JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Approach for Cancer Risk Estimation of Acrylamide in Food on the Basis of Animal Cancer Tests and in Vivo Dosimetry

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The question about the contribution from acrylamide (AA) in food to the cancer risk in the general population has not yet had a satisfactory answer. One point of discussion is whether AA constitutes a cancer risk through its genotoxic metabolite, glycidamide (GA), or whether other mechanism(s) could be operating. Using a relative cancer risk model, an improvement of the cancer risk estimate for dietary AA can be obtained by estimation of the genotoxic contribution to the risk. One cornerstone in this model is the in vivo dose of the causative genotoxic agent. This paper presents an evaluation, according to this model, of published AA cancer tests on the basis of in vivo doses of GA in rats exposed in the cancer tests. The present status regarding data with importance for an improved estimation of the contribution from GA to the cancer risk of AA, such as in vivo doses measured in humans, is discussed.

KEYWORDS: Acrylamide; glycidamide; cancer tests; hemoglobin adducts; cancer risk estimation; genotoxicity

INTRODUCTION

The demonstration of a positive association between cancer and exposure in cancer epidemiological studies is usually taken as the ultimate proof that the exposure constitutes a cancer risk in humans. However, cancer epidemiology is not a sensitive method and does not usually have the power to detect unacceptable risks of low magnitude (*1, 2*). Therefore, cancer risk estimation on the basis of toxicological data is important for the detection and assessment of cancer risk factors, despite the fact that this involves large uncertainties in high- to low-dose extrapolations. Usually, extrapolations are done from experimental studies of toxic effects at concentrations 10^3-10^5 higher than the human exposure levels, as in the case with toxicological studies of health risks associated with the exposure to AA in food (*3*).

In work aimed at improving methods for cancer risk assessment of genotoxic chemicals, an approach for risk assessment is being developed, which is based on experience from ionizing radiation (*4, 5*). According to this approach the cancer risk increment is proportional to the dose in vivo of the causative genotoxic agent and its genotoxic potency. The in vivo dose is here defined as the time integral of the concentration of the genotoxic compound (*6*), which is the same as the "area under the concentration−time curve" (AUC). For the purpose of in vivo dosimetry of short-lived genotoxic chemicals, specific methods have been developed for the measurement of stable reaction products (adducts) with hemoglobin (Hb) (reviewed in ref *7*).

Evaluations of animal cancer tests using this approach indicate that a relative cancer risk model previously used for ionizing radiation could be applied to genotoxic chemicals (*8, 9*). The relative risk model implies that the cancer risk increment is proportional to the in vivo dose of the genotoxic agent and to the background cancer incidence in the studied species, strain, organ, etc. In addition, it is indicated that the risk increment according to this model is common for the different responding sites and approximately the same independent of species. Thus, using this approach animal cancer tests should be evaluated according to the relative cancer risk model, and the extrapolation to humans should be based on the measured in vivo doses. This model has been shown to provide a way to predict the tumor incidence in long-term animal tests with ionizing radiation, and it is used for the projection of human cancer risk from ionizing radiation, showing approximately the same risk coefficient in different species (reviewed in ref *8*). The applicability of the relative risk model to cancer test data for ethylene oxide (*8*) and butadiene (*9*) has been validated. It has been demonstrated that on the basis of in vivo doses of the potential causative agents the observed tumor incidences in the cancer tests are in agreement with the incidences predicted from the relative risk model and the relative genotoxic potencies of the causative agents (*8, 9*).

AA is metabolized to GA (*10, 11*), which in contrast to AA has been shown to give DNA adducts in vivo (*12, 13*). GA is

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10.1021/jf800490s CCC: $40.75
Published on Web 07/15/2008

mutagenic [for a review see Rice (*14*)] and could therefore be assumed to constitute a cancer risk already from low exposure doses of AA. However, it has been discussed whether a nongenotoxic mechanism, with nonlinearity, could operate and contribute to the observed tumor frequency in the cancer tests with AA in rats (see e.g., refs *15* and *16*).

In our ongoing work AA is evaluated according to the described relative model for cancer risk assessment of genotoxic carcinogens. This work was initiated in 1997 when a leakage of AA in a tunnel construction work at Hallandsås in Sweden urged the estimation of cancer risks of exposed tunnel workers, as well as of potentially exposed residents in the area (*17, 18*). The estimation of cancer risks relied on earlier studies of in vivo doses of AA and GA in occupationally exposed humans and in rats and on published cancer tests (*19*). These studies of the AA leakage at Hallandsås, including the cancer risk assessment of AA, initiated studies of the background exposure to AA in humans and tracing of food as the exposure source (*20, 21*).

The aim of the present work is to improve the cancer risk estimation of AA to comprise the low exposure doses from AA in food, based on the approach indicated above. This approach concerns the evaluation of the genotoxic contribution to risk, and therefore GA, the genotoxic metabolite of AA, is in focus.

This paper presents the measurement of in vivo doses of AA and GA in rats exposed in a short-term test at similar conditions as in the published 2-year cancer tests with AA. The main aim is the evaluation of the published data from the cancer tests according to the relative cancer risk model, on the basis of the in vivo doses of GA, as obtained from the short-term tests. Furthermore, other data of relevance for the risk estimation are summarized, and the present status of the cancer risk estimation of AA, according to this model, is discussed.

MATERIALS AND METHODS

Published Cancer Test Data for Acrylamide. In the present evaluation, published data from 2 year cancer tests with Fischer 344 rats exposed to AA in drinking water are used (*22, 23*). The daily doses were 0, 0.01, 0.1, 0.5, or 2 mg/kg body weight (bw) to female and male rats in the study by Johnson et al. (*22*). In the study by Friedman et al. (*23*), daily doses of 0, 1.0, and 3.0 mg/kg bw and 0.1, 0.5, or 2.0 mg/kg bw were given to female and male rats, respectively. For details see refs *22* and *23* or the review by Rice (*14*).

Short-Term Exposure of Rats to Acrylamide. Fisher 344 rats, 9−10 weeks old, females (n = 12, 150−160 g bw) and males (n = 11, 215−255 g bw), were given AA in the drinking water for 1 week. The animals, supplied from Scanbur-BK AB (Sollentuna, Sweden), were kept at the animal house at Stockholm University with free access to standard animal dry feed. The study was approved by the Ethical Committee on Animal Experiments, Stockholm North (no. N/56/02 and N/228/03).

The rats were divided into three exposure groups for females and two exposure groups for males (three animals per group) that were given AA doses in drinking water corresponding to a daily dose of 0.1, 0.5, and 2.0 mg/kg bw, that is, at similar concentrations as in the cancer tests referred to above. Control groups (three females and five males) were given ordinary tap water. On the basis of the fact that rats eating dry feed drink 100 mL water/kg bw per day (*24*), water with AA concentrations of 1, 5, and 20 mg/L were prepared to obtain the desired doses. The drinking water for the exposures was prepared twice during the time of the experiment, and the AA concentrations were monitored by LC-MS/MS (performed at Eurofins Food & Agro Sweden AB, Lidköping, Sweden). The increase in body weights during the study (6 g for females and 16 g for males) showed no significant difference between exposed animals and controls. After 7 days, the animals were weighed and sacrificed. Blood samples were collected for the analysis of Hb adducts from AA and GA, as a measure of the corresponding in vivo doses.

Analysis of Hemoglobin Adducts. In vivo doses from AA and GA in exposed rats were measured by Hb adducts. A modified Edman degradation method was used for the analysis of adducts to N-terminal valines in Hb (*25, 26*). Globin was precipitated from the samples of hemolyzed red blood cells (*27*). Globin samples were treated with pentafluorophenyl isothiocyanate, which leads to specific detachment of adducts to N termini, through the formation of pentafluorophenylthiohydantoin (PFPTH) derivatives of the N-substituted valines. The detachment through ring closure is facilitated by a Thorpe−Ingold effect (*28*). The method has been adapted for the analysis of the adduct from AA (*29*) and the adduct from GA, which has to be further derivatized (*30*). Internal standards of corresponding deuterium-substituted PFPTHs were used (*29, 30*). Calibration curves were established from samples prepared from standard globins with known adduct levels from AA and GA mixed with myoglobin. Analysis was carried out using a triple-quadrupole GC-MS/MS instrument as described earlier (*30*).

Calculation of in Vivo Dose from Hemoglobin Adduct Levels. For the application in risk estimation procedures, the in vivo dose (D; in M × h) is defined as the time integral of the concentration, that is, the AUC as in eq 1. This dose expression reflects absorption or formation, and rate of elimination in vivo (*6*).

$$D = \int_t C(t)\,\mathrm{d}t \tag{1}$$

The AUC in blood could be calculated from the measured Hb adduct level (A; in mol g^{-1}), if the reaction rate constant of the electrophilic agent toward the nucleophile is known (e.g., the rate constant for the formation of a valine adduct k_{val}; in L g^{-1} h^{-1}; in these calculations 1 kg ∼ 1 L). In a situation with an acute exposure the dose in blood of an electrophilic compound could be inferred from the adduct level to N-terminal valine in Hb measured a short time after exposure according to eq 2:

$$D = \frac{1}{k_{val}} \times A \tag{2}$$

The kinetics for elimination of Hb (lifetime of erythrocytes) have to be considered for the calculation of the dose in blood from an adduct level measured a longer time after termination of exposure or after prolonged exposure (*31*). The measured adduct level in this study was adjusted to the daily incremental adduct level by multiplying by a factor of 1.07 (*31*) and dividing by 7 (days).

The rate constants for the reaction of AA and GA with N-terminal valines in Hb from rats were determined in an experiment in which blood samples from nonexposed rats were incubated with 40 μM AA and 10 μM GA, respectively, at 37 °C during 1−3 h (cf. ref *32*). The Hb adduct levels were measured as described above.

Cancer Risk Model. In the present study the relative cancer risk model has been fitted to the tumor incidence data from the published cancer tests on rats with AA (*22, 23*). Data for the following sites were included in the evaluation: in the study by Johnson et al. (*22*), (i) females, mammary gland, CNS, thyroid gland, oral tissue, and uterus, and (ii) males, thyroid gland, testes, and adrenal gland; in the study by Friedman et al. (*23*), (i) females, mammary gland, CNS, and thyroid gland, and (ii) males, thyroid gland and testes.

The evaluation was first carried out on the basis of the lifetime exposure dose of AA (in mg/kg bw) and then on the lifetime doses in blood (in mMh) of GA and of AA, as obtained from the short-term exposure of rats in the present study. Strictly, the dose in the relative risk model assigns the target dose in tumor sites. Hb adducts measure the dose in blood, and the target dose has then to be approximated by considering the dose distribution of the genotoxic agent. For the evaluation of AA exposure, the dose of GA in blood is a good approximation of the target dose of GA (see Discussion).

The relative risk model (*8*) implies that the fraction of animals in the cancer test with tumors in target tissue i, that is, P_i (D), can be described according to eq 3:

$$P_i(D) = 1 - \mathrm{e}^{-P_i^0(1+\beta D)} \tag{3}$$

D then denotes the dose in target tissues of the genotoxic agent. P_i^0 represents the cumulative hazard for tumor formation in the target tissue

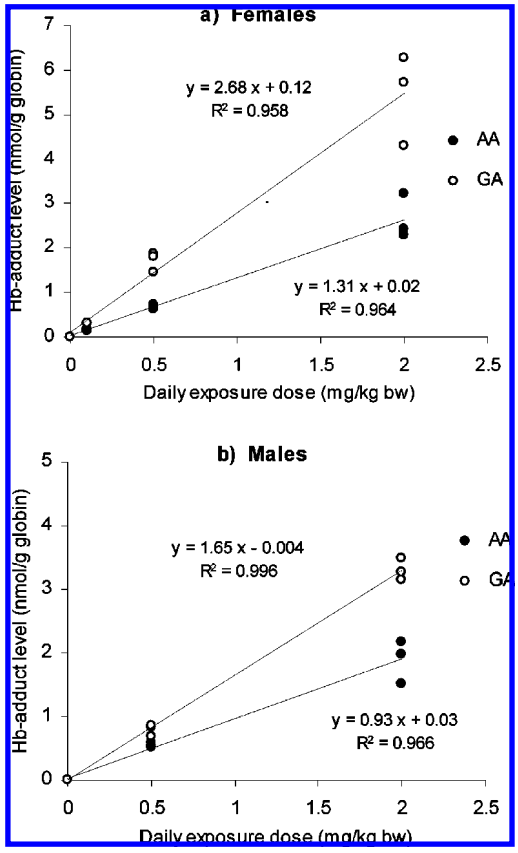

Figure 1. Hb adduct levels from acrylamide (AA) and glycidamide (GA) measured in blood from rats treated with different daily doses of AA in their drinking water for 7 days: (**a**) females; (**b**) males.

i among unexposed animals, and β is the common relative excess risk per unit of dose. The relative potency is expressed as the doubling dose (the same as $1/\beta$), that is, the dose yielding a cumulative hazard of $2P_i^0$.

The parameters of this nonlinear function were estimated by a binomial regression model. The 95% confidence intervals for the estimated doubling doses were obtained by the profile likelihood method.

RESULTS

In Vivo Doses in Rats Exposed to Acrylamide through Drinking Water. The in vivo doses of AA and GA are inferred from measured Hb adduct levels. The in vivo dose, that is, the concentration over time, is expressed in, for example, μM × h (cf. Materials and Methods). Hb adduct levels up to 2.6 and 5.4 nmol/g for AA and GA, respectively, were found in the exposed rats. There were linear relationships between the Hb adduct levels, for both AA and GA in female and male rats, and the administered dose of AA (**Figure 1**). This means that there was no significant saturation of the metabolism observed within the tested dose range. In females the increases in the adduct levels from both compounds were somewhat higher than in males (**Figure 1** and **Table 1**).

The rate constants for the reaction of AA and GA, respectively, with N-terminal valine in rat Hb, were determined from the in vitro incubations. The respective rate constants were determined to be

$$k_{\text{val-AA}} = 4.6 \times 10^{-6}\ (\text{L g}^{-1}\ \text{h}^{-1})$$

$$k_{\text{val-GA}} = 13.6 \times 10^{-6}\ (\text{L g}^{-1}\ \text{h}^{-1})$$

Using the determined values of $k_{\text{val-AA}}$ and $k_{\text{val-GA}}$ the daily in vivo dose of the respective compound was calculated according to eq 2 from the daily adduct increment (obtained after adjustment of adduct levels, see Materials and Methods). The measured adduct levels and the calculated daily in vivo doses are summarized in **Table 1**. **Table 1** also shows the obtained in vivo doses per administered dose of AA. For GA, the focus of this study, these are ca. 34 and 18 μMh GA per mg AA/kg bw, for females and males, respectively.

Evaluation of the Cancer Tests with the Relative Risk Model. The in vivo doses of AA and GA obtained in the short-term exposures to AA were used to calculate the lifetime in vivo doses in the 2-year cancer tests, which were then used in the evaluation of the cancer tests. In this evaluation according to the relative risk model, the data for sites with significant response are evaluated together, that is, all sites, doses, and genders, as well as the data from both cancer tests. The fit of the model (eq 3) is displayed as observed versus predicted tumor incidence. The doubling dose, the dose giving an incidence corresponding to a doubling of the background incidence in a tumor site, is obtained as $1/\beta$ from eq 3.

The 2-year cancer test data were first evaluated according to eq 3 with the dose expressed as lifetime exposure dose of AA (in mg/kg bw) during the cancer tests. Then the model was adapted to the lifetime in vivo doses, in mMh, of both AA and GA calculated from the short-term exposure in the present study. The goodness of fit of the model (eq 3) when using in vivo doses of GA (plotted in **Figure 2**), was similar to the goodness of fit obtained for AA exposure doses and in vivo doses of AA. This follows from the linear dose−response obtained in **Figure 1** (as Hb adduct levels).

The doubling dose obtained when using the exposure dose in the evaluation of the risk model according to eq 3 was 510 mg AA/kg bw (**Table 2**). The doubling doses obtained when the in vivo doses were applied were 12.7 mMh GA and 19.3 mMh AA, respectively. For the estimated doubling doses no significant difference was observed between the cancer tests (**Table 2**). No significant difference between males and females was detected (data not shown).

DISCUSSION

In the evaluation according to the outlined approach, aiming at an improved cancer risk estimation of the exposure to AA in food, the following are considered essential:

- the in vivo dose of GA in animal cancer tests with AA
- the applicability of the relative risk model to animal cancer tests on the basis of in vivo dose
- the average exposure dose to AA and the average in vivo dose of GA, their relationship, and their variation in the general population
- dose−response relationships at low exposure doses of AA

In this study the first two points have been evaluated. In addition, knowledge about in vivo doses of GA in humans is summarized below, and some views are given on the dose−response relationships at low exposure doses of AA.

Applicability of the Cancer Tests with Acrylamide According to the Relative Risk Model. The evaluation of the data from the cancer tests according to the relative risk model (eq 3) gave a doubling dose of GA of about 13 mMh, with a fairly narrow confidence interval. This model with a common

Table 1. Measured Hb Adduct Levels of Acrylamide (AA) and Glycidamide (GA) and Calculated Internal Doses in Rats Treated with Different Exposure Doses of AA in Their Drinking Water

		Hb adduct level (after 7 days) (nmol/g globin)		internal dose[a]			
				μMh/day		μMh per mg AA/kg bw	
daily exposure dose (mg AA/kg bw per day)	*N*	AA (mean ± SD)	GA (mean ± SD)	AA	GA	AA	GA
females							
0.0	3	0.006 ± 0.002	0.008 ± 0.004				
0.1	3	0.16 ± 0.01	0.32 ± 0.000	5.4	3.5	54	35
0.5	3	0.68 ± 0.04	1.71 ± 0.22	23	19	46	38
2.0	3	2.64 ± 0.49	5.43 ± 1.78	88	61	44	30
average						48	34
males							
0.0	5	0.007 ± 0.001	0.010 ± 0.002				
0.5	3	0.54 ± 0.04	0.79 ± 0.09	18	8.9	36	18
2.0	3	1.89 ± 0.33	3.30 ± 0.18	63	37	32	19
average						34	18

[a] Calculated according to eq 2 when accumulated adduct level has been adjusted to the daily adduct level increment according to Materials and Methods.

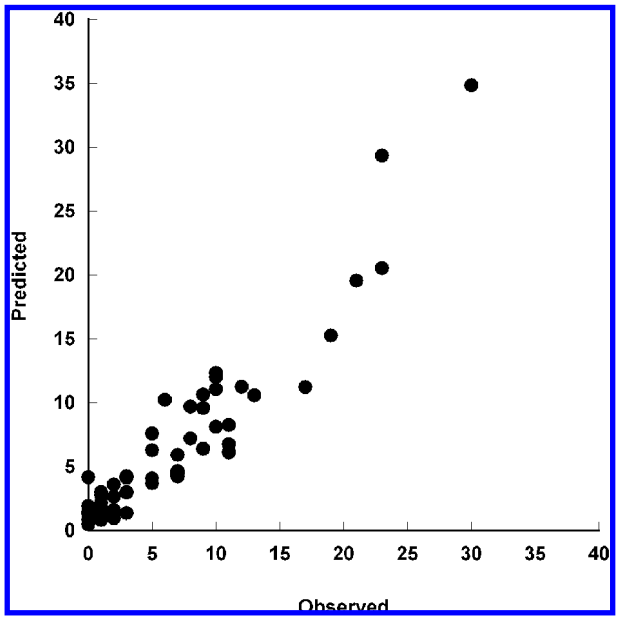

Figure 2. Predicted versus observed tumor frequency in cancer tests with acrylamide, using internal doses of glycidamide (in mMh) in eq 3.

risk coefficient for all responding sites yields an appropriate fit to the observed data for all sites. However, the estimate is relatively strongly influenced by the response in mammary and thyroid glands, and it can therefore not be excluded that a potential non-genotoxic action (*15, 16*) influenced the estimate.

Ethylene oxide, another simple epoxide, used as a model in the outlined approach for cancer risk estimation and for which cancer test data have been evaluated with the relative risk model, could be used for a comparison with GA. For ethylene oxide a doubling dose of ca. 11 mMh has been obtained in the evaluation of data from published cancer tests (in mice and rats) (*8*). The applicability of the relative risk model, eq 3, to the data from the cancer tests with ethylene oxide has been validated (*8*). Similar results, in congruence with data for ethylene oxide, have been obtained for butadiene and its epoxy metabolites (*9*).

The outcome from the present evaluation could be discussed in light of a comparison of the genotoxic potency of GA and ethylene oxide. In our approach the relative genotoxic potencies for simple alkylating agents have been estimated in two different ways (see ref *33*). It is possible to estimate the relative genotoxic potency from mutation tests through comparison with a standard agent, under the condition that the dose of the genotoxic compound/metabolite during the treatment in the mutation test is determined as AUC (mMh). For alkylating agents also the reaction rate constants toward model nucleophiles have been used to estimate the relative reactivity toward assumed critical sites in DNA. In a comparison on the basis of reaction rates, GA is expected to have 2−3.5 times higher mutagenic potency than ethylene oxide (*33*). Preliminary mutation tests show ca. 7 times higher potency of GA than of ethylene oxide (*33, 34*) (ongoing work indicates a somewhat lower value, personal communication from Dag Jenssen, Stockholm University). According to this preliminary comparison with ethylene oxide we expect a somewhat lower doubling dose of GA in the cancer tests with AA than obtained in this evaluation; that is, the tumor incidence increments are somewhat lower than expected from estimates of the genotoxic potency of GA in vitro.

The ongoing cancer studies with AA and GA within the National Toxicology Program (NTP) (*35*) will give important information with regard to the issue of genotoxic and/or non-genotoxic contribution to outcomes in cancer tests with AA. If GA is the sole carcinogen arising from AA exposure, our approach predicts the outcome in these experiments, that is, the same responding sites as, for example, ethylene oxide and butadiene, and that the estimated doubling dose expressed as the in vivo dose of GA would be in agreement with what we have found in the present study. According to estimates by Doerge et al. the AUC of GA per exposure dose of AA is about 50% higher in the rat compared to the mouse strain used in the cancer test ($B6C3F_1$ mice) (*36, 37*). According to the relative risk model the same doubling dose per in vivo dose of GA is expected in mice. We judge that the data from other types of cancer tests of AA in mice that is already published are not useful for a quantitative comparison with the rat cancer tests, because the exposure is of short-term nature and with uncertain in vivo doses (cf. refs *19* and *38*).

In Vivo Doses in Rats Exposed to Acrylamide as in Cancer Tests. Since our preliminary evaluation of the cancer risk associated with exposure to AA (*19*), many studies with exposure of rodents have been published that are potentially useful for improvements of the estimate of in vivo doses of GA. Several studies concern

Table 2. Doubling Doses in Published Cancer Tests with Acrylamide (AA), Obtained after Evaluation According to the Relative Cancer Risk Model (Equation 3), Expressed as Exposure Dose of AA and Internal Doses of AA or Glycidamide (GA)

	doubling doses (95% confidence intervals)		
evaluated cancer test data	exposure dose AA (mg/kg)	internal dose AA (mMh)	internal dose GA (mMh)
both cancer tests	510 (364−730)	19.3 (13.7−27.8)	12.7 (9.0−18.4)
Johnson et al. (*22*)	590 (373−989)	22.7 (14.3−38.1)	15.4 (9.7−25.9)
Friedman et al. (*23*)	427 (259−723)	15.6 (9.3−27.0)	9.7 (5.8−17.1)

AA exposure of rats through different routes of administration, including oral exposure, and the determination of biomarkers useful for the calculation of in vivo doses (*37, 39–42*). Three studies (*37, 39, 42*) with oral exposure at low doses are the most relevant for comparisons with the present study of in vivo doses in AA-exposed rats (cf. **Table 1**).

Fennell et al. (*39*) administered AA to F344 rats (males) by gavage at a single dose of 3 mg/kg bw. The in vivo doses (calculated from the Hb adduct levels to N termini and reaction rate constants) per administered dose (μMh per mg AA/kg bw) were higher than in the present study—about 5 times higher for AA and 3.5 times higher for GA.

Doerge and collaborators have recently published data on Fischer 344 rats exposed to low doses of AA. One experiment concerns administration through drinking water of daily doses of 1 mg AA/kg bw during 42 days and the determination of adducts to N-terminal valine in Hb (*42*). In agreement with the present results higher adduct levels were found in females compared with males. The reported adduct levels per administered dose of AA, estimated from figures in the paper, indicated about 2 times higher in vivo doses of GA compared to the present study.

Both of these cited studies used different versions of the method for measurement of the Hb adduct levels to N termini. As interlaboratory calibrations have not been done, there are uncertainties in comparisons of data on adduct levels from different laboratories, as also indicated in the figures for the reported rate constants for adduct formation (*39, 42*). The calculation of in vivo dose from measured adduct levels could be used to reduce these uncertainties.

Doerge et al. have also measured AUC of AA and GA in exposed rodents through analysis of serum concentrations over time (*37*). In F344 rats exposed to single doses of 0.1 mg/kg bw through diet, the AUC was determined to 15 and 19 μMh GA per mg AA/kg bw for males and females, respectively. The result for males is compatible with that observed in our study. The in vivo dose of GA observed for the females is about 50% lower than in our study (cf. **Table 1**). The reported AUC for AA, however, was 2−3 times lower than in the present study.

In the present study rats (F344, females and males) were exposed to AA through drinking water for 7 days at doses similar to those in the cancer tests. The data obtained have high relevance (route of administration, dose rates, duration of exposure, and accuracy of method) for an accurate calculation of the in vivo doses in the cancer tests. Our preliminary risk estimation (*19*) was based on in vivo doses from studies with exposure through intraperitoneal injections (3.3 mg AA/kg bw per day, 30 days) in the rat (Sprague−Dawley, males), from measurements of Hb adducts to cysteine (*43, 44*). The doses obtained were, in μMh per mg AA/kg bw, 28.2 for AA and 16.3 for GA. This is in good agreement with the data for male rats in the present study (cf. **Table 1**).

In conclusion, the accuracy of the in vivo dose determination in this study is supported by the agreement with the early data (*43, 44*) and by the study using a completely different method for measurement of AUC by Doerge et al. (*37*). The gender difference is also supported by the studies by Doerge et al. and Tareke et al. (*37, 42*).

In the relative risk model (eq 3) the target dose in the different tumor sites is the exact parameter of in vivo dose. In the present evaluation of the cancer test data the target dose was approximated by the dose in blood. The volumes of distribution (V_d) for both AA and GA have been approximated to 1 in the present evaluation. Doerge et al. (*12, 37*) have recently determined V_d in rats to be 0.77−0.87 for AA and 0.68−0.77 for GA. This means that the compounds have somewhat higher concentrations in blood than the average concentration in the tissues. In addition, DNA adduct levels to N-7 guanine have been observed in all examined organs in rodents (*12, 13*). These data show that it is reasonable to approximate the target dose of GA with the dose of GA measured in blood.

In Vivo Doses of Acrylamide and Glycidamide in Humans. The average exposure to AA from food has been estimated to about 0.5 μg/kg bw in several countries (*45*). If GA is the cancer-risk-increasing agent in AA exposure, it is important to know the relationship between AA exposure and the in vivo doses of GA in humans. The steady-state levels of adducts to N termini in Hb, which reflects the average in vivo dose over the previous months, have been used to characterize the AA exposure in the general population in many recent studies. These studies are discussed below.

In a pioneer study of workers from an AA-producing plant, Hb−AA adduct levels up to 34000 pmol/g globin (*46*) were measured. In later studies of occupational exposure more moderate levels have been observed (*17, 29, 47–50*) (reviewed in ref *51*). Another known source of AA is tobacco smoke (*52*), and in smokers increased AA adduct levels up to 420 pmol/g globin (*29, 53–59*) have been observed. A rough estimate of the AA adduct increment (steady-state level) in smokers is about 5 pmol/g globin per smoked cigarette/day (*29, 56, 60, 61*).

The tracing of AA in food was initiated by the observations of the AA adduct observed in Hb from nonsmokers (*17, 29*), which was low or absent in animals (*62*). Subsequently, several studies have presented AA adduct levels in nonsmokers (*30, 50, 53–56, 58, 61, 63, 64*). The published studies are from different laboratories with no published intercalibration, which makes comparisons uncertain. The two most comprehensive studies show AA adduct levels of 5−100 pmol/g globin (estimated from a figure in the paper, $n = 828$) (*60*) and 18−90 pmol/g globin ($n = 484$) in nonsmokers (*57*). A pilot study ($n = 60$) assessing the variation in the levels of AA−Hb adducts in nonsmokers from five European countries indicated that there are no large differences between the studied countries (*65*).

Fewer data are available on the corresponding GA adduct levels. The first studies showed GA adduct levels in the same range as AA adducts, with a ratio of the adduct levels of ~1, both at high occupational exposures and background exposures (*30, 46, 66*). Recent studies present GA adduct levels in the ranges of 10−50 pmol/g globin for nonsmokers and 20−130

Table 3. Estimations of Internal Dose of Glycidamide (GA) from Exposure to Acrylamide (AA): Estimated for AA Exposure Corresponding to Exposure in the Cancer Tests with Rats and for AA Exposure in Humans (M, Males; F, Females)

	internal dose of GA (μMh per mg AA/kg bw)	
	rat	human
from 1998(*19*)	16 (M)[a]	28[b]
later data	34 (F);[c] 18 (M)[c]	(64) (M)[d]

[a] From Bergmark et al. (*44*); Bergmark (*43*); ip injections in Sprague−Dawley rats. [b] Calculated by Calleman (*72*); high occupational or accidental exposures. [c] Obtained from the present study; exposure in drinking water of Fisher 344 rats as in cancer tests. [d] From Fennell et al. (*39*), high experimental exposures 0.5−3 mg AA/kg bw.

pmol/g globin for smokers (*53, 54, 56, 57*). Larger ranges of GA adduct levels have been reported by Vesper et al. (*67, 68*), who also observed large variations in the GA-to-AA adduct ratios, from 0.03 (*67*) up to 3.12 (*68*). The measured ratio indicates that the metabolism of AA to GA is more effective at low levels of background exposure to AA (*56, 68*).

In summary, the information gained from these studies is more relative than absolute. The study by Kütting et al. (*60*) indicates a large interindividual difference in AA adduct levels with a factor of 20, which is considerably higher than the factor of 5 observed in other studies (*57, 63, 69*). There does not seem to be any large difference in the ratio between in vivo doses of GA and AA from high occupational exposures down to moderate low exposures. However, surprisingly, a relatively higher GA dose is indicated at low background doses of AA. Despite this, we judge that the large interindividual difference of a factor of about 40 observed in the ratio between the adduct levels from GA to AA (*67*) probably is an overestimation.

Other information, from a study for the validation of questionnaires, indicated that the estimated AA intake showed a considerably higher interindividual variation (a factor of 50) than the measured AA adduct levels (a factor of 5) (*63, 69*).

In conclusion, the published data on background levels are not yet sufficient for exact calculation of in vivo doses of GA at low exposure doses and its relation to AA exposure levels. The most exact data available are from a human experiment with oral exposure of males to AA at very high doses (single dose in drinking water of 0.5, 1, or 3 mg [$^{13}C_3$]AA/kg bw), performed by Fennell et al. (*39*). This study, at about 1000 times higher doses than from the average daily AA exposure via food, showed GA-to-AA adduct ratios of about 0.4 and related to AA intake, for AA about 25 and for GA about 60 μMh per mg AA/kg bw (*39*). This is 2−3 times higher GA dose per exposure dose of AA than observed in female and male rats, respectively, in the present study (cf. **Table 1**).

Additional information is obtained from a human study (n = 6) with intake of a single dose of ca. 1 mg AA in the diet (ca. 30 times the estimated average daily dose), followed by measurement of AA in urine (*70*). This study points toward a shorter half-life of AA ($t_{1/2}$ ca. 2.4 h) than in Fennell et al.'s human exposure study (*71*) ($t_{1/2}$ = 3.1−3.5) and the half-life ($t_{1/2}$ ca. 4.6) previously estimated by Calleman (*72*). The shorter half-life of AA at the lower exposure dose obtained by Fuhr et al. (*70*) would correspond to a lower in vivo dose of AA, due to faster detoxification through conjugation with glutathione and/or oxygenation to GA. Fuhr et al. (*70*) also suggest that GA doses are relatively higher in humans than in rodents.

Table 3 summarizes the current status of the estimates of the in vivo dose of GA compared to the early estimations in 1998 (*19*).

Dose−Response Relationships. The studies of biological effects of AA like all toxicological studies have the general difficulty of high doses in the tests, compared to human exposures at low doses. One of the studies at lowest exposure doses of AA concerns induction of micronuclei in mice at doses down to 2.5 mg/kg bw (*73*). Furthermore, both AA and its metabolite GA are electrophilically reactive and have toxic potency. Therefore, it is of great importance to be able to discriminate between effects of AA and GA in studies with AA exposure. For instance, this has been obtained in studies with CYP2E1 null mice (*74*) or by in vivo dosimetry of AA and GA (*75*).

One way to aid extrapolations from high to low exposure doses is to measure in vivo doses by biomarkers, such as Hb adducts. Nonlinearity at high exposure doses has been observed for Hb adduct level increments in AA-exposed rats, as expected from saturation kinetics in the metabolism. Already from about 5 mg AA/kg bw there was a nonlinearity in incremental Hb adduct levels from GA (*44*). In recent animal studies at daily exposure doses close to the human AA exposure levels from food, linear relationships have been observed. In a subchronic feeding experiment in mice at 3−50 μg AA/kg bw per day, linearity was observed for the increment of adduct levels from both AA and GA (*76*). In a study in swine measured AA adduct levels showed a linear increment at AA doses of 0.8 or 8 μg/kg bw per day (*77*).

The indicated nonlinearity of in vivo doses of GA at low AA exposures in humans discussed above is unexpected from the animal experiments. This could be a consequence of the human situation being much more complex. For example, food substances, etc., with an influence on the metabolism might have an impact on the internal dose of AA and GA. This concerns possible influence on enzymes involved in the detoxification or activation of AA, such as CYP2E1, which is involved in the oxygenation of AA to GA (*10*). CYP2E1 is known to be induced by ethanol. For instance, a study on HepG2 cells with the Comet assay showed that treatment with ethanol and AA in combination gave a higher genotoxic potential compared to AA treatment alone (*78*). CYP2E1 induction and glutathione depletion were observed (*78*). Diallyl sulfide, an ingredient in garlic, was shown to inhibit CYP2E1 (rat liver, in vitro) and the following bioactivation of AA to GA (*79*).

Possible influence on the metabolism due to polymorphisms would be a plausible explanation for the differences in GA-to-AA in vivo dose ratios. However, a study on the detoxifying enzymes glutathione transferases GSTT1 and GSTM1 and epoxide hydrolase showed no significant influence in vitro on the blood dose (*32*). During the conditions of the study, detoxification by noncatalyzed chemical reactions seemed to dominate (*32*). Still there is no information whether polymorphisms in the genes coding for CYP2E1 have an influence on the in vivo doses of AA or GA.

For other compounds, such as ethylene oxide, background adduct levels due to endogenous formation of the precursor electrophile have been demonstrated (*80*). So far, it has not been demonstrated that AA is formed endogenously even though it cannot be excluded.

As discussed above the dose−response as a function of exposure dose of AA may be influenced by factors that modify the ultimate target dose. Measurement of in vivo doses can bypass problems with factors that could modify the metabolism and hence the in vivo dose. A more difficult aspect of the dose−response is related to mechanisms that may modify the biological effects at low target doses. The projections of risks to low doses based on observed

linearity at higher doses are inherently uncertain. The sensitivities of biological test systems here constitute a limitation. A linear dose−response relationship for genotoxic carcinogens is at the present state of knowledge the best assumption, even though it is a subject for discussion.

Cancer Risk from Acrylamide: Status of the Evaluation Using Dosimetry and a Relative Risk Model. The aim of this paper has been to evaluate the published cancer tests on rats with AA on the basis of in vivo doses and the relative cancer risk model. A final goal is to improve the cancer risk estimation of AA in food. In vivo doses from GA in F344 rats, exposed at conditions corresponding to the published cancer tests, have been determined and used in the evaluation according to the relative cancer risk model (eq 3). The evaluation of the published cancer test data shows that the relative risk model is applicable to the data and gives a result of a doubling dose of ca. 13 mMh for GA. Comparison with the well-studied model compound ethylene oxide indicates that the tumor incidence increments in the cancer tests with AA are not higher than expected from the in vivo doses of GA and the genotoxic potency of GA. According to the relative risk model ionizing radiation has approximately the same risk coefficient per dose unit in humans and a few tested animal species (*8*), which indicates that the doubling dose obtained from a genotoxic agent in animal cancer tests should be approximately the same in humans.

The preliminary risk estimation of AA according to this model (*19*), without the accurate in vivo dose measurements, arrived at a lifetime risk of 16×10^{-3} per μg AA/kg bw, which is 3.4 times higher than the published U.S. EPA figure (*81*). Our present accurate determination of GA doses in the rat, exposed as in the published cancer tests, does not deviate much from the earlier data (**Table 3**). Recent data on GA doses in humans from experimental studies (**Table 3**) and measured Hb adducts at very low AA exposures in the general population indicate relatively higher in vivo GA dose per exposure dose of AA in humans than earlier estimated. Taking the data together we could not show that the early cancer risk figure for AA is an overestimation. Another re-evaluation of the cancer tests has arrived at a lower estimate of $1.4-1.6 \times 10^{-3}$ per μg AA/kg bw, which is 10 times lower than our estimate (*82*).

The applicability of the relative risk model to the cancer test data for AA has not yet been validated by independent data. In the ongoing work the involved parameters, such as the relative genotoxic potency of GA, will be further scrutinized. A comparison with the outcomes of the ongoing National Toxicology Program (NTP) (*35*) cancer tests with AA and GA will be of great interest.

Validation in humans of risk estimates derived from animal experiments is only exceptionally possible, as in the case of ionizing radiation (cf. ref *8*). For a substance like AA in food, the possibility to achieve a validation is extremely small, above all as the compound occurs naturally in a range of foods and constitutes a general exposure with a relatively limited range of exposure levels. Recently, two nutritional epidemiological studies of AA (*57, 83*) have indicated a positive association with cancer risk. Even given our relatively high risk estimates, the magnitude of the risks found in these studies seems to be too high. Considering associations between different foodstuffs and cancer found in many epidemiological studies, it seems difficult to identify the effect of a single substance such as AA, where the groups with high and low intake are very likely to differ in other ways with respect to eating habits. Quantitative toxicological data should be used to calculate the expected magnitudes of cancer risks from epidemiological studies, when possible. It is important that agreement between approaches for risk estimation is obtained to reach as close as possible to the true figures.

It is indicated that AA could not be ruled out as a risk factor of a nonacceptable magnitude. However, it should be realized that AA occurs as a background exposure simultaneously as many other known and unknown natural risk factors, of which some compounds are probably posing risks of the same magnitude as AA.

ACKNOWLEDGMENT

The late Prof. Lars Ehrenberg was the initiator of the development of the cancer risk model and a great source of inspiration. We are grateful to Dr. Emma Bergmark for contributions to the initial work, Dr. Sune Eriksson for support with acrylamide analysis, and Ioannis Athanassiadis for assistance with GC-MS/MS analysis.

LITERATURE CITED

(1) Granath, F.; Törnqvist, M. Who knows whether acrylamide in food is hazardous to humans? *J. Natl. Cancer Inst.* **2003**, *95*, 842–843.

(2) Hagmar, L.; Törnqvist, M. Inconclusive results from an epidemiological study on dietary acrylamide and cancer. *Br. J. Cancer* **2003**, *89*, 774–776.

(3) Törnqvist, M.; Osterman-Golkar, S.; Paulsson, B. Preface in Special Issue: Acrylamide: Genetic Toxicity and Exposure Assessment. *Mutat. Res.* **2005**, *580*, 1–2.

(4) Ehrenberg, L. Genetic toxicology of environmental chemicals. *Acta Biol. Iugosl. Ser. F. Genet.* **1974**, *6*, 367.

(5) Ehrenberg, L.; Granath, F.; Törnqvist, M. Macromolecule adducts as biomarkers of exposure to environmental mutagens in human population. *Environ. Health Perspect.* **1996**, *104* (Suppl. 3), 423–428.

(6) Ehrenberg, L.; Moustacchi, E.; Osterman-Golkar, S.; Ekman, G. Dosimetry of genotoxic agents and dose-response relationships of their effects. *Mutat. Res.* **1983**, *123*, 121–182.

(7) Törnqvist, M.; Fred, C.; Haglund, J.; Helleberg, H.; Paulsson, B.; Rydberg, P. Protein adducts: quantitative and qualitative aspects of their formation, analysis and applications. *J. Chromatogr., B* **2002**, *778*, 279–308.

(8) Granath, F.; Vaca, C.; Ehrenberg, L.; Törnqvist, M. Cancer risk estimation of genotoxic chemicals based on target dose and a multiplicative model. *Risk Anal.* **1999**, *19*, 309–320.

(9) Fred, C.; Törnqvist, M.; Granath, F. Evaluation of cancer tests of 1,3-butadiene using internal dose, genotoxic potency and a multiplicative risk model *Cancer Res.* **2008**, in press.

(10) Sumner, S. C. J.; Fennell, T. R.; Moore, T. A.; Chanas, B.; Gonzalez, F.; Ghanayem, B. I. Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. *Chem. Res. Toxicol.* **1999**, *12*, 1110–1116.

(11) Calleman, C.-J.; Bergmark, E.; Costa, L. G. Acrylamide is metabolized to glycidamide in the rat: evidence from hemoglobin adduct formation. *Chem. Res. Toxicol.* **1990**, *3*, 406–412.

(12) 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*, 131–141.

(13) 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.

(14) Rice, J. M. The carcinogenicity of acrylamide. *Mutat. Res.* **2005**, *580*, 3–20.

(15) Allen, B.; Zeiger, E.; Lawrence, G.; Friedman, M.; Shipp, A. Dose-response modeling of in vivo genotoxicity data for use in risk assessment: some approaches illustrated by an analysis of acrylamide. *Regul. Toxicol. Pharmacol.* **2005**, *41*, 6–27.

(16) Besaratinia, A.; Pfeifer, G. P. A review of mechanisms of acrylamide carcinogenicity. *Carcinogenesis* **2007**, *28*, 519–528.
(17) Hagmar, L.; Törnqvist, M.; Nordander, C.; Rosén, 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.
(18) Törnqvist, M. Acrylamide in food: the discovery and its implications—a historical perspective. In *Chemistry and Safety of Acrylamide in Food*; Friedman, M., Mottram, D., Eds.; Springer: New York, 2005; Vol. 561, pp 1−19.
(19) Törnqvist, M.; Bergmark, E.; Ehrenberg, L; Granath, F. Risk assessment of acrylamide (in Swedish). *Natl. Chem. Inspectorate, Sweden* **1998**, 7–98.
(20) Tareke, E.; Rydberg, P.; Karlsson, P.; Eriksson, S.; Törnqvist, M. Acrylamide: a cooking carcinogen. *Chem. Res. Toxicol.* **2000**, *13*, 517–522.
(21) 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.
(22) Johnson, K. A.; Gorzinski, S. J.; Bodner, K. M.; Campbell, R. A.; Wolf, C. H.; Friedman, M. A.; Mast, R. W. Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fisher 344 rats. *Toxicol. Appl. Pharmacol.* **1986**, *85*, 154–168.
(23) Friedman, M. A.; Dulak, L. H.; Stedham, M. A. A lifetime oncogenicity study in rats with acrylamide. *Fundam. Appl. Toxicol.* **1995**, *27*, 95–105.
(24) Havenaar, R.; Meijer, J. C.; Morton, D. B.; Ritskes-Hoitinga, J.; Zwart, P. Biology and husbandry of laboratory animals. In *Principles of Laboratory Animal Science*; van Zutphen, L. F. M., Baumans, V., Beynen, A. C., Eds.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1993; pp 17−74.
(25) Törnqvist, M.; Mowrer, J.; Jensen, S.; Ehrenberg, L. Monitoring of environmental cancer initiators through hemoglobin adducts by a modified Edman degradation method. *Anal. Biochem.* **1986**, *154*, 255–266.
(26) Törnqvist, M. Epoxide adducts to N-terminal valine of hemoglobin. In *Methods in Enzymology*; Everse, J., Vandegriff, K. D., Winslow, R. W., Eds.; Academic Press: New York, 1994; Vol. 231, pp 650−657.
(27) Mowrer, J.; Törnqvist, M.; Jensen, S.; Ehrenberg, L. Modified Edman degradation applied to hemoglobin for monitoring occupational exposure to alkylating agents. *Toxicol. Environ. Chem.* **1986**, *11*, 215–231.
(28) Rydberg, P.; Lüning, B.; Wachtmeister, C. A.; Eriksson, L.; Törnqvist, M. Applicability of a modified Edman procedure for measurement of protein adducts: mechanisms of formation and degradation of phenylthiohydantoins. *Chem. Res. Toxicol.* **2002**, *15*, 570–581.
(29) Bergmark, E. Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers, and nonsmokers. *Chem. Res. Toxicol.* **1997**, *10*, 78–84.
(30) Paulsson, B.; Athanassiadis, I.; Rydberg, P.; Törnqvist, M. Hemoglobin adducts from glycidamide: acetonization of hydrophilic groups for reproducible gas chromatography/tandem mass spectrometric analysis. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 1859–1865.
(31) Granath, F.; Ehrenberg, L.; Törnqvist, M. Degree of alkylation of macromolecules in vivo from variable exposure. *Mutat. Res.* **1992**, *284*, 297–306.
(32) Paulsson, B.; Rannug, A.; Henderson, A. P.; Golding, B. T.; Törnqvist, M.; Warholm, M. In vitro studies of the influence of glutathione transferases and epoxide hydrolase on the detoxification of acrylamide and glycidamide in blood. *Mutat. Res.* **2005**, *580*, 53–59.
(33) Silvari, V.; Haglund, J.; Jenssen, D.; Golding, B. T.; Ehrenberg, L.; Törnqvist, M. Reaction-kinetic parameters of glycidamide as determinants of mutagenic potency. *Mutat. Res.* **2005**, *580*, 91–101.
(34) Johansson, F.; Lundell, T.; Rydberg, P.; Erixon, K.; Jenssen, D. Mutagenicity and DNA repair of glycidamide-induced adducts in mammalian cells. *Mutat. Res.* **2005**, *580*, 81–89.
(35) U.S. National Toxicology Program (NTP) (available on NTP homepage http://ntp.niehs.nih.gov/), 2008.
(36) 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.
(37) 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.
(38) Paulsson, B.; Granath, F.; Grawé, J.; Ehrenberg, L.; Törnqvist, M. The multiplicative model for cancer risk assessment: applicability to acrylamide. *Carcinogenesis* **2001**, *22*, 817–819.
(39) 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.
(40) Paulsson, B.; Grawé, J.; Törnqvist, M. Hemoglobin adducts and micronucleus frequencies in mouse and rat after acrylamide or *N*-methylolacrylamide treatment. *Mutat. Res.* **2002**, *516*, 101–111.
(41) Sumner, S. C. J.; Williams, C. C.; Snyder, R. W.; Krol, W. L.; Asgharian, B.; Fennell, T. R. Acrylamide: a comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal, oral, or inhalation exposure. *Toxicol. Sci.* **2003**, *75*, 260–270.
(42) Tareke, E.; Twaddle, N. C.; McDaniel, L. P.; Churchwell, M. I.; Young, J. F.; Doerge, D. R. Relationships between biomarkers of exposure and toxicokinetics in Fischer 344 rats and B6C3F$_1$ mice administered single doses of acrylamide and glycidamide and multiple doses of acrylamide. *Toxicol. Appl. Pharmacol.* **2006**, 63–75.
(43) Bergmark, E. Hemoglobin Dosimetry and Comparative Toxicity of Acrylamide and its Metabolite Glycidamide. Ph.D. thesis, Department of Radiobiology, Stockholm University, 1992.
(44) Bergmark, E.; Calleman, C. J.; Costa, L. Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. *Toxicol. Appl. Pharmacol.* **1991**, *111*, 352–363.
(45) Dybing, E.; Farmer, P. B.; Andersen, M.; Fennell, T. R.; Lalljie, S. P. D.; Müller, D. J. G.; Olin, S.; Petersen, B. J.; Schlatter, J.; Scholz, G.; Scimeca, J. A.; Slimani, N.; Törnqvist, M.; Tuijtelaars, S.; Verger, P. ILSI Report: human exposure and internal dose assessments of acrylamide in food. *Food Chem. Toxicol.* **2005**, *43*, 365–410.
(46) Bergmark, E.; Calleman, C. J.; He, F.; Costa, L. G. Determination of hemoglobin adducts in humans occupationally exposed to acrylamide. *Toxicol. Appl. Pharmacol.* **1993**, *120*, 45–54.
(47) Jones, K.; Garfitt, S.; Emms, V.; Warren, N.; Cocker, J.; Farmer, P. Correlation of haemoglobin−acrylamide adducts with airborne exposure: an occupational survey. *Toxicol. Lett.* **2006**, *162*, 174–180.
(48) Kjuus, H.; Goffeng, L.-O.; Skard Heier, M.; Hansteen, I.-L.; Øvrebø, S.; Skaug, V.; Ryberg, D.; Sjöholm, H.; Törnqvist, M.; Paulsson, B.; Langeland, B. T.; Brudal, S. *Examination of Nervous System Effects and Other Health Effects in Tunnel Workers Exposed to Acrylamide and N-Methylolacrylamide in Romeriksporten, Norway*; STAMI Rapport 5; National Institute of Occupational Health, **2002**.
(49) Licea Pérez, H.; Cheong, H. K.; Sun Yang, J.; Osterman-Golkar, S. Simultaneous analysis of hemoglobin adducts of acrylamide and glycidamide by gas chromatography−mass spectrometry. *Anal. Biochem.* **1999**, *274* (1), 59–68.
(50) Paulsson, B.; Larsen, K.-O.; Törnqvist, M. Hemoglobin adducts for assessment of potential occupational exposure to acrylamides—three case studies. *Scand. J. Work Environ. Health* **2006**, *32* (2), 154–159.
(51) Törnqvist, M.; Paulsson, B.; Osterman-Golkar, S. Biomonitoring of acrylamide. In *Acrylamide and Other Health Hazardous*

Compounds in Heat-Treated Foods; Skog, K., Alexander, J., Eds.; Woodhead Publishing: Cambridge, U.K., 2006; pp 164−194.
(52) Schumacher, J. N.; Green, C. R.; Best, F. W.; Newell, M. P. Smoke composition. An extensive investigation of the water-soluble portion of cigarette smoke. *J. Agric. Food Chem.* **1977**, *35*, 310–320.
(53) Bjellaas, T.; Olesen, P. T.; Frandsen, H.; Haugen, M.; Stolen, H.; Paulsen, J. E.; Alexander, J.; Lundanes, E.; Becher, G. Comparison of estimated dietary intake of acrylamide with haemoglobin adducts of acrylamide and glycidamide. *Toxicol. Sci.* **2007**, *98*, 110–117.
(54) Chevolleau, S.; Jacques, C.; Canlet, C.; Tulliez, J.; Debrauwer, L. Analysis of hemoglobin adducts of acrylamide and glycidamide by liquid chromatography−electrospray ionization tandem mass spectrometry, as exposure biomarkers in French population. *J. Chromatogr., A* **2007**, *1167*, 125–134.
(55) Kütting, B.; Schettgen, T.; Beckmann, M. W.; Angerer, J.; Drexler, H. Influence of diet on exposure to acrylamide—reflections on the validity of a questionnaire. *Ann. Nutr. Metab.* **2005**, *49*, 173–177.
(56) Schettgen, T.; Rossbach, B.; Kütting, B.; Letzel, S.; Drexler, H.; Angerer, J. Determination of haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the general population. *Int. J. Hyg. Environ. Health* **2004**, *207*, 531–539.
(57) Thonning Olesen, P.; Olsen, A.; Frandsen, H.; Frederiksen, K.; Overvad, K.; Tjonneland, A. Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. *Int. J. Cancer* **2008**, *122*, 2094–2100.
(58) Urban, M.; Kavvadias, D.; Riedel, K.; Scherer, G.; Tricker, A. R. Urinary mercapturic acids and a hemoglobin adduct for the dosimetry of acrylamide exposure in smokers and nonsmokers. *Inhalation Toxicol.* **2006**, *18*, 831–839.
(59) Scherer, G.; Engl, J.; Urban, M.; Gilch, G.; Janket, D.; Riedel, K. Relationship between machine-derived smoke yields and biomarkers in cigarette smokers in Germany. *Regul. Toxicol. Pharmacol.* **2007**, *47*, 171–183.
(60) Kütting, B.; Uter, W.; Drexler, H. The association between self-reported acrylamide intake and hemoglobin adducts as biomarkers of exposure. *Cancer Causes Control* **2007**, *19*, 273–281.
(61) Scherer, G.; Engl, J.; Urban, M.; Gilch, G.; Janket, D.; Riedel, K. Relationship between machine-derived smoke yields and biomarkers in cigarette smokers in Germany. *Regul. Toxicol. Pharmacol.* **2007**, *47*, 171–183.
(62) Godin, A. C.; Bengtsson, B.; Niskanen, R.; Tareke, E.; Törnqvist, M.; Forslund, K. Acrylamide and *N*-methylolacrylamide poisoning in a herd of Charolais crossbreed cattle. *Vet. Rec.* **2002**, *151*, 724–728.
(63) Hagmar, L.; Wirfält, E.; Paulsson, B.; Törnqvist, M. Differences in hemoglobin adduct levels of acrylamide in the general population with respect to dietary intake, smoking habits and gender. *Mutat. Res.* **2005**, *580*, 157–165.
(64) Abramsson-Zetterberg, L.; Vikström, A. C.; Törnqvist, M.; Hellenäs, K.-E. Differences in the frequency of micronucleated erythrocytes in humans with respect to consumption of fried carbohydrate-rich food. *Mutat. Res.* **2008**, *653*, 50–56.
(65) Vikström, A.; Paulsson, B.; Davies, R.; Rydberg, P.; Aston, P.; Scheepers, P.; Törnqvist, M. Hemoglobin adducts as biomarker of background exposure to acrylamide in five European countries—a pilot study. Poster presentation, Annual Meeting of the European Environmental Mutagen Society (EEMS). Programme and Abstracts, July 2−6, Prague, Czech Republic, 2006.
(66) Paulsson, B. Dose monitoring for health risk assessment of exposure to acrylamides. Ph.D. thesis, Department of Environmental Chemistry, Stockholm University, 2003.
(67) Vesper, H. W.; Bernert, J. T.; Ospina, M.; Meyers, T.; Ingham, L.; Smith, A.; Myers, G. L. Assessment of the relation between biomarkers for smoking and biomarkers for arylamide exposure in humans. *Cancer Epidemiol. Biomarkers Prev.* **2007**, *16*, 2471–2478.
(68) Vesper, H. W.; Ospina, M.; Meyers, T.; Ingham, L.; Smith, A.; Gray, J. G.; Myers, G. L. Automated method for measuring globin adducts of acrylamide and glycidamide at optimized Edman reaction conditions. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 959–964.
(69) Wirfält, E.; Paulsson, B.; Törnqvist, M.; Axmon, A.; Hagmar, L. Associations between estimated acrylamide (AA) intakes, and Hb AA-adducts in a sample from the Malmö Diet and Cancer cohort. *Eur. J. Clin. Nutr.* **2008**, *62*, 314–323.
(70) Fuhr, U.; Boettcher, M. I.; Kinzig-Schippers, M.; Weyer, A.; Jetter, A.; Lazar, A.; Taubert, D.; Tomalik-Scharte, D.; Pournara, P.; Jakob, V.; Harlfinger, S.; Klaassen, T.; Berkessel, A.; Angerer, J.; Sörgel, F.; Schömig, E. Toxicokinetics of acrylamide in humans after ingestion of a defined dose in a test meal to improve risk assessment for acrylamide carcinogenicity. *Cancer Epidemiol. Biomarkers Prev.* **2006**, *15*, 266–271.
(71) Fennell, T. R.; Sumner, S. C. J.; Snyder, R. W.; Burgess, J.; Friedman, M. A. Kinetics of elimination of urinary metabolites of acrylamide in humans. *Toxicol. Sci.* **2006**, *93*, 256–267.
(72) Calleman, C.-J. The metabolism and pharmacokinetics of acrylamide: implications for mechanisms of toxicity and human risk. *Drug Metab. Rev.* **1996**, *28*, 527–590.
(73) Abramsson-Zetterberg, L.; Grawé, J.; Zetterberg, G. Flow cytometric analysis of micronucleus induction in mice by internal exposure to ^{137}Cs at very low dose rates. *Int. J. Radiat. Biol.* **1995**, *67*, 29–36.
(74) 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.
(75) Paulsson, B.; Kotova, N.; Grawé, 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.
(76) Vikström, A. C.; Eriksson, S.; Paulsson, B.; Karlsson, P.; Athanassiadis, I.; Törnqvist, M. Internal doses of acrylamide and glycidamide in mice fed diets with low acrylamide contents. *Mol. Nutr. Food Res.* **2008**, doi: 10.1002/mnfr.200700341.
(77) Aureli, F.; Di Pasquale, M.; Lucchetti, D.; Aureli, P.; Coni, E. An absorption study of dietary administered acrylamide in swine. *Food Chem. Toxicol.* **2007**, *45*, 1202–1209.
(78) Lamy, E.; Völkel, Y.; Roos, P. H.; Kassie, F.; Mersch-Sundermann, V. Ethanol enhanced the genotoxicity of acrylamide in human, metabolically competent HepG2 cells by CYP2E1 induction and glutathione depletion. *Int. J. Hyg. Environ. Health* **2007**, *211*, 74–81.
(79) Taubert, D.; Glöckner, R.; Müller, D.; Schömig, E. The garlic ingredient diallyl sulfide inhibits cytochrome P450 2E1 dependent bioactivation of acrylamide to glycidamide. *Toxicol. Lett.* **2006**, *164*, 1–5.
(80) Törnqvist, M.; Kautiainen, A. Adducted proteins for identification of endogenous electrophiles. *Environ. Health Perspect.* **1993**, *99*, 39–44.
(81) U.S. EPA. Acrylamide. 7-1-1993, http://www.epa.gov./iris/subst/0286.htm, 1993.
(82) Alexander, J. Risk assessment techniques for acrylamide. In *Acrylamide and Other Health Hazardous Compounds in Heat-Treated Foods*; Skog, K., Alexander, J., Eds.; Woodhead Publishing: Cambridge, U.K., 2006; pp 275−295.
(83) Hogervorst, J. C.; Schouten, L. J.; Konings, E. J.; Goldbohm, R. A.; van den Brandt, P. A. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. *Cancer Epidemiol. Biomarkers Prev.* **2007**, *16*, 2304–2313.

Received for review February 15, 2008. Revised manuscript received April 22, 2008. Accepted May 20, 2008. This work was supported financially by the Swedish Cancer Society and the European Commission (Contract FOOD-CT-2003-506820 "Heat-generated food toxicants—identification, characterisation and risk minimisation"), the Swedish Agency for Animal Welfare, and the Swedish Cancer and Allergy Fund.

JF800490S