# Exposure and Biomarkers Working Group
# White Paper
# April 2004

## I. Background

Since Swedish scientists discovered the presence of acrylamide in foods produced for human consumption in April 2002, considerable progress has been made toward determining acrylamide levels in various foods and food groups. Progress has also been made in establishing biomarkers of acrylamide exposure, although no biomarker is currently able to determine the source of the exposure (e.g. food versus smoking versus workplace). Acrylamide has been linked to increased risk of cancer and reproductive problems in some laboratory animals at high doses. Although acrylamide is acutely neurotoxic to humans at high concentrations, the health effects of chronic, low-dose acrylamide exposure is still being debated.

In October, 2002, the Exposure and Biomarkers Working Group concluded that gaps in the scientific knowledge surrounding acrylamide were substantial and proposed the following:

1. To expand the database of acrylamide levels in U.S. foods through a clearinghouse for data and through collection of additional data to fill gaps.
2. To establish the relationship between biomarkers, such as hemoglobin (Hb) adducts, and acrylamide in foodstuffs. This research should include completion of the proposed Centers for Disease Control and Prevention (CDC) study of both acrylamide and glycidamide and of an on-going human study assessing the relationship between oral administration of acrylamide and acrylamide and glycidamide Hb adduct levels.
3. To determine the bioavailability of acrylamide found in selected foods via appropriate animal or human studies.
4. To determine glycidamide levels in foods that also contain high concentrations of acrylamide.

## II. Areas of Progress

Acrylamide levels in many additional foods have been determined since the first JIFSAN/NCFST Workshop on Acrylamide in Food in October, 2002, and previously unknown food sources of acrylamide have been discovered (e.g. black olives, prune juice, certain coffee alternatives).

Ongoing research has also improved our understanding of how food storage, preparation, and cooking processes affect acrylamide concentrations in

foods, including foods prepared at home. Acrylamide concentration data and food consumption data have been used to generate reasonably reliable estimates of acrylamide intake from foods and food groups.

Various adducts (e.g. Hb, DNA) have been identified as useful biomarkers of overall acrylamide exposure, and ongoing research has improved our understanding of the bioavailability of acrylamide in foods, although many questions still remain.

## III. Current Research

Home Food Preparation

In collaboration with food industry representatives, the National Center for Food Safety and Technology (NCFST) and the U.S. Food and Drug Administration (FDA) examined the acrylamide concentration variability within one brand and type of French fry designed by the manufacturer for home preparation. Jackson et al. chose to prepare the French fries using the deep fat frying option provided on the product label and varied either the cooking time or the cooking temperature. The researchers discovered that the degree of browning was a good indicator of acrylamide levels in French fries prepared in deep fat fryers. The higher the degree of browning attained in the French fries, the higher their acrylamide concentration. In addition, the same degree of browning yielded the same acrylamide levels, regardless of which cooking temperature or cooking time used.

Jackson et al. also analyzed how various preparation and storage methods of raw potatoes influence acrylamide levels in the cooked product. Two varieties of raw potatoes (Russett and Klondike Rose) were cut into French fries and cooked in deep fat fryers. Acrylamide levels were most effectively reduced by presoaking the raw cut fries in water for at least 15 minutes or by pretreating them with an acidic “wash,” either ascorbic or acetic acid. Storing the raw potatoes at room temperature versus refrigeration prior to frying was also very effective at reducing acrylamide levels in the cooked product. No taste tests were simultaneously performed to demonstrate the acceptability to consumers of the cooked potato product after being treated with presoaking in water or with an acidic “wash.” However, other research has suggested that consumers find the flavor of potato products subjected to acidic washes to be unacceptable.

Jackson et al. acknowledged that their research did not address other factors either known or suspected to affect acrylamide levels in French fries prepared in deep fat fryers, such as the cut/size of the French fries, any precooking done by the manufacturer, or the type of oil used for frying. Some of these variables are being considered for future analyses.

The researchers also analyzed toasted bread for acrylamide levels and discovered, not surprisingly, that toasted potato breads contain higher acrylamide levels than other toasted bread varieties. As with French fries prepared in deep fat fryers, the degree of browning in toasted bread is a good indicator of acrylamide levels. Acrylamide levels in toasted bread can be reduced by up to 2/3 by scraping away some of the darkest portions from the surface.

Epidemiology

With a few exceptions, epidemiology studies have not found a statistically significant association between acrylamide exposure and risk of cancer. Three separate prospective cohort studies examining two different cohorts have attempted to address the relationship between acrylamide exposure and various types of cancer. In 1986, Sobel et al. published a study that examined the relationship between acrylamide exposure and cancers of the central nervous system, thyroid gland, other endocrine glands, and mesothelium in a cohort of 371 employees assigned to acrylamide monomer and polymerization operations. Out of an expected 38 deaths, only 29 deaths were observed. The authors concluded that acrylamide exposure is not related to overall mortality, total malignant neoplasms, or any specific cancers.

In 1988, Collins et al. published their observations of the relationship between all-cause and cause-specific mortality rates and acrylamide exposure in a cohort of 8854 men, 2293 of which were exposed to acrylamide. The cohort consisted of four factory populations in the United States and the Netherlands. No relationship between acrylamide exposure and risk of mortality from several cancer sites was observed. Marsh et al. reexamined the findings from the Collins et al. study by focusing on specific types of cancer (central nervous system, thyroid gland, testis and other male genital organs, respiratory system, and pancreas). A significant 2.26-fold risk was found for pancreatic cancer among workers with cumulative exposure to acrylamide. However, no consistent exposure-response relationship was observed after adjusting for time since first exposure to acrylamide. Based on the inconsistent exposure-response relationship, the researchers concluded that there was no relationship between acrylamide exposure and pancreatic cancer risk. When Schulz et al. reanalyzed the data by regrouping the acrylamide dose levels and calculating standardized mortality ratios (SMR) for pancreatic cancer, a monotonic, positive dose-response relationship was found.

An unpublished study by Mucci et al. examined the relationship between colon/rectal cancer and acrylamide in food in a cohort of 60,000 Swedish women for a period of 12 years. Another unpublished study by Mucci et al. examined the relationship between breast cancer and acrylamide in food in a cohort of 49,000 Swedish women for a period of 11 years. Neither study

found an association between either colon/rectal or breast cancer and acrylamide in food.

Several case-control studies have been performed, but these studies show no conclusive relationship between cancer development and acrylamide exposure. Mucci et al. examined a population-based Swedish case-control study that included cases with either large bowel (n=591), bladder (n=263), or kidney (n=133) cancer matched to 538 healthy controls. They found no association between acrylamide exposure from food and these types of cancers. Acrylamide exposure from food was determined by linking extensive food frequency data with acrylamide levels in certain food items recorded by the Swedish National Food Administration. A follow-up study by Mucci et al. utilized the Swedish case-control study to examine the relationship between acrylamide exposure from food and renal cell cancer. No relationship was found.

Pelucchi et al. found no association between fried/baked potato consumption and cancer risk in a series of hospital-based case-control studies conducted in Italy and Switzerland. The cancer sites examined included oral cavity/pharynx, esophagus, larynx, large bowel, breast, and ovary. However, a study by Bosetti et al. did find a significant and positive association between laryngeal cancer and consumption of several types of fried foods--meat (OR=1.6), fish (OR= 3.1), eggs (OR=1.9), and potatoes (OR=1.9)--in their analysis of the same hospital-based case-control study.

Even though epidemiology studies do not provide strong, conclusive evidence for an association between acrylamide exposure and cancer risk, this does not necessarily indicate that no relationship exists. Existing epidemiological studies do not have the statistical power to detect cancer risk from acrylamide exposure at the levels suggested by toxicology studies. Much larger sample sizes, along with the corresponding increase in financial and organizational resources, would be required to achieve this level of statistical power.

Epidemiology studies are also subject to many confounders. Some of the potential confounders for cohort studies examining the relationship between acrylamide exposure in the workplace and cancer risk include inexact exposure estimates due to changes in the workplace environment over time, failure to control for smoking or other (non-workplace) sources of acrylamide, and reporting errors in cause of death.

Potential confounders also exist for cohort and case-control studies examining the relationship between acrylamide exposure from food and cancer risk. If the range of acrylamide exposure from food in the study population is very narrow, then statistical significance can not be determined. Also, hospital-based studies may be subject to selection bias.

Other confounders include problems with the dietary assessment tools (e.g. food frequency questionnaire, 24-hour dietary recall). Not only are these assessment tools subject to recall bias, but they are also not designed to target the foods or food preparation/storage methods most likely to increase acrylamide exposure.

Future epidemiology studies investigating the relationship between acrylamide exposure and cancer risk should take precautions to minimize selection bias by focusing primarily on cohort, nested case-control, and population-based case-control studies. Controlling for known confounders, such as smoking and other dietary components (e.g. heterocyclic amines, polycyclic aromatic hydrocarbons), is essential. Efforts to understand how biomarker data might be utilized to validate dietary assessment tools or to improve exposure assessment should also be examined. In addition, it may be useful to examine population differences in acrylamide metabolism via enzyme (e.g. CYP2E1) activity.

Exposure Assessments

The U.S. Food and Drug Administration (FDA) continues to refine its estimates of acrylamide exposure from food by applying updated food acrylamide levels to food consumption data.

The FDA's Center for Food Safety and Applied Nutrition (CFSAN) estimated acrylamide exposure from food using a Monte Carlo simulation approach and food consumption data from the Continuing Survey of Food Intakes by Individuals (CSFII) 1989-1992; CSFII 1994-1996, 1998; and the Marketing Research Corporation of America (MRCA) 1982-1987. CSFII 1989-92 included three 24-hour dietary recalls (24HRs); CSFII 1994-1996, 1998 included two 24HRs; and MRCA included a 14-day frequency survey.

Exposure to acrylamide from food was calculated using the Simplified Exposure Equation (SSE) that is widely used by the FDA to estimate the exposure of a wide range of substances in food.

**Simplified Exposure Equation**

$$EDI_x = \sum_{f=1}^{F} \frac{Freq_f \bullet Port_f \bullet Conc_{xf}}{N}$$

where,

$EDI_x$ = The Estimated Daily Intake of Substance x

F = Total number of foods in which x can be found
$Freq_f$ = Number of eating occasions for food f over N survey days
$Port_f$ = Average portion size for food f
$Conc_{xf}$ = Concentration of the substance x in the food f
N = Number of survey days

$Freq_f$ is estimated from the relevant survey (CSFII or MRCA). $Port_f$ is estimated from the relevant survey for the two CSFII surveys. MRCA did not include data on portion sizes, so data from the USDA/Nationwide Food Consumption Survey for portion size was used with the MRCA survey. $Conc_{xf}$ is determined experimentally based on the FDA exploratory data on acrylamide in foods. These concentration values can be updated as more data become available.

Acrylamide intake was also modeled using a probabilistic process to allow for the examination of the entire range of distributions of the parameters and to facilitate sensitivity testing of various "what if" scenarios. The probabilistic model was based on the following equation:

**AA Intake = [Eaters(yes or no)] x (Food Amount) x (AA Level)**

where,

Eaters(yes or no) = Either 0 or 1 in Proportion to Percent Eaters

Food Amount = Food Consumption Value from Survey Data

AA Level = Acrylamide Value from Laboratory Data (Each Value Equally Likely on Each Iteration)

In this model, the probability of consuming a food that may contain acrylamide is based on the frequency of consumption in the dietary survey. For each

iteration of the simulation, the outcome of the Eaters variable is binary—the food is consumed or it is not consumed—based on the underlying frequency of consumption. The food amount is randomly determined based on the underlying distribution of food consumption, usually smoothed to a lognormal distribution. The acrylamide level is based on the laboratory data using a uniform distribution, making each value (lowest to highest) equally likely.

Each iteration of the model represents a "virtual consumer," and 25,000 iterations were generated in the computer simulation. The model assumed that there was no correlation between the foods (consuming any one food did not make a virtual consumer any more or less likely to consume any of the other foods). The distributions of food amounts were truncated at the 99$^{th}$ percentile to eliminate irrationally high consumption values, such as 13 liters of coffee consumed by a single individual.

Using the CSFII 1994-1996, 1998, the simulation estimated that mean acrylamide intake for the entire population (2+ years) was 0.43 μg/kg body weight/day, and the 90$^{th}$ percentile of acrylamide intake was 0.92 μg/kg body weight/day. Acrylamide intake among children was higher. Again using the CSFII 1994-96, 1998, the mean acrylamide intake for children (2-5 years) was 1.06 μg/kg body weight/day, and the 90$^{th}$ percentile of intake was 2.31 μg/kg body weight/day.

Sensitivity tests provided "what if" scenarios that simulated reducing acrylamide concentration levels to 0 μg/g for selected food items. These scenarios showed that no one food accounts for the majority of the mean population acrylamide intake.

## What-If Scenarios

### CSFII, 1994-96, 98, 2+ Years Population

- Mean = 0.43 μg/kg body weight/day, 90th=0.92 μg/kg body weight/day
- Remove AA from French Fries
  - Mean = 0.37 μg/kg body weight/day; 90$^{th}$=0.78 μg/kg body weight/day
- Remove AA from Snack Foods
  - Mean = 0.38 μg/kg body weight/day; 90$^{th}$=0.85 μg/kg body weight/day
- Remove AA from Breakfast Cereal
  - Mean = 0.38 μg/kg body weight/day; 90$^{th}$=0.84 μg/kg body weight/day
- Remove AA from Coffee
  - Mean = 0.40 μg/kg body weight/day; 90$^{th}$=0.88 μg/kg body weight/day

The mean population acrylamide intakes from the "what if" scenarios are consistent with previous exposure estimates that estimated acrylamide exposure for adults in the range of 0.3 to 0.8 µg/kg body weight/day. The greatest contributors to mean population acrylamide intakes are the same for all surveys. Some foods with lower acrylamide levels contribute appreciably to the overall mean population acrylamide intake because these foods are commonly consumed. No one food accounts for the majority of the mean population acrylamide intake.

An earlier version of this research was presented to the FDA Food Advisory Committee Meeting on February 24-25, 2003 and is available online at http://vm.cfsan.fda.gov/~dms/acryrob2.html.

Adduct Studies

Adduct studies tend to focus mainly on hemoglobin (Hb) and DNA adducts. Due to the relatively short-term and consistent life span of red blood cells (120 days), Hb adducts are primarily used to determine day-to-day differences in acrylamide exposure. Hemoglobin adducts are also easier to access than DNA adducts. DNA adducts are better indicators of long-term acrylamide exposure. DNA adducts illustrate how much glycidamide, a metabolite of acrylamide, eventually progresses to DNA in tissues after acrylamide exposure. Due to the long-term stability of DNA adducts, they are used to evaluate relationships between acrylamide exposure and disease. No adduct can currently determine the source of acrylamide exposure.

Acrylamide and glycidamide hemoglobin adducts are currently being monitored in the National Health and Nutrition Examination Survey (NHANES). This monitoring is expected to be completed by the end of 2004. The results should provide data on acrylamide exposure from all sources for a nationally representative sample.

A preliminary exposure study conducted by Vesper et al. at the Center for Disease Control and Prevention's National Center for Environmental Health examined changes in acrylamide and glycidamide hemoglobin adduct concentrations in 6 individuals (3 males, 3 females). The subjects consumed 3 ounces of potato chips per day (approximately 1.6 µg/kg body weight/day) for one week in addition to their normal diets. Only one of the six subjects showed an increase in acrylamide hemoglobin adduct levels, while four subjects showed an increase in glycidamide hemoglobin adduct levels. Interestingly, the individual who showed an increase in acrylamide hemoglobin adduct levels did not also show an increase in glycidamide hemoglobin adduct levels.

Currently, plans are underway with the U.S. Food and Drug Administration (FDA) and other potential collaborators to conduct a more in-depth study examining the relationship between acrylamide and glycidamide hemoglobin

adduct levels and defined changes in specific food sources of acrylamide in a larger study population. The relationship between acrylamide and glycidamide hemoglobin adduct levels and glycidamide DNA adduct levels in leukocytes will also be examined.

Acrylamide exposure is often measured by mass spectrometric detection of the specific adduct, N-(2-carbamoylethyl)valine (CEV), to the N-termini of hemoglobin. Several studies have noted a CEV background level of about 40 pmol/g of globin in humans without known exposure to acrylamide. According to Tareke et al., the average acrylamide hemoglobin adduct level measured in Swedish adults corresponds to an acrylamide intake of approximately 100 µg/day. However, the typical consumption of foods containing acrylamide only accounts for a daily intake in the tens of micrograms. Svensson et al. also noted that the estimated dietary intake of acrylamide for an individual in the 95$^{th}$ percentile of acrylamide consumption was 62 µg/day. Some debate exists as to whether or not this discrepancy is scientifically relevant, although determining the reason for this discrepancy may lead to an increased understanding of the relationship between acrylamide exposure and its subsequent biomarkers.

## IV. Research Priorities

Although significant progress has been made towards analyzing acrylamide levels in foods; the biomarkers associated with acrylamide exposure; and the bioavailability of acrylamide from foods, more progress needs to be made.

The highest priority needs to be placed on integrating the scientific knowledge concerning food exposure levels, biomarkers, and toxicity of acrylamide. This integration will provide a better understanding of acrylamide risk assessment, management, and communication.

Other priorities should include ongoing data collection of acrylamide levels in food in order to strengthen the existing data, including the assessment of acrylamide levels from various home preparation and storage methods. “Specialty” foods (seasonal, ethnic, diabetic, kosher) should also be examined to determine if there are specific populations that are regularly exposed to high levels of acrylamide in foods.

Increased data sharing is essential to the expansion of scientific knowledge of acrylamide risk, as well as to the prevention of unnecessary duplication of work and waste of valuable resources. In addition to promoting cooperation between industry and government, there should also be a concerted effort to encourage international data sharing. This will require detailed food specifications to eliminate confusion resulting from the various national and regional terminologies. However, increased access to advances in

acrylamide exposure and biomarker assessments will be a significant benefit to an increasingly global scientific community.

The applicability to humans of acrylamide exposure and resulting disease in laboratory animals must be further examined. Animal studies typically utilize some form of pure acrylamide dosing either via diet, drinking water, injection, gavage, or intravenous drip. However, humans are typically exposed to acrylamide via the workplace, smoking, or the consumption of certain foods. Significant physiologic and metabolic differences may also exist between humans and laboratory animals after treatment with acrylamide. No definitive information exists concerning the quantity of acrylamide absorbed from food or where acrylamide metabolism occurs in the body. Even results from different animal studies are difficult to compare due to study design variations in acrylamide administration and dosing.

Most, but not all, acrylamide found in food is the result of the Maillard Reaction, or non-enzymatic browning. The Maillard Reaction is an extremely complex set of chemical reactions that occurs between amines (e.g. proteins, carbonyl compounds) and reducing sugars (e.g. glucose, fructose, maltose, lactose) in food when sufficiently high cooking temperatures are attained. Not only does the Maillard Reaction affect the physical qualities of food, such as flavor, texture, aroma, and color, it also causes the formation of many new compounds that influence the nutritional value of food. More research should be directed toward understanding acrylamide's role in the Maillard Reaction. Other compounds produced during the Maillard Reaction may either promote or hinder the bioavailability or metabolism of acrylamide in humans. Conversely, acrylamide may influence how other harmful or beneficial compounds react within biological systems. Other methods of acrylamide formation in food should also be investigated.

## References


1. Bosetti, C, Talamini, R, Levi, F, Negri, E, Franceschi, S, Airoldi, L, and La Vecchia, C. “Fried foods: a risk factor for laryngeal cancer?” *British Journal of Cancer* 2002; 87:1230-3.

2. Collins, J, Swaen, G, Marsh, G, Utidjian, H, Caporossi, J, and Lucas, L. “Mortality patterns among workers exposed to acrylamide.” *Journal of Occupational Medicine* 1989; 31:614-7.

3. Marsh, G, Lucas, L, Youk, A, and Schall, L. “Mortality patterns among workers exposed to acrylamide: 1994 follow up.” *Occupational and Environmental Medicine* 1999; 56:181-90.

4. Mucci, L, Dickman, P, Steineck, G, Adami, H, and Augustsson, K. “Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden.” *British Journal of Cancer* 2003; 88:84-9.

5. Mucci, L, Lindblad, P, Steineck, G, and Adami, H. “Dietary acrylamide and risk of renal cell cancer.” *International Journal of Cancer* 2004; 109:774-6.

6. Pelucchi, C, Franceschi, S, Levi, F, Trichopoulos, D, Bosetti, C, Negri, E, and La Vecchia, C. “Fried potatoes and human cancer.” *International Journal of Cancer* 2003; 105:558-60.

7. Schulz, M, Hertz-Picciotto, I, van Wijngaarden, E, Hernandez, J, and Ball, L. “Dose-response relation between acrylamide and pancreatic cancer.“ *Occupational and Environmental Medicine* 2001; 58:609.

8. Sobel, W, Bond, G, Parsons, T, and Brenner, F. ”Acrylamide cohort mortality study.” *British Journal of Industrial Medicine* 1986; 43:785-8.

9. Svensson, K, Abramsson, L, Becker, W, Glynn, A, Hellenas, K, Lind, Y, and Rosen, J. “Dietary intake of acrylamide in Sweden.” *Food and Chemical Toxicology* 2003; 41:1581-6.

10. Tareke, E, Rydberg, P, Karlsson, P, Eriksson, S, and Tornqvist, M. “Analysis of acrylamide, a carcinogen formed in heated foodstuffs.” *Journal of Agricultural and Food Chemistry* 2002; 50:4998-5006.

11. Tareke, E, Rydberg, P, Karlsson, P, Eriksson, S, and Tornqvist, M. “Acrylamide: a cooking carcinogen?” *Chemical Research in Toxicology* 2000; 13:517-22.