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Autoclave Sterilization Produces Acrylamide in Rodent Diets: Implications for Toxicity Testing

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Acrylamide (AA) is a neurotoxic and carcinogenic contaminant that is formed during the cooking of starchy foods. Assessment of human risks from toxicants is routinely performed using laboratory rodents, and such testing requires careful control of unintended exposures, particularly through the diet. This study describes an analytical method based on liquid chromatography with electrospray tandem mass spectrometry that was used to measure endogenous AA in rodent diets and to survey a number of commercial products for contamination. Method sensitivity permitted accurate quantification of endogenous levels of AA in raw diets below 20 ppb. Autoclaving a standard rodent diet (NIH-31) increased the AA content 14-fold, from 17 to 240 ppb. A nutritionally equivalent diet that was sterilized by irradiation was found to contain ~10 ppb of AA (NIH-31IR). A toxicokinetic study of AA and its epoxide metabolite, glycidamide, was performed by switching mice from NIH-31IR to the autoclaved diet for a 30 min feeding period (average AA dose administered was 4.5 μg/kg of body weight). The concentrations of AA and glycidamide were measured in serum collected at various times. The elimination half-lives and the areas under the respective concentration−time curves were similar for AA and glycidamide. Mice maintained on autoclaved NIH-31 diet, but otherwise untreated, showed elevated steady state levels of a glycidamide-derived DNA adduct in liver relative to mice maintained on the irradiated diet. This study demonstrates that a heat sterilization procedure used in laboratory animal husbandry (i.e., autoclaving) can lead to the formation of significant levels of AA in basal diets used for toxicity testing. AA in rodent diets is bioavailable, is distributed to tissues, and is metabolically activated to a genotoxic metabolite, which produces quantifiable cumulative DNA damage. Although the contribution of endogenous AA to the incidence of tumors in multiple organs of rodents otherwise untreated in chronic carcinogenicity bioassays (i.e., control groups) is not known, the reduction of endogenous AA through the use of a suitable irradiated diet was deemed to be critical for ongoing studies of AA carcinogenicity and neurotoxicity.

KEYWORDS: Acrylamide; glycidamide; DNA adducts; toxicokinetics; mass spectrometry

INTRODUCTION

Acrylamide (AA, see structure in **Figure 1**) is an important industrial chemical with annual worldwide production estimated at >200 Gg. Concern about human toxicity from AA arises from the observations that AA is neurotoxic in experimental animals (reviewed in ref *1*) and in humans (*2*), is mutagenic to male germ cells (reviewed in ref *3*), shows a wide variety of evidence for genotoxicity in vitro and in vivo (reviewed in ref *4*), and is carcinogenic in several organs of experimental animals following chronic exposure (reviewed in ref *4*).

Human exposures to acrylamide from industrial production, commercial uses, cigarette smoking, and polyacrylamide gel electrophoresis (PAGE) analysis of proteins have been well-documented, primarily by measurements of AA−hemoglobin (Hb) adducts present in blood (*5*). Initial biomonitoring studies in human subjects consistently reported measurable AA−Hb adduct levels in seemingly unexposed individuals (i.e., nonsmokers with no known occupational exposure to AA), albeit at low levels. This finding was striking by comparison with acrylonitrile, a related toxic environmental chemical that also forms a circulating Hb adduct, which was not observed in nonsmokers with no known occupational exposure (*5*). This finding was replicated in experimental animal studies in which

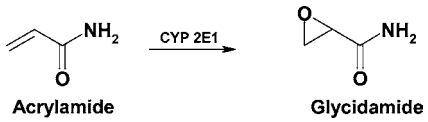

Figure 1. Structures of acrylamide and glycidamide.

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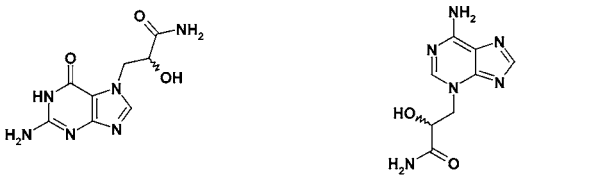

Figure 2. Structures of glycidamide–DNA adducts observed in mouse tissues.

untreated rats, consuming only commercial rodent diets, had measurable AA−Hb adducts (*6*). A clear link between the diet and AA−Hb adduct formation was made by Tareke et al. (*6*) when they showed that frying the rodent diet caused an 8−14-fold increase in AA−Hb adduct level. Analysis of the rodent diet showed AA levels below the method limit of detection (10 ppb) in the normal diet but 110−200 ppb levels in samples of fried diet.

A conclusive link between AA in foods and human exposures was made when Tareke et al. (*7*) quantified AA at parts per million levels in a number of fried and baked starchy foods, including potato chips, French fries, bread, and crackers. The mechanism for formation of AA in such foods was recently elucidated and involves the Maillard reaction of asparagine and reducing sugars, which are present in high levels in potatoes and cereals, at elevated temperatures (*8*−*10*). The total daily intake of AA from foodstuffs has been estimated by the World Health Organization to be in the range of 0.3−0.8 μg/kg of body weight (*11*).

In a recent study, we (*12*) characterized several DNA adducts arising from reaction of DNA with glycidamide (GA, **Figure 1**), a prominent metabolite of AA predominantly produced by the action of cytochrome P450 2E1 (CYP 2E1, *13*). That study also quantified GA−DNA adduct levels in tissues from untreated mice and AA- and GA-treated mice (see **Figure 2** for structures). Similar to the findings of Tareke et al. (*6*), contamination of the standard diet (NIH-31) by AA was inferred from the finding that GA−DNA adducts were consistently observed in tissues from mice consuming only the basal diet. The steady state level of GA−DNA adducts measured in mice was equivalent to that predicted from a single intraperitoneal injection of ∼100 μg/kg (*12*). Measurable internal exposures to AA and GA in otherwise untreated rodents were also reported by Sumner et al. (*14*) by using measurements of AA−Hb and GA−Hb adducts.

All of these studies provided strong evidence for the presence of AA in commercial rodent diets. Contamination by AA of basal diets typically used at this institution complicated our plans for rodent carcinogenicity and neurotoxicity testing of AA and could have implications for toxicological testing in general. For this reason, this study was initiated to quantify the endogenous AA content in commercial rodent diets, to determine processing procedures that promote AA formation, and to identify a commercially available diet with sufficiently low AA content to proceed confidently with rodent toxicity testing of AA.

MATERIALS AND METHODS

Reagents. Sigma Chemical Co. (St. Louis, MO) supplied the AA and all biochemical reagents. Labeled [$^{13}C_3$]AA (99 atom %) was obtained from Cambridge Isotope Laboratories (Andover, MA); both unlabeled and [$^{13}C_3$]GA (99 atom %) were obtained from Toronto Research Chemicals (North York, ON, Canada). All solvents were of HPLC grade, and Milli-Q water was used throughout.

Diets. Irradiated rodent diets and their autoclavable equivalents were obtained from Harlan-Teklad (Madison, WI), Zeigler Brothers (Gardner, PA), and Purina Mills Inc. (St. Louis, MO). Rodent grain-based reward pellets (45 mg, F0165) were obtained from Bioserve Co. (Frenchtown, NJ). Most diets were supplied as pellets that were ground into meal prior to analysis; an irradiated meal diet was also tested.

Liquid Chromatography (LC). A Waters Alliance 2795 separation module (Waters Co., Milford, MA) was used at a flow rate of 300 μL/min to deliver a mobile phase step gradient beginning at 0.5% (v/v) methanol in aqueous 0.01% formic acid (v/v) for 3.5 min, followed by 30% acetonitrile in aqueous 0.01% formic acid for 1.5 min, and a final reequilibration for 6 min (total run time = 11 min). Separations were conducted using an Aquasil column (2.1 × 150 mm, 5 μm particle size; Thermo Hypersil-Keystone, Bellefonte, PA) maintained at ambient temperature. A SpectraFocus UV detector (ThermoSeparation Products, San Jose, CA) was used to assess chemical purity and to quantify internal standards (200 nm). For analyses requiring additional chromatographic resolution, a second column was included for a total column length of 300 mm, and an isocratic mobile phase consisting of 0.5% (v/v) methanol in aqueous 0.01% formic acid (v/v) and a flow rate of 200 μL/min was used (total run time = 15 min).

Mass Spectrometry (MS). A Quattro Ultima (Waters Co.) triple-quadrupole mass spectrometer equipped with an electrospray probe was used in multiple reaction monitoring (MRM) mode for analysis of positive ions. The optimal MRM transitions (i.e., those giving the maximal responses) for AA and [$^{13}C_3$]AA were determined to be *m*/*z* 72 → 55 and 75 → 58, respectively (i.e., loss of NH_3), and those for GA and [$^{13}C_3$]GA were *m*/*z* 88 → 44 and 91 → 47, respectively (i.e., loss of $CONH_2$). Other MS parameters included dwell times of 0.25 s, argon collision gas at 2.7 × 10^{-3} bar, nitrogen as both cone gas (300 L/h) and desolvation gas (742 L/h), with source and desolvation temperatures of 120 and 450 °C, respectively. An optimized sampling cone-skimmer potential of 31 V was used throughout, and a collision energy of 11 or 16 eV was used for AA and GA transitions, respectively. Resolution was set to give peak widths at half-height of 0.9 Th for product and precursor ions.

Solid-Phase Extraction (SPE). Representative portions of ground diet samples were accurately weighed (2 g) and transferred to a laboratory blender; 20 mL of water was added, and the mixture was blended for three 1 min intervals. The resulting suspension was transferred to glass culture tubes and was centrifuged for 5 min at approximately 1600*g*. A 3 mL aliquot of the supernatant was then passed through an 0.45 μm poly(tetrafluoroethylene) (PTFE) syringe filter (Lida Manufacturing, Kenosha, WI) to remove particulates. The eluent from the syringe filter was then diluted 1:10 (v/v) with water. A 100 μL aliquot from the diluted solution was transferred to a glass culture tube, and 100 μL of water containing $^{13}C_3$-labeled AA internal standard (0.1 pmol/μL) was added. Reverse phase SPE was performed in 96-well plates using ENV+ (50 mg, Argonaut Technologies, Foster City, CA) cartridges processed with minimal vacuum. The cartridges were activated with 1 mL of methanol followed by conditioning with 2 × 1 mL portions of water. The sample was loaded and washed with 2 × 100 μL of water followed by 1 × 200 μL of 2% methanol in water (v/v). AA was eluted with 4 × 100 μL of 10% aqueous acetonitrile. The eluent then either was transferred to culture tubes and concentrated under a stream of nitrogen or was left in the 96-well tray and concentrated in a nitrogen jet array evaporator (J-KEM Scientific Inc., St. Louis, MO) designed for the 96-well format. Care was taken not to allow the samples to come to complete dryness. Finally, all samples were reconstituted with water to ∼100 μL.

Preparation of AA-Fortified Diet. Meal diet (3.8 kg of NIH-31IR) was spiked with AA (4 mg dissolved in 200 mL of water) at 1000 ppb using a V-blender equipped with a rotating intensifier bar (Patterson-Kelley, East Stroudsburg, PA). The feed was mixed for 20 min. Samples were taken from the top and middle parts of the blend to determine variability in mixing, and the remaining feed was placed in a refrigerator or under ambient conditions for stability determination under different storage conditions. To determine interday and intraday method variability, a single sample was separated further into three portions and analyzed on two different days using the method described above.

Animal Handling Procedures. Procedures involving care and handling of mice were reviewed and approved by the NCTR Laboratory Animal Care and Use Committee. All mice were obtained from the

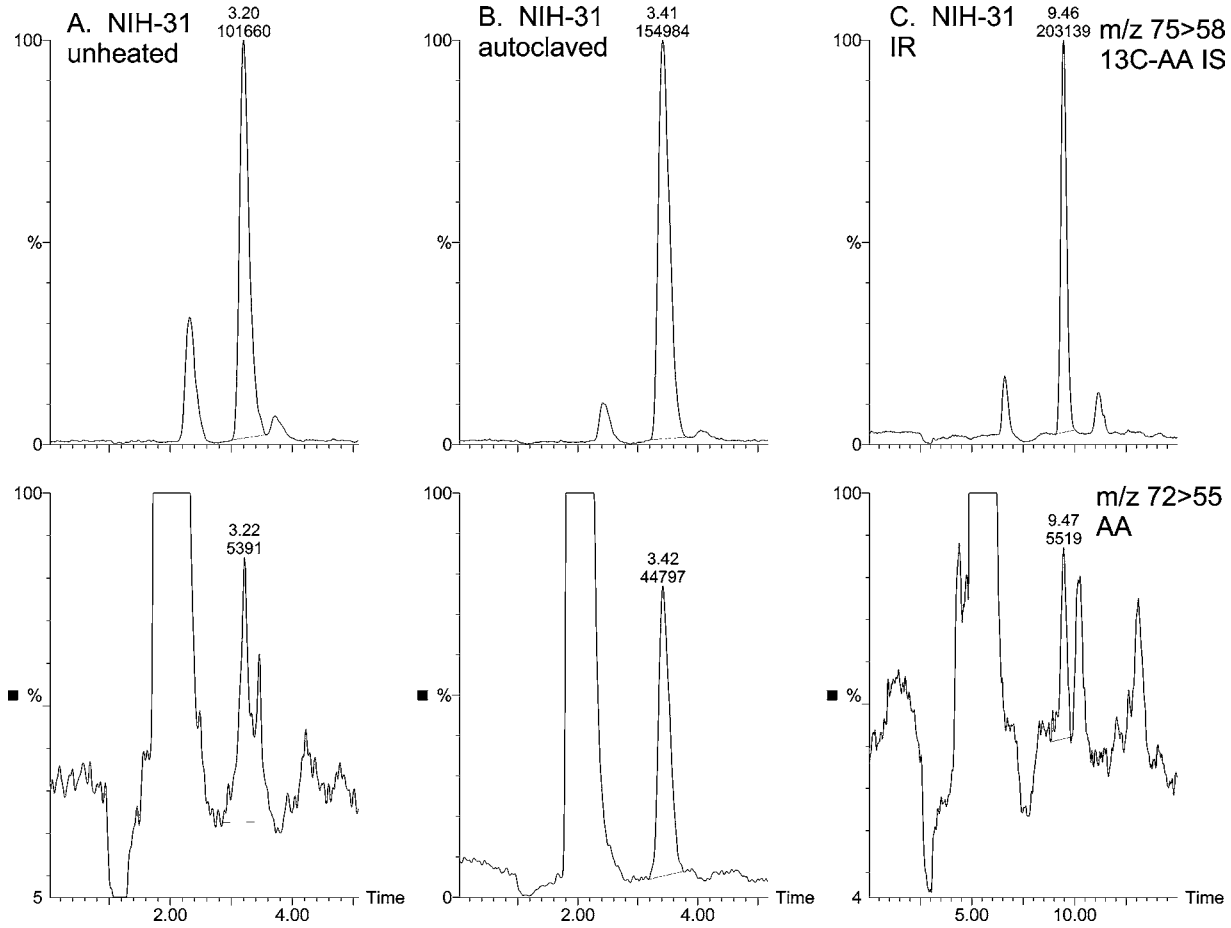

Figure 3. LC-ES/MS/MS chromatograms for acrylamide in NIH-31 diet before and after autoclaving and the equivalent irradiated diet: (**A**) unheated NIH-31 feed containing 17 ppb; (**B**) autoclaved NIH-31 feed containing 240 ppb; (**C**) NIH-31IR feed containing 11 ppb analyzed using a 300 mm column length. Upper traces correspond to [$^{13}C_3$]acrylamide internal standard (*m/z* 75 → 58) and bottom traces to unlabeled acrylamide (*m/z* 72 → 55).

NCTR colony. For the toxicokinetic study, male B6C3F$_1$ mice were placed on the basal diet (NIH-31IR meal) at weaning on postnatal day 21 until ~50 days of age. At that time, mice were presented with autoclaved NIH-31 pellets containing 240 ± 28 ppb of AA for a single acute exposure. To ensure consistent feed consumption, singly housed mice were trained for ~1 week to consume a bolus of feed in a 30 min period early in the morning (8:00−9:00 a.m.) following an overnight fast. After all training sessions, mice were given ad libitum access to the basal diet until that evening. For the single dietary exposure to AA, fasted mice were presented the pelleted autoclaved diet, and consumption by individuals was determined at the end of a 30 min feeding period by computing differences in pellet weight (0.41 ± 0.14 g of feed consumed). Individual mouse body weights (21.3 ± 1.4 g) were determined at sacrifice for individual mice (three per time point and controls). Blood was collected by cardiac puncture from three mice at each of the five time points (30−360 min) and allowed to clot on ice. Serum was produced by centrifugation, and samples were stored at −80 °C until analyzed in a single batch. Analysis of AA and GA in serum was performed by using a validated LC-ES/MS/MS method previously described (*15*).

For determination of DNA adducts in adult mouse liver (~75−120 days of age), the basal diet was autoclaved NIH-31 and GA−DNA adducts were quantified using LC-ES/MS/MS as previously described (*12*). Alternatively, liver DNA adducts were measured in male and female B6C3F$_1$ mice (~50 days of age) maintained exclusively on NIH-31IR diet. Statistical differences were assessed using the two-tailed *t* test and deemed to be significant for $p < 0.05$.

RESULTS

Method Performance. The method described was optimized for recovery of AA and removal of interferences by determining effects of the SPE and HPLC solvents, extraction cartridges, and hardware for use in high-throughput sample processing. It was necessary to use step gradient conditions in the LC separation to elute interference from the diet matrix and thereby minimize ion suppression during subsequent injections. Suppression of AA signals by diet interferences was determined to be 39% on the basis of the differences in responses for labeled and unlabeled standards fortified into a blank diet extract compared to neat standards. Recovery of 710 ppb of AA from spiked samples was determined to be 87 ± 14% ($n = 7$) on the basis of a comparison of $^{13}C_3$ signals from spiked diet processed through the entire method with those from a blank similarly processed and fortified with standards just prior to analysis. **Figure 3** shows representative chromatograms from samples of unheated NIH-31, autoclaved NIH-31, and NIH-31IR diets. It should be noted that the first two diets, which contained relatively higher levels of AA relative to the interference peak, were analyzed using an LC column length of 150 mm and the last diet, which contained a relatively low level of AA, was analyzed using an LC column length of 300 mm.

Method Validation. The method was validated using the method described above using NIH-31IR spiked with 25, 50, 100, and 1000 ppb of AA on two separate days ($n = 5$). This diet contained a measurable endogenous level of AA that was subtracted from each spiked diet determination to provide net AA values. The inter- and intraday precision and accuracy data are shown in **Table 1**. On the basis of the observed deviation from nominal accuracy of ≤20%, the limit of quantification for added AA in rodent diets was <25 ppb for analysis of a 1

Table 1. Intra- and Interday Precision and Accuracy for LC-ES/MS/MS Analysis of Acrylamide in NIH-31IR Rodent Diet[a]

AA added (ppb)	AA measured day 1	AA measured day 2	accuracy (%)
blank	18 ± 5 (28%)[b]	15 ± 3 (18%)	
25	24 ± 5 (22%)	27 ± 3 (10%)	102
50	60 ± 13 (22%)	55 ± 4 (7%)	115
100	91 ± 14 (16%)	105 ± 8 (7%)	98
1000	950 ± 26 (3%)	1060 ± 66 (6%)	101

[a] Meal extract was spiked with different amounts of acrylamide and analyzed on two separate days as described under Materials and Methods ($n = 5$ for each analysis). [b] Data are presented as mean ± SD and (RSD).

Table 2. Acrylamide Content in Commercial Rodent Diets

diet	AA (ppb)
Teklad 7913 IR	20
Zeigler NIH31-M 18-5	3
Teklad 7913	15
PMI 5K52	14
PMI 5K54	11
PMI 5002	130
PMI 5K96	12
Bioserve F0165 reward pellets	22 ± 3.3 (15%, $n = 4$)[a]
PMI NIH-31 (unheated)	17 ± 2.0 (12%, $n = 3$)[a]
PMI NIH-31 (autoclaved)	240 ± 28 (12%, $n = 21$)[a]
PMI NIH-31IR	7.5 ± 1.1 (15%, $n = 4$)[a]
	11 ± 0.8 (7%, $n = 4$)[b]

[a] Data are presented as mean ± SD and (RSD). If the number of analyses (n) is not specified, a single analysis was performed. [b] Analysis of a second lot of NIH-31IR diet.

mg of diet sample size and a 50 μL injection volume; the limit of detection was estimated to be 0.5 ppb (signal/noise ratio = 3). An interfering peak eluting closely after the AA peak was observed only in samples containing small amounts of AA (equivalent to ~10 ppb, **Figure 3A**) and the peak overlap was presumably a significant contribution to the aforementioned accuracy deviations at low levels of AA in NIH-31IR feed (**Table 1**). Therefore, to quantify AA levels near 10 ppb in the NIH-31IR feed, it was necessary to increase chromatographic resolution by adding an identical Aquasil column in series (i.e., 300 mm column length). This increased resolution produced baseline separation of the AA and contaminant peaks (**Figure 3C**) and permitted accurate quantification at ~10 ppb with good precision (RSD = 7−15% for two batches of feed, **Table 2**).

Analysis of AA-Fortified Diet. Fortified NIH-31IR diet that had been spiked with AA (4 mg in 4 kg) was analyzed as described, and the measured AA content was 1100 ± 50 ppb ($n = 5$). Inter- and intraday RSDs were determined to range from 4 to 9% and from 0 to 6%, respectively, from analyses conducted over a 3 day period. Stability of AA in the diet was also evaluated over the course of 28 days after either ambient temperature (~21 °C) or refrigerator (4 °C) storage. The feed stored at room temperature showed a slow decline in AA concentration ($t_{1/2} = 41$ days), whereas refrigerated feed showed no change over 28 days (data not shown). A similar evaluation of AA stability in water (1 mg/mL) at room temperature indicated no change in content over a 28 day period (data not shown).

Survey of Commercial Rodent Diets. Table 2 shows the results from analysis of a variety of rodent diets to determine those appropriate for toxicological testing of AA. In addition, a feed-based rodent reward pellet was analyzed to determine its suitability for use in behavioral studies of AA neurotoxicity

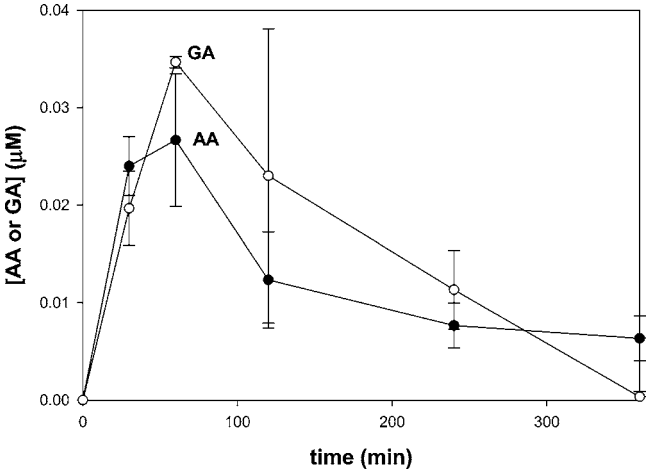

Figure 4. Time–serum concentration profiles for acrylamide (●) and glycidamide (○) following ingestion by adult male B6C3F$_1$ mice of an acrylamide dose equivalent to 4.5 ± 1.5 μg/kg. Data represent means ± SD ($n = 3$ mice per time point) for blood collection times after the 30 min feeding period.

at this institution. The criteria for a suitable basal diet included minimal AA content and identical nutritional composition to NIH-31, the predominant diet used for chronic toxicity studies at this institution. The diets, as supplied from the manufacturers, had AA content below 20 ppb, with one notable exception of 130 ppb. All but one diet, PMI 5K96, were supplied as pellets. Except for the single irradiated meal diet, none of the diets had been specifically treated to reduce microbial pathogens, and some institutions, including this one, require further sterilization before diets are used. This institution uses autoclaving to sterilize diets, and **Table 2** shows that heating by superheated steam and subsequent drying, all at 275 °F (135 °C), increased the AA content in NIH-31 by ~14-fold. The effect of location on AA formation was investigated by sampling feed pellets throughout positions in the autoclave (data not shown). The formation of AA was remarkably consistent in feed pellets located throughout the autoclave and even within individual feed pellets (i.e., AA levels in exterior and interior portions were essentially identical). The value reported in **Table 2** is the mean ± SD of all sample determinations ($n = 21$).

Toxicokinetic Analysis. Both AA and GA were observed in serum from male mice after being dosed with autoclaved NIH-31 diet containing 240 ppb of AA for a 30 min feeding period (**Figure 4**). The AA consumed was equivalent to an average total administered dose of 4.5 ± 1.5 μg/kg of body weight (mean ± SD, $n = 15$). By contrast, mice maintained on the NIH-31IR diet showed no measurable AA or GA in serum (not shown). **Figure 4** shows the subsequent time−serum concentration profiles for AA and GA. Maximal serum concentrations (C_{max}) for GA and AA were observed at 60 min after the end of the feeding period and concentrations were similar (0.035 and 0.027 μM, respectively, C_{max} ratio = 1.4). Toxicokinetic analysis showed that the areas under the concentration−time curves (AUCs from 0 to infinity) and elimination half-times were similar (5.3 vs 5.7 μM·min and 107 vs 112 min for AA and GA, respectively. The formation half-time for GA was ~24 min.

DNA Adduct Analysis. Liver DNA from untreated adult male and female B6C3F$_1$ mice, which had been maintained exclusively on NIH-31IR diet since weaning, contained measurable levels of N7-GA-Gua (1.0 ± 0.2 adducts in 10^8 nucleotides, **Table 3**). This steady-state DNA adduct level is ~7-fold lower than that previously observed in livers from untreated mice, which had been maintained exclusively on autoclaved NIH-31

Table 3. Comparison of Glycidamide–DNA Adducts in Mouse Liver Produced by either Acrylamide Present in Basal Diets or Administration of a Single Dose of Acrylamide

source of acrylamide	liver N7-GA-Gua (adducts in 10^8 nucleotides)	estimated acrylamide dose
NIH-31IR	1 ± 0.2[a]	1.4 μg/kg/day[d]
NIH-31 autoclaved	7 ± 4[b]	7–32 μg/kg/day[d]
gavage	5 ± 1[c]	100 μg/kg
intraperitoneal injection	67 ± 12[b]	1000 μg/kg

[a] This study. [b] Mice maintained on NIH-31 autoclaved basal diet (12). [c] Mice maintained on NIH-31IR basal diet (Doerge et al., unpublished results). [d] Estimated total daily dose based on consumption of 0.135 g of diet/g of mouse body weight observed in a recent bioassay conducted at this institution and data in **Table 2**.

diet (*12*). No N3-GA-Ade was observed because this adduct is formed in ~100-fold lower amounts relative to N7-GA-Gua (12). The concentration of AA in the latter diet was not specifically monitored throughout the exposure period, but a single contemporaneous sample of unknown age contained 49 ppb compared with 240 ppb in the samples analyzed immediately after autoclaving in this study (**Table 2**). The lower levels could result from between-batch variability in AA formation or, more likely, from degradation of AA content during use in the animal facility (half-life = 1.4 months), which can be for as long as 6 months.

DISCUSSION

The recent identification of significant levels of AA in a number of commonly consumed starchy foods has provided the impetus for additional research to define the risks to humans from the neurotoxic and carcinogenic effects of AA. The basis for human risk assessment remains chronic testing in laboratory animals, primarily rodents. In the controlled environments of modern animal toxicity testing facilities, the basal diet is a critical control point for introduction of uncertainty into such studies. The planning of toxicity testing for AA at this institution requires scrutiny of diets to define and minimize unintentional contamination.

This paper describes the adaptation of a method, based on previous LC-ES/MS/MS methods for analysis of AA in foods and serum (*7*, *15*, *16*), and its validation for use in measuring AA in rodent diets. The analytical sensitivity is comparable to that reported previously (*7*, *15*, *16*) and was sufficient for its intended use in surveying AA contamination in rodent feed.

Products from several commercial feed vendors were surveyed, and **Table 2** shows that all diets, with one exception, contained ~20 ppb AA or less. The preparation of pelleted diets, which involves steam extrusion, apparently does not produce AA because similar levels were observed in most pelleted diets compared with a meal diet. However, heating a pelleted diet at an autoclave temperature of 135 °C (275 °F) for ~1.5 h was sufficient to increase the AA content in NIH-31 diet ~14-fold to ~240 ppb. This level of AA contamination is also observed in many popular human foods, where levels can often exceed 1000 ppb (*7*, *16*).

Neither the AA level in autoclaved NIH-31 diet nor the level of microbes in the raw diet was acceptable for long-term rodent toxicity studies, so an alternative diet or sterilization process was required. Purina Mills also produces an irradiated diet that is nutritionally equivalent to NIH-31 with only an adjustment of heat-labile vitamin levels to compensate for losses incurred during autoclaving. This diet, which is referred to as NIH-31IR, was found to contain sufficiently low levels of AA (**Table 2**) and microbes (<200 colony-forming units, data not shown) to proceed with rodent toxicity testing. Analyses of AA content in two separate lots of NIH-31IR gave similar results, which are listed in **Table 2**.

Formation of AA in model Maillard reactions (*8*, *9*) and in foods (*7*, *17*) is known to be temperature-dependent. For example, boiled potatoes have very low AA levels when compared with those fried or baked (*7*). Whereas the temperatures associated with AA formation are usually >140 °C (*7*, *8*, *17*), significant formation of AA has been reported in microwaved foods, during which the cooking temperature presumably does not exceed 100 °C (*7*, *16*). The formation of AA in rodent feed during autoclaving is consistent with these observations.

The ~10^4-fold difference in dietary dose of AA administered to mice in this study relative to our previous study of mice treated by oral gavage with 50 mg/kg AA (*15*) produced some changes in toxicokinetic parameters. The elimination half-life for AA at the dose of 4.5 μg/kg was approximately double (i.e., slower), the GA elimination half-time was essentially unchanged, but the half-time for GA formation was approximately half (i.e., faster) relative to that observed in our previous high-dose study (*15*). These results at a much lower dose confirm our previous conclusion that conversion to GA is the predominant pathway for elimination of AA in the mouse (*15*).

This study demonstrates that heat sterilization of laboratory animal feeds can lead to the formation of elevated levels of AA in basal diets used for toxicity testing. The significance of such contamination in rodent diets is underscored by the observation that AA ingested from one limited feeding of an autoclaved diet, at intake levels comparable to those consumed by humans, is efficiently absorbed and metabolically converted to GA in mice (**Figure 4**). Furthermore, measurable steady-state levels of GA-derived DNA adducts are present in tissues from mice maintained on autoclaved NIH-31 diet (5−11 N7-GA-Gua adducts in 10^8 nucleotides from lung, liver, and kidney; *12*), and hemoglobin adducts with AA and GA have been measured in rats maintained on NIH-07 diet (*14*). **Table 3** shows a comparison of steady-state AA-derived liver DNA adducts in mice fed NIH-31 autoclaved and NIH-31IR basal diets with adduct levels observed following acute dosing with AA from other studies. Levels of N7-GA-Gua DNA adducts formed in liver appear to be proportional to the respective level of AA contamination in the basal diet. The total dose of AA administered to mice from consumption of the NIH-31IR diet was calculated from the 28 day exposure period and the estimated daily intake to be ~39 μg/kg. The adducts measured in the liver of these mice can be compared with the maximal adduct level produced by a single oral dose of 100 μg/kg or an intraperitoneal injection of 1000 μg/kg (**Table 3**). The estimated N7-GA-Gua formed in liver per unit dose was remarkably similar for both acute doses (0.04−0.06 adduct in 10^8 nucleotides/μg/kg) and for chronic exposure through NIH-31IR diet (0.03 adduct in 10^8 nucleotides/μg/kg). This observation suggests that GA−DNA adducts can accumulate from chronic exposures.

These observations demonstrate the bioavailability of AA from standard laboratory animal diets, distribution to tissues, extensive metabolic activation to a genotoxic metabolite, and quantifiable DNA damage that accumulates with repeat dosing. Although the contribution of unquantified amounts of AA in basal diets to the incidence of tumors in multiple organs of rodents otherwise untreated in chronic carcinogenicity tests (i.e., control groups) is not known, AA contamination in an auto-

claved diet was deemed to be incompatible with the conduct of our ongoing studies of AA carcinogenicity and neurotoxicity.

ABBREVIATIONS USED

AA, acrylamide; CYP 2E1, cytochrome P450 2E1; ES, electrospray; GA, glycidamide; Hb, hemoglobin; N7-GA-Gua, *N*7-(2-carbamoyl-2-hydroxyethyl)guanine; NCTR, National Center for Toxicological Research; ppb, parts per billion (ng/g); ppm, parts per million (μg/g); RSD, relative standard deviation (SD/mean $\times$ 100).

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