FOOD ADVISORY COMMITTEE
February 24–25, 2003 Meeting
Acrylamide

Sheraton College Park Hotel
4095 Powder Mill Road
Beltsville, Maryland 20705

Participating Food Advisory Committee Members
Sanford A. Miller, Ph.D., Chairman; Food Advisory Committee (FAC); Catherine DeRoever, Executive Secretary, FAC; Frank Busta, Ph.D.; Annette Dickinson, Ph.D.; Johanna Dwyer, D.Sc., R.D.; Brandon Scholz

Temporary Voting Members
Jean Halloran; Ken Lee, Ph.D.; Harihara Mehendale, Ph.D.; Robert Russell, M.D.; Clifford Scherer, Ph.D.; J. Antonio Torres, Ph.D.

FDA Staff
Lester Crawford, D.V.M., Ph.D., Deputy Commissioner; Joseph A. Levitt, Director, Center for Food Safety and Applied Nutrition (CFSAN); David Acheson, M.D., Office of Science, CFSAN; Donna Robie, Ph.D., Office of Food Additive Safety, CFSAN; Terry Troxell, Ph.D. Director, Office of Plant and Dairy Foods and Beverages, (OPDFB) CFSAN; Henry Kim, Ph.D., Executive Secretary, Contaminants and Natural Toxicants Subcommittee (CNTS); Richard Canady, Ph.D.; Office of the Commissioner

Guest Speakers
Robert Brown (substituting for Steve Saunders, Ph.D.), Frito Lay, Inc.; Tim Fennell, Ph.D., RTI International; Stephen S. Olin, Ph.D., ILSI Risk Science Institute; Sorell Schwartz, Ph.D., Georgetown University School of Medicine; David Zyzak, Ph.D., Procter & Gamble

Monday, February 24, 2003

Welcome and Introductions—Sanford Miller

Committee Chairman Miller called the meeting to order at 8:30 a.m. He then welcomed the committee and guests, introduced the members of the committee, and announced the meeting's purpose: to review the FDA's acrylamide Action Plan and confirm that this plan is on the right track.

Conflict of Interest Statement—Catherine DeRoever

Committee Secretary DeRoever noted that the topic of the meeting, acrylamide, affects the entire food industry, and in light of this broad reach, Dr. Busta, Dwyer and Miller's associations with the food industry did not present a conflict of interest for the purpose of this meeting. She also noted that two of the guest speakers are employed by the regulated industry, Drs. Robert Brown and David Zyzak.

Opening Comments—Joseph A. Levitt and Lester Crawford

Mr. Levitt reported on the activities of the previous 6 months by the international food research community following publication of the April 2002 Swedish study reporting that acrylamide had been found in food. He concluded by noting that while a lot had been completed in less than one year, much remained to be done, and what the FDA wanted was the committee to offer their best advise to optimize the Action Plan.

Deputy Commissioner Crawford declared that the FDA is fully committed to developing better knowledge of acrylamide in foods and sharing this information with the public as soon as possible. He declared that the public health risks of the levels of acrylamide found in foods is unknown, although it is known to cause cancer in rats and neurotoxic damage in humans at very high doses. FDA drafted an Action Plan in order to assess what is known and identify what needs to be learned. Since then the Agency has learned more about acrylamide levels and how it forms in food.

Presently the FDA upholds its previous recommendations that people eat a balanced diet containing a variety of foods, particularly low-fat and high-fiber foods, and a variety of grains, fruits, and vegetables. The purpose of the meeting is for the committee members to offer their input into the Action Plan, particularly in light of research accomplished in the previous 6 months. Dr. Crawford noted that the FDA soon would be releasing test results for another 110 samples and that these results do not vary dramatically from previous test results, including the propensity for two items from the same food group to produce different acrylamide levels.

The FDA also has prepared a preliminary exposure assessment, which does not vary significantly from those calculated by the Food and Agriculture Organization (FAO) and other groups. Many foods contribute to the total exposure, but no one single food accounts for the majority of the exposure. Also, certain foods may have low acrylamide levels, yet significantly contribute to exposure, if these foods make up a significantly large share of a person's diet. The food industry is investigating ways to reduce acrylamide in processed foods; researchers have reported interesting leads but no conclusive results.

Chairman Miller reminded the committee that the meeting's purpose was to offer advice to revise the Action Plan and the conclusions made by the Contaminants and Natural Toxicants Subcommittee (CNTS), not resolve the hazardousness of food-borne acrylamide. He then listed the three issues shaping the Action Plan:

- Toxicology—data on acrylamide's known effects at certain doses and what risk the doses in food present
- Exposure
- Available technologies—could acrylamide be blocked from forming or, once formed, be removed from the food?

Development of the Acrylamide Action Plan—Terry Troxell

Dr. Troxell reviewed the charge to the committee:

1. Does the revised Action Plan meet its intended goal of serving as a tool for providing a scientific basis to assess the significance of acrylamide in foods and its potential public health consequences?
2. New findings on acrylamide levels, exposure, and potential mitigation strategies have become available in recent months. Does the revised Action Plan accommodate these new data? Please comment on the new data, including the exposure assessment and the potential interventions.
3. The FDA's consumer message stresses the importance of eating a balanced diet. Given the uncertainties associated with the current state of scientific knowledge, the FDA has concluded that there are insufficient data to revise this message. Please comment.

Dr. Troxell reviewed the history leading to the drafting of the Action Plan, starting with the April 2002 Swedish studies that first reported the detection of acrylamide in food—a great surprise, as acrylamide had not been detected in previous studies. Researchers throughout the world immediately began to develop a test to confirm the Swedish findings; the FDA first posted its method in June 2002 and has updated it twice. National and international meetings followed, bringing together concerned parties. A World Health Organization/Food and Agriculture Organization (WHO/FAO) consultation, which was attended by three FDA experts, concluded that food-borne acrylamide was a substance of serious concern. FDA held an interagency roundtable and a public meeting in September. The Joint Institute for Food Safety and Applied Nutrition (JIFSAN) and the National Center for Food Safety and Technology (NCFST), consortia between FDA and the University of Maryland and the Illinois Institute of Technology, respectively, held a workshop in October. JIFSAN is also operating the WHO/FAO acrylamide in food information network and assuming a coordinating role for international research.

In December 2002 the CNTS reviewed the FDA's original draft Action Plan, outlining goals and planned activities for the next 2–3 years, and FDA's plans to work with other federal agencies and participate in international activities. Dr. Troxell said the FDA knew there would be a lot of work and that leveraging and coordination would be important.

He then reviewed the original Draft Action Plan's component sections:

- Testing foods
- Toxicology
- Formation
- Methodologies
- Meetings and collaborative projects
- Consumer messages and regulatory options.

He noted that the revised plan covers the same areas in more depth, with the addition of several sections. He said that although WHO/FAO did not note neurotoxicity as a problem at the food exposure levels, subsequent discussions suggested more work needed to be done to characterize

neurodevelopmental effects and chronic exposure. He reviewed several factors involved in developing the action plan, including the amounts consumed, research gaps, and occurrence through standard cooking practices. The agency's overall goal is to: "Through scientific investigation and risk management decision making, prevent and/or reduce the potential risk of acrylamide in foods to the greatest extent feasible."

The CNTS reviewed and recommended changes to this plan. The FDA has since revised the Action Plan, to accommodate both these recommendations and other ongoing activities, including studies mentioned in the plan and upcoming meetings.

Report of the CTNS Findings—Henry Kim

CNTS Executive secretary, Dr. Kim, summarized the December 2002 meeting, during which the subcommittee reviewed and commented on the Draft Action Plan. The subcommittee had been charged to assess the FDA's Draft Action Plan, given what the scientific community knew about acrylamide (toxicology, occurrence, formation, exposure, and risks), at the time of the December 2002 meeting.

The Subcommittee was asked:

1. Are the research steps appropriate to describe and address the public health significance of acrylamide in food?
2. Are there gaps in the research plan or areas where emphasis should be increased?
3. Are there priority research needs that should be addressed first?

Dr. Kim then summarized the presentations made at that meeting:

1. Toxicology (Richard Canady, Ph.D., FDA)—what data are known, what data are needed, and what research is ongoing? Dr. Canady surveyed what is known about acrylamide's toxicokinetics; that it's known to be an animal carcinogen and a human neurotoxicant at high doses, but what about these or reproductive or development effects at the very low food-borne doses? What sort of safety or risk assessment can be made of the levels found in food?
2. Occurrence (Steve Musser, Ph.D., FDA)—in what foods and at what levels is acrylamide found? Dr. Musser reported on the analytical method the FDA developed and the exploratory survey.
3. Formation (Lauren Jackson, Ph.D., FDA)—what mechanisms, precursors, and factors affect acrylamide formation, and can this formation be prevented or reduced? Dr. Jackson summarized research to date on acrylamide formation and discussed outstanding research needs.
4. Exposure (Michael DiNovi, Ph.D., FDA)—Dr. DiNovi described the sources from which acrylamide exposure would be assessed (consumption data and acrylamide levels in given foods), and reported that the FDA's initial exposure estimate had begun.
5. Consumer Risk (David Acheson, M.D., FDA)—Dr. Acheson talked about achieving a balance with respect to the importance of a balanced diet, risk from exposure to acrylamide in food, and potential dangers of inadequate cooking.

In response to the three questions the subcommittee said the research steps were appropriate. They supported the overall Draft Action Plan and the research steps outlined in the Plan. When asked if the research plan had any gaps or areas where emphasis should be increased, the subcommittee suggested the inclusion of more detailed information on risk assessment, human toxicology and epidemiology studies, animal toxicology studies, sampling and analytical variability, and food consumption data for various population groups. As for question 3, research priorities, the subcommittee felt that the described approach was appropriate (methodology, occurrence, formation, exposure, and then risk); toxicology and risk assessment studies should move forward quickly; rapid, inexpensive testing methods should be a priority; and science-based risk communication is also important. The subcommittee's recommendations were as follows:

1. Toxicology:
   a) Physiological studies in humans (absorption, metabolism, distribution, excretion)
   b) Toxicokinetic studies of ingested acrylamide in humans
   c) Animal neurotoxicity and genotoxicity studies
   d) Animal bioassay studies for carcinogenicity (short-term studies; dose-response relationship at lower levels, especially between no observed adverse effect level (NOAEL) and tumor-producing doses; mechanism of action)
   e) Biomarker-exposure relationship in smokers.
2. Epidemiology:
   a) Highlight a separate section on epidemiology in the Draft Action Plan
   b) More epidemiology studies in human populations (identify and study populations with higher or lower exposure—confounding factors may limit the validity of study; investigate epidemiology studies of occupational exposures—are these applicable to food exposures?)
3. Exposure Assessment:
   a) Highlight exposure assessment element
   b) Provide information about databases
   c) Improve ability to blind data to facilitate data sharing
   d) Statistically determine sampling sufficiency
   e) Obtain exposure assessors input on type of foods sampled
   f) Use food consumption data by various population groups
4. Risk Assessment:
   a) Incorporate more information into the Draft Action Plan (importance of risk assessment; methology for conducting risk assessment; methods for incorporating developing data).
5. Risk Communication:
   a) Importance of FDA's risk communication activities
   b) Provide science-based information for dietary choices
   c) Involve dietetic and nutrition associations in communication efforts (e.g., American Dietetic Association, American College of Nutrition)
   d) Disseminate consumer and cooking messages through extension services.

The CNTS spoke positively about the Draft Action Plan, generally agreed with the FDA's approach and planned research activities, and provided recommendations on certain plan aspects.

Dr. Busta, the Chairman of the CNTS, complimented Dr. Kim on his summation of the subcommittee meeting and concurred with the report.

Revised Action Plan—Terry Troxell

Dr. Troxell noted that the draft Action Plan had been revised based on previous public and CNTS meetings, and now the FDA wanted the committee's input in order to finalize the plan. Major changes made to the plan were a reorganized structure, intended to provide a more logical flow, and, as per CNTS recommendations, new sections on exposure assessment, epidemiology, and risk assessment, more details, and updated information.

The plan has seven sub-goals:

(1) to develop rapid or inexpensive screening methods and validate confirmatory methods of analysis;
(2) to identify the mechanisms of acrylamide formation in food and identify means to reduce acrylamide exposure;
(3) to assess U.S. consumers' dietary acrylamide exposure, by measuring various foods' acrylamide levels and estimating dietary exposure;
(4) to characterize the potential risks and uncertainties associated with dietary acrylamide by assessing available information, expanding research into acrylamide toxicology in order to reduce uncertainty, and performing a quantitative risk assessment based on the new information;
(5) to develop and foster public/private partnerships to gather scientific and technological information and data for assessing the human risk;
(6) to inform and educate consumers and processors about the potential risks associated with acrylamide, throughout the assessment process and as knowledge is gained; and
(7) to provide the fundamental elements of risk analysis—risk assessment, risk communication, and risk management.

There are nine sections detailing the actions toward accomplishing these goals:

- Methodologies—The LC/MS/MS method was posted on the web in June 2002 and recently updated. Dr. Musser's laboratory has analyzed approximately 400 samples, to date. Dr. Troxell also mentioned other groups at FDA that have been involved in laboratory analysis. Revisions in this section from the previous plan are the publication of a second update on the FDA method, explicit discussion of AOAC validation, and the statement that the FDA is looking at LC/UV as a screening method.
- Formation research—Research on formation is in the planning stages at NCFST. Formation research by academic and industry investigators is also crucial. Revisions in this section include NCFST plans to investigate home cooking practices, particularly toasting and frying.
- Measuring exposure—Dr. Troxell discussed current analyses being done at CFSAN, preliminary analyses of Total Diet Study foods, plans for a contract with JIFSAN for testing of nationally collected foods, the need to fill in gaps in data, which will draw in part on JIFSAN database efforts, and potentially on European data relevant to U.S. processing conditions. The revised Action Plan contains more details on testing plans and a new section

on the exposure assessment process, including information on modeling and databases.

- Toxicology and health effects—Dr. Troxell summarized four areas that FDA wants information on from the toxicology work: (1) a better understanding of carcinogenicity, neurotoxicity, and germ cell toxicity in rodents, (2) the bioavailability of acrylamide in food, (3) the difference in metabolism between high and low doses, and (4) the difference between the metabolism and processing of acrylamide between animals and humans. Under carcinogenicity, he mentioned hemoglobin adducts and the relation between hemoglobin adducts and DNA adducts, and studies relating rat high-dose and human low-dose adduct data. He said that data for these key needs may be available in a relatively short timeframe, one to two years, including bioavailability and adduct studies at the National Center for Toxicological Research (NCTR). Short-term studies by NCTR are exploring acrylamide bioavailability in food and the range of DNA and protein adducts (DNA adducts are associated with cancer, and hemoglobin adducts, once the dose-adduct relationship has been established, are expected to serve as a biological marker). In the meantime, NCTR will do a National Toxicology Program (NTP) rodent carcinogenicity bioassay, the subchronic and mechanistic components of which will be done fairly fast and used for physiologically-based pharmacokenetics (PBPK) modeling. Neurodevelopmental effects are also being studied at NCTR, and mechanistic research on germ cell toxicity and neurotoxicity is being done at the National Institute of Environmental Health Sciences (NIEHS).

  As for human toxicology and health effects, NCTR and the Centers for Disease Control and Prevention's (CDC's) National Center for Environmental Health (NCEH) are investigating rodent-human adduct correlations, while the FDA and NCEH are studying diet-related adducts in humans. Potential outside sources of information include the National Health and Nutrition Examination Survey (NHANES) general population study, the National Institute for Occupational Safety and Health (NIOSH) worker study, and a major acrylamide manufacturer's toxicokinetics studies. New toxicology material in the Revised Action Plan includes new study areas, particularly neurotoxicity, germ cell toxicity, and industry toxicokinetics; PBPK and dose-response elements; and more details on NTP studies, including chronic carcinogenicity.

- Epidemiology—Dr. Troxell noted that some of the work on adducts is viewed as epidemiological as well as toxicological, so it is cross-cutting work. Plans include exploring the feasibility of prospective studies and relevant literature studies. He explained that there are two avenues of epidemiological studies, occupational and food exposure. Occupational studies are most likely to say whether high doses can cause cancer in humans. So far, these studies have not demonstrated carcinogenicity in humans, but it is possible that they covered too short a time. Food exposure studies may have limited ability to detect the risk levels expected with acrylamide. Problems include diet misclassification, difficulties in finding nonexposed groups because acrylamide is widespread in the diet, and the array of chemicals other than acrylamide that are found in food. The main change in the Revised Action Plan on epidemiology is the addition of this new section, which explains how epidemiological data could benefit FDA work on acrylamide and details a number of studies and collaborations that are under consideration.

- Risk assessment—Dr. Troxell pointed out that when adequate information is available, FDA wants to characterize the potential risk of acrylamide in food, including doing an uncertainty analysis. Key toxicology data needs include bioavailability, biomarkers, metabolism, and toxicokinetics. FDA will revise the risk assessment when significant developments yield enough information to materially change the assessment. The Risk Assessment section is a new feature of the Revised Action Plan.

- Meetings—Since April 2002 FDA personnel have participated in and convened meetings; this will continue as appropriate.
- Inform and educate the public—Early in the process the FDA committed itself to communicate with the public on this issue; this has included public meetings, Web page postings, and an article on acrylamide in the January/February 2003 issue of the magazine *FDA Consumer*. The FDA does not believe that there is enough science at this point to change its dietary message—eat a balanced diet containing a variety of foods that are low in trans fat and saturated fat and rich in high-fiber grains, fruits, and vegetables. New in this section of the Action Plan is that the previous consumer messages section has been expanded, including a summary of risk communication efforts to date, and the plan to collaborate with other organizations is made explicit.

Further actions—The FDA plans to develop and revise regulatory options as the agency receives data on food-borne acrylamide, and will encourage the food industry to adopt feasible, practical, and safe practices that successfully reduce acrylamide, as needed.

Mechanisms of Formation—David Zyzak

Dr. Zyzak reported on Procter & Gamble's research that identified the most likely means by which acrylamide develops in food. In the wake of the April 2002 report on acrylamide in food, Proctor & Gamble tested a number of their food products, and found various acrylamide levels. Based on acrylamide's molecular structure, researchers identified several possible precursors, including acrylic acid, acrolein, and asparagine and other amino acids. They then tested these substances in a model system, cooking them with potato starch and water, with and without dextrose, a simple sugar associated with the Maillard browning process. Only the asparagine-dextrose combination yielded significant amounts of acrylamide.

About 50 percent of a potato's amino acids are in a free (not bound to protein) state, and about half of these free amino acids are asparagine. Protein-bound asparagine was ruled out by testing it in the model system as no acrylamide formation was observed.

The researchers used isotopes to identify the mechanism of acrylamide formation from the reaction of asparagine with glucose. Then, to confirm that asparagine was the primary precursor, researchers treated a potato with the enzyme asparaginase. Asparagine levels in the potato product treated with asparaginase were less than 99 percent of those in the control.

The identification of the acrylamide precursors—asparagine and reducing sugars—has suggested possible interventions in the formation process. The dose-response curve suggests that acrylamide production corresponds with the amount of free asparagine and simple sugars—

reducing one or the other results in lower acrylamide levels. The amount of asparagine and reducing sugars in a potato depend on the variety and the conditions under which it was stored; researchers are investigating how this can impact acrylamide formation in cooked potatoes.

Dr. Zyzak concluded by saying that asparagine is the primary precursor to the formation of acrylamide in foods; that the reaction requires a carbonyl source (reducing sugars); and oil oxidation products, oil quality, and starch do not appear to be significant factors in acrylamide formation.

Reduction Strategies—Robert Brown

Dr. Brown appeared on behalf of Frito-Lay's, Dr. Steve Saunders, who could not appear before the committee. After summarizing the likely mechanism for the formation of acrylamide from asparagine, he described the chemical pathway leading to acrylamide formation as "a low-yield pathway with high activation energy. He also noted that the foods that contain acrylamide constitute a significant portion of a diet's calories, fiber, vitamins, minerals, and other micronutrients. He listed the three proposed approaches to managing acrylamide:

1. Remove the reactants
2. Disrupt the reaction
3. Remove the acrylamide after formation.

Dr. Brown noted that the presence of acrylamide in cooked food is directly mediated by the presence of asparagine in the raw food, and that the amount of asparagine in crops can vary dramatically. He also noted that the chemical reaction of asparagine and glucose is second order when the substrates are approximately equal; when one is substantially lower it becomes rate limiting.

As for possibly disrupting the formation of acrylamide, He noted that acrylamide levels increase substantially as a food is browned—overcooked oven fries have much higher levels than the same type of fries cooked to a less brown color. The acrylamide formation process appears to begin at 120°–130°C, and accelerate at 150°C. Dr. Brown observed that it probably would not be possible to cook food without creating at least some acrylamide; and studies into controlling the food's surface temperature as it cooks probably will be critical. pH levels under 7, particularly under 5, inhibit acrylamide formation somewhat, and researchers are investigating other potential food-safe reaction inhibitors, such as cysteine, rosemary and flavonoids, but there is no prospective "magic bullet." Frito-Lay has studied di-and trivalent cations and found that they inhibit acrylamide formation, but the large amounts required may not be practical. Other potential sites of intervention include using other free amino acids to deplete glucose, and looking at pH, time, and temperature variables.

As for removing acrylamide from cooked food, UV light was ineffective, while supercritical CO_2 removed everything but destroyed the product in the process.

Dr. Brown noted that even if a person eliminates potatoes from his or her diet, he or she still would consume 15–20 µg of acrylamide per day. He also observed that the concept of

carcinogens in food is not new; that humans have been eating starchy foods for millennia; and there are no prospective quick fixes.

Exposure Assessment—Donna Robie

Dr. Robie opened her presentation by reviewing acrylamide exposure assessments. In the April 2002 article reporting on acrylamide in food, the Swedish researchers tested about 100 foods and used medians for eight food categories to estimate mean acrylamide exposures of 40 µg per person per day (assuming 0.67 µg/kgbw-day, 60 kg bw per person). This included "expected" values for food groups not included in their sampling, some of which have turned out to not be significant acrylamide sources.

- In June 2002, the FAO and WHO drew on acrylamide residue data from the Swedish study and food consumption data from Australia, the Netherlands, Norway, Sweden, the United States, and the IARC's EPIC (International Agency for Research on Cancer's European Prospective Investigation into Cancer and Nutrition) study. The WHO/FAO then used probabilistic modeling and point estimate methods to calculate an exposure of 0.3–0.8 µg/kgbw-day.

Dr. Robie described FDA's exposure assessment. In general, the estimated daily intake of substance X is a factor of the total number of foods in which substance X is found (F), the number of occasions on which food F is eaten during days in a survey period (N), the average portion size of food F, and the concentration of substance X in food F.

The food consumption surveys the FDA used were:

- Continuing Survey of Food Intakes by Individuals (CSFII)—this compiled 3-day consumption records during 1989–1992 and 2-day consumption records during 1994–1996 and 1998, based on surveys of approximately 20,000 participants. It is expected to overestimate eaters' intakes and underestimate the percent of eaters, relative to intake records covering a longer survey period.
- Marketing Research Corporation of America (MRCA)—this was based on 14-day consumption records during 1982–1987 and surveyed approximately 26,000 participants. This study did not record portion sizes, but this was estimated from the USDA/Nationwide Food Consumption Survey.

The MRCA classifies foods consumed into broader groups; the CSFII is more specific as to what foods were consumed.

The simplest exposure assessment model is a factor of food consumption times a substance's concentration in that food, summed over foods and individuals and expressed as point estimates. It is useful only for substances in a few foods, when the estimated daily intake (EDI) is very different from the acceptable daily intake/tolerable daily intake (ADI/TDI); this model is not applicable to acrylamide. Therefore, FDA applied probabilistic modeling. This modeling is an iterative process.—For each point ("virual consumer") on the acrylamide distribution, the computer calculates values for food consumption, acrylamide level, and percentage of eaters, based on random selections from the underlying distributions for all the foods in the assessment.

Plotting these values produces a curve showing acrylamide exposure and the likelihood of exposure. According to this model, acrylamide intake is a function of the eater's likelihood to eat a given food times the amount of that food times that food's acrylamide level.

Dr. Robie then referred to a handout listing estimated food consumption for several "virtual consumers." The model lists which of a selection of foods this consumer ate, how much of those foods he ate and how much acrylamide those foods contain, to calculate one day's acrylamide intake. The researchers prepared 25,000 virtual consumers, each randomly generated. Robie advised that this model does not account for correlations between food selections, and that the distributions were truncated to remove irrationally high values—for example, the 100th percentile of coffee consumption would be 13 liters per day. Other model limitations are the surveys' different durations and food classifications, and uncertainty surrounding the laboratory data. Some food types are represented by fewer than five samples, and detected acrylamide levels are known to vary from lot to lot, brand to brand, and product to product. Also, how a food is prepared will affect its acrylamide levels.

Dr. Robie then presented a series of slides listing the seven or eight primary dietary acrylamide sources based on mean acrylamide intake. These tables were broken down by survey source and population (total population ages 2 and older and ages 2–5; noting that children typically consume about one half of what adults eat, but weigh about one-quarter of adults, which results in higher acrylamide intake per kilogram of body weight). A series of slides showing the distribution of acrylamide intake followed.

She then showed a table listing acrylamide intake by single serving. She also showed results demonstrating that if acrylamide could be completely eliminated from a single food, mean acrylamide consumption level would drop only from the present estimated 0.37 µg/kgbw per day to 0.34–0.26 µg/kgbw-d.

Dr. Robie then listed work to be done:

- Modeling of longer-term food consumption, to expand consumption duration for individuals beyond 2-day surveys; and more accurately model chronic acrylamide intake.
- As the food industry develops acrylamide mitigation strategies, run what-if scenarios to calculate the effect on mean acrylamide consumption.
- Sensitivity analysis, to identify uncertainties and investigate how to accommodate these.

She summarized her presentation by observing that mean population acrylamide intakes are consistent with previous exposure estimates; the greatest contributors to mean population acrylamide intake are the same across the surveys; some foods with lower acrylamide levels contribute appreciably to the population intake because they are commonly consumed; and no one food accounts for the majority of the mean population acrylamide intake..

Adduct Studies—Tim Fennell

Dr. Fennell reviewed what is known about acrylamide metabolism and its adducts with hemoglobin and DNA:

- A reactive chemical. Undergoes Michael addition. Very reactive with –SH groups. Also reacts with amino groups.
- Very reactive with proteins.
- Reacts very slowly with DNA.

He also discussed glycidamide, which is formed by the oxidation of acrylamide. It is a reactive epoxide, which reacts with protein, and reacts with DNA; this is associated with mutations and carcinogenicity.

Dr. Fennell listed the basic principles of metabolism and pharmacokinetics:

- Most chemicals undergo metabolism to be converted to a form that can be readily excreted.
- While most metabolites are unreactive and readily excreted, some are more reactive.
- Reactive chemicals or metabolites can react with tissue molecules, including glutathione, protein, RNA, and DNA, and this may produce a toxic or carcinogenic effect.
- Metabolism can occur by more than one route, producing both reactive and stable metabolites. Reactive metabolites can undergo further conversion into stable metabolites.
- The balance of the various metabolic processes and their relative rates can be an important determinant in toxicity, and can differ between species and between high and low doses.

Risk assessment issues include the relationship between exposure and internal dose (dose in blood and at the target site); dose-response (is it linear in range of effects; does it compare with bioassays); difference between species; and measures of dose for reactive chemicals or metabolites. With both acrylamide and glycidamide, researchers are concerned with metabolites excreted, presence in blood and tissues, adducts with proteins, and adducts with DNA—and with the balance of all of these.

Researchers regard hemoglobin adducts to be a useful indicator of acrylamide exposure:

- Adducts in hemoglobin are proportional to the area under the curve (AUC) for reactive chemicals or metabolites.
- Hemoglobin is readily available from a blood sample.
- Hemoglobin has a number of amino acid residues that can react with chemicals and their metabolites—cysteine, histidine, N-terminal valine, and carboxyl groups.
- The red cell has a long lifetime in circulation (120 days), and is removed with 0 order kinetics.
- With repeated exposure, accumulate and reach a steady state when the duration of exposure exceeds the red cell lifespan. This makes hemoglobin adducts useful when studying dietary, occupational, smoking, or other long-term exposures.

Dr. Fennell briefly summarized several studies of hemoglobin adducts in rats, which suggested a nonlinear dose-response. Of particular interest was one study describing a saturable metabolic process, the oxidation of acrylamide to glycidamide. It implied greater risk per unit exposure at lower doses, if glycidamide is the metabolite that generates adverse effects. He also reported on studies of valine adducts, which even before the April 2002 Swedish studies were suggesting a baseline presence of acrylamide in people who had no known industrial exposure, noting that at least one researcher in these studies would later co-author the April 2002 findings.

Recent studies on acrylamide metabolism have sought to understand, in different species, how internal doses relate to exposure via different routes; and whether the glycidamide conjugation-oxidation ratio is altered by exposure route and dose. This research includes comparisons of dermal, inhaled, and intraperitoneal exposure in rats; comparison of inhaled acrylamide exposure in rats and mice; and an evaluation of the role of *Cyp 2E1* in the metabolism of mice. The findings indicate a dose-response correlation, although differences are found in different species.

Dr. Fennell reported that his laboratory was working on a new, selectively cleaving method of adduct analysis that produces a higher sample throughput, greater sensitivity, and the ability to process smaller amounts of globin and distinguish adducts from natural abundance and labeled acrylamide.

The conclusions were offered:

- Route differences translate into different internal doses and metabolism to glycidamide. Dermal exposure resulted in a low percentage of exposure (3–22 percent).
- Different species metabolize acrylamide into glycidamide differently.
- Researchers can readily measure acrylamide- and glycidamide-valine adducts, have found background levels of both, and can distinguish these from labeled acrylamide.
- The ratio of acrylamide-valine adducts to glycidamide-valine adducts depends on dose, route of exposure, and species (more glycidamide adducts were found in mice, compared to rats).
- A good correlation between metabolism data and hemoglobin adduct data has been formed.

The outstanding data gaps are the metabolism and route of action of glycidamide versus acrylamide; how is acrylamide taken up and metabolized in humans; what is the relationship between exposure and hemoglobin adducts in humans; and how good are the data regarding DNA adducts.

Studies in progress include research on measurement of adducts in human volunteers, to assess uptake, metabolism, and adduct formation before and after oral or dermal exposure. The findings from this study are expected within 1 or 2 months. One published paper on acrylamide-DNA adducts in rats and mice used labeled acrylamide to assess adduct formation and found one species of glycidamide-DNA adduct; Dr. Fennell reported that similar studies to assess DNA adducts are underway, e.g., at NCTR.

He concluded by summarizing the FAO/WHO Consultation research recommendations:

- Evaluation and calculation of exposure biomarkers.
- Data on the absorption, metabolism, distribution, and excretion of food-borne acrylamide in humans.
- A better understanding of the formation of glycidamide and its binding to DNA as a marker of toxicity and carcinogenicity.
- A better assessment of the dose-response characteristics of acrylamide and glycidamide relative to toxicity, disposition, and binding to DNA and macromolecules.
- A more thorough exploration of the relationship between hemoglobin and DNA adducts in different organs.

Public Comment

No public comments were offered.
Adjourn

Dr. Miller adjourned the meeting at 3:55 p.m.

Tuesday, February 25, 2003

Welcome and Introductions—Sanford Miller

Committee Chairman Miller called the meeting to order at 8:30 a.m.

Animal Studies and Human Health Consequences—Sorell Schwartz

Dr. Schwartz opened his presentation by advising the committee that his presentation was not on acrylamide, but rather whether and how findings from animal studies can be extrapolated to humans. Saying that a substance that has been shown to be carcinogenic in animals is therefore risky to humans would be a gross rather than a more specific extrapolation, based on the assumption that humans are as sensitive as animals are to that substance. It may not be very scientific, but in the name of public health and safety, researchers generally take the no observable adverse effects level (NOAEL) from animal studies and divide that by 100 or 1,000 to calculate the maximum permitted level for humans.

He distinguished between pharmacokinetics and pharmacodynamics:

- Pharmacokinetics: The action of the body on the chemical
  - System: Absorption, distribution, metabolism, elimination
  - Output: Concentration-time relationship

- Pharmacodynamics: The action of the chemical on the body
  - System: Biological ligands or other targets in the biophase (site of action)
  - Output: Biological response

However, carcinogenic and teratogenic responses are not so easily extrapolated.

This interspecies scaling can be either isometric (most organs [except for the human brain], blood and tidal volume, and vital capacity have a roughly constant proportion to body weight across species) or allometric (proportion to body weight varies exponentially across species, and formulas to translate animal heart rate, circulation time, respiratory rate, basal metabolic rate, blood flow, and clearance into expected values for humans exist).

However, a number of pharmacokinetic factors affect the efficacy of interspecies extrapolations:

- Volume of distribution. This is essentially defined as the volume the chemical would be distributed in if it were distributed throughout the body in the same concentration it is in the blood.
  - This quantitatively describes the distribution of the chemical throughout the body and ultimately at the site of action. The greater the volume of distribution the greater the substance's biological half-life.
  - This value is scalable based on interspecies composition relationships and physical chemical factors.
- Clearance. This is a factor of blood flow times extraction ratio (ER).
  - This refers to the volume of blood per unit time from which the chemical is completely extracted. The higher the clearance the shorter the half-life.
  - Blood flow is allometrically scalable across mammalian species.
  - Extraction can occur by diffusion mechanism (e.g., in the kidney) or by metabolic mechanism (e.g., in the liver).
  - Clearance can be flow-limited (high ER) or capacity-limited (low ER). Flow-limited clearance across species is more likely to be scalable than capacity-limited clearance.
- Absorption and bioavailability. Bioavailability is a function of the fraction absorbed from the GI lumen, the fraction not metabolized by GI tissue, and the fraction that gets by the liver metabolism. A higher hepatic ER increases the likelihood that interspecies differences in absorbed dose will be magnified, and this factor is not always taken into consideration.

Allometric extrapolation based on GI absorption, volume of distribution, blood flow, clearance (where clearance is flow-limited and hepatic ER is high), and bioavailability (where hepatic ER islow) is likely to be more reliable. Extrapolation is likely to be less reliable when clearance capacity is limited (as the hepatic ER is low) or bioavailability is affect by a high hepatic ER, across species.

Researchers have proposed a number of formulas to try to extrapolate clearance rates; and in vitro cultivation of hepatocytes has helped predict actual clearance rates in humans. However, another approach is PBPK modeling. This seeks to estimate the concentration of a chemical in

each of the body's various organs, at the biophase. This model can translate intake into concentration in the human target tissue corresponding to the animal tissue associated with the observed response.

Dr. Schwartz concluded by listing the applications of PBPK modeling to risk assessment:

- Interspecies extrapolation
- Prediction of biophase concentration
- Dose extrapolation in cases of nonlinear pharmacokinetics
- Low-dose extrapolation
- Route of exposure extrapolation
- Relative risk from multiple routes of exposure
- Estimation of exposure based on biological markers.

Acrylamide Toxicity: Research to Address Key Data Gaps—Stephen Olin

Dr. Olin reviewed the October 2002 JIFSAN/NCFST workshop on acrylamide in food, at which he co-chaired the working group for toxicology and metabolic consequences. He listed the toxicity focus areas the group identified as having data gaps and research needs for the toxicology component of a risk assessment:

- Kinetics and metabolism
- Genetic toxicity
- Reproductive and developmental toxicity
- Carcinogenicity
- Neurotoxicity
- Epidemiology

The primary research objectives are to:

1. Assess the significance of adverse effects observed at high doses in animals (and in the case of neurotoxicity, in humans) for low-level human exposures in foods.
2. Assess the significance for humans of effects observed *in vitro* or *in vivo* in rodents.

Kinetics, metabolism, and modes of action. Priority research needs identified by the group were critical events and dose metrics related to modes of action (MoA) for key acrylamide toxicities; acrylamide's fate and kinetics in humans; and PBPK models. Dr. Olin reported on some ongoing and planned research: the FDA/NCTR, and NIEHS are studying critical events, dose metrics and MoA; several groups are exploring acrylamide metabolism and kinetics in humans; and one recently published study applied a PBPK model to rats.

Genetic toxicity. Priority research needs include the identification and characterization of acrylamide and glycidamide adducts with DNA and significant nuclear proteins (biological relevance, *in vitro* and *in vivo* study of species and dose dependence); and an investigation of the mechanisms of specific effects (e.g., chromosomal effects, cell transformation). The FDA and

NCTR are studying DNA and protein adducts and dose response, while industry is studying DNA adducts *in vitro* and *in vivo*. The FDA and NCTR also are studying *in vivo* mutagencity in Big Blue rats and *tk+/-* mice, while industry is studying interaction with kinesin-related proteins.

Reproductive and developmental toxicity. Research is needed on dose-response data for germ cell toxicity in rodents, particularly the role of acrylamide versus glycidamide; and further examination of the potential for developmental neurotoxicity. NIEHS has announced a planned *CYP 2E1* null mouse dominant lethal study to assess germ cell toxicity. As for developmental neurotoxicity, some academics have started work in this sector, while NCTR has shown signs of interest.

Carcinogenicity. Priority research needs are: confirm and clarify carcinogenicity in standard rodent models (pathology working group review, assess effects of perinatal exposure, develop enhanced data for dose-response assessment); and determine the induction mechanisms of key tumors. NTP and NCTR have drafted plans to study acrylamide-related carcinogenicity in rats and mice over 2 years, and a study of acrylamide and glycidamide's effects on neonatal mice. NIEHS may be planning a review of critical slides from previous neonatal mouse studies. As for mechanisms, NTP and NCTR are expected to study this as part of their 2-year studies, and industry has already begun investigation into thyroid and brain tumors and cell proliferation.

Neurotoxicity: Acrylamide's neurotoxic effects at high doses are one of the few things known about the chemical. Research needs are: the relationships between dose, duration, effect levels, and onset of neurotoxicity (effects of low-level, long-term dietary exposures, links between damage at the cellular or tissue level and functional changes); and the mechanisms of neurotoxicity (the role of acrylamide versus glycidamide versus any other acrylamide metabolites or adducts; bridging the effects of these in animals and humans). The announced 2-year FDA/NCTR rodent bioassay studies could provide an opportunity to study cumulative damage from low-level dietary exposures. As for the mechanisms, some researchers have shown interest in studying issues such as nerve terminal damage, axonal transport, and key proteins; NIEHS's *CYP 2E1* null mouse study also is relevant; and NIOSH is studying markers in exposed workers.

Epidemiology. Research needs are: study on new or previously evaluated exposed worker cohorts for specific effects; link exposure biomarkers to effects in workers; and assess feasibility and design criteria for studying acrylamide exposure and effects in nonoccupationally exposed populations. NIOSH and industry are interested in acrylamide's specific effects in workers; NIOSH has included biomarkers in their work; and studies like CDC/NHANES and EPIC and a 2003 study by Mucci et al. are looking at or have looked at nonoccupationally exposed populations.

Dr. Olin concluded his presentation by noting that ongoing and planned research, particularly by the FDA and NCTR, will address many important toxicology research needs. He also reminded the committee that although results from some of these studies are due within the next year, others will take longer.

Potential Implications—David Acheson

Dr. Acheson began by stating FDA's goal -- to prevent or reduce the potential risk of acrylamide in foods to the greatest possible extent, on the basis of scientific investigation and risk-management decision making. Integral to this is the need to continue to inform and educate consumers and processors about the potential risks, throughout the assessment process and as additional knowledge is gained.

He noted that consumers may have questions—will certain foods cause cancer, what is safe to eat, should they stop eating certain foods, should they cook those foods differently, should they do anything differently now to protect themselves and their families. The FDA's goal is to provide a consistent, evidence-based answer. Because so many questions are outstanding, the agency presently upholds its previous messages on the importance of a balanced diet—but does current scientific knowledge support this?

The current areas of scientific interest are:

- Acrylamide formation and ways to diminish this process
- Acrylamide levels in food
- Dietary intake of various foods (based on chronic consumption, rather than 2 days' or 2 weeks' worth)
- Exposure assessment
- Epidemiology—the impacts of acrylamide exposure on human health.

The implication of this discussion is that understanding the acrylamide formation process and developing mitigation strategies could lead to reduced acrylamide levels; and the key need is to understand the health implications of acrylamide exposure at the levels found in food.

Exposure assessment. Based on analyses to date a small number of foods (but no one single food) contribute the most to daily acrylamide exposure. The average daily acrylamide exposure is 0.3–0.5 µg/kg, although this varies according to a person's dietary choices. The question is whether these acrylamide levels have an impact on human health, in neurologic, germ cell, or carcinogenic outcomes.

The evidence that food-borne acrylamide may be hazardous to human health are based on:

- Animal studies (in mg/kg doses, as compared to the µg/kg doses found in food)
- Human dosing studies to explore acrylamide's kinetic effects in human (data from these studies are not yet available but will be highly valuable in understanding the issue)
- Human epidemiological studies, which are complex and cumbersome (occupational exposure has shown no links with cancer; only one study has investigated exposure via food).

Human epidemiological studies will need to consider dose, length of exposure, age, genetic susceptibility, synergistic factors, and types of tumors. The first such study, published earlier this year in the *British Journal of Cancer*, surveyed patients who had been diagnosed with large bowel, bladder, or kidney cancer and a control group as to their consumption of certain foods

during the previous 5 years. The authors concluded that they did not find a positive association between dietary exposure to acrylamide and risks of bowel, bladder, or kidney cancer. The authors acknowledged that many but not all of the known high-acrylamide foods were included in their survey. Dr. Acheson also noted the limited sample size and consideration of only certain cancers.

The current implications that researchers are addressing are:

- The strength of the link between animal toxicity (at acrylamide doses of mg per kg) and human exposure (at doses of µg per kg).
- The data to indicate that this level of exposure poses a significant human health risk are lacking.
- Consuming certain types of food will increase a person's exposure to acrylamide.

Risk management. As the data to support these assumptions are weak, what should the FDA recommend to consumers? The agency does not want to create a new problem by solving a previous one—for example, reducing intake of a high-fiber food in order to avoid acrylamide would not be a satisfactory outcome. Maintaining objectivity and balance is critical. Therefore, the FDA presently upholds its dietary guidelines—eat a variety of grains (especially whole grains), fruits, and vegetables; limit consumption of sugars, salt, and foods high in cholesterol, trans fat, and saturated fat (as well as having a diet moderate in total fat); aim for a healthy weight; and include physical activity in your day. However, the agency also will continue to reassess these messages based on the emerging science.

Summary, Charge, and Questions—Terry Troxell

Dr. Troxell reviewed FDA's charge to the Committee—"Evaluate the revised Action Plan as a tool for providing the scientific basis from which to assess the significance of acrylamide in foods and potential public health consequences."—and the specific questions the FDA wanted the committee to answer.

Public Comment

No public comments were offered.

Committee Recommendations:

Question 1 Does the revised Action Plan meet its intended goal of serving as a tool for providing the scientific basis to assess the significance of acrylamide in foods and its potential public health consequences?

The Committee agreed that the Action Plan is useful tool. They did, however, stress the importance of leadership and coordination. The multiple activities incorporated into the plan will require that there be a designated lead for the efforts, as well as time lines with which to accomplish items. The Committee applauded the collaborative nature of the Action Plan but

encouraged additional efforts with other government agencies and the academic community. The Committee members also expressed the view that various subpopulations and consumption patterns be incorporated into the equation.

Question 2: New data on acrylamide levels, exposure and potential interventions have become available in recent months. Does the Action Plan accommodate these new data? Please comment on the new data, including the exposure assessment and the potential interventions.

The Committee felt that the Action Plan was flexible enough to accommodate new data and that this is critical given the ongoing research. The Committee agreed that leveraging resources was also important and suggested ongoing large-scale human studies that might be resources for the overall acrylamide effort. The Committee stressed the importance of standardization of data, e.g., methods, and reporting.

Question 3: FDA's consumer message stresses the importance of eating a balanced diet. Given the uncertainties associated with the current state of scientific knowledge, FDA has concluded that there is insufficient data to revise this message. Please comment.

The Committee felt very strongly that consumers must be provided with information that is useful, timely and updated routinely.

Several suggestions were made for making information readily available, i.e., web postings, collaborative efforts with professional associations and colleagues. It was recommended that a risk communication strategy be developed that would take into consideration the potential range of research results and how those results could best be communicated.

Respectfully Submitted:

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Certified:

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