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Using Dietary Exposure and Physiologically Based Pharmacokinetic/Pharmacodynamic Modeling in Human Risk Extrapolations for Acrylamide Toxicity

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The discovery of acrylamide (AA) in many common cooked starchy foods has presented significant challenges to toxicologists, food scientists, and national regulatory and public health organizations because of the potential for producing neurotoxicity and cancer. This paper reviews some of the underlying experimental bases for AA toxicity and earlier risk assessments. Then, dietary exposure modeling is used to estimate probable AA intake in the U.S. population, and physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling is used to integrate the findings of rodent neurotoxicity and cancer into estimates of risks from human AA exposure through the diet. The goal of these modeling techniques is to reduce the uncertainty inherent in extrapolating toxicological findings across species and dose by comparing common exposure biomarkers. PBPK/PD modeling estimated population-based lifetime excess cancer risks from average AA consumption in the diet in the range of $1-4 \times 10^{-4}$; however, modeling did not support a link between dietary AA exposure and human neurotoxicity because marginal exposure ratios were 50−300 lower than in rodents. In addition, dietary exposure modeling suggests that because AA is found in so many common foods, even big changes in concentration for single foods or groups of foods would probably have a small impact on overall population-based intake and risk. These results suggest that a more holistic analysis of dietary cancer risks may be appropriate, by which potential risks from AA should be considered in conjunction with other risks and benefits from foods.

KEYWORDS: Acrylamide; glycidamide; risk assessment; cancer; neurotoxicity; PBPK modeling; DNA adducts

INTRODUCTION

Acrylamide (AA) is an important industrial chemical that has received considerable regulatory scrutiny because of its neurotoxicity in many animals species (reviewed in ref *1*), including humans (*2*), and its rodent carcinogenicity at multiple sites (*3–6*). The challenge of risk assessment was significantly expanded recently when it was discovered that typical cooking of many starchy foods produces significant amounts of AA (*7, 8*). Several important international bodies have evaluated the carcinogenicity of AA, including the International Agency for Research on Cancer (*9*), the U.S. National Toxicology Program (*10*), the U.S. Environmental Protection Agency (*11*), and the World Health Organization/Food and Agriculture Organization (*12*). In all cases, AA was deemed to be a likely human carcinogen based on rodent carcinogenicity studies through formation of a DNA-reactive metabolite, glycidamide (GA). Additional cancer risk assessments have been reported for AA with the preponderance coming to a very similar conclusion (reviewed in ref *13*). Even though most regulatory bodies currently agree that AA is probably carcinogenic in humans, there is less agreement on the means to quantify population-based cancer risk, particularly from dietary exposure to typical commercial and home-cooked foods.

Review of Existing Cancer Risk Assessments for Acrylamide. The carcinogenic risks to humans from AA exposure have been evaluated by a number of regulatory and international scientific groups. These risk assessments are summarized in **Table 1**, modified from ref *13*. Ruden developed a carcinogenicity risk assessment index to describe and compare the overall conclusions drawn in the AA carcinogenic risk assessments. Organizations whose determinations are summarized in **Table 1** include the German MAK Commission, the American Conference of Governmental Industrial Hygienists, the Swedish National Chemicals Inspectorate, the Arbeitsmiljoinstiitutet, the

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Table 1. Categorization of the AA Risk Assessment Documents (Modified from Reference *13*)[a]

− − −	+ − −	+ − +	+ + +
NIOSH (1976)	none	MAK (1985)	none
WHO (1985)		IARC (1986)	
IRPTC (1988)		KEMI (1989)	
		MAK (1990)	
		Molak (1991)	
		ACGHI (1991)	
		AMI (1992)	
		U.S. EPA (1993)	
		IARC (1994)	
		IMM (1998)	
		U.S. FDA (1998)	
		ACGIH (2001)	
		EU (2002)	
		U.S. NTP (2002)	
		NFCA (2002)	
		NICNAS (2002)	
		FAO/WHO (2002)	
		Konings et al. (2003)	
		Dybing and Sanner (2003)	
		McClure et al. (2004)	
		U.S. EPA/IRIS (draft 2004)	
		JECFA (2006)	

[a] − − −, not carcinogenic in animals, negative epidemiology no/implausible human cancer risk. + − −, carcinogenic in animals, negative epidemiology, no/implausible human cancer risk. + − +, carcinogenic in animals, negative epidemiology a plausible human cancer risk. + + +, carcinogenic in animals, positive epidemiology, a plausible human cancer risk.

Institutet for Miljomedicin, and the European Union. The U.S. EPA, through the Integrated Risk Information System (IRIS), estimated risks for AA in drinking water (*11*). The Norwegian Food Control Authority, the Australian assessment (National Industrial Chemicals Notification and Assessment Scheme), the FAO/WHO Consultation on Acrylamide in foods, and the Dutch assessment by Konings et al. (*14*) were performed more recently.

Some organizations whose assessments were performed before 1988 concluded that AA was not carcinogenic to either experimental animals or humans; however, the cancer bioassay data of Johnson et al. (*5*) were not generally available to the authors of these early risk assessments. After 1995, the Friedman et al. (*6*) study was also available for later risk assessments. Most of the risk assessments in **Table 1** concluded that AA is carcinogenic to experimental animals, that epidemiology data are negative, and that AA is likely or "reasonably anticipated to be a human carcinogen" (*10*).

The U.S. FDA (*15*) performed a risk assessment for AA as a contaminant of copolymers, retention aids, drainage aids, stabilizer or fixing agents in paper and paperboard contacting foods. The FDA's estimate of carcinogenic potency was based on the Johnson et al. (*5*) study; tumor incidences selected were male rats with thyroid follicular adenomas, male rats with testicular mesotheliomas, female rats with mammary tumors (adenomas or adenocarcinomas, fibromas or fibroadenomas, adenocarcinomas alone), and female rats with central nervous system tumors (brain astrocytomas, brain or spinal cord glial tumors). FDA used a simple linear extrapolation from the dose of AA that showed an effect (not necessarily statistically significant) to zero dose (*16*). Unit risks (carcinogenic potencies) were determined to be in the range from 5×10^{-2} to 5×10^{-1} per mg/kg of body weight (bw)/day. Ranges of relative risks were estimated based on several tumor types while the most sensitive tumor type in the Johnson et al. (*5*) study, that is, the mammary tumors, yielded the highest risk. To estimate cancer risks from dietary AA, FDA combined the unit risks (slope of dose response curve) with estimated AA exposure of 0.4 μg/kg of bw/day to determine upper bound lifetime cancer risks in the range from 2×10^{-5} to 2×10^{-4} (risk $\simeq$ unit risk $\times$ dose). These estimates are quite similar to those quoted in the literature, given the many uncertainties in the risk estimation process (*11, 17*). The FDA has presented its cancer risk estimates for various products on its Website for acrylamide (www.cfsan.fda.gov/dms/acrydata.html).

In 2005, the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization/Food and Agriculture Organization reviewed all available data from member countries all over the world and performed risk assessments for AA (*12*). JECFA chose to use Benchmark Dose (BMD)/Margin of Exposure (MOE) methodology and estimated the BMD and the Benchmark Dose Lower Confidence Limit (BMDL) values based on a 10% extra risk (BMDL10), defined as

$$\text{extra risk} = \frac{R(\text{BMD}) - R(0)}{1 - R(0)} \quad (1)$$

which represents the additional response fraction divided by the tumor-free fraction in the control animals. Use of the BMDL in risk assessment is often favored because, among other reasons, it does not require extrapolation beyond observed toxicity and incorporates a measure of data uncertainty. These calculations were performed with the dose−response software package PROAST, version V07. JECFA combined mammary gland fibroadenomas from both Johnson et al. (*5*) and Freidman et al. (*6*). The Committee noted that although both studies showed a dose-related increase, the dose−response information in the data was limited with high background response relative to the maximum response. MOEs were estimated at intakes of AA of 0.001 mg/kg of bw/day, to represent the *average dietary intake* of AA for the general international population and a dietary intake for the *high consumer* of 0.004 mg/kg of bw/day. When the value of 0.001 mg/kg of bw/day was compared to the BMDL10 of 0.30 mg/kg of bw/day for induction of mammary tumors in rat, the MOE was 300; for consumers with a high level of AA intake, the MOE was 75. JECFA concluded "these MOEs to be low for a compound that is genotoxic and carcinogenic" and that "this may indicate a human health concern. Appropriate efforts to reduce concentrations of AA in food and beverage should be continued".

Epidemiological Studies of Dietary Acrylamide. Epidemiological investigations searching for possible linkage between AA exposure through the diet and risks of cancer at several sites have been reported (large bowel, urinary bladder, kidney; reviewed in ref *18*). In general, population-based data previously collected for other research purposes were reanalyzed by using more recently available information about AA levels in selected foods (e.g., coffee, fried or baked potatoes). Although these analyses have consistently shown no increased cancer risks, significant questions about statistical power and the potential for nondifferential misclassification of AA intake have been raised (*19, 20*). Food-frequency questionnaires are central to the exposure assessment in many population-based studies of diet and cancer, but significant reliability issues have been raised with respect to their usefulness in epidemiological studies of AA. Specifically (1) the very wide distribution of AA in many common foods makes it unlikely that focusing on one or even several food types can suffice to distinguish high and low exposure populations (see dietary exposure modeling discussion below); (2) food frequency questionnaires have been shown to poorly correlate with measured biomarkers of AA and GA internal exposure, even when sampled concurrently (*21–23*); (3) food frequency questionnaires cannot effectively capture the

inherent variability of AA content in individual foods that result from lot-to-lot variation in commercially prepared foods, agronomic factors (e.g., soil, seasonal, varietal, or storage conditions) that affect levels of AA precursors (i.e., asparagine plus reducing sugars) in crops, and particularly the high variation of AA formation in home-cooked foods; (4) AA intake has been shown to change with age of subjects (*22*); (5) food frequency questionnaires cannot predict internal exposures to the putative genotoxic metabolite, GA (see below), because of variability in CYP 2E1 expression across the population, particularly when enzyme induction can vary between individuals and within individuals across time on the basis of age, lifestyle, and disease state factors; and (6) specific human cancers caused by AA are unknown and poorly predicted by the observed organotropy for tumorigenesis in rodents. These potential inaccuracies in estimation of AA dose, both administered and internal, would serve to decrease the slope of the dose−response curve. Consequently, such studies are unlikely to reject the null hypothesis for an association between the surrogate measure of dietary AA and cancer risks. Given the many difficulties in exposure assessment noted above, a definitive association between dietary AA intake and increased incidences of site-specific human cancers may not be forthcoming from epidemiological studies, even with large numbers of subjects.

Genotoxic Mechanisms for AA Carcinogenicity. A significant body of experimental evidence has accumulated supporting a genotoxic (i.e., DNA-damaging) mechanism for AA carcinogenicity that requires metabolic conversion to GA. GA is structurally related to other known epoxide carcinogens, including the human/rodent carcinogen, ethylene oxide (*24*), and rodent carcinogen, glycidol (*25*). Lifetime exposure to these compounds induces tumors at similar sites in F344 rats, including the central nervous system and peritesticular mesothelium. The reactivity of GA with nucleophilic sites on DNA is much greater than that for AA (*26–28*) and GA−DNA adducts [*N*3-(2-carbamoyl-2-hydroxyethyl)adenine, *N*3-GA-Ade, and *N*7-(2-carbamoyl-2-hydroxyethyl)guanine, N7-GA-Gua)] have been quantified in every rat and mouse tissue examined (*28, 28*). GA is mutagenic in *Salmonella* tester strains without activation, but AA is not (*30*) and GA is more mutagenic than AA in Big Blue mouse embryonic fibroblasts, primarily by inducing G:C to T:A transversions (*31*). The association of clastogenicity (i.e., micronuclei formation in reticulocytes) with internal exposures to GA has also been published (*32*). Additional strong and consistent evidence for the importance of AA metabolism to GA comes from studies comparing toxicity in wild-type mice with CYP 2E1 knockout mice, which eliminates the predominant enzyme responsible for AA oxidation. Virtually all DNA adduct formation is dependent on CYP 2E1-mediated metabolism of AA to GA because serum GA and GA adduct formation is decreased by >95% in knockout mice (*33*); similarly, virtually all increased incidences of micronuclei and DNA damage detected by using the Comet assay require GA formation because they are observed in only wild-type mice (*34*); finally, male germ cell mutagenicity of AA, measured using the dominant lethality assay, also requires metabolism to GA because it is observed in only wild-type mice (*35*). Mutation assays in vivo have demonstrated that oral administration of AA or GA increases mutant frequencies in lymphocyte *Hprt* and liver *cII* genes of adult Big Blue mice by inducing primarily G:C to T:A transversions (*36*). This finding is consistent with that reported for mutagenicity of GA in vitro (*31*) and links formation of N7-GA-Gua, the major AA-derived DNA adduct, with mutations in vivo. In addition, GA, but not AA, is a genotoxic mutagen in neonatal $Tk^{\pm}$ mice at *Hprt* and *Tk* loci, presumably because of undeveloped CYP 2E1 activity (Beland et al., unpublished data). Finally, a structurally related compound, *N*-methylolacrylamide, which is apparently partially converted to AA and GA in vivo (*37, 38*), induced significantly increased incidences of tumors (liver, lung, and hardarian gland) in $B6C3F_1$ mice but not in F344 rats (*39*).

Non-genotoxic Mechanisms for AA Carcinogenicity. Alternative mechanisms for AA-induced carcinogenesis in male and female Fischer 344 rats have been proposed, often on the basis of the results of in vitro studies conducted at concentrations well above those relevant to internal doses for AA cancer bioassays. These include hormonal dysregulation (*40*), oxidative stress (*41*), and modification of critical sulfhydryl residues on kinesin proteins that function in chromosome separation (*42*). Moreover, these alternate hypotheses do not account for the significant body of evidence from two rodent species supporting a genotoxic mechanism for tumorigenesis in multiple tissues as described above. Furthermore, cancer risk assessments conducted by several prominent regulatory organizations have consistently disregarded these non-genotoxic mechanisms as largely unsubstantiated (*9, 11, 12*).

DNA Adduct−Tumorigenesis Correlations in Experimental Animals. Formation of covalent adducts between DNA and chemical carcinogens or their metabolites is generally regarded as one of the earliest cellular changes in tumor initiation (reviewed in ref *43*). Although the formation of such adducts is assumed to be necessary, but not sufficient, for tumor initiation in many animal species, it is widely accepted as an indication of biologically effective dose in experimental animals and humans. Chronic exposure to carcinogens in the diet leads to accumulation of DNA adducts to steady state levels, which reflect the balance between formation and loss. Poirier and Beland (*43*) examined several animal studies conducted with genotoxic carcinogens from several important chemical classes [2-aminofluorene; 4-aminobiphenyl; aflatoxin B_1; *N*,*N*-diethylnitrosamine; and 4-(*N*-methyl-*N*-nitrosoamino)-1-(3-pyridyl)-1-butanone] in which steady state DNA adduct levels were measured after 1−2 months and tumor incidences after a lifetime of continuous exposure (i.e., 2 years). In most, but not all cases, the dose−response relationship for steady state levels of DNA adducts correlated directly with that observed for increased tumor incidences. In addition, at the low end of the dose−response curve, steady state DNA adduct levels were linear with administered dose for these chemicals. Therefore, within an animal model, extrapolation from high to low dose for the increases in tumor incidences can often be predicted by the respective steady state DNA adduct levels. In those cases when direct correlation is not observed, it is likely that additional factors are required for tumorigenesis, including cell proliferation or hormonal influences.

Use of Modeling To Reduce Uncertainty in Risk Assessments of AA. This assessment uses dual modeling approaches to characterize population-based dietary cancer and neurotoxicity risks from AA. First, extensive measurements of AA content in important foods comprising the U.S. diet and consumption/frequency/portion size estimates were used as a basis for Monte Carlo simulations that provide a reliable population-based estimate of total daily AA consumption. Second, PBPK/PD modeling was used to estimate tissue GA−DNA adducts and nervous system AA levels in people consuming an average amount of dietary AA and to use relative levels as metrics to connect demonstrable rodent carcinogenicity and neurotoxicity with human risks. The goal of this approach is to reduce the uncertainty inherent in default assumptions regarding pharma-

cokinetic and pharmacodynamic differences between rodents and humans. This approach can improve confidence in extrapolations between the relatively high doses of AA required to produce statistically significant increases in toxicity observed in small groups of rodents (~50) and the very low dietary levels to which very large numbers of people are exposed.

MATERIALS AND METHODS

Dietary Exposure Modeling for AA. The U.S. Food and Drug Administration's Center for Food Safety and Applied Nutrition (CFSAN) has modeled probable human dietary exposure to AA from consumption of 66 food and beverage types for which data have been collected on AA levels. This type of modeling provides estimates of AA exposure levels for use in risk assessments, which can also be used to examine the possible effects of mitigation strategies on AA levels in food. The data sources for the AA levels in these foods and beverages are individual food product surveys conducted by FDA in 2002−2004, as well as surveys of selected foods from FDA's Total Diet Study in 2003−2006. All food collection and most testing were performed by FDA staff, although some testing was done at a nongovernmental laboratory under contract to the FDA. The data are available at http://www.cfsan.fda.gov/~dms/acrydata.html and http://www.cfsan.fda.gov/~dms/acrydat2.html. These surveys were conducted in regions throughout the country, primarily urban locations. Foods thought likely to contain high or variable levels of AA, such as oven-baked or restaurant French fries and cereals, were sampled extensively. Foods were mostly analyzed under ready-to-consume conditions, either purchased precooked or prepared prior to analyses in the laboratory, when appropriate. The FDA believes that the scope and depth of the surveys are sufficient to provide adequate information for deriving distributional estimates of probable dietary exposure to AA.

An individual's dietary exposure to a substance can be estimated by combining the consumption of a food containing the substance with the concentration of the substance in that food. The summation of the contributions from all of the foods containing the substance yields the individual's dietary exposure. The generalized relationship of food consumption (based on food consumption frequency and portion size) and substance concentration to the estimated daily consumption (EDI) of a substance x is captured in the following equation. It is assumed that food consumption data are taken from a survey of short duration (2−14 days), which is then representative of chronic, or lifetime, consumption of the foods.

$$\mathrm{EDI}_x = \sum_{f=1}^{F} \frac{\mathrm{Freq}_f \times \mathrm{Port}_f \times \mathrm{Conc}_{xf}}{N} \qquad (2)$$

where F = total number of foods in which substance x can be found, Freq_f = number of eating occasions of food f over N survey days, Port_f = average portion size for food f, Conc_{xf} = concentration of the substance x in food f, and N = number of survey days.

The population distribution of probable dietary exposures is then prepared by repeating the analysis for every individual in the population of interest.

The distribution of probable AA dietary exposures was derived via a Monte Carlo analysis using @Risk software (Palisade, Inc., Ithaca, NY). The Monte Carlo simulation sums incremental AA exposure values calculated using food intake and AA concentration levels randomly drawn from survey-derived distributions of possible values for each food to yield a total exposure for an individual. Each iterative individual exposure value results from the multiplication of a food or beverage consumption value with an AA residue value sampled from a discrete uniform distribution of AA residue values, taking into consideration the likelihood that a person eats that particular food (taken from the percent eaters for each food in the food consumption survey). Food and beverage consumption values were taken from the U.S. Department of Agriculture (USDA) Continuing Survey of Food Intake by Individuals (CSFII, 1994−1996 and 1998 Supplemental Children's Survey). A total of 5000 iterations was performed, enough to provide a stable exposure estimate, that is, <0.5% change in the mean, standard deviation, and percentiles (in 5% increments from 5 to 95).

This type of modeling of dietary exposure has limitations. The use of random sampling of food consumption distributions results in the loss of correlations of consumption of food types, both positive and negative, in individuals' diets. Although this would not affect the mean of the resulting dietary exposure distribution for an individual, it might have effects at the high-consumption end of the distribution. The large number and broad distribution in the diet of foods found to contain AA result in a very high likelihood that the whole population is exposed to AA via the diet. This, in turn, results in the minimizing of any effects of correlations on the high-consumption end of the population distribution of AA dietary exposure. This minimization was verified by combining the mean level of AA contamination with the actual dietary records of the more than 5000 individuals sampled in the CSFII and comparing the 90th percentile exposure of the resulting distribution with that derived from the modeling.

PBPK/PD Modeling of AA in Rodents and Humans. A physiologically based pharmacokinetic model was developed for AA and three of its metabolites: GA and the glutathione conjugates AA−GS and GA−GS. GA−DNA adducts and hemoglobin (Hb) adducts with AA and GA were included as pharmacodynamic components of the model (*44*). Serum/tissue AA and GA concentrations, adduct levels, and urinary elimination levels for all four components from male and female, mice and rats, were simulated using data obtained from iv and oral administration of 0.1 mgof AA/kg or 0.12 mg of GA/kg (*45–48*) and other data sets from the literature. Adduct formation and decay rates were determined from a 6 week repeated-dose exposure to approximately 1 mg/kg of bw/day AA in drinking water and subsequent 6 week nonexposure period, respectively (*28, 47*). Measurements of both urinary mercapturates and Hb adducts from a group of nonsmoking humans were used to extrapolate to a human PBPK/PD model (*49*). For the simulations of human levels for steady state GA−DNA adducts in different tissues (brain, thyroid, mammary, testes) and steady state concentrations of AA in nervous tissue (brain), the PBPK/PD parameters used were those previously published for the human model (*44*) based on six nonsmokers [three men and three women (*49*)] along with a dietary exposure level of 0.4 μg/kg of bw/day. For the simulations of rat levels for steady state GA−DNA adducts in different tissues (brain, thyroid, mammary, testes) and steady state concentrations of AA in nervous tissue (brain), the PBPK/PD parameters used were those previously published in the rodent model (*44*) and the desired oral dose of AA.

Dose−Response Modeling. BMD estimation was performed using generalized multistage modeling of rodent tumor or neurotoxicity data for AA as indicated below. The generalized multistage model is available in the U.S. EPA Benchmark Dose Software (www.epa.gov/ncea/bmds.htm).

A 10% level of excess adverse effects over those in the controls was chosen as benchmark response, and the BMDL10 was taken as the point of departure for risk extrapolation.

RESULTS AND DISCUSSION

Dietary Exposure Modeling for AA. AA levels were found to be highest in potato-based foods, such as French fries, potato chips, and potato skins, with mean levels as high as 700 μg/kg. Flavored and fabricated potato crisps contained AA at levels as high as 2700 μg/kg. Roasted and instant coffees were also consistently high in AA, averaging 190 and 340 μg/kg, respectively, in the ground or powdered nonbrewed products. The single highest food containing AA was a sample of a coffee substitute (in powdered, premixed form) at 5400 μg/kg. Levels for coffee or coffee substitutes were substantially lower in the brewed or prepared drinks.

The database on AA levels in food is large, with over 2500 analyses from 66 food types. The mean estimate of dietary exposure to AA was found to be 0.44 μg/kg of bw/day, with a 90th percentile of 0.95 μg/kg of bw/day for the population aged 2 years and older. The mean estimate of dietary exposure to AA for children aged 2−5 years old was 1.1 μg/kg of bw/day, with a corresponding 90th percentile exposure of 2.3 μg/kg of

Table 2. Top 20 Foods by Mean Acrylamide Intake in U.S. Taken from the 2006 Updated Exposure Assessment for Acrylamide Available at http://www.cfsan.fda.gov/~dms/acryexpo.html

food	mean AA intake (μg/kg of bw/day)	cumulative fraction
French fries (restaurant)	0.070	0.16
French fries (oven-baked)	0.051	0.28
potato chips	0.045	0.38
breakfast cereal	0.040	0.47
cookies	0.028	0.53
brewed coffee	0.027	0.60
toast	0.023	0.65
pies and cakes	0.018	0.69
crackers	0.017	0.73
soft bread	0.014	0.77
chile con carne	0.014	0.80
corn snacks	0.011	0.82
popcorn	0.007	0.84
pretzels	0.007	0.86
pizza	0.006	0.87
burrito/tostada	0.006	0.88
peanut butter	0.003	0.89
breaded chicken	0.003	0.90
bagels	0.003	0.90
soup mix	0.003	0.91

bw/day, with the higher estimates primarily due to children's lower body weights. The top 20 foods contributing to the population mean for AA exposure are listed in **Table 2** along with the cumulative contribution that each makes to the total mean estimated daily AA intake of 0.44 μg/kg of bw/day.

In 2002, shortly after the discovery of AA in foods, Swedish researchers estimated a possible dietary exposure to AA of 40 μg/person/day (equivalent to 0.7 μg/kg of bw/day for a 60 kg individual). A June 2002 WHO expert consultation expanded on this original estimate, to include a range of possible dietary exposures of 0.3−0.8 μg/kg of bw/day. FDA's original estimate of mean dietary exposure for AA for the U.S. population 2 years and older, made in 2003 and based on data from fewer than 30 food types known at that time to contain AA, was 0.37 μg/kg of bw/day. Subsequent updates, based on additional analyses resulting in a total of more than 2000 samples, were made in 2004 and 2006. The current mean estimate of dietary exposure to AA, 0.44 μg/kg of bw/day, has not changed significantly from the original estimate. It is not anticipated that information on AA levels in additional foods will result in any significant change to these estimates. A population-based estimate of mean dietary AA intake in The Netherlands recently reported a quite similar value (0.4 μg/kg of bw/day) (*50*).

The widespread presence of AA in heated foodstuffs results in a dietary exposure pattern that may make it challenging to change overall population exposure, although individuals could certainly reduce exposure by careful adherence to more restrictive diets. That is, mitigating AA in one or a few food types does not affect the population-mean dietary exposure significantly. In the 2006 FDA estimates of AA consumption (**Table 2**) "what-if" scenarios were examined. The complete "removal" of AA from snack foods reduced the mean exposure from 0.44 to 0.37 μg/kg of bw/day (a 16% reduction), whereas the largest effect noted for removal of all kinds of French fried potatoes was a reduction of the mean to 0.32 μg/kg of bw/day (a 28% reduction). In the real-world situation, such complete removal scenarios are not practicable and any partial reduction would result in an even smaller effect on the mean dietary exposure. Future technological advances by the food-processing industry (e.g., asparaginase pretreatment) could conceivably reduce levels of precursors and subsequent formation of AA in foods, although doing so without affecting consumer acceptance is a significant challenge.

Table 3. Selected Published Measurements of Acrylamide-Derived Hemoglobin Adducts and Urinary Metabolites in Groups of Nonsmokers[a]

study	group size (*n*)	AAMA (μg/L)	GAMA (μg/L)	AA-Val (fmol/mg)	GA-Val (fmol/mg)
Paulsson et al. (*54*)	5	–[b]	–	27	26
Boettcher et al. (*49*)	16	29	5	19	17
Bjellaas et al. (*55*)	65	39	31	–	–
Bjellaas et al. (*22*)	44	–	–	38	20
Urban et al. (*56*)	60	73	16	28	3
Vesper et al. (*57*)	6	–	–	43	26
Fennell et al. (*58*)	24	ND[c]	ND	76	29
Kellert et al. (*59*)	13	26	3	–	–
Chevolleau et al. (*60*)	52	–	–	27	22
Vesper et al. (*61*)	61	–	–	51	34

[a] Urinary concentrations of acrylamide- and glycidamide-derived mercapturic acids in urine (AAMA and GAMA, respectively) and N-terminal valine adducts of hemoglobin with acrylamide and glycidamide (AA-Val and GA-Val, respectively) are reported from the respective studies of nonsmoking humans. [b] –, not measured. [c] Not detected.

Using PBPK/PD for AA Risk Assessment. PBPK/PD modeling of human and rodent exposures to AA was undertaken because it is a recognized way to reduce the uncertainty in extrapolations across both species and dose for toxic effects seen only in rodent bioassays at exposure levels that are typically much higher than those in people. Internal exposures to people consuming AA only through the diet were modeled on the basis of urinary metabolite and Hb adduct data from nonsmokers (*49*) by using the modeling procedures previously described (*44*). The ranges of AA-derived biomarker measurements from several recent studies of nonsmokers are shown in **Table 3**. The PBPK/PD simulations for humans used a daily exposure rate of 0.4 μg/kg of bw, which is the population estimate described above for the U.S. and The Netherlands (*50*), and simulations of rat exposures at oral doses equivalent to the respective BMDL10 were performed as previously described (*45*).

For cancer risk estimation, a linear relationship was assumed between N7-GA-Gua adduct levels and cancer incidences. The rationale of using this approach for estimating cancer risks from a genotoxic carcinogen acting by a specific mutagenic DNA adduct was discussed above. In this way, the excess human cancer incidence from dietary AA was estimated from the following relationship: human incidence/human DNA adducts = rodent incidence (10% at BMDL10)/rat DNA adducts.

Table 4 shows the results of N7-GA-Gua adduct simulations at the respective BMDL for several tissues with excess tumor incidences in the Johnson et al. study of AA carcinogenicity in male and female F344 rats (*5*). In addition, **Table 4** shows the simulated human DNA adduct levels for the same tissues and the calculated excess cancer risk for each site. These BMDLs were in the same range as those previously published by JECFA (*12*), and the lifetime excess cancer risks ranged from 1 to 4×10^{-4}. This range is similar to that published in a previous quantitative risk assessment from which an estimated lifetime cancer risk from dietary AA was reported to be approximately 6×10^{-4} (*17*). In a historical context in which de minimis level of excess cancer risk is often taken as one in a million, this risk from dietary AA exposure would be considered to be significant. This notion is similar to that of an international risk assessment by JECFA (*12*) based on much of the same rodent cancer bioassay data, which stated "The Committee considered these MOEs (margins of exposure 300 and 75 for mean and 90th percentile consumers, respectively) to be low for a

Table 4. Using PBPK/PD-Simulated DNA Adduct Levels To Estimate Excess Tumor Incidences in Humans Exposed to Acrylamide in the Diet

tissue, sex	BMDL10 for excess tumor incidence (mg/kg of bw/day)[a]	N7-GA-Gua (at BMDL10)[b]	N7-GA-Gua (av human)[c]	tumor incidence (av human)[d]
mammary, F	0.40	119	0.46	3.9×10^{-4}
mesothelioma, M	0.66	164	0.41	2.5×10^{-4}
thyroid, M	1.2	292	0.41	1.4×10^{-4}
CNS, F	1.3	387	0.47	1.2×10^{-4}
thyroid, F	1.5	451	0.47	1.0×10^{-4}

[a] Lower confidence limit dose (mg/kg of bw/day) for 10% excess tumor incidence in Fischer 344 rats due to lifetime acrylamide exposure in drinking water determined from data of Johnson et al. (*5*), using multistage cancer model with 95% confidence limits. [b] DNA adduct levels (per 10^8 nucleotides) in Fischer 344 rats simulated using the PBPK/PD model using a dose equal to the BMDL. [c] Average DNA adduct levels (per 10^8 nucleotides) in nonsmoking humans simulated using the PBPK/PD model with a dose of 0.4 μg/ kg of bw/day. [d] Excess tumor incidence in nonsmoking humans exposed to dietary acrylamide calculated using the following proportional relationship: rat tumor incidence at BMDL (10%)/rat DNA adduct level at BMDL $\times$ human DNA adduct level.

Table 5. Using PBPK/PD-Simulated Brain Concentrations of Acrylamide To Estimate Neuropathy in Humans Exposed to Acrylamide in the Diet

study	BMDL10 (mg/kg of bw/day)[a]	brain [AA] at BMDL10 (μM)[b]	brain [AA] (av human) (μM)[c]	marginal exposure ratio[d]
Johnson et al., 1986-M	0.65	0.48	0.0028	170
Johnson et al., 1986-F	0.60	0.27	0.0021	130
Friedman et al., 1995-M	0.37	0.61	0.0028	220
Friedman et al., 1995-F	0.90	0.67	0.0021	320
Burek et al., 1980-M	0.2 (NOAEL)	0.15	0.0028	54-fold lower[e]

[a] Lower confidence limit dose (mg/kg of bw/day) for 10% increased peripheral nerve degeneration in male and female Fischer 344 rats due to either lifetime acrylamide exposure determined from data of Johnson et al. (*5*) (microscopic tibial nerve degeneration; moderate to severe for males, slight to moderate for females) and Friedman et al. (*6*) (microscopic sciatic nerve degeneration). [b] Acrylamide concentration in male and female Fischer 344 rat brain simulated using the PBPK/PD model using a dose equal to the BMDL10. [c] Average [AA] concentration in brain from nonsmoking men and women simulated using the PBPK/PD model with a dose of 0.4 μg/kg of bw/day. [d] Ratio between male and female Fischer 344 rat brain [AA] at BMDL simulated using the PBPK/PD model and average [AA] concentration in brain from nonsmoking men and women simulated using the PBPK/PD model with a dose of 0.4 μg/kg of bw/day. [e] Ratio between [AA] in male Fischer 344 rat brain simulated using the PBPK/PD model using a dose equal to the NOAEL from the data of Burek et al. (*51*) (electron microscopic sciatic nerve alterations from subgroups of rats at different doses) and simulated [AA] in brain from nonsmoking men and women using a dose of 0.4 μg/kg of bw/day.

compound that is genotoxic and carcinogenic and that this may indicate a human health concern". It should be noted that the relative risks reported here could represent upper bounds of true risks and are best used for comparison with other risk factors.

For neurotoxicity risk estimation, a linear relationship was assumed between nervous tissue levels and incidences. Although there is uncertainty about the identity of neurotoxic species (AA, GA, or both), this modeling exercise demonstrates the process for AA. **Table 5** shows the simulations of nervous tissue AA concentration (using whole brain as the tissue compartment) at the respective BMDL or NOAEL for several studies of F344 rat neurotoxicity (*6, 7, 51*). Similarly, nervous tissue levels of AA in people consuming the average dietary dose of 0.4 μg/kg of bw were simulated. The margin of exposure, defined as rat nervous tissue [AA] at BMDL/human nervous tissue [AA], was 130−320; for the NOAEL level of Burek et al. (*51*), the ratio was 54. When one considers that the neurotoxicity reported for lifetime exposure of rats to AA was mild to severe microscopically detected peripheral nerve degeneration (*5, 6*) or any electron microscopic changes in rat peripheral nerves that were largely reversible after 90 days of exposure (*51*), the calculated margins of exposure suggest that the risks for neurotoxicity from dietary AA would be minimal. This conclusion is similar to that of JECFA (*12*), which used the NOAEL from Burek et al. (*51*) to conclude that "Based on these MOEs (2000 and 500 for mean and 90th percentile consumers, respectively), the Committee concluded that adverse effects were unlikely at the estimated average intakes, but that morphological changes in nerves could not be excluded for some individuals with a very high intake". This conclusion does not consider possible differences in the pharmacodynamics of neurotoxicity between humans and rats or alternative targets in the central nervous system that have not yet been identified, especially during fetal or neonatal development. However, a study in which human neurotoxicity produced by acute occupational exposure to AA, characterized as generally mild peripheral neuropathy that in almost all cases was reversible, was correlated with exposure biomarkers (Hb adducts) does not support the notion that humans are uniquely susceptible (*2*).

Cooking Carcinogens and Dietary Cancer Risk. Two implications can be drawn from this work, namely that substantial reductions in AA consumption from typical cooked foods comprising the modern diet will be difficult to achieve and that theoretical cancer risks from dietary AA might be significant at the population level. Such a conclusion would be particularly troubling given the high percentage of worldwide total caloric content that is represented by cereals and tubers and the large, but imprecise, proportion of human cancers that have long been associated with the diet, in general (*52*). Moreover, this paper focuses on but one of many cooking carcinogens, which include polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene, heterocyclic aromatic amines such as PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine), furan, and others still unknown. Finally, this discussion does not take into account the many reasons why foods are cooked, particularly the critical role in eliminating microbial pathogens but also the formation of numerous desirable flavor and colored components. The possibility that cooking can produce chemopreventative effects via induction of detoxification enzymes (e.g., glutathione-*S*-transferases, UDP-glucuronyl-transferases) has also been suggested (*53*), which could be relevant to the prominent glutathione conjugation of AA and GA prior to excretion in humans (*49, 54, 56, 58, 59*). The complex exposure to numerous pyrolysis products in the diet suggests that a more holistic approach to assessing dietary cancer risks may be necessary; that is, the difficulty in effectively removing or accepting risks must be weighed alongside the many real benefits from consuming nutritious cooked foods with reduced microbial contamination. Thus, the seemingly generic dietary advice promulgated by the U.S. FDA to moderate AA exposure may in the end prove to be the best overall, that is, "FDA continues to advise consumers to eat a balanced diet, choosing a variety of foods that are low in trans and saturated fat and rich in high fiber grains, fruits and vegetables" (http://www.fda.gov/bbs/topics/news/2004/NEW01040.html).

ABBREVIATIONS USED

AA, acrylamide; BMD, benchmark dose; BMDL, lower confidence limit for benchmark dose; BW, body weight; EPA,

U.S. Environmental Protection Agency; FDA, U.S. Food and Drug Administration; GA, glycidamide; GS, glutathione; Hb, hemoglobin; IRIS, integrated risk information system; IARC, International Agency for Research on Cancer; JECFA, Joint Expert Committee on Food Additives; MOE, margin of exposure; N3-GA-Ade, *N*3-(2-carbamoyl-2-hydroxyethyl)adenine; N7-GA-Gua, *N*7-(2-carbamoyl-2-hydroxyethyl)guanine; NOAEL, no observed adverse effect level; NTP, National Toxicology Program; PBPK/PD, physiologically based pharmacokinetic/pharmacodynamic; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; UDP, uridine diphosphate.

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LITERATURE CITED

(1) LoPachin, R. M.; Balaban, C. D.; Ross, J. F. Acrylamide axonopathy revisited. *Toxicol. Appl. Pharmacol.* **2003**, *188*, 135–153.

(2) Hagmar, L.; Törnqvist, 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.

(3) Bull, R.; Robinson, M.; Laurie, R.; Stoner, G. D.; Greisinger, E.; Meier, J. R.; Stober, J. Carcinogenic effects of acrylamide in Sencar and A/J mice. *Cancer Res.* **1984**, *44*, 107–111.

(4) Bull, R.; Robinson, M.; Stober, J. Carcinogenic activity of acrylamide in the skin and lung of Swiss-ICR mice. *Cancer Lett.* **1984**, *24*, 209–212.

(5) Johnson, K. A.; Gorzinski, S. J.; Bodner, K. M.; Campbell, R. A.; Wolf, C. H.; Friedman, M. A.; Mast, R. Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. *Toxicol. Appl. Pharmacol.* **1986**, *85*, 154–168.

(6) Friedman, M. A.; Dulak, L. H.; Stedman, M. A. A lifetime oncogenicity study in rats with acrylamide. *Fundam. Appl. Toxicol.* **1995**, *27*, 95–105.

(7) Rosen, J.; Hellenas, K. E. Analysis of acrylamide in cooked foods by liquid chromatography tandem mass spectrometry. *Analyst* **2002**, *127*, 880–882.

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

(9) International Agency for Research on Cancer. Acrylamide. In *Some Industrial Chemicals*; IARC Monograph on the Evaluation of Carcinogenic Risks to Humans 60; IARC Press: Lyon, France, 1994; pp 1−45.

(10) U.S. National Toxicology Program. 10th Report on Carcinogens; U.S. Department of Health and Human Services, online version available at http://ntp.niehs.nih.gov/ntp, 2002.

(11) U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS); Acrylamide; available at http://www.epa.gov/iris/subst/0286.htm, 1993.

(12) JECFA. Acrylamide in Safety Evaluation of Certain Contaminants in Food. Prepared by the 64th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA); FAO Food and Nutrition Paper 82, pp 1−778; available online at http://whqlibdoc.who.int/publications/2006/9241660554_ACR_eng.pdf, 2006.

(13) Ruden, C. Acrylamide and cancer risk−expert risk assessments and the public debate. *Food Chem. Toxicol.* **2004**, *42*, 335–349.

(14) Konings, E. J.; Baars, A. J.; van Klaveren, J. D.; Spanjer, M. C.; Rensen, P. M.; Hiemstra, M.; van Kooij, J. A.; Peters, P. W. Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. *Food Chem. Toxicol.* **2003**, *41*, 1569–1579.

(15) U.S. Food and Drug Administration. Report of the Quantitative Risk Assessment Committee, Assessment of carcinogenic upper-bound lifetime risk resulting from contamination by acrylamide of copolymers, retention aids, drainage aids, stabilizer or fixing agents in paper and paperboard contacting foods, 1998.

(16) Gaylor, D. W.; Axelroad, J. A.; Brown, R. P.; Cavagnaro, J. A.; Cyr, W. H.; Hulebak, K. L.; Lorentzen, R. J.; Miller, M. A.; Mulligan, L. T.; Schwetz, B. A. Health risk assessment practices in the U.S. Food and Drug Administration. *Reg. Toxicol. Pharmacol.* **1997**, *26*, 301–321.

(17) Dybing, E.; Sanner, T. Risk assessment of acrylamide in foods. *Toxicol. Sci.* **2003**, *75*, 7–15.

(18) Mucci, L. A.; Adami, H.-O. The role of epidemiology in understanding the relationship between dietary acrylamide and cancer risk in humans. *Adv. Exp. Med. Biol.* **2005**, *561*, 39–47.

(19) Hagmar, L.; Törnqvist, M. Inconclusive results from an epidemiological study on dietary acrylamide and cancer. *Br. J. Cancer* **2003**, *89*, 774–775.

(20) Vainio, H. Acrylamide in heat-processed foods−a carcinogen looking for human cancer. *Eur. J. Epidemiol.* **2003**, *18*, 1105–1106.

(21) Wirfält, E.; Paulsson, B.; Törnqvist, M.; Axmon, A.; Hagmar, L. Associations between estimated acrylamide intakes, and hemoglobin AA adducts in a sample from the Malmö Diet and Cancer cohort. *Eur. J. Clin. Nutr.* **2007**, in press.

(22) Bjellaas, T.; Oleson, P. T.; Frandsen, H.; Haugen, M.; Stolen, L. H.; Paulsen, J. E.; Alexander, J.; Lundanes, E.; Becher, G. Comparison of estimated dietary intake of acrylamide with hemoglobin adducts of acrylamide and glycidamide. *Toxicol. Sci.* **2007**, *98*, 110–117.

(23) Kütting, B.; Uter, W.; Drexler, H. The association between self-reported acrylamide exposure intake and hemoglobin adducts as biomarkers of exposure. *Cancer Causes Control* **2007**, in press.

(24) International Agency for Research on Cancer. Ethylene oxide. In *Some Industrial Chemicals*; IARC Monographs on the Evaluation of Carcinogenic Risks to Humans 60; IARC Press: Lyon, France, 1994; pp 45−71.

(25) Irwin, R. D.; Eustis, S. L.; Stefanski, S.; Haseman, J. K. Carcinogenicity of glycidol in F344 rats and B6C3F1 mice. *J. Appl. Toxicol.* **1996**, *16*, 201–209.

(26) 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.

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

(28) Gamboa da Costa, G.; Churchwell, M. I.; Hamilton, L. P.; Beland, F. A.; Marques, M. M.; Doerge, D. R. DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. *Chem. Res. Toxicol.* **2003**, *16*, 1328–1337.

(29) Doerge, D. R.; Gamboa da Costa, G.; McDaniel, L. P.; Churchwell, M. I.; Twaddle, N. C.; Beland, F. A. DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. *Mutat. Res.* **2005**, *580*, 131–142.

(30) Hashimoto, K.; Tanii, H. Mutagenicity of acrylamide and its analogues in Salmonella typhimurium. *Mutat. Res.* **1985**, *158*, 129–133.

(31) Besaratinia, A.; Pfeifer, G. P. Genotoxicity of acrylamide and glycidamide. *J. Nat. Cancer Inst.* **2004**, *96*, 1023–1029.

(32) Paulsson, B.; Kotova, N.; Grawe, J.; Henderson, A.; Granath, F.; Golding, B.; Törnqvist, M. Induction of micronuclei in mouse and rat by glycidamide, genotoxic metabolite of acrylamide. *Mutat. Res.* **2003**, *535*, 15–24.

(33) Ghanayem, B. I.; Hamilton, L. P.; Churchwell, M. I.; Twaddle, N. C.; Snyder, R.; Fennell, T. R.; Doerge, D. R. Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA and hemoglobin adducts. *Toxicol. Sci.* **2005**, *88*, 311–318.

(34) Ghanayem, B. I.; Witt, K. L.; Kissling, G. E.; Tice, R. R.; Recio, L. Absence of acrylamide-induced genotoxicity in CYP2E1-null mice: evidence consistent with a glycidamide-mediated effect. *Mutat. Res.* **2005**, *578*, 284–297.

(35) Ghanayem, B. I.; Witt, K. L.; El-Hadri, L.; Hoffler, U.; Kissling, G. E.; Shelby, M. D.; Bishop, J. B. Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with acrylamide: evidence supporting a glycidamide-mediated effect. *Biol. Reprod.* **2005**, *72*, 157–163.

(36) Manjanatha, M. G.; Aidoo, A.; Shelton, S. D.; Bishop, M. E.; McDaniel, L. P.; Lyn-Cook, L. E.; Doerge, D. R. Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to male and female Big Blue mice. *Environ. Mol. Mutagen.* **2006**, *47*, 6–17.

(37) Paulsson, B.; Grawe, J.; Törnqvist, M. Hemoglobin adducts and micronucleus frequencies in mouse and rat after acrylamide or *N*-methylolacrylamide treatment. *Mutat. Res.* **2002**, *516*, 101–111.

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

(39) Bucher, J. R.; Huff, J.; Haseman, J. K.; Eustis, S. L.; Peters, A.; Toft, J. D. Neurotoxicity and carcinogenicity of *N*-methylolacrylamide in F344 rats and B6C3F1 mice. *J. Toxicol. Environ. Health* **1990**, *31*, 161–177.

(40) Shipp, A.; Lawrence, G.; Gentry, R.; McDonald, T.; Bartow, H.; Bounds, J.; MacDonald, M.; Clewell, H.; Allen, B.; Van Landingham, C. Acrylamide: review of toxicity data and dose-response analyses for cancer and noncancer effects. *Crit. Rev. Toxicol.* **2006**, *36*, 481–608.

(41) Park, J.; Kamendulis, L. M.; Friedman, M. A.; Klaunig, J. E. Acrylamide-induced cellular transformation. *Toxicol. Sci.* **2002**, *65*, 177–183.

(42) Sickles, D. W.; Sperry, A. O.; Testino, A.; Friedman, M. Acrylamide effects on kinesin-related proteins of the mitotic/meiotic spindle. *Toxicol. Appl. Pharmacol.* **2007**, *222*, 111–121.

(43) Poirier, M. C.; Beland, F. A. DNA adduct measurements and tumor incidence during chronic carcinogen exposure in animal models: implications for DNA adduct-based human cancer risk assessment. *Chem. Res. Toxicol.* **1992**, *5*, 749–755.

(44) Young, J. F.; Luecke, R. H.; Doerge, D. R. Physiologically based pharmacokinetic/pharmacodynamic model for acrylamide and its metabolites in mice, rats and humans. *Chem. Res. Toxicol.* **2007**, *20*, 388–399.

(45) Doerge, D. R.; Young, J. F.; McDaniel, L. P.; Twaddle, N. C.; Churchwell, M. I. Toxicokinetics of acrylamide and glycidamide in B6C3F$_1$ mice. *Toxicol. Appl. Pharmacol.* **2005**, *202*, 258–267.

(46) Doerge, D. R.; Young, J. F.; McDaniel, L. P.; Twaddle, N. C.; Churchwell, M. I. Toxicokinetics of acrylamide and glycidamide in Fischer 344 rats. *Toxicol. Appl. Pharmacol.* **2005**, *208*, 199–209.

(47) Tareke, E.; McDaniel, L. P.; Churchwell, M. I.; Twaddle, N. C.; Doerge, D. R. Relationship between biomarkers of exposure and toxicokinetics in Fischer 344 rats and B6C3F1 mice administered single doses of acrylamide and glycidamide and multiple doses of acrylamide. *Toxicol. Appl. Pharmacol.* **2006**, *217*, 63–75.

(48) Doerge, D. R.; Twaddle, N. C.; McDaniel, L. P.; Boettcher, M. I.; Angerer, J. Urinary excretion of acrylamide and metabolites in Fischer 344 rats and B6C3F$_1$ mice administered a single dose of acrylamide. *Toxicol. Lett.* **2007**, *169*, 34–42.

(49) Boettcher, M. I.; Schettgen, T.; Kutting, B.; Pischetsrieder, M.; Angerer, J. Mercapturic acids of acrylamide and glycidamide as biomarkers of the internal exposure to acrylamide in the general population. *Mutat. Res.* **2005**, *580*, 167–176.

(50) Boon, P. E.; de Mul, A.; van der Voet, H.; van Donkersgoed, G.; Brette, M.; van Klavern, J. Calculations of dietary exposure to acrylamide. *Mutat. Res.* **2005**, *580*, 143–155.

(51) Burek, J. D.; Albee, R. R.; Beyer, J. E.; Bell, T. J.; Carreon, R. M.; Morden, D. C.; Wade, C. E.; Hermann, E. A.; Gorzinski, S. J. Subchronic toxicity of acrylamide administered to rats in the drinking water followed by up to 144 days of recovery. *J. Environ. Pathol. Toxicol.* **1980**, *4*, 157–182.

(52) Doll, R.; Peto, R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *J. Natl. Cancer Inst.* **1981**, *66*, 1191–1308.

(53) Somoza, V.; Wenzel, E.; Lindenmeier, M.; Grothe, D.; Erbersdobler, H. F.; Hofmann, T. Influence of feeding malt, bread crust, and a pronylated protein on the activity of chemopreventive enzymes and antioxidative defense parameters in vivo. *J. Agric. Food Chem.* **2005**, *53*, 8176–8182.

(54) Paulsson, B.; Athanassiadis, I.; Rydgerg, P.; Törnqvist, M. Hemoglobin adducts from glycidamide: acetonization of hydrophilic groups for reproducible gas chromatography/tandem mass spectrometric analysis. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1859–1865.

(55) Bjellaas, T.; Janak, K.; Lundanes, E.; Kronberg, L.; Becher, G. Determination and quantification of urinary metabolites after dietary exposure to acrylamide. *Xenobiotica* **2005**, *35*, 1003–1018.

(56) Urban, M.; Kavvadias, D.; Reidel, K.; Scherer, G.; Tricker, A. R. Urinary mercapturic acids and a hemoglobin adduct for the dosimetry of acrylamide exposure in smokers and nonsmokers. *Inhal. Toxicol.* **2006**, *18*, 831–839.

(57) Vesper, H. W.; Licea-Perez, H.; Meyers, T.; Ospina, M.; Meyers, G. L. Pilot study on the impact of potato chips consumption on biomarkers of acrylamide exposure. *Adv. Exp. Med. Biol.* **2005**, *561*, 89–96.

(58) Fennell, T. R.; Sumner, S. C. J.; Snyder, R. W.; Burgess, J.; Spicer, R.; Bridson, W. E.; Friedman, M. A. Metabolism and hemoglobin adduct formation of acrylamide in humans. *Toxicol. Sci.* **2005**, *85*, 447–459.

(59) Kellert, M.; Scholz, K.; Wagner, S.; Dekant, W.; Völkel, W. Quantitation of mercapturic acids from acrylamide and glycidamide in human urine using a column switching tool with two trap columns and electrospray tandem mass spectrometry. *J. Chromatogr., A* **2006**, *1131*, 58.

(60) Chevolleau, S.; Jacques, C.; Canlet, C.; Tulliez, J.; Debrauwer, L. Analysis of hemoglobin adducts of acrylamide and glycidamide by liquid chromatography-electrospray tandem mass spectrometry, as exposure biomarkers in French population. *J. Chromatogr., A* **2007**, *1167*, 125–134.

(61) Vesper, H. W.; Bernert, J. T.; Ospina, M.; Meyers, T.; Ingram, L.; Smith, A.; Meyers, G. L. Assessment of the relation between biomarkers for smoking and biomarkers for acrylamide exposure in humans. *Cancer Epidemiol. Biomarkers Prev.* **2007**, *16*, 2471–2478.

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