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A review of mechanisms of acrylamide carcinogenicity

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The fact that acrylamide, a proven rodent carcinogen, is present in significant quantities (up to several mg/kg of foodstuff) in a wide range of commonly consumed human foods is alarming. Attempts to determine a possible involvement of dietary acrylamide in human cancers have not been conclusive, however. To resolve the carcinogenicity of acrylamide to humans, the as yet unknown mechanism of action of acrylamide needs to be unraveled. The present review is a synopsis of research on the known and hypothetical modes of action of acrylamide of relevance for carcinogenesis. Both genotoxic and non-genotoxic modes of action of acrylamide are discussed with special emphasis on DNA adduct-targeted mutagenesis. Mechanistic data are presented from various experimental systems including *in vitro* experiments and *in vivo* rodent and human studies with special focus on mouse models. Human exposure data, including estimates of daily intake of dietary acrylamide in different populations and the corresponding cancer risk assessments are provided. The significant gaps in knowledge, which currently preclude a more definitive evaluation of human cancer risk due to exposure to dietary acrylamide, are highlighted. Future directions for research on acrylamide and cancer are outlined, and potential challenges are underscored.

Introduction

Acrylamide is a low molecular weight vinylic compound (Figure 1). This colorless and odorless crystalline substance is highly water soluble, easily reactive in air and rapidly polymerizable, i.e. single molecules of acrylamide (monomers) can bind together and form a larger molecule (polymer) with new properties. Polymers of acrylamide (polyacrylamide) are extensively used in modern chemical technology for a variety of purposes. These include their use as flocculants for sewage and wastewater treatment, as coagulants for clarifying drinking water, as sealants for construction of dams, tunnels and water reservoirs, as soil stabilizers in roadways construction, as binders in paper and pulp industry and as additives/adhesives/fixatives for manufacturing various industrial and cosmetic products. In analytical biochemistry, polyacrylamides are widely used for chromatography and electrophoresis, e.g. for separation and purification of proteins (1).

The vinyl group of acrylamide is electron deficient and can be easily attacked by nucleophiles. This characteristic electrophilicity of acrylamide enables it to interact with nucleophilic biological macromolecules (2). The biological consequences of acrylamide exposure have chiefly centered on neurotoxicity ever since this effect was observed in humans occupationally exposed to this compound (3). Subsequently, experimental exposure of rodents to acrylamide has also revealed a carcinogenic mode of action for this chemical [reviewed in (1,4)]. However, epidemiologic studies in work settings have not established an unambiguous link between acrylamide exposure and human cancers (5–7).

Research on carcinogenicity of acrylamide to humans regained momentum consequent to a tunnel construction accident in Sweden (8,9). Floating dead fish in an aquaculture pond downstream of a brook, wherein tunnel drainage water was pumped out, paralyzed cows that had grazed in the vicinity of the brook and tunnel construction workers with symptoms of neurotoxicity led the local authorities to declare a state of emergency (9). 'Rhoca Gil', an acrylamide-based grouting agent, which had been used to seal the tunnel leakage, was found in high concentrations in the water from the brook and the wells nearby (9). Elevation of acrylamide–hemoglobin (Hb) adducts in the blood of the affected cows and construction workers suggested the involvement of acrylamide in this disastrous chain of events (10,11). Surprisingly, considerable levels of Hb-bound acrylamide (adducts) were detected in the blood of non-exposed control individuals. The latter finding confirmed an unexplained detection of acrylamide–Hb adducts previously reported in a control population of non-smokers (12). The background levels of acrylamide in the control populations were suspected to have arisen from dietary sources (8,9). This theory was substantiated by demonstration that rats fed with fried chow had significantly higher levels of acrylamide–Hb adducts relative to controls. In confirmation, chemical analysis showed substantial levels of acrylamide in the fried chow, whereas negligible amounts of acrylamide were detectable in the non-fried chow (13).

Public health concern about acrylamide escalated after the provocative announcement of the presence of acrylamide in a host of commonly consumed foods particularly in fried, roasted and baked starch-based goods (14). Subsequent biochemical analysis identified the origin of dietary acrylamide by demonstration that the heating of free amino acids, in particular asparagine, and sugars during food processing (120–180°C) results in the formation of acrylamide (15,16). Following these findings, a few epidemiologic studies have attempted to link dietary acrylamide to human cancers (17,18). However, such questionnaire/interview-based studies have been inconclusive because of the ubiquity of acrylamide in the human diet and its variable generation even within the same brands of food (dependent on preparation method and ingredients used) (4). A comprehensive and updated summary of acrylamide content of various food products is shown in Table I.

To address the involvement of dietary acrylamide in human cancers, it would be more appropriate to conduct dietary intervention studies in which human volunteers are supplemented with foods rich in acrylamide and monitored for relevant biomarkers of exposure and effects pre- and post-supplementation. A prerequisite for such studies is the availability of biomarkers both specific for acrylamide and pertinent to cancer. Identifying these biomarkers can be achieved by unraveling the as yet unknown mechanism of acrylamide carcinogenicity (19). This review article summarizes the current knowledge of acrylamide-induced carcinogenesis, and discusses the uncertainties surrounding this field of research. Emphasis is placed on acrylamide genotoxicity and tumorigenicity with special focus on DNA adduct-targeted mutagenesis, an important area of research for which a growing body of evidence is available (19). Mechanistic data are recapitulated from experimental systems, including *in vitro* experiments and *in vivo* rodent and human studies. Highlights are provided from mouse models, for which the available data are most comprehensive for many aspects of DNA adduction, mutagenesis and tumorigenesis.

Biological properties of acrylamide

The low molecular weight and high water solubility of acrylamide enable this compound to easily pass through various biological membranes (1,2,4). Subsequently, the characteristic chemical structure of acrylamide and its ability to undergo metabolic transformation (20) make it react with different (sub)cellular targets (1,2,4). The chemical structure of acrylamide distinctive by an α,β-unsaturated carbonyl

Abbreviations: CYP2E1, cytochrome P450 2E1; GSH, glutathione; Hb, hemoglobin; *hprt*, *hypoxanthine-guanine phosphoribosyl transferase*; i.p., intraperitoneal; N7-GA-Gua, 7-(2-carbamoyl-2-hydroxyethyl)-guanine.

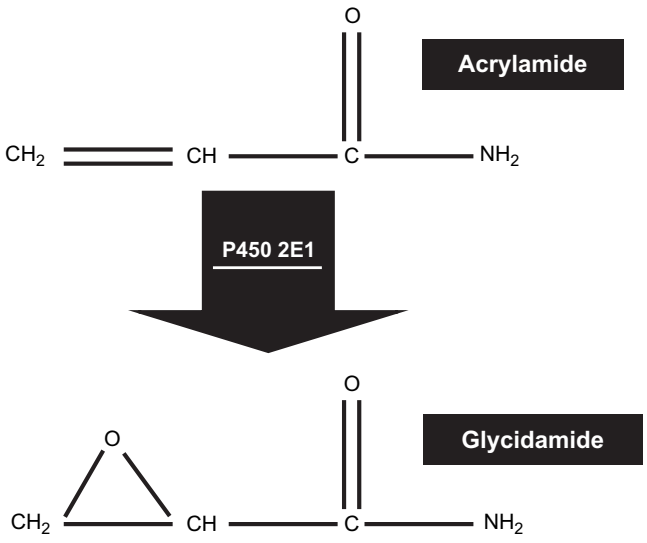

Fig. 1. Chemical structures of acrylamide and its epoxide glycidamide and the respective oxidative biotransformation by CYP2E1. The schematic metabolic pathway through which acrylamide is converted to glycidamide is shown.

group (Figure 1) constitutes its 'Michael-type' reactivity toward thiol, hydroxyl or amino groups, and to a lesser extent the nucleophilic centers in DNA. The Michael-type addition of acrylamide to thiols of glutathione (GSH) occurs most efficiently, and results in urinary excreted mercapturic acid conjugates, thereby representing a detoxification pathway (1,2,4). However, the addition of acrylamide to thiols of proteins such as protamines can cause alkylation, thus potentially causing epigenetic effects (4). Acrylamide can also bind to plasma proteins, primarily Hb, with an as yet undefined biological consequence. To date, Hb-bound acrylamide is essentially considered as the internal dose marker of exposure to acrylamide. Furthermore, acrylamide can undergo oxidative biotransformation by cytochrome P450 2E1 (CYP2E1) (20). The resulting metabolite is an epoxide derivative, i.e. glycidamide (Figure 1), which is more reactive toward DNA and proteins than the parent compound, acrylamide (2).

Acrylamide genotoxic versus non-genotoxic modes of action

Binding of acrylamide to plasma proteins and its conjugation with GSH compete against acrylamide genotoxicity, which arises from the reaction of this compound or its metabolite, glycidamide, with DNA (4). The conjugation of acrylamide with GSH can also result in depletion of cellular GSH stores, thereby changing the redox status of the cell (21). The latter can potentially affect gene expression directly or through regulating various transcription factors, which are redox dependent (22–25). Consequently, cell transformation or proliferation and apoptosis might occur independently of acrylamide-induced genotoxicity (22–25). A hormonal mode of action for acrylamide has also been hypothesized (26–31) in view of tumorigenicity of acrylamide in rat endocrine (thyroid) and mammary glands (1,2,4). This hypothesis, however, remains to be substantiated by mechanistic studies (32). Also, critics have argued that many genotoxins induce tumorigenesis at the same organ sites without causing any hormonal imbalance/dysregulation (32). Admittedly, however, despite the large body of evidence supporting a genotoxic mode of action for acrylamide, the observed tissue-specific tumorigenicity of acrylamide cannot solely be explained by its DNA damaging and mutagenic effects (see Acrylamide-induced tumorigenesis).

Synopsis of research on acrylamide carcinogenicity

To date, scant data are available on the involvement of a non-genotoxic mode of action in acrylamide carcinogenicity. However, a large body of evidence exists on acrylamide genotoxicity in relation to its carcinogenicity. In the following sections, we will summarize the existing data on metabolism and bioavailability, DNA adduct formation, mutagenicity and tumorigenicity of acrylamide, and also discuss the interdependence of these events insomuch as the available information permits. The compiled data are from *in vitro* and/or *in vivo* studies using various experimental model systems, including rodents and humans. Because the data obtained from mouse model studies are most exhaustive, we will highlight the findings of these studies at the end of each section.

Toxicokinetics of acrylamide

Toxicokinetic studies in humans have shown a terminal elimination half-life of 2.4–7.0 h for acrylamide (33,34). Over 60% of acrylamide taken up by the body can be recovered from the urine (34,35), of which ~86% are GSH conjugates (36). These include mercapturic acid metabolites of acrylamide, i.e. *N*-acetyl-*S*-(2-carbamoylethyl)-cysteine, and glycidamide, i.e. *N*-acetyl-*S*-(2-hydroxy-2-carbamoylethyl)cysteine, and minor amounts of other derivatives (36). The ratio of glycidamide- to acrylamide–GSH conjugates excreted in human urine is ~0.1 (34,35). Unchanged acrylamide in the urinary excrete accounts for 4.4% of the uptaken acrylamide, whereas unchanged glycidamide can only be found in marginal quantities (34,35). Both acrylamide and glycidamide have a significant affinity for binding to plasma proteins, in particular, to Hb (4). The Hb-bound acrylamide and glycidamide are N-terminal valine adducts, which are widely recognized as the internal dose markers of 'exposure' to acrylamide (4). Acrylamide can be found in human placenta (37) and breast milk (33), thus easily transferable to fetus or newborn infants. Transplacental exposure to acrylamide in humans has been shown by the detection of acrylamide–Hb adducts in the blood of pregnant women as well as the umbilical cord blood of their respective neonates (37). Given the shorter lifespan of neonatal erythrocytes and the lower body weight of the infants relative to adults, the internal dose of acrylamide in neonates is estimated to be at least equal to that in the mothers (37).

Toxicokinetic studies of acrylamide in rodents have shown a lower detoxification of acrylamide in mice than in rats by demonstration that per administered dose of acrylamide, mice had considerably lower levels of urinary excreted acrylamide–GSH conjugates relative to rats. However, the metabolic activation of acrylamide to glycidamide was more efficient in mice than in rats because the levels of free and GSH-conjugated glycidamide were significantly higher in the mice than in the rats (38). Also, the extent of glycidamide–Hb adduct formation per administered dose of acrylamide was 3–10 times greater in mice than in rats (39). The ratio of glycidamide- to acrylamide–GSH conjugates in rats and mice was 0.2 and 0.5, respectively (35). Thus, it can be inferred that rodents metabolize acrylamide to glycidamide more efficiently than humans. It is estimated that at equimolar dose of acrylamide, humans have ~2- and 4-fold, respectively, lower internal exposure to glycidamide than rats and mice (34,35).

Acrylamide metabolism and bioavailability in mice

Toxicokinetic studies of acrylamide in male and female B6C3F1 mice following acute dosing by intravenous, gavage and dietary routes at 0.1 mg/kg/body wt showed that acrylamide was rapidly absorbed after oral dosing, widely distributed throughout various tissues and efficiently converted to glycidamide (40). Increased levels of glycidamide–DNA adducts were observed in liver after complete elimination of acrylamide from serum. Intravenous and gavage dosing with an equimolar amount of glycidamide also resulted in rapid absorption and wide distribution to tissues, and produced liver DNA adduct levels that were ~40% higher than those from an equimolar dose of acrylamide (40). While oral administration was found to attenuate acrylamide bioavailability to 23% from the diet and 32–52% from aqueous gavage, a first-pass effect or other kinetic change resulted in higher relative internal exposure to glycidamide when compared with the intravenous route (40). A similar effect on relative glycidamide

Table I. Acrylamide content of various food products

Food category	Food item	Number of samples[a]	Mean concentration (μg/kg)	Maximum concentration (μg/kg)	CV (%)[b]
Cereals and cereal-based products		3304 (12 346)	343	7834	156
	Cereals and pasta (raw and boiled)	113 (372)	15	47	71
	Cereals and pasta (processed: toasted, fried, grilled)	200 (634)	123	820	110
	Cereal-based processed products (all)	2991 (11 327)	366	7834	151
	Breads and rolls	1294 (5145)	446	3436	130
	Pastry and biscuits (US = cookies)	1270 (4980)	350	7834	162
	Breakfast cereals	369 (1130)	96	1346	131
	Pizza	58 (85)	33	763	270
Fish and seafood (breaded, fried, baked)		52 (107)	25	233	180
Meat and offal (coated, cooked, fried)		138 (325)	19	313	174
Milk and dairy products		62 (147)	5.8	36	119
Nuts and oilseeds		81 (203)	84	1925	233
Pulses		44 (93)	51	320	137
Root and tubers		2068 (10 077)	477	5312	108
	Potato purees/mashed/boiled	33 (66)	16	69	92
	Potato baked	22 (99)	169	1270	150
	Potato crisps (US = chips)	874 (3555)	752	4080	73
	Potato chips (US = french fries)	1097 (6309)	334	5312	128
	Potato chips, croquettes (frozen, not ready-to-serve)	42 (48)	110	750	145
Stimulants and analogues		469 (1455)	509	7300	120
	Coffee (brewed), ready-to-drink	93 (101)	13	116	100
	Coffee (ground, instant or roasted, not brewed)	205 (709)	288	1291	51
	Coffee extracts	20 (119)	1100	4948	93
	Coffee decaffeinate	26 (34)	668	5399	169
	Coffee substitutes	73 (368)	845	7300	90
	Cocoa products	23 (23)	220	909	111
	Green tea (roasted)	29 (101)	306	660	67
Sugars and honey (mainly chocolate)		58 (133)	24	112	87
Vegetables		84 (193)	17	202	206
	Raw, boiled and canned	45 (146)	4.2	25	103
	Processed (toasted, baked, fried, grilled)	39 (47)	59	202	109
	Fruits (fresh)	11 (57)	<1	10	188
	Fruits (processed: dried, fried)	37 (49)	131	770	125
Miscellaneous	Alcoholic beverages (beer, gin, wine)	66 (99)	6.6	46	143
	Condiments and sauces	19 (22)	71	1168	345
	Infant formula	82 (117)	<5	15	82
	Baby food (canned, jarred)	96 (226)	22	121	82
	Baby food (dry powder)	24 (34)	16	73	125
	Baby food (biscuits, rusks, etc.)	32 (58)	181	1217	106
	Dried food	13 (13)	121	1184	266

Data were obtained from the Summary Report of the 64th Meeting of the Joint Food and Agriculture Organization of the United Nations/World Health Organization Expert Committee on Food Additives (http://www.who.int/). Acrylamide occurrence data for different food items analyzed from 2002 to 2004 were provided from 24 countries (see ref. 74). The total number of analytical results (single or composite samples) was 6752 with 67.6% from Europe, 21.9% from North America, 8.9% from Asia and 1.6% from Pacific. No data from Latin America and Africa were submitted.

[a]Number of analytical results for individual plus composite samples. In parentheses, the total numbers of individual samples are shown.

[b]CV, coefficient of variation.

exposure was also evident as the administered dose was reduced, which suggests that as dosing rate decreases, the conversion of acrylamide to glycidamide is more efficient (40).

Kinetic studies by Sumner *et al.* (20,38) have established a major role of CYP2E1 in biotransformation of acrylamide to glycidamide. These investigators have shown that administration of acrylamide (50 mg/kg/body wt single oral gavage) to wild-type mice pre-treated with an inhibitor of P450, 1-aminobenzotriazole, as well as to mice deficient of CYP2E1 resulted in the urinary excretion of only acrylamide–GSH conjugates (20,38). However, similar administration of acrylamide to wild-type mice without any 1-aminobenzotriazole pre-treatment yielded both glycidamide- and acrylamide-conjugated urinary metabolites (20,38). Gamboa da Costa *et al.* (41) have demonstrated that consistent with the known deficiency of P450 activity in neonatal mice (42), formation of DNA adducts induced by glycidamide [50 mg/kg/body wt, single intra-peritoneal (i.p.) injection] in mouse neonates was 5- to 7-fold higher than that in their counterparts treated with an equimolar dose of acrylamide. Adult mice, however, showed only 1.2- to 1.5-fold higher formation of DNA adducts after treatment with glycidamide relative to acrylamide (41).

Acrylamide-induced DNA adduction and mutagenesis

Direct interaction of acrylamide with DNA through Michael-type addition reaction is considerably slow (4). *In vitro* treatment of calf thymus DNA with acrylamide at physiological pH and temperature for prolonged periods of time (up to 40 days) revealed a slow reactivity of acrylamide toward DNA. The reaction products were alkylated

2′-deoxynucleoside adducts (43) of adenine, cytosine and guanine (43). Addition of S9 liver extracts to the reaction mix of acrylamide and DNA, however, resulted predominantly in the formation of 7-(2-carbamoyl-2-hydroxyethyl)-guanine (N7-GA-Gua), a glycidamide-derived DNA adduct (Figure 2a) (41,44). Similarly, *in vivo* oral or topical administration of acrylamide to mice and rats induced primarily N7-GA-Gua adducts, which were evenly distributed throughout the animals' organs (41,44). The latter finding was consistent with the uniform and ubiquitous presence of the labeled acrylamide administered to the animals (44). The omnipresence of acrylamide and glycidamide and the respective DNA adducts (41,44) is consistent with the chemical properties of acrylamide, i.e. being a small and highly water-soluble molecule that can easily pass through biological membranes and reach various organs (1,2,4). Further characterization of the induced DNA adducts in acrylamide- and glycidamide-treated mice identified a minor glycidamide–DNA adduct, i.e. 3-(2-carbamoyl-2-hydroxyethyl)-adenine (Figure 2b). The formation of another minor glycidamide-based DNA adduct, i.e. 1-(2-carboxy-2-hydroxyethyl)-2′-deoxyadenosine, was also deduced (Figure 2c) (41).

Acrylamide was non-mutagenic in bacterial Ames assays with or without an exogenous activation system (1,45). Acrylamide was tested negative in assays using different strains of *Salmonella typhimurium*, including TA1535, TA1537, TA1538, TA97, TA98, TA100 and TA102 in the plate incorporation and/or liquid pre-incubation procedures in the presence or the absence of an exogenous activating system (1,45). Glycidamide was, however, mutagenic in the same assays even in the absence of an exogenous activating system (45). The observed non-mutagenicity of acrylamide has been ascribed to a suboptimal conversion of acrylamide to glycidamide by the utilized activating system (1,2,4). The widely used Aroclor-induced S9 mix is known to be competent in the epoxidation of a variety of chemical carcinogens, including polycyclic aromatic hydrocarbons, e.g. dimethylbenzo[*a*]anthracene and benzo[*a*]pyrene, as well as olefinic compounds, e.g. vinyl chloride and butadiene (2). Specifically, however, the presence of CYP2E1, the enzyme required for epoxidation of acrylamide to glycidamide (20), in this activating system remains to be determined.

Acrylamide was shown to be mutagenic at the *hypoxanthine–guanine phosphoribosyl transferase (hprt)* locus in cultured Chinese hamster ovary cells in the presence or the absence of exogenous activating systems. Acrylamide was also mutagenic and/or clastogenic at the *thymidine kinase* locus in mouse lymphoma cells even in the absence of an exogenous activation system (1). Acrylamide clastogenicity has been verified independently by other studies (46–49). Acrylamide caused meiotic mutations in mouse germ cells assayed by the morphological specific-locus test (50). Acrylamide also induced spots of genetic relevance, indicative of point mutations/chromosomal loss/somatic recombination, quantified by a mammalian spot test in mouse offspring borne from embryos exposed to acrylamide at 10–12 days post-gestation (51). Acrylamide was mutagenic in the *lacZ* transgene in Muta mice treated i.p. with daily doses of acrylamide (50 mg/kg/body wt) for five consecutive days (52). A recent mutagenicity experiment in Big Blue mice has also shown that animals receiving acrylamide or glycidamide at equimolar doses of up to 500 mg/l in drinking water for 3–4 weeks had significant increases in mutant frequencies of the *hprt* and *cII* genes 21 days following the last treatments (49).

Acrylamide genotoxicity in mice

A single i.p. injection of acrylamide (50–53 mg/kg/body wt) to male adult mice of two different strains, i.e. BALB/c and C3H/HeNMTV, induced significantly acrylamide–DNA adduction in various organs 6 h post-treatment (41,44). The induced DNA adducts detected by liquid chromatography with tandem mass spectrometry were all of glycidamide nature, including a predominant adduct, N7-GA-Gua, and a minor adduct, 3-(2-carbamoyl-2-hydroxyethyl)-adenine, with another minor adduct, 1-(2-carboxy-2-hydroxyethyl)-2′-deoxyadenosine, being also implicated (Figure 2). The two minor adducts comprised roughly ∼1% of the overall detected adducts. The predominant adduct was elevated on average a few 100-fold over background in all investigated organs, e.g. ∼380-, 240- and 110-fold increases in lung, liver and kidney, respectively (41,44). The background level of N7-GA-Gua adducts varied in the range of 5–11 × 10^{-8} nucleotides in different organs, and was ascribed to the acrylamide content of standard rodent chow and drinking water (13). The administered amounts of acrylamide yielded doses of 606–642 μM/kg/body wt per mouse, and were well tolerated without causing any severe adverse health effects (41).

Adler *et al.* (53) have shown that pre-treatment of mice (102/E1 × C3H/E1: F1) with 1-aminobenzotriazole for three consecutive days (50 mg/kg/body wt daily) inhibited or significantly reduced the dominant lethal mutagenic effects in germ cells induced by a single i.p. injection of acrylamide (125 mg/kg/body wt) at day 4. To explore the role of CYP2E1 metabolism in the germ cell mutagenicity of acrylamide, *CYP2E1*-null and wild-type male mice were treated daily with acrylamide by i.p. injection (0, 12.5, 25 or 50 mg/kg/body wt) for five consecutive days (46). At defined times after exposure, males were mated to untreated B6C3F1 females. Females were killed in late gestation and uterine contents were examined. Dose-dependent increases in dominant lethal mutations were detected in uterine contents of female mice mated to acrylamide-treated wild-type males but not *CYP2E1*-null males (46). A follow-up study examined the induction of somatic cell damage assessed in leukocytes, liver and lung of female wild-type and *CYP2E1*-null mice administered acrylamide i.p. (0, 25, 50 mg/kg/body wt) once daily for five consecutive days using the alkaline single-cell gel electrophoresis assay (54). The frequency of micronucleus formation was also determined in erythrocytes of the mice 24 h after the last treatments using the flow cytometry assay. Significant dose-related increases in induction of DNA damage in somatic cells and formation of micronucleated

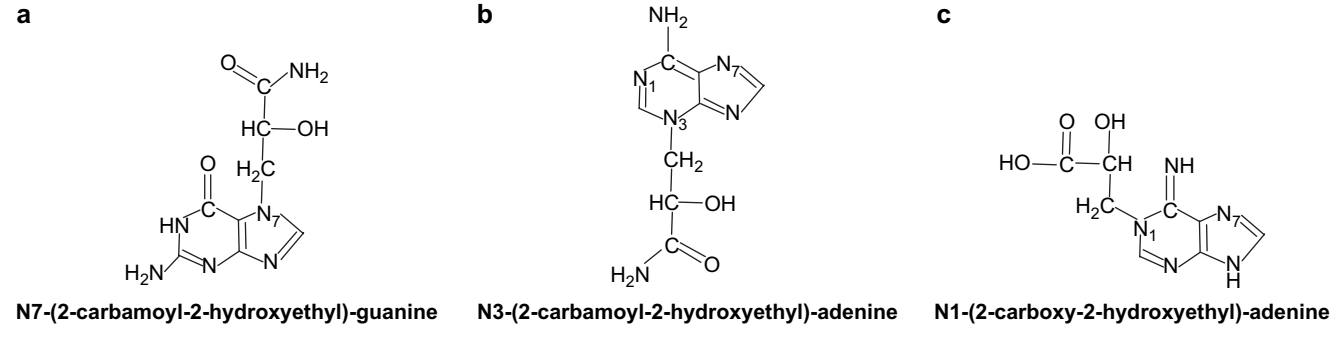

Fig. 2. Chemical structures of acrylamide-induced DNA adducts. The major adduct (**a**) is identified as N7-GA-Gua, and two minor adducts are identified as (**b**) 3-(2-carbamoyl-2-hydroxyethyl)-adenine and (**c**) 1-(2-carboxy-2-hydroxyethyl)-2′-deoxyadenosine. The predominant N7-GA-Gua adduct was formed ∼100-fold more extensively than 3-(2-carbamoyl-2-hydroxyethyl)-adenine adduct in all tested organs, i.e. liver, lung and kidney. The formation of 1-(2-carboxy-2-hydroxyethyl)-2′-deoxyadenosine adduct *in vitro* was estimated to be at least 11-fold higher than that of 3-(2-carbamoyl-2-hydroxyethyl)-adenine adduct (41).

erythrocytes were observed in acrylamide-treated wild-type but not in the *CYP2E1*-null mice (54). The overall findings support the notion that genetic damage in somatic and germ cells of mice treated with acrylamide is dependent upon metabolism of the parent acrylamide to glycidamide by CYP2E1 (46,54).

Recently, *in vivo* genotoxicity of acrylamide was investigated in groups of male and female Big Blue mice administered 0, 100 or 500 mg/l of acrylamide or equimolar doses of glycidamide (via drinking water) for 3–4 weeks (49). The frequency of micronucleated reticulocytes was significantly increased in males treated with the high doses of acrylamide and glycidamide within 24 h of the last treatment (1.7- to 3.3-fold higher than control). Both doses of acrylamide and glycidamide significantly elevated lymphocyte *hprt* mutant frequencies in both male and female mice 21 days following the last treatments, with the high doses producing responses 16- to 25-fold higher than those of the respective controls. The high doses of acrylamide and glycidamide significantly increased liver *cII* mutant frequency in both males and females 21 days post-treatment (2- to 2.5-fold higher than the respective controls) (49). The more pronounced mutagenic response induced by both acrylamide and glycidamide in the *hprt* gene relative to the *cII* transgene can be ascribed to the greater number of mutable sites as well as a lower spontaneous mutant frequency rate (~10-fold) in the former gene (55). Unlike the *hprt* gene whose mutation analysis can only be performed on limited organs, i.e. bone marrow and peripheral blood, the *cII* mutation analysis can, however, be done in various target and non-target organs without any restriction (55).

Acrylamide-induced tumorigenesis

Acrylamide has shown a characteristic organ-specific tumorigenicity in both mice and rats, although with distinct site specificity in each species (1,2,4). Mid/long-term carcinogenicity experiments in rodents have documented that administration of acrylamide through various routes, e.g. orally, topically and systemically, can increase the incidence of lung and skin adenomas and carcinomas in mice, as well as induce scrotal mesotheliomas (malignant), thyroid adenomas and/or adenocarcinoma (benign and malignant), mammary gland tumors (benign and malignant), uterine adenocarcinomas (malignant), central nervous system tumors (malignant), clitoral gland adenomas (benign) and oral papillomas (benign) in rats (1,2,4). However, the site-specific tumorigenicity of acrylamide in both species has not been accounted for by any underlying mechanisms, and remains to be elucidated.

Acrylamide tumorigenicity in mice

Acrylamide initiated skin tumorigenesis in two different strains of mice, i.e. Sencar and the ICR–Swiss mice (56,57). Using an 'initiation–promotion protocol', acrylamide administered via various routes, i.e. oral gavage, i.p. and topically (up to 50 mg/kg/body wt triweekly for 2 weeks) followed by a 'promotion' regimen of 12-*O*-tetradecanoyl-phorbol-13-acetate for 20 weeks, induced significantly papillomas and squamous cell carcinomas of the skin in a dose-dependent fashion in both strains (56,57). The greatest initiating effect of acrylamide in both the Sencar and ICR–Swiss mice was observed when it was administered orally (56,57). In the ICR–Swiss mice, oral administration of acrylamide, even in the absence of 12-*O*-tetradecanoyl-phorbol-13-acetate as the 'promoting' agent, also led to a significant formation of alveolar bronchiolar adenomas and carcinomas (57). Similar complete lung carcinogenicity of acrylamide was observed in the A/J mice treated with acrylamide through both oral and i.p. routes of exposure at concentrations up to 30 mg/kg/body wt thrice a week for a total period of 8 weeks (56). The A/J and ICR–Swiss mice killed at the age of 1 year and 8–9 months, respectively, had survival rates of 94 and 90%. In the respective strains, 30 out of 32 animals and 36 out of 40 animals, which received acrylamide orally, were available for autopsy without unacceptable levels of autolysis at the end of each experiment. The percentages of tumor-bearing animals in the A/J and ICR–Swiss mice after oral administration of acrylamide were significantly increased relative to solvent-treated controls, i.e. 93 versus 10% and 39 versus 11%, in the respective strains (detailed information on acrylamide tumorigenicity in the Sencar, ICR–Swiss, and A/J mice is available in Table II, Table III and

Table II. Tumor-initiating effects of acrylamide in the skin of female Sencar mice

Dose (mg/kg)	Route of administration	TPA	Cumulative number of tumor-bearing animals[a]/number of animals initiated	Number of squamous cell papillomas/number of animals examined[b]	Number of squamous cell carcinomas/number of animals examined[b]	Percentage of animals bearing squamous cell carcinomas
0	p.o.	+	2/40	0/34	0/34	0
75	p.o.	+	12/40	3/35	2/35	5.7
150	p.o.	+	23/40	8/33	7/33	21.2
300	p.o.	+	30/40	11/38	6/38	15.8
300	p.o.	−	0/20	0/17	0/17	0
0[c]	i.p.	+	0/40	0/35	0/35	0
75	i.p.	+	10/40	2/38	2/38	5.2
150	i.p.	+	13/40	3/36	4/36	11.1
300	i.p.	+	21/40	6/35	4/35	11.4
300	i.p.	−	0/20	0/17	0/17	0
0[d]	Topical	+	7/40	5/36	0/36	0
75	Topical	+	4/40	3/38	1/38	2.6
150	Topical	+	11/40	3/35	2/35	5.7
300	Topical	+	18/40	2/34	3/34	8.8
300	Topical	−	0/20	0/20	0/20	0

Acrylamide was dissolved in distilled deionized water for the p.o. and i.p. routes and in ethanol for topical application. Female Sencar mice (6–8 weeks of age) were treated with acrylamide at doses of 12.5, 25.0 or 50.0 mg/kg/body wt for six applications over a 2 week period for total doses of 75, 150 or 300 mg/kg/body wt, respectively, via different routes, i.e. gastric intubation (p.o.), i.p. injection and topical administration to the shaved back of the mice. Two weeks following the tumor-initiating doses of acrylamide, a tumor promotion regimen was begun, wherein 1.0 μg 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) dissolved in 0.2 ml acetone was applied to the shaved back of each animal three times per week for 20 weeks. Tumor incidences were charted from weekly observations. All animals were killed at 52 weeks in the study, and histopathological evaluations were performed on all gross lesions. Data were obtained from ref. (56).

[a]To be included in the cumulative count, an animal must have had a tumor of >1 mm diameter at the same location for three consecutive observations.

[b]Number of animals available for histopathological examination following death or at termination of the experiment at 1 year (excluding heavily autolyzed and cannibalized animals).

[c]Distilled deionized water was administered in the same volume (0.2 ml/mouse) and at the same frequency as administered to experimental groups.

[d]Ethanol was administered topically in the same volume (0.2 ml/mouse) and at the same frequency as administered to experimental groups.

Table III. Tumorigenicity of acrylamide in the skin and lung of female ICR–Swiss mice

Dose (mg/kg)	TPA	Skin				Lung			
		Cumulative number of tumor-bearing animals[a]/total number of treated animals	Cumulative number of tumors/animal	Histological classification of squamous cell tumors at autopsy		Number of animals available for autopsy	Histological classification of alveolar bronchiolar tumors		
				Papilloma	Carcinoma		Adenoma	Carcinoma	Total
0[b]	+	0/40 (35)	0	0	0	36	3	1	4
75	+	4/40 (34)	0.10	1	1	34	6	2	8
150	+	4/40 (32)	0.13	0	3	36	5	1	6
300	+	13/40 (32)	0.43	6	4	34	10	1	11
300	−	10/40 (33)	0.03	0	1	36	4	10	14

Female ICR–Swiss mice were administered acrylamide orally by gavage at doses of 12.5, 25.0 or 50.0 mg/kg/body wt for six applications over a 2 week period for total doses of 75, 150 or 300 mg/kg/body wt, respectively. Two weeks following the tumor-initiating doses of acrylamide, a tumor promotion regimen was begun, wherein 2.5 μg 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) dissolved in 0.2 ml acetone was applied to the shaved back of each animal three times per week for 20 weeks. Tumor incidences were charted from weekly observations. All animals were killed at 52 weeks in the study, and histopathological evaluations were performed on all gross lesions. Data were obtained from ref. (57).
[a]To be included in the cumulative count, an animal must have had a tumor of >1 mm diameter at the same location for three consecutive observations.
[b]Distilled deionized water was administered in the same volume (0.2 ml/mouse) and at the same frequency as administered to experimental groups.

Table IV. Acrylamide-induced lung adenomas in A/J mice

Dose (mg/kg)	Sex	Number of surviving mice/initial number of mice	Percentage of mice with lung adenomas[b]	Average number of lung adenomas /mouse[c]
NT[a]	M	16/16	31	0.31 ± 0.48
	F	14/16	50	0.50 ± 0.52
	M + F	30/32	40	0.40 ± 0.50
0[b]	M	16/16	13	0.06 ± 0.25
	F	15/16	8	0.13 ± 0.35
	M + F	31/32	10	0.10 ± 0.30
24	M	16/16	50	0.75 ± 0.93
	F	17/17	35	0.35 ± 0.60
	M + F	33/33	42	0.55 ± 0.78
72	M	16/16	38	0.69 ± 1.03
	F	17/17	53	0.88 ± 1.11
	M + F	33/33	46	0.79 ± 1.05
240	M	17/17	59	0.88 ± 0.99
	F	14/15	79	1.57 ± 1.79
	M + F	31/32	68	1.19 ± 1.40
720	M	15/16	93	1.87 ± 1.55
	F	15/16	93	2.53 ± 1.46
	M + F	30/32	93	2.20 ± 1.52

Male (M) and female (F) A/J mice (8 weeks old) were treated i.p. with acrylamide at doses of 1, 3, 10 or 30 mg/kg/body wt three times per week for 8 weeks for total doses of 24, 72, 240 or 720 mg/kg/body wt, respectively. Two base-line control groups were included: an untreated group (NT[a]), and a vehicle control group receiving solvent distilled deionized water (0[b]). Animals were killed at 8 months of age, their lungs were fixed in Tellyesniczky's solution and the surface adenomas were counted after 24 h. Data were obtained from ref. (56).
[c]Mean ± SD.

Table IV, respectively) (56,57). Historically, the A/J mice develop spontaneously 1–2 lung tumors per animal around 1.5 years of age (58–60), whereas the ICR–Swiss mice have a much lower incidence of the respective tumors, which occur at 19–24 months of age (61–63).

On the basis of neurotoxicity and carcinogenicity data, it is now established that acrylamide causes appreciable toxicity when it is administered at doses exceeding 50 mg/kg/body wt (1,2,4). Most recently, a mutagenicity experiment in Big Blue mice (49) has shown that animals receiving acrylamide in drinking water at a concentration of 100 mg/l, which yielded an average daily dose of 19–25 mg/kg/body wt, exhibited no signs of toxicity after 4 weeks of continuous dosing followed by an additional post-treatment period of 3 weeks. Acrylamide at a concentration of 500 mg/l, which resulted in an average daily dose of 98–107 mg/kg/body wt, caused neurotoxicity after 3 weeks of continuous dosing. However, cessation of the treatment ameliorated the neurological symptoms and the mice recovered thereafter (49).

DNA adduct-targeted mutagenicity of acrylamide

Repair resistance and promutagenicity of a DNA adduct determine its biological significance (55). The predominant DNA adduct induced by acrylamide, i.e. N7-GA-Gua, and one of its two minor adducts, i.e. 3-(2-carbamoyl-2-hydroxyethyl)-adenine, are both depurinating adducts, capable of generating apurinic/apyrimidinic (abasic) sites (64,65). The produced apurinic/apyrimidinic (abasic) sites are likely to give rise to 2′-deoxyadenosine incorporation during DNA replication, thereby leading to G → T transversions (65–68). Other minor adduct of acrylamide, i.e. N1-GA-Ade, has also significant miscoding potential, thus being highly promutagenic (64). We have documented a persistence of induced DNA adducts at specific locations along the *cII* transgene in Big Blue mouse embryonic fibroblasts treated *in vitro* with acrylamide or glycidamide (69). We have also demonstrated a characteristic G → T transversion mutations in the *cII* transgene induced by glycidamide in the same model system (Figure 3a and b) (69). The signature mutation of G → T transversions was not, however, as pronounced in the spectrum of mutations induced by acrylamide, although its frequency was still elevated relative to control (70).

Manjanatha *et al.* (49) have recently shown that Big Blue mice treated *in vivo* with acrylamide or equimolar doses of glycidamide through drinking water have similar induced mutation spectra in the *cII* transgene, both spectra being significantly different from that of the control (49). The predominant types of *cII* mutations induced by both acrylamide and glycidamide were G:C → T:A transversions and −1/+1 frameshifts (Figure 3c and d). The majority of these frameshift mutations were 'jackpot' mutations, particularly, G insertions/deletions at nucleotide positions 179–184. These jackpot mutations are common phenomena in transgenic rodents, and are thought to occur during early stages of development and undergo clonal expansion. Consequently, many cells from various organs of (un) treated animals may harbor the exact same mutation (71). To rule out the over-representation of jackpot mutations, it is methodologically appropriate to exclude these mutations from all spectra analyses. Excluding the jackpot mutations, the spectra of mutation induced by both acrylamide and glycidamide *in vivo* (49) resembled closely that produced by glycidamide *in vitro* (69) (see Figure 3a and e).

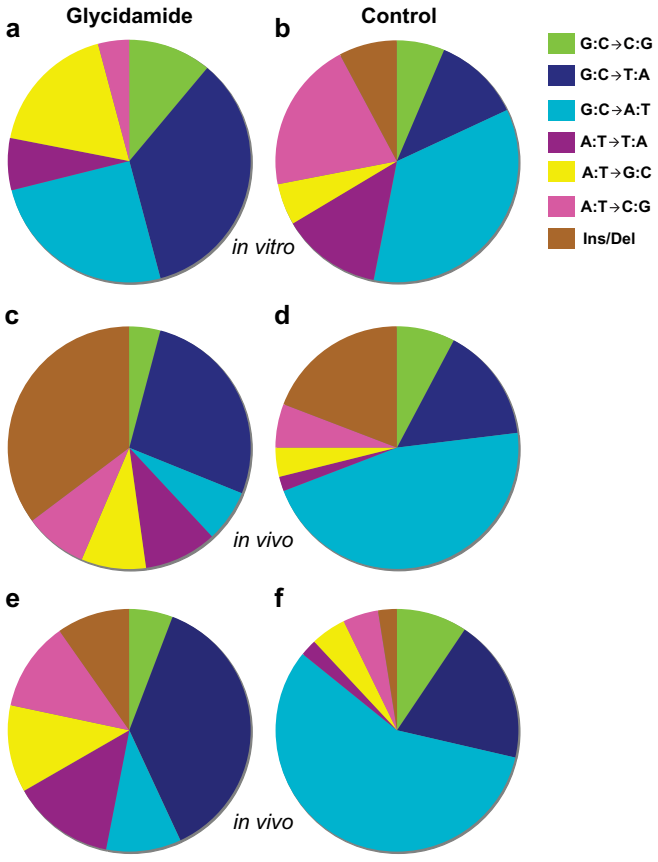

Fig. 3. Mutation spectrometry data of glycidamide *in vitro* and *in vivo* in the *cII* transgene in Big Blue mouse system [data are adopted from (69) and (49), respectively]. (**a**,**b**) Early stage Big Blue mouse embryonic fibroblasts were treated *in vitro* with 500 μM glycidamide or control solvent (double-distilled water). Mutations were quantified 8 days after treatment using the lambda select-*cII* mutation detection system for Big Blue rodents (Stratagene, La Jolla, CA). Verified mutant plaques were subsequently subjected to DNA sequence analysis. All jackpot mutations found in control-treated cells, i.e. G insertion/deletion at nucleotide positions 179–184, G → A transition at nucleotide position 196, G → C transversion at nucleotide position 211 and T → G transversion at nucleotide position 221, were excluded from the analyses. Total numbers of sequenced plaques were 134 and 173 from glycidamide-treated and control samples, respectively. Ins, insertion; Del, deletion. (**c**–**f**) Male and female adult Big Blue mice were treated *in vivo* with glycidamide in drinking water at a concentration of 600 mg/l, which yielded an average daily dose of 88–111 mg/kg/body wt, for up to 4 weeks. Mutations in liver *cII* were determined 21 days post-treatment. The *cII* mutant plaques were verified and subjected to DNA sequence analysis. Total numbers of sequenced plaques were 75 and 58 from glycidamide-treated and control samples, respectively. Because there was no significant difference in the spectra of mutations between males and females within each group ($P \geq 0.67$), the data were combined in the respective groups (49). For comparability purposes, the *in vivo* data are presented both with (c–d) and without (e–f) the exclusion of the above-mentioned jackpot mutations.

The similarity in mutation spectra induced *in vivo* by acrylamide and glycidamide, respectively, can explain our *in vitro* finding of the less-explicit signature mutation of G → T transversions being induced by acrylamide relative to glycidamide in early stage mouse embryonic fibroblasts with immature metabolic machinery (69,70). Our findings could also be explained, at least partially, by the known deficiency of P450 activity in neonatal mice (42).

Dietary acrylamide and cancer

The intentional introduction of genotoxic and carcinogenic compounds into food during manufacturing is generally prohibited by regulation (72). However, unintentional generation of process-induced contaminants can occur both at industrial and at domestic settings. Complete elimination of all these contaminants may not be feasible, especially because of the ever-increasing sensitivity of the analytical methods, which enables detection of trace amounts of specific contaminants in food matrices (72). Acrylamide like other classes of carcinogens, including heterocyclic amines, polycyclic aromatic hydrocarbons and *N*-nitroso compounds, can be formed during cooking or other thermal processing in a wide range of food products (73). High formation of acrylamide can occur in plant commodities rich in carbohydrates and low in proteins, as a result of heating at temperatures exceeding 120°C (15,16). Besides the composition of food items, i.e. content of free amino acid, e.g. asparagine, and reducing sugars, e.g. fructose and glucose, formation of acrylamide is dependent on the modality of cooking, particularly temperature and duration (14–16). As such, large variations exist in acrylamide content of different brands of the same food product as well as among various batches of the exact same brand (see Table I).

The World Health Organization estimates a daily intake of dietary acrylamide in the range of 0.3–2.0 μg/kg/body wt for the general population (74). For high-percentile consumers (90th to 97.5th), daily intakes of dietary acrylamide vary in the range of 0.6–3.5 μg/kg/body wt, and as high as 5.1 μg/kg/body wt for the 99th-percentile consumers. The daily intakes of dietary acrylamide in children are estimated to be 2–3 times those of adults based on average body weight ratios. The daily intakes of dietary acrylamide for the general population and high consumers (including children) are estimated to be on average 1 and 4 μg/kg/body wt, respectively. The main sources of dietary acrylamide are potato chips (16–30%), potato crisps (6–46%), coffee (13–39%), pastry and sweet biscuits (10–20%) and bread and rolls/toasts (10–30%). Other food products can account for <10% of the total intake of dietary acrylamide (see Table I) (74).

So far, several epidemiologic studies have attempted to find a link between dietary acrylamide exposure and human cancers (17,18,75–78). However, no association has been found between the intake of dietary acrylamide and the risk for development of any type of cancer (75,79–81). The absence of positive results in these observational studies, however, cannot be interpreted as proof of no carcinogenicity of acrylamide to humans (75,79–81). Obviously, the conducted studies have potential limitations, including inadequate statistical power due to the small size of study populations and the narrow range of exposure between cases and controls. By virtue of design, case–control studies require a reasonable difference in exposure levels between cases and controls; yet, acrylamide is abundantly present in foods eaten nearly universally, e.g. bread products. Also, the observational studies are prone to misclassification of acrylamide exposure as a result of reliance on crude estimates of dietary intake using food frequency questionnaires. Notwithstanding is that the administered questionnaires of some of these studies have been originally tailored to elicit information regarding exposure to carcinogens other than acrylamide, e.g. heterocyclic amines (17); at the original design of these studies, the presence of acrylamide in foods was not even discovered. Other drawbacks of these studies include selection and recall biases, especially in hospital-based case–control studies in which cases tend to modify their dietary habits soon after the appearance of early symptoms of the disease, as well as report their dietary intakes more accurately than controls (75,79–81).

As a default assumption, genotoxic carcinogens are often considered to have no threshold limit of exposure, i.e. a single exposure to one molecule of carcinogen can trigger the biological process leading to cancer (82). In the food safety area, such compounds are mainly dealt with by the principle of 'as low as reasonably achievable', as is the case for acrylamide (72). Regulatory agencies also perform (semi) quantitative risk assessment for acrylamide exposure using tumorigenicity data, which are largely available from a few mid/long-term bioassays of acrylamide in rodents (56,57,83,84). Inherent in the latter approach, however, are major uncertainties caused by the extrapolation of animal data at exposure levels that are 3–5 orders of magnitude higher than those to which humans are generally exposed (74).

The growing body of evidence on inter-species differences in acrylamide bioavailability and metabolism and sensitivity to tumor induction at specific organ sites also casts shadow on extrapolating data from rodents to humans (74). Thus far, several cancer risk assessments have been performed for dietary acrylamide using various dose–response computer modelings, which have led to different carcinogenic potency estimates (82). For example, utilizing a linearized multi-stage model, which does not account for inter-species variabilities, a lifelong daily intake of 1 μg acrylamide has been estimated to lead to a lifetime cancer risk of 1×10^{-5} (85). Another model based on the scaling of dose per unit of body surface area has estimated a corresponding risk of 6×10^{-5} (86).

Comparing the average daily intake of 1 μg/kg/body wt of dietary acrylamide by the general population and a dose of 0.30 mg/kg body wt/day for induction of mammary tumors in rats, the margin of exposure for dietary acrylamide in humans is 300 (74). The respective margin of exposure for high-consumer groups with a daily intake of 4 μg/kg/body wt of dietary acrylamide is 75 (74). The estimated margin of exposures are considered to be low for a genotoxic and carcinogenic substance that is so omnipresent in human food chain (74). Admittedly, there are still significant knowledge gaps, which currently preclude a more reliable estimate of the cancer risk due to acrylamide intake via foods. Nevertheless, the significant presence of a known rodent carcinogen in commonly consumed human foods is a legitimate health concern. It is recommended that appropriate efforts be continued to minimize human exposure to acrylamide, especially through reducing acrylamide concentrations in frequently consumed food products (74). For example, efficient reduction in acrylamide formation has been achieved by using the enzyme asparaginase to selectively remove asparagine prior to heating of cereal and potato products (74). However, this approach is only applicable to certain foods prepared from liquidized or slurried materials. Formulation changes such as increasing the concentrations of other amino acids or proteins, which compete with asparagine in the Maillard reaction or react with formed acrylamide, may also lower the content of acrylamide in foods (74). Genetic or chemical manipulation of enzymes involved in acrylamide biotransformation, e.g. CYP2E1 induction or inhibition (by flavonoids), is also an important area of research, which awaits further exploration.

Concluding remarks

The provocative announcement that acrylamide, a proven rodent carcinogen (87), is found in a variety of commonly consumed human foods has raised public health concerns (14–16). Thus far, the involvement of dietary acrylamide in human cancers has been investigated using conventional exposure assessment in relation to cancer incidence rating (19). However, such questionnaire/interview-based investigations are prone to inconclusiveness because of the ubiquity of acrylamide in the human diet and its variable generation even within the same individual food (4). In addition, the presence of acrylamide in the environment, i.e. in occupational settings or in the ambient air, e.g. as a constituent of environmental tobacco smoke (88), confounds the profile of human exposure to acrylamide.

A more reliable approach is to monitor well-defined human populations using biological markers, which are both specific for acrylamide and pertinent to cancer (19). This requires an understanding of the as yet unknown mechanism of acrylamide carcinogenicity. The underlying mechanism of acrylamide carcinogenicity can be resolved by investigating a multi-stage continuum that starts with exposure to acrylamide and ends with tumorigenesis (19). Of significance in this continuum are acrylamide-induced DNA adduction and mutagenesis, as well as possible epigenetic alterations (19). Many chemical carcinogens display a characteristic DNA adduct-targeted mutagenicity in cancer-related genes, i.e. oncogenes and tumor suppressor genes (55). Also, a range of environmental carcinogens can cause heritable epigenetic modifications to the DNA, thus changing gene expression while the primary DNA sequence being conserved (89–96). To date, the engagement of an epigenetic pathway in acrylamide-induced carcinogenesis is unclear, and remains to be determined. However, substantial data are available on the genotoxic mode of action of acrylamide of relevance for carcinogenesis (4,32).

So far, all available data point to an involvement of glycidamide in acrylamide-induced DNA adduction and mutagenesis (4,32). However, the interrelated chain of events starting from glycidamide–DNA adduct formation and leading to mutation has not been directly investigated. The correlative nature of events contributing to acrylamide-induced mutagenesis and tumorigenesis is also another area of research, which remains to be investigated. These investigations should help unravel the underlying mechanism of action of acrylamide carcinogenicity. Increasing the mechanistic knowledge of acrylamide-induced carcinogenesis will help identify biological markers that can be used for monitoring of human exposure to acrylamide, in particular, to determine a possible link between dietary acrylamide and human cancers.

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