A catechol derivative (4-methylcatechol) accelerates the recovery from experimental acrylamide-induced neuropathy. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 231–237.
- (199) Shukla, P. K.; Khanna, V. K.; Ali, M. M.; Maurya, R. R.; Handa, S. S.; Srimal, R. C. Protective effect of *Acorus calamus* against acrylamide induced neurotoxicity. *Phytother. Res.* **2002**, *16*, 256–260.
- (200) Ashok, A.; Atwood, W. J. Contrasting roles of endosomal pH and the cytoskeleton in infection of human glial cells by JC virus and simian virus 40. *J. Virol.* **2003**, *77*, 1347–1356.
- (201) El-Khairi, L.; Ueland, P. M.; Nygard, O.; Refsum, H.; Vollster, S. E. Lifestyle and cardiovascular disease risk factors as determinants of total cysteine in plasma: the Hordaland Homocysteine Study. *Am. J. Clin. Nutr.* **1999**, *70*, 1016–1024.
- (202) Shelby, M. D.; Cain, K. T.; Cornett, C. V.; Genoroso, W. M. Acrylamide: induction of heritable translocations in male mice. *Environ. Mutagen.* **1987**, *9*, 363–368.
- (203) Adler, I. D.; Schmid, T. E.; Baumgartner, A. Induction of aneuploidy in male mouse germ cells detected by the sperm-FISH assay: a review of the present data base. *Mutat. Res.-Fundam. Mol. Mech. Mutagen.* **2002**, *504*, 173–182.
- (204) Abramsson-Zetterberg, L. The dose-response relationship at very low doses of acrylamide linear in the flow cytometer-based mouse micronucleus assay. *Mutat. Res.* **2003**, *535*, 215–222.
- (205) Zenick, H.; Hope, E.; Smith, M. K. Reproductive toxicity associated with acrylamide treatment in male and female mice. *J. Toxicol. Environ. Health* **1986**, *17*, 457–472.
- (206) Field, E. A.; Price, C. J.; Sleet, R. B.; Marr, M. C.; Schwetz, B. A.; Morissey, R. E. Developmental toxicity evaluation of acrylamide in rats and mice. *Fundam. Appl. Toxicol.* **1990**, *14*, 502–512.
- (207) Chapin, R. E.; Fail, P. A.; George, J. D.; Grizzle, T. B.; Heindel, J. J.; Harry, G. J.; Collins, B. J.; Teague, J. The reproductive and neural toxicities of acrylamide and three analogues in Swiss mice, evaluated using the continuous breeding protocol. *Fundam. Appl. Toxicol.* **1995**, *27*, 9–24.
- (208) Sakamoto, J.; Hashimoto, K. Reproductive toxicity of acrylamide and related compounds in mice—effects on fertility and sperm morphology. *Arch. Toxicol.* **1986**, *59*, 201–205.
- (209) Wise, L. D.; Gordon, L. R.; Soper, K. A.; Duchai, D. M.; Morissey, R. E. Developmental neurotoxicity evaluation of acrylamide in Sprague-Dawley rats. *Neurotoxicol. Teratol.* **1995**, *17*, 189–198.
- (210) Gutierrez-Espleta, G. A.; Hughes, L. A.; Piegorsch, W. W.; Shelby, M. D.; Genoroso, W. M. Acrylamide: dermal exposure produces genetic damage in male mouse germ cells. *Fundam. Appl. Toxicol.* **1992**, *18*, 189–192.
- (211) Tyl, R. W.; Marr, M. C.; Myers, C. B.; Ross, W. P.; Friedman, M. A. Relationship between acrylamide reproductive and neurotoxicity in male rats. *Reprod. Toxicol.* **2000**, *14*, 147–157.
- (212) Tyl, R. W.; Friedman, M. A. Effects of acrylamide on rodent reproductive performance. *Reprod. Toxicol.* **2003**, *17*, 1–13.
- (213) Holland, N.; Ahlborn, T.; Turteltaub, K.; Markee, C.; Moore, D., 2nd; Wyrobek, A. J.; Smith, M. T. Acrylamide causes preimplantation abnormalities in embryos and induces chromatin-adducts in male germ cells of mice. *Reprod. Toxicol.* **1999**, *13*, 167–178.
- (214) Friedman, M. A.; Tyl, R. W.; Marr, M. C.; Myers, C. B.; Gerling, F. S.; Ross, W. P. Effects of lactational administration of acrylamide on rat dams and offspring. *Reprod. Toxicol.* **1999**, *13*, 511–520.
- (215) NTP. NTP Toxicology and Carcinogenesis Studies of *N*-Methylolacrylamide (CAS No. 924-42-5) in F344/N Rats and B6C3F1 Mice (Gavage Studies). *Natl. Toxicol. Program Tech. Rep. Ser.* **1989**, *352*, 1–204.
- (216) Bull, R. J.; Robinson, M.; Laurie, R. D.; Stoner, G. D.; Greisiger, E.; Meier, J. R. J.; Stober, J. Carcinogenic effects of acrylamide in Sencar and A/J mice. *Cancer Res.* **1984**, *44*, 107–111.
- (217) Friedman, M. A.; Duak, L. H.; Stedham, M. A. A lifetime oncogenicity study in rats with acrylamide. *Fundam. Appl. Toxicol.* **1995**, *27*, 95–105.
- (218) Granath, F. N.; Vaca, C. E.; Ehrenberg, L. G.; Tornqvist, M. A. Cancer risk estimation of genotoxic chemicals based on target dose and a multiplicative model. *Risk Anal.* **1999**, *19*, 309–320.
- (219) Paulsson, B.; Granath, F.; Grawe, J.; Ehrenberg, L.; Tornqvist, M. The multiplicative model for cancer risk assessment: applicability to acrylamide. *Carcinogenesis* **2001**, *22*, 817–819.
- (220) Waddell, W. J.; Leach, J. J.; Marlowe, C.; Kleinow, K. M.; Friedman, M. A. The distribution of [¹⁴C]acrylamide in rainbow trout studied by whole-body autoradiography. *Fundam. Appl. Toxicol.* **1990**, *14*, 84–87.
- (221) Solomon, J. J.; Fedyk, J.; Mukai, F.; Segal, A. Direct alkylation of 2'-deoxynucleosides and DNA following in vitro reaction with acrylamide. *Cancer Res.* **1985**, *45*, 3465–3470.
- (222) Solomon, J. J. Cyclic adducts and intermediates induced by simple epoxides. *IARC Sci. Publ.* **1999**, 123–135.
- (223) Carlson, G. P.; Fossa, A. A.; Morse, M. A.; Weaver, P. M. Binding and distribution studies in the SENCAR mouse of compounds demonstrating a route-dependent tumorigenic effect. *Environ. Health Perspect.* **1986**, *68*, 53–60.
- (224) Hashimoto, K.; Tani, H. Mutagenicity of acrylamide and its analogues in *Salmonella typhimurium*. *Mutat. Res.* **1985**, *158*, 129–133.
- (225) Segerback, 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 [¹⁴C]acrylamide. *Carcinogenesis* **1995**, *16*, 1161–1165.
- (226) Bolt, H. M. Genotoxicity—threshold or not? Introduction of cases of industrial chemicals. *Toxicol. Lett.* **2003**, *140–141*, 43–51.
- (227) Friedman, M.; Wehr, C. M.; Schade, J. E.; MacGregor, J. T. Inactivation of aflatoxin B₁ mutagenicity by thiols. *Food Chem. Toxicol.* **1982**, *20*, 887–892.
- (228) Agnes, V. F.; Akbarsha, M. A. Spermatotoxic effect of aflatoxin B₁ in the albino mouse. *Food Chem. Toxicol.* **2003**, *41*, 119–130.
- (229) De Flora, S.; Benicelli, C.; Serra, D.; Izzotti, A.; Cesarone, C. F. Role of glutathione and *N*-acetylcysteine as inhibitors of mutagenesis and carcinogenesis. In *Absorption and Utilization of Amino Acids*; Friedman, M., Ed.; CRC: Boca Raton, FL, 1989; pp 19–53.

- (230) Valdivia, A. G.; Martinez, A.; Damian, F. J.; Quezada, T.; Ortiz, R.; Martinez, C.; Llamas, J.; Rodriguez, M. L.; Yamamoto, L.; Jaramillo, F.; Loarca-Pina, M. G.; Reyes, J. L. Efficacy of *N*-acetylcysteine to reduce the effects of aflatoxin B₁ intoxication in broiler chickens. *Poult. Sci.* **2001**, *80*, 727–734.
- (231) Stevens, K. L.; Wilson, R. E.; Friedman, M. Inactivation of a tetrachloroimide mutagen from simulated processing water. *J. Agric. Food Chem.* **1995**, *43*, 2424–2427.
- (232) Friedman, M.; Rayburn, J. R.; Bantle, J. A. Developmental toxicology of potato alkaloids in the frog embryo teratogenesis assay-*Xenopus* (FETAX). *Food Chem. Toxicol.* **1991**, *29*, 537–547.
- (233) Friedman, M.; Burns, C. F.; Butchko, C. A.; Blankemeyer, J. T. Folic acid protects against potato glycoalkaloid α -chaconine-induced disruption of frog embryo cell membranes and developmental toxicity. *J. Agric. Food Chem.* **1997**, *45*, 3990–3994.
- (234) Friedman, M.; Henika, P. R.; Mackey, B. E. Effect of feeding solanidine, solasodine and tomatidine to non-pregnant and pregnant mice. *Food Chem. Toxicol.* **2003**, *41*, 61–71.
- (235) Friedman, M.; Henika, P. R. Absence of genotoxicity of potato alkaloids α -chaconine, and α -solanine and solanidine in the Ames *Salmonella* and adult and foetal erythrocyte micronucleus assays. *Food Chem. Toxicol.* **1992**, *30*, 689–694.
- (236) Friedman, M.; Roitman, J. N.; Kozukue, N. Glycoalkaloid and calystegine contents of eight potato cultivars. *J. Agric. Food Chem.* **2003**, *51*, 2964–2973.
- (237) Rachmilewitz, D.; Karmeli, F.; Okon, E. Sulfhydryl blocker-induced rat colonic inflammation is ameliorated by inhibition of nitric oxide synthase. *Gastroenterology* **1995**, *109*, 98–106.
- (238) Sherwood, P. V.; Wibawa, J. I. D.; Atherton, J. C.; Jordan, N.; Jenkins, D.; Barrett, D. A.; Shaw, P. N.; Spiller, R. C. Impact of acid secretion, gastritis, and mucus thickness on gastric transfer of antibiotics in rats. *Gut* **2002**, *51*, 490–495.
- (239) Aalto-Korte, K.; Jolanki, R.; Suuronen, K.; Eastlander, T. Biochemist's occupational allergic contact dermatitis from iodoacetamide and acrylamide. *Contact Dermatitis* **2002**, *47*, 361–364.
- (240) Jiang, M.-C.; Liao, C.-F.; Tai, C.-C. CAS/CSE 1 stimulates E-cadherin-dependent cell polarity in HT-29 human colon epithelial cells. *Biochem. Biophys. Res. Commun.* **2002**, *294*, 900–905.
- (241) Strega, P. R.; Holm, A. N.; Rich, A.; Miller, S. M.; Ou, Y. J.; Sarr, M. G.; Farrugia, G. Cytoskeletal modulation of sodium current in human jejunal circular smooth muscle cells. *Am. J. Physiol.—Cell Physiol.* **2003**, *284*, C60–C66.
- (242) Shibolet, O.; Karmeli, F.; Eliakim, R.; Swennen, E.; Brigidi, P.; Gionchetti, P.; Campieri, M.; Morgenstern, S.; Rachmilewitz, D. Variable response to probiotics in two models of experimental colitis in rats. *Inflamm. Bowel Dis.* **2002**, *8*, 399–406.
- (243) Friedman, M. Inhibition of lysinoalanine synthesis by protein acylation. *Adv. Exp. Med. Biol.* **1978**, *105*, 613–648.
- (244) Friedman, M. Nutritional and health benefits of soy proteins. *J. Agric. Food Chem.* **2001**, *49*, 1069–1086.
- (245) Fernandez, S.; Kurppa, L.; Hyvonen, L. Content of acrylamide decreased in potato chips with addition of proprietary flavonoid spice mix (Flavormare) in frying. *Innovations Food Technol. (Helsinki)* **2003**, 2–3.
- (246) Namiki, M. Chemistry of Maillard reactions: recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. *Adv. Food Res.* **1988**, *32*, 115–184.
- (247) Chauffee, L.; Windle, J. J.; Friedman, M. An electron spin resonance study of melanin treated with reducing agents. *Biophys. J.* **1975**, *15*, 565–572.
- (248) Krogull, M. K.; Fennema, O. Oxidation of tryptophan in the presence of oxidizing methyl linoleate. *J. Agric. Food Chem.* **1987**, *35*, 66–72.
- (249) German, J. B.; Kinsella, J. E. Hydroperoxide metabolism in trout gill tissue: effect of glutathione on lipoxygenase products generated from arachidonic and docosahexenoic acids. *Biochim. Biophys. Acta* **1986**, *879*, 378–386.
- (250) Molnar Perl, I.; Friedman, M. Inhibition of browning by sulfur amino acids. Part 3. Apples and potatoes. *J. Agric. Food Chem.* **1990**, *38*, 1652–1656.
- (251) Finley, J. W.; Snow, J. T.; Johnston, P.; Friedman, M. Inhibition of lysinoalanine formation in food proteins. *J. Food Sci.* **1978**, *43*, 619–621.
- (252) Seron, L. H.; Poveda, E. G.; Moya, M. S. P.; Carratala, M. L. M.; Berguer-Navarro, V.; Grane, T., N. Characterisation of 19 almond cultivars on the basis of their free amino acid composition. *Food Chem.* **1994**, *61*, 451–455.
- (253) Dizey, M.; Martin Alvarez, P. J.; Cabezudo, M. D.; Polo, M. C. Grape, apple and pineapple juice characterisation and detection of mixtures. *J. Sci. Food Agric.* **1992**, *60*, 47–53.
- (254) Eppendorfer, W. H.; Bille, S. W. Free and total amino acid composition of edible parts of beans, kale, spinach, cauliflower and potatoes as influenced by nitrogen fertilisation and phosphorus and potassium deficiency. *J. Sci. Food Agric.* **1996**, *71*, 449–458.
- (255) Gomes, M. H.; Rosa, E. Free amino acid composition in primary and secondary inflorescences of 11 broccoli (*Brassica oleracea var italica*) cultivars and its variation between growing seasons. *J. Sci. Food Agric.* **2001**, *81*, 295–299.
- (256) Murcia, M. A.; Lopez Ayerra, B.; Martinez Tome, M.; Garcia Carmona, F. Effect of industrial processing on amino acid content of broccoli. *J. Sci. Food Agric.* **2001**, *81*, 1299–1305.
- (257) Ngudi, D. D.; Kuo, Y. H.; Lambein, F. Food safety and amino acid balance in processed cassava “cossettes”. *J. Agric. Food Chem.* **2002**, *50*, 3042–3049.
- (258) Bonvehí, J. S.; Coll, F. V. Factors affecting the formation of alkylypyrazines during roasting treatment in natural and alkalinized cocoa powder. *J. Agric. Food Chem.* **2002**, *50*, 3743–3750.
- (259) Feidt, C.; Petit, A.; F., B. R.; Brun B., J. Release of free amino acids during ageing in bovine meat. *Meat Sci.* **1996**, *44*, 19–25.
- (260) Flores, M.; Moya, V. J.; Aristoy, M. C.; Toldra, F. Nitrogen compounds as potential biochemical markers of pork meat quality. *Food Chem.* **2000**, *69*, 371–377.
- (261) Rodriguez-Saona, L. E.; Wroslstad, R. E. Influence of potato composition on chip quality. *Am. Potato J.* **1997**, *74*, 87–106.
- (262) Oruna-Concha, M. J.; Duckham, S. C.; Ames, J. M. Comparison of volatile compounds isolated from the skin and flesh of four potato cultivars after baking. *J. Agric. Food Chem.* **2001**, *49*, 2414–2421.
- (263) Yang, J.; Powers, J. R.; Boylston, T. D.; Weller, K. M. Sugars and free amino acids in stored Russet Burbank potatoes treated with CIPC and alternative sprout inhibitors. *J. Food Sci.* **1999**, *64*, 592–596.
- (264) Saikusa, T.; Horino, T.; Mori, Y. Distribution of free amino acids in the rice kernel and kernel fractions and the effect of water soaking on the distribution. *J. Agric. Food Chem.* **1994**, *42*, 1122–1125.
- (265) Tkachuk, R. Free amino acids in germinated wheat. *J. Sci. Food Agric.* **1979**, *30*, 53–58.
- (266) Pripis Nicolau, L.; Revel, G. D.; Marchand, S.; Beloqui, A. A.; Bertrand, A. Automated HPLC method for the measurement of free amino acids including cysteine in musts and wines; first applications. *J. Sci. Food Agric.* **2001**, *81*, 731–738.
- (267) Jung, M. Y.; Choi, D. S.; Ju, J. W. A novel technique for limitation of acrylamide formation in fried and baked corn chips and in French fries. *J. Food Sci.* **2003**, *68*, 1287–1290.
- (268) Besaratinia, A.; Pfeifer, G. P. Weak yet distinct mutagenicity of acrylamide in mammalian cells. *J. Nat. Cancer Inst.* **2003**, *95*, 842–843.
- (269) Modification of human serum albumin by acrylamide at cysteine 64: a basis for a rapid biomonitoring procedure. *Arch. Toxicol.* **2003**, *77*, in press.

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