FAO/WHO Consultation on the Health Implications of Acrylamide in Food Geneva, 25-27 June 2002

Summary Report[1]

INTRODUCTION

In April 2002 the Swedish National Food Administration (NFA) and researchers from Stockholm University announced their findings that acrylamide, a toxic and potentially cancer-causing chemical, is formed in many types of food prepared/cooked at high temperatures. The NFA informed regional and international authorities and organizations about their findings in order to initiate international collaboration as a priority concern. Moreover, international initiatives to commence multidisciplinary research were viewed as urgently needed as the formation of acrylamide during the cooking process may be a widespread phenomenon.

In light of concern expressed by member countries, a Consultation was convened jointly by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO). The Consultation was held at WHO Headquarters in Geneva, Switzerland on 25-27 June 2002. A list of participants and agenda as adopted are provided in Annexes 1 and 2, respectively. Dr Dieter Arnold, Acting Director, Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany served as Chairman.

The Consultation was opened by Dr David Nabarro, Executive Director of the Cluster on Sustainable Development and Healthy Environments and Senior Policy Adviser to the WHO Director General, who emphasized that in addition to the evaluation of specific scientific aspects of acrylamide in food, governments, industry and consumers were looking forward to any interim advice that could be offered, particularly in the light of the lack of adequate data and the limited understanding of many of the processes involved.

[1] This summary report contains the collective views of an international group of experts, and does not necessarily represent the decisions or the stated policy of the Food and Agriculture Organization of the United Nations and the World Health Organization. The full report will be issued in due course.

The objectives of the Consultation were:

1. To review and evaluate new and existing data and research on acrylamide relevant to:

- toxicology, especially carcinogenicity and neurotoxicity;
- epidemiology;
- exposure assessment;
- analytical methodology; and
- formation, fate and bioavailability of acrylamide in cooked food.

2. To identify needs for further information and studies; and

3. To develop and suggest possible interim advice for governments, industry and consumers.

The Consultation reviewed the health significance of the presence of acrylamide in foods on the basis of known international assessment reports, specific background papers prepared in advance by invited experts and on the available new data and publications. A list of the major documents available to the Consultation can be accessed on the WHO Web site (http://who.int/fsf). Note that individual documents are not specifically referred to in these texts, nor are they exhaustively summarized in this report.

EXECUTIVE SUMMARY

The FAO/WHO Consultation on Health Implications of Acrylamide in Food has undertaken a preliminary evaluation of new and existing data and research on acrylamide. The following main conclusions were reached:

Methods of analysis for acrylamide

By current standards of analytical science, the recent findings of acrylamide in foodstuffs are reliable. None of the methods used to measure acrylamide in foodstuffs has yet been fully validated by inter-laboratory collaborative trials. However, most methods fulfil the requirements of single-laboratory ("in-house") validation and accreditation.

Formation and fate of acrylamide in food

Acrylamide has been found in certain foods that have been cooked and processed at high temperatures, and the levels of acrylamide increase with the time of heating. However, the mechanisms of formation of acrylamide in food are poorly understood.

Exposure assessment

Based on the available data, food is estimated to make a significant contribution to total exposure of the general public to acrylamide. Average intakes for the general population were estimated to be in the range of 0.3 to 0.8 microgram of acrylamide intake per kilogram of body weight per day. Within a population, it is anticipated that children will generally have intakes that are two to three times those of adults when expressed on a body weight basis. Dietary intakes of acrylamide by some consumers may be several times higher than the average.

Non-cancer toxicology

Neurotoxicity is the key non-cancer, non- genotoxic effect of acrylamide in humans and animals. No neurotoxic effects are to be expected from the levels of acrylamide encountered in food.

Genotoxicity

Acrylamide may induce heritable damage.

Carcinogenicity

Acrylamide has a carcinogenic potency in rats that is similar to that of other carcinogens in food, but the intake levels for acrylamide are likely to be higher. For humans, the relative potencies of cancer-causing agents in food are not known. Only limited human population data are available for acrylamide and these provide no evidence of cancer risk from occupational exposure. All such studies have limited power to detect small increases in tumour incidence. The Consultation recognized the presence of acrylamide in food as a major concern in humans based on the ability to induce cancer and heritable mutations in laboratory animals.

Need for further information and provision of interim advice

The Consultation provided a range of recommendations for further information and new studies to better understand the risk to human health posed by acrylamide in food. The Consultation also provided some advice to minimize whatever risk exists, including avoiding excessive cooking of food[*], choosing healthy eating, investigating possibilities for reducing levels of acrylamide in food, and establishing an international network on acrylamide in food.

[*] However, all food - particularly meat and meat products - should be cooked thoroughly to destroy foodborne pathogens.

CONCLUSIONS AND RECOMMENDATIONS

Methods of analysis

Sensitive and reliable methods are available to identify and measure acrylamide in foodstuffs. The measurement uncertainty of the methods is small in relation to the between-sample and the within-lot variability expected for acrylamide levels. Methods are also available to determine biomarker adducts as an alternative means to assess exposure. Interlaboratory validation of analytical methods and the preparation of reference materials and standards for proficiency testing, is desirable. There is a need to develop simple low-cost method(s) to be used for routine monitoring.

- Interlaboratory validation of analytical methods covering a range of different food types should be conducted.
- Reference materials and standards for proficiency testing should be prepared and distributed.
- Low-cost and simple method(s) for routine monitoring of acrylamide in food should be developed.

Modes of formation, fate and levels of acrylamide in food

Acrylamide is formed when some foods are cooked or processed at high temperatures. It seems to arise when different food components react together. These may be carbohydrates, proteins and amino acids, lipids, and possibly other minor food components also. The reaction is promoted by heating and increases with the time of heating. It is not yet clear what combinations of food components are involved and it may well be that the situation is complex with many mechanisms operating. The situation is further complicated by the fact that acrylamide is a volatile and reactive substance that could itself be partially lost after formation. With the limited data available so far, it is not possible to identify any specific routes of formation nor exclude any possibilities. To understand completely the formation and fate of acrylamide in heated foods it will be necessary to conduct hypothesis-driven model studies coupled with a systematic examination of the relation between acrylamide levels and processing/cooking conditions. This understanding would allow formulation, processing and cooking conditions to be optimised to minimise and possibly eliminate acrylamide levels in heated foods.

- The relation between acrylamide levels and processing/cooking conditions should be systematically examined.
- Hypothesis-driven model studies are needed to elucidate sources, mechanism(s) of formation and fate of acrylamide in heated foodstuffs
- Optimization of formulation, processing and cooking conditions to minimize and possibly eliminate acrylamide levels in foods prepared industrially and at home should be investigated
- The range of foods investigated needs to be extended to include staple foods from different regions and diets.

Dietary intake

The range of levels of acrylamide found in foods was broad and the determinants of variability unknown. The foods that have been analysed to date represent only a portion of the total diet and do not include foods representative of those consumed in developing countries (see Annex 3). Nonetheless, based on the available data, food appears to contribute a significant proportion of total exposure. Based on the estimates of biomarkers of exposure (haemoglobin adducts), it seems likely that there are other important sources as well. Additional foodstuffs may be found to contain residues. The available data allowed the Consultation to make only an order-of-magnitude estimate of average long-term dietary intakes of acrylamide in developed countries, which would be 0.3 to 0.8 µg/kg body weight/day. Within a population, it is anticipated that children will generally have exposures two to three times those of adult consumers when expressed on a body weight basis. Although there was inadequate data to reliably estimate exposure for high consumers, their exposure could be several times the mean exposure.

- Further data on the levels of acrylamide in food, particularly staple foods consumed in developing countries, needs to be obtained in order to refine the estimates of dietary exposure.
- An understanding of the mechanisms of formation and fate of acrylamide in foods would help identify those foods (in addition to the starchy foods analysed to date) that are likely to make a major contribution to dietary intakes of acrylamide.
- Information on how food is cooked and processed (domestic and industrial) should be collected to permit reliable estimation of acrylamide intake.
- In collecting data the emphasis should be on foodstuffs contributing most to exposure. In addition to food with the highest values, foods with lower values but high levels of consumption should be sampled. Attention should be paid to the sampling procedures to ensure that representative data are obtained.
- A consistent system for collecting and describing the available data should be used. The GEMS/Food Programme could provide a structure for data collection and reports and the GEMS/Food Regional Diets (http://who.int/fsf/GEMS/index.htm) could provide an indication of important staple foods in each of the regions of the world. National governments may collect data with additional details.
- Developing and other countries with insufficient information for determining population-level dietary exposures to acrylamide should consider generating interim information relevant to their own circumstances. This could include analysing total diet study samples, where they are available, for acrylamide, as the basis for estimating per capita dietary intake estimate; determining levels of acrylamide in a limited range of staple foods prepared in ways that reflect common domestic practice; and, analysing blood or urinary biomarkers of exposure.
- Given the state of knowledge on methods of formation and levels of acrylamide in food, biomarkers of exposure are likely to provide the most direct means of evaluating exposures to acrylamide from food and other sources. These biomarkers need to evaluated and calibrated, and their correlation with dietary intakes should be investigated.
- Other sources of exposure to humans to acrylamide should be investigated to better define the relative contribution of food, smoking and other sources including the potential for endogenous formation of acrylamide.

Toxicology of acrylamide

Considered collectively, data on the absorption, metabolism, distribution and excretion of acrylamide suggest that toxicological findings in animals should be assumed to be relevant for extrapolation to humans.

The Consultation recognized neurotoxicity as the key non-cancer, non- genotoxic effect of acrylamide in humans. Effects on fertility have also been recognized in animals. Single exposures to large doses of acrylamide to humans and animals induce changes in the central nervous system while prolonged exposure to low levels (of relevance to the present risk assessment) result in peripheral neuropathy in the presence or absence of central nervous system involvement. Given the lack of dose-response data for human neurotoxicity, the risk assessment was based on rodent studies, and supported by primate studies of acrylamide neuropathy. Based on these data, the Consultation concluded that the no observed adverse effect level (NOAEL) for acrylamide neuropathy is 0.5 mg/kg body weight/day. The NOAEL for fertility changes is four times higher than for peripheral neuropathy. On the basis of current knowledge, controlling for peripheral neuropathy is expected to control for effects on fertility. The estimated average chronic human dietary intake is in the order of 1 µg/kg body weight/day. This provides a margin between exposure and the NOAEL of 500.

Greater understanding of the hierarchy of target organ toxicity would permit a refinement of the risk assessment for the non-cancer effects of acrylamide. In particular, the relative impacts of acrylamide on the peripheral nervous system, and the central nervous system and fertility would be helpful. Assessment of the impact of acrylamide on the endocrine system also warrants further investigation.

Acrylamide is genotoxic in vivo in somatic cells and germ cells, therefore acrylamide has the potential to induce heritable damage at gene and chromosome level. It is known to be metabolised to glycidamide, a chemically reactive epoxide that forms DNA adducts. The findings that acrylamide induces tumours both in rats and mice at a number of different sites are consistent with a genotoxic mode of action of the chemical. While suggestions have been made that additional modes of action might contribute to the observed spectrum of tumours seen in acrylamide treated rats, especially tumours of hormone-responsive tissues, these suggestions are speculative only. In conclusion, the Consultation endorsed the IARC classification Group 2A that acrylamide is probably carcinogenic to humans.

Generally, introduction of genotoxic and carcinogenic substances into food during manufacturing is prohibited by regulations. However, certain carcinogens are formed in food as a result of cooking, such as benzo[*a*]pyrene and heterocyclic aromatic amines, and because of their formation in domestic settings such chemicals cannot always be controlled. It has recently been discovered that acrylamide is also formed in food cooked in certain ways. These are all genotoxic and carcinogenic substances and are considered to be without a threshold for their action on DNA. For such compounds it is generally recommended that exposures should be as low as reasonably achievable (ALARA). Another approach is to estimate carcinogenic risks. Ideally, such an assessment should be based on extensive epidemiological data that contain both accurate determinations of exposure and the tumour incidence in the exposed human population. Such data are rarely available.

All epidemiological studies have limited power to detect small increases in tumour incidence. Negative epidemiological studies may therefore provide an upper-bound to possible carcinogenic

effects, rather than proof that no such effects exist. Only limited epidemiological data are available for acrylamide, and these provided no evidence of increased cancer risk from occupational exposures.

If experimental animal carcinogenicity data are to be used to estimate human cancer risk, extrapolation has usually to be done over several orders of magnitude down to the human exposure level arising from food. To do so, different mathematical models have been used. The Consultation noted, however, that it is not known whether a given model actually reflects the underlying biological processes. The numerical estimate of risk obtained is critically dependent on which model is used. The Consultation noted that several efforts have been made to use such models to quantify the risk posed by acrylamide in food. The Consultation did not reach consensus on how quantitative risk assessment based on animal data should be used to estimate human cancer risk from acrylamide in food.

Acrylamide has a carcinogenic potency in rats that is similar to that of other carcinogens in food as mentioned above, but the intake levels for acrylamide are likely to be higher. For humans, the relative potencies of cancer-causing agents in food are not known. The Consultation recognized the presence of acrylamide in food as a major concern in humans, given its ability to induce cancers and heritable mutations in laboratory animals.

- More data are required on the absorption, metabolism, distribution and excretion of acrylamide in humans by the oral route to permit more informed estimates of risk to humans
- The formation of glycidamide and binding to DNA as a marker of toxicity and carcinogenicity risk needs to be better defined.
- The bioavailability of acrylamide from food should be determined.
- Risk factors of susceptibility such as genetically-based differences in metabolism and the impact of age, sex or other factors that contribute to risk should be characterized.
- Cancer epidemiology and testicular toxicity in populations of known high exposure, such as occupationally exposed workers with neurotoxic signs and high levels of haemoglobin adducts, should be studied.
- Quantitative risk assessment models should be investigated on the basis of scientific merit and uncertainty of estimates.
- The toxicity and carcinogenicity of glycidamide need to be studied.
- The dose-response characteristics of acrylamide and glycidamide relative to toxicity, disposition, and binding to DNA and macromolecules need to be further assessed.
- Mechanisms of action and dose response characteristics for the effects of acrylamide and glycidamide on germ cell damage should be studied.
- Genotoxic effects on somatic and germ cells using genome-wide expression profiling should be studied.
- The relationship between adducts with haemoglobin and DNA in different organs should be explored.
- Application of new methods in biological research may be helpful in clarifying whether it is possible to establish a threshold for the genotoxicity of acrylamide.

Interim advice

The information on the levels of acrylamide in food is far from complete. Although the magnitude of the cancer risk posed by acrylamide in food was not quantified, the Consultation noted that several principles can be applied now to minimize whatever risk exists:

- Food should not be cooked excessively, i.e. for too long or at too high a temperature. However, all food - particularly meat and meat products - should be cooked thoroughly to destroy foodborne pathogens.
- The information available on acrylamide so far reinforces general advice on healthy eating. People should eat a balanced and varied diet, which includes plenty of fruit and vegetables, and should moderate their consumption of fried and fatty foods.
- The possibilities for reducing the levels of acrylamide in food by changes in formulation, processing and other practices should be investigated.
- An international network "Acrylamide in Food" should be established inviting all interested parties to share relevant data as well as ongoing investigations.

Risk communication

The Consultation would encourage transparent and open risk assessment and risk management processes and recognises the importance of involving interested parties (consumer, industry, retail etc.) in this process at some stages. Risk communication policy could facilitate the crucial communication process between risk assessor and risk manager and among all parties involved.

ANNEX 1

FAO/WHO Consultation on the
Health Implications of Acrylamide in Food
25 - 27 June 2002, Geneva, Switzerland

List of Participants

Dr G. Adegoke, Professor, Department of Food Science and Technology, University of Ibadan, Ibadan, Nigeria

Dr D. Arnold, Acting Director, Federal Institute for Health Protection of Consumers and Veterinary Medicine, Berlin, Germany (Chairman)

Dr R.A. Canady, Toxicologist, Hazard Assessment Branch, Division of Risk Assessment, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, College Park, Maryland, USA

Dr A. Carere, Istituto Superiore di Sanita, Laboratorio de Tossicologia comparata ed Ecotossicologia, Rome, Italy

Dr R. Cary, Health and Safety Executive, Industrial Chemicals Unit, Bootle, Merseyside, England

Mr L. Castle, Food Safety and Quality, Central Science Laboratory, Sand Hutton, York, England

Dr G.W. Diachenko, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, College Park, Maryland, USA

Dr P.B. Farmer, Cancer Biomarkers and Prevention Group, University of Leicester, Leicester, England

Dr M.A. Friedman, Consultant Toxicologist, Oviedo, Florida, USA

Dr K.-E. Hellenäs, National Food Administration, Uppsala, Sweden

Dr M. Hirose, National Institute of Health Sciences, Tokyo, Japan

Dr A.G.A.C. Knaap, Centre for Substances and Risk Assessment, National Institute of Public Health and the Environment, Bilthoven, Netherlands

Dr H. Lingnert, Swedish Institute for Food and Biotechnology, Goteborg, Sweden

Dr B. Petersen, Exponent Inc., Washington, DC, USA

Dr T. Sanner, Department of Environmental and Occupational Cancer, Institute for Cancer Research, Norwegian Radium Hospital, Oslo, Norway

Dr J. Schlatter, Food Toxicology Section, Swiss Federal Office of Public Health, Zurich, Switzerland

Dr B.A. Schlwetz, Office of the Commissioner, US Food and Drug Administration, Rockville, Maryland, USA

Dr F. Shahidi,n, Department of Biochemistry, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

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Dr M. Törnqvist, Department of Environmental Chemistry, Wallenberg Laboratory, Stockholm University, Stockholm, Sweden

Dr J. Van Klaveren, State Institute for Quality Control of Agricultural Products, Wageningen, The Netherlands

Dr P. Verger, Scientific Directorate on Human Nutrition and Food Safety, National Institute for Agricultural Research (INRA), Paris, France

Mr S. Wearne, Head, Chemical Contaminants and Animal Feed Division, Food Standards Agency, London, England (Rapporteur)

Secretariat

Dr J. Herrman, Chemical Safety, World Health Organization, Geneva, Switzerland

Dr M. Luetzow, Food Quality and Standards Service, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, Italy (FAO Secretary)

Ms A. Matula, Chemical Safety, World Health Organization, Geneva, Switzerland

Dr G.G. Moy, Food Safety, World Health Organization, Geneva, Switzerland (WHO Co-Secretary)

Mrs H. Odden Reksnes, Oslo, Norway (WHO Temporary Adviser)

Dr J.M. Rice, International Agency for Research on Cancer, Lyon, France

Dr J. Schlundt, Coordinator, Food Safety, World Health Organization, Geneva, Switzerland

Dr N. Slimani, International Agency for Research on Cancer, Lyon, France

Ms C. Vickers, Chemical Safety, World Health Organization, Geneva, Switzerland (WHO Co-Secretary)

ANNEX 2

FAO/WHO Consultation on the
Health Implications of Acrylamide in Food
25 - 27 June 2002, Geneva, Switzerland

Agenda

Tuesday, 25 June

9:00 - 12:30

Welcome
Introduction of Participants
Election of Chair and Appointment of Rapporteur
Housekeeping announcements
Adoption of Agenda
Introduction of Background Papers
Briefing for Working Groups

14:00 - 17:30 Working Groups on:

- Toxicology, in particular neurotoxicology
- Carcinogenicity, including epidemiology
- Methods of analysis, formation and fate of acrylamide in food
- Dietary exposure, including levels in food, as well as other potential exposures

Wednesday, 26 June

9:00 – 9.30
Plenary progress report from Working Groups

9.30 ¬12:30
Working Groups continued

14:00 - 17:30
Plenary discussion of draft Working Group reports and interim risk management advice to governments, industry and consumers

Thursday, 27 June

9:30 - 12:30
Plenary discussion of recommendations, including data needs and further studies

14:00 - 15:30
Adoption of report and other communication outputs of the meeting
Next Steps

ANNEX 3

Acrylamide levels in different foods and food product groups from Norway, Sweden, Switzerland, the United Kingdom and the United States of America

Food/Product Group	Acrylamide levels (µg/kg) [1]			
	Mean[2]	Median[2]	Minimum – Maximum	Number of samples
Crisps, potato/sweet potato[3]	1312	1343	170 - 2287	38
Chips, potato[4]	537	330	<50 - 3500	39
Batter based products	36	36	<30 - 42	2
Bakery products	112	<50	<50 - 450	19
Biscuits, crackers, toast, bread crisps	423	142	<30 - 3200	58
Breakfast cereals	298	150	<30 - 1346	29
Crisps, corn	218	167	34 - 416	7
Bread, soft	50	30	<30 - 162	41
Fish and seafood products, crumbed, battered	35	35	30 - 39	4
Poultry or game, crumbed, battered	52	52	39 - 64	2
Instant malt drinks	50	50	<50 - 70	3
Chocolate powder	75	75	<50 - 100	2
Coffee powder	200	200	170 - 230	3
Beer	<30	<30	<30	1

[1] The limits of detection and quantification varied among laboratories; values reported as less than a value are below the limit reported by the laboratory.

[2] Mean and median were calculated where individual data were available; samples sizes were extremely small particularly for some food categories; where the mean and median are different it reflects the skewed distribution of the underlying data that were collected in different countries and may represent different food items within the larger category.

[3] Products that are thinly sliced and fried (Includes foods called potato chips in some regions including North America)

[4] Products that are more thickly sliced (Includes foods called French fries in some regions including North America)

MEETING SUMMARY AND RESEARCH NEEDS

Federal Interagency Acrylamide Research Meeting

September 24, 2002
Center for Food Safety and Applied Nutrition
Food and Drug Administration, College Park, MD

Welcome and Introduction, Desired Meeting Outcomes

Dr. Bern Schwetz, Senior Science Adviser, Food and Drug Administration (FDA), opened the meeting with background on acrylamide (AC). He was followed by Dr. Terry Troxell, Director, Office of Plant and Dairy Foods and Beverages, Center for Food Safety and Applied Nutrition, FDA, who discussed desired meeting outcomes, especially the need to coordinate federal research on acrylamide to maximize results and effectively use scarce resources.

Environmental Protection Agency, Research Triangle Park, N.C.

Dr. Rob DeWoskin, National Center for Environmental Assessment, Environmental Protection Agency (EPA) spoke briefly about the IRIS review of AC in 1988. Development of IRIS files under the current EPA program includes preparation of comprehensive toxicological reviews. EPA has begun an IRIS review update in September 2002; internal review will be completed by August 2003, and release to the public is anticipated in June 2004.

National Institute for Occupational Safety and Health, Cincinnati, Ohio

Mr. William J. Moorman summarized NIOSH research activities with acrylamide. NIOSH has performed six Health Hazard Evaluations in the past where AC was suspected as a worker problem. (These may be accessed at http://dshefs.niosh.cdc.gov/hetab/). Coal preparation plant workers have reported neurotoxic symptoms and there is concern regarding AC exposures associated with polyacrylamide flocculents used to precipitate coal particles. NIOSH's study in coal preparation plants will describe and evaluate worker exposure to AC, solvents and manganese, and develop a database of neurotoxic chemicals based on the National Occupational Health Survey of Mining. Information will be obtained by focus group discussions with chemical suppliers, mine operators, union and non-union workers.

NIOSH is also studying potential reproductive and neurological effects of exposure to AC. Worker exposure to AC and congeners will be evaluated using ambient area and personal sampling, dermal sampling, reported exposure data and exposure biomarkers (urinary metabolites, hemoglobin (Hb) adduct levels). In addition, male reproductive health will be assessed (semen quality and sperm DNA integrity, hormone levels, PSA levels and reported reproductive health history). Neurobehavioral parameters will be assessed. Protocol is available from Mr. Moorman of NIOSH.

Mr. Moorman pointed out several aspects of NIOSH research potentially relevant to FDA. NIOSH will evaluate exposure to hemoglobin (Hb) adducts in non-occupationally exposed people, attempting to distinguish between smokers and those with regular dietary uptake of foods containing high amounts of AC. NIOSH will also assess the relative sensitivity of reproductive and neurological effects. The study will analyze levels of a B6 metabolite in urine, as B6 supplementation has been shown to antagonize AC neurotoxicity in rats. Genetic differences (*i.e.*, polymorphisms for enzymes in the pathway) affecting AC metabolism will be evaluated.

Center for Disease Control and Prevention, National Center for Environmental Health

Dr. Hubert Vesper described CDC's AC research. Currently CDC is developing a method to analyze Hb adducts (N-Val) of AC and its metabolite, glycidamide (GC). Peptide-based standards and calibrators will be developed and characterized. AC and GC adducts in people will be assessed in special studies and in NHANES. CDC's method will be based on procedures described by Springer *et al* (J. Tox. Environmen. Health 1993; 40:161-176) and Jeppsson *et al.* (Clin. Chem. Lab. Med. 2002; 40:78-89). The method currently in development is based on a well-established procedure which uses a well defined, specific and stable analyte, shows a good correlation between exposure and health risk, and reflects exposure over the last 3 months. The method also shows good precision and accuracy and is independent of fasting status and diurnal variation. In the future, CDC hopes to create reference materials, perform method comparisons, and establish relationships between other analytes (*i.e.*, DNA adducts, free serum AC) and Hb adducts.

National Center for Toxicological Research, FDA, Little Rock, Arkansas

Dr. Daniel Doerge presented a brief review of AC metabolism and disposition, and carcinogenicity. NCTR proposes to study AC DNA adducts using stable labeled analogs, a validated LC-/MS/MS method, and DNA from a short-term rodent exposure (leukocytes and target tissues). In an *in vivo* mutagenicity study in transgenic rats and mice (Big Blue), administered AC and GC (short-term exposure in drinking water), target tissues will be identified and correlated with GC-DNA adducts.

NCTR also proposes to develop and validate a LC/MS/MS method for serum AC/GC and perform a toxicokinetic analysis for AC and GC, including looking at AC bioavailability (*i.v.* vs. oral gavage studies) in an AC-fortified diet. AC/GC Hb adducts (N-Val) will be determined in rodents after short-term exposure and correlated with rodent GC-DNA adducts.

In human volunteers, "background" GC-DNA and AC/GC Hb adducts will be measured and compared to those in cigarette smokers. These data will be compared with rodent dose-responses for exposure estimation.

In addition, Dr. Fred Beland will be leading a two-year rodent carcinogenicity bioassay using drinking water exposures to AC and GC in male and female F344 rats and B6C3F1 mice. The benefits and need for using feed-incorporation as the delivery mechanism will also be evaluated. The study will be designed especially to yield a dose-response relationship. GC-DNA adducts levels in target tissues will be correlated with tumor incidences.

National Institute for Environmental Health and Safety, Research Triangle Park, N.C.

Dr. Jack Bishop presented NIEHS/NTP GeneTox data on AC. A reproductive assessment using continuous breeding has been conducted on AC as well as several AC congeners (N-hydroxymethylacrylamide, methacrylamide, and methylene bisacrylamide). Significant adverse reproductive effects were seen in the absence of overt neurotoxicity. Germ cell assays including the dominant lethal test, the heritable translocation test, PAINT/DAPI 1st-cleavage embryo chromosome damage (developed by Dr. Francesco Marchetti, Lawrence Livermore National Laboratory, Livermore, CA), the specific locus test and adduct binding, have been conducted on AC. The NIEHS/NTP study showed that paternal exposure to AC significantly increased the frequencies of zygotes with chromosomal abnormalities especially during the last two weeks of spermatogenesis. There was no selection against unstable

aberrations between the first and second metaphase stage. PAINT/DAPI analysis of zygotes and 2-cell embryos showed that unstable aberrations are associated with embryo loss during pregnancy and that stable aberrations are associated with heritable translocations at birth.

Dr. Bishop also noted that, in a 13-week, multidose, drinking water dominant lethal study on N-hydroxymethylacrylamide conducted by the NTP, the induction of germ cell mutations appeared to be associated with attainment of a total accumulated exposure dose of greater than 1000 mg/kg. This could have important biological relevance for chronic low dose AC exposures in food

Dr. Bishop noted that the review by Dearfield (Mutation Res. 1995 330:71-99) has summarized information showing that AC is negative in Salmonella, causes chromosome aberrations *in vitro* and *in vivo*, is positive in the rat and mouse dominant lethal test, is positive in the mouse heritable translocation test, and generally causes reproductive and developmental toxicity. However, most of the *in vivo* tests of AC have been conducted in mice using the *i.p.* route of exposure and at relatively high doses of 50-150 mg/kg.

Dr. Bishop recommended that human epidemiology studies (for example, those under development by NIOSH) should include collection of sperm for sperm FISH analysis and measure of adducts, protamine and DNA . A low-dose PAINT/DAPI study should also be conducted.

Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD

Dr. Richard Canady summarized CFSAN's data on AC. The agency's initial response was to perform a hazard assessment using the Swedish data on AC levels in foods, U.S. consumption rates and FDA dose-response evaluations developed for AC in food packaging contact issues. This assessment has indicated that the Swedish data is probably correct, further action is needed, and that the hazard is not clearly insignificant.

FDA's occurrence data shows that the range of AC levels is similar to that reported previously at the WHO Consultation, and that cooking time and temperature make a difference in AC levels.

In 2003, the Total Diet Study will include AC monitoring. FDA will encourage collaboration between government, trade groups, consumer groups, and academia to achieve public health improvements.

FDA will hold a public meeting on September 30, 2002 and a Joint Institute for Food Safety and Applied Nutrition (U of MD/CFSAN consortium) meeting October 28-30 in Chicago in which members of the food industry will participate.

Since AC levels seem to increase with frying or baking, the need exists to clarify nutritional needs vs. risk aversion choices. For example, if we reduce exposure, what negative impact will this action cause on nutrition, microbial risk and other added risk factors? It is important to FDA that these risk management alternatives be discussed in public meetings, such as the meeting planned September 30, 2002.

Dr. Canady also summarized the conclusions of the World Health Organization Consultation on Acrylamide. Analytical methods are judged adequate to confirm occurrence. The formation mechanism of AC in foods in unknown. Exposure is in the sub to low mcg/kg/day range, with children possible receiving several fold higher levels. Neurotoxicity lowest observed adverse effect levels (LOAELS) are well above current observed consumer exposures. AC is an animal carcinogen and may also induce heritable damage. The consumer message to date is to reinforce dietary guidelines (*i.e.*, consumption of a balanced diet) with limited advice on cooking.

The WHO Consultation listed the following research needs: 1) define GC-DNA binding as a marker of toxicity/risk; 2) describe the relationship of Hb adduct to DNA adducts in different organs; 3) describe the susceptibility to AC, as influenced by metabolism variations,

age, gender, other, etc.; 4) evaluate human exposure using biomarkers which are correlated and calibrated with intake; 5) look at other sources of exposure; 6) acquire toxicity/carcinogenicity data for GC; 7) identify mechanisms for germ cell damage and linearity/non-linearity of genotoxicity; and 8) conduct an epidemiology study of cancer and testicular effects in workers that had neurotoxic signs and high AC-Hb adducts measures.

SUMMMARY OF DISCUSSIONS OF BREAKOUT GROUPS

BREAKOUT GROUP I. Toxicity, Toxicokinetics and Bioavailability of Acrylamide (AC)

Question 1: What research is needed to improve the risk characterization of AC relevant to food exposures?

The group stated the need to better define germ cell mutations generated by AC and/or glycidamide (GC). Low and multiple dose exposure effects need to be defined; a dose response needs to be established. Results from acute, subchronic and chronic exposures should be assessed; out comes should be compared when AC is administered in a food matrix or in the drinking water. Past routes of exposure to AC have largely been i.p. and/or skin painting.

It was pointed out that FDA/CFSAN has never regulated a substance based on positive germ cell mutations in animals. The group recommended that other types of mutations resulting from AC and particularly GC, be re-assessed. Some members believed that the Big Blue Mouse, Big Blue Rat and Tk+/- animal models would be appropriate to use in such assessments along with standard assays, such as micronuclei, etc.

The group agreed that neurotoxicity had been well documented for AC. However, when conducting the chronic studies with AC and GC, notice should be taken of neurotoxic endpoints, such as distal peripheral axonopathy or synaptopathy, energy pathways, e.g., mitochondrial energy pathways.

All members thought that hemoglobin (Hb) adduct determination was an excellent way to assess exposure, in both animal models and in humans. The need to measure protamine adducts in germ cells was also discussed. Every effort should be made to involve the NHANES database and to make certain that measurements for AC be included in future NHANES as soon as possible. Though NHANES is a cross-sectional study that provides only limited information on dietary behavior, it allows the assessment of the general AC exposure of the U.S. population and provides data on smoking and occupation, two other possible sources of AC exposure. Furthermore, it provides information on diseases and health conditions such as anemia (low Hb concentrations in blood) or diabetes, (formation of Hb adducts of glucose that may compete with AC adduct formation) which could confound results obtained with Hb adducts. However, the importance of these possible confounders needs to be assessed. Further specialized studies investigating AC exposure from food need to be performed. Perhaps susceptible populations or populations known to consume large amounts of fried foods should be over-sampled. Many group members believed that an epidemiology study would be extremely difficult given the wide variety of "rodent carcinogens" and other "tumor promoter" substances known to be in most American diets.

The determination and identification of DNA adducts by NCTR in upcoming rodent studies was strongly supported, and some believed it important to attempt to determine if such adducts are present in humans, perhaps using human lymphocytes.

The importance of comparing and contrasting metabolic and pharmacokinetic studies of AC and GC in rodents was discussed. The issue of polymorphism was discussed. It was noted that only induction of particular isozymes, *e.g.*, 2E1, was likely in rodents, but that polymorphism would certainly be an issued for humans exposed to AC. Some members of the group believed that human metabolism and pharmacokinetic studies should be considered.

The group supported the need to conduct a well-controlled chronic toxicity/carcinogenicity study that would establish a dose response, thus providing CFSAN with data that would be the basis for

conducting a scientifically sound risk assessment. Many believed that it was particularly important to conduct cancer studies in the mouse because of metabolic similarities to humans. Parallel studies with AC and GC were deemed important, both in the rat and in the mouse model. All agreed that it was important to determine differences in bioavailability between AC/GC administration in drinking water vs. a food matrix. The consensus of the group was that FDA's nomination of AC and GC to the National Toxicology Program as FDA's Fiscal Year '03 priority chemical was appropriate. It was agreed that assessment of germ cell damage (adduct and structural/numerical chromosome damage) should be included in the NTP study.

It was also agreed that the FDA-sponsored studies should include hormone level assessment, particularly those hormones that modulate organ and cell growth in the sex organs. Several discussants mentioned work being done by researchers outside government to assess the effect of AC on endocrine modulation, focusing on LH/FSH levels and on prolactin, estrogen and progesterone levels. Hormone studies should also be done in humans; NIOSH intends to study hormones in the male reproductive studies in workers exposed to AC.

Research needs may be summarized as follows:

Germ cell mutations:

- There is a need to define more completely germ cell mutations generated by AC and/or GC. The current mouse germ cell data (dominant lethal, heritable translocation, morphological specific locus and Paint/Dapi) define well the type of damage induced by AC and GC at high doses. The issue is only relevant to low dose exposures.
    - Assess low dose and multiple dose exposure effects;
    - Establish a dose response;
    - Assess the results from acute, subchronic and chronic exposures, and
    - Compare the outcomes when administered in a food matrix or in drinking water.
- Oral route of exposure judged to be most relevant, as opposed to past studies which used i.p. and or skin painting.
- Incorporation of germ cell toxicity into NIOSH epidemiology study.
- Accumulation of defects over time from repeated exposures.

Regulatory importance of this endpoint: Germ cells may be the most sensitive target. Exposure assessments may demonstrate significant germ cell accumulations that extrapolate to significant germ cell damage and/or significant germ cell damage in chronic low dose exposure studies may be demonstrable. If these prove to be true, is CFSAN willing to regulate a compound based on positive germ cell mutations in animals?

Other Types of Mutations:

- Re-assess the types of mutations that result from AC and particularly GC.
    - Use Big Blue Mouse, Big Blue Rat and Tk+/- animal models.
    - Use in addition standard Salmonella tests, micronuclei, chromosomal aberrations, etc. models.

Neurotoxicity

Include neurotoxic endpoints, such as distal peripheral axonopathy, synaptopathy, energy pathways, (*e.g.*, mitochondrial) when conducting the chronic studies with AC and particularly with GC.

DNA and Hemoglobin Adducts:

- Use Hb adduct determination as best way to assess exposure, in both animal models and in humans.
- Include measurements for AC in future NHANES assessments as soon as possible.
- Determine if an epidemiology study is feasible, given the difficulty of distinguishing between effects caused by AC/GC and other "rodent carcinogens" and "tumor promoters" in the American diet.
- Determine if such adducts are present in humans, perhaps using human lymphocytes.
- Measure protamine adducts in sperm.

Metabolism and Pharmacokinetic Studies:

- Compare and contrast general metabolism and pharmacokinetic studies of AC and GC in rodents.
- Evaluate AC induction of particular isozymes (*e.g.*, CYP2E1) in rodents to help interpretation of relevance of induction (by AC and other inducers) in humans.
- Evaluate likely effect of CYP2E1 polymorphism for humans exposed to AC. Consider performing human metabolism and pharmacokinetic studies.

Subchronic and Chronic Studies:

- There is a need for a well-controlled chronic toxicity/carcinogenicity study, establishing a dose response.
- Recognizing the past two industry-sponsored studies in rats, cancer studies in the mouse are particularly important to conduct because of metabolic similarities to humans.
- Parallel studies with AC and GC are important, both in the rat and the mouse model.
- Differences between AC/GC administered in drinking water and in a food matrix should be determined.
- Both AC and GC are being nominated by FDA to the National Toxicology Program as FDA's Fiscal Year 03 priority chemicals. Such studies will be conducted at NCTR under an interagency agreement between the FDA/NCTR and the NIEHS/NTP.

Alternative Modes of Action for Carcinogenicity:

There is ongoing research outside government to assess the effect of AC on endocrine modulation, focusing on LH/FSH levels and on prolactin, estrogen and progesterone levels. Studies conducted by the government should include hormone level assessments, particularly those hormones that modulate organ and cell growth in the sex organs. Interface with non-governmental researchers should be maintained.

Reproductive and Developmental Studies:

It was generally agreed that measurement of protamine and DNA adducts in sperm, and Paint/Dapi and sperm FISH assessment of numerical and structural chromosome damage in sperm would be included in any NTP bioassay of AC or GC.

Question 2. What are the priority needs; what sequencing of research is needed?

The first four research projects may be viewed as basic to the other suggested research areas and are therefore ranked as top priority.

Cooperation and data sharing:

Communication and sharing of data between government, academia and industry, as well as European research groups, are top priorities.

Method of analysis for Hb adducts:

Hb adducts appear to be the current most appropriate biomarker of AC exposure. A method for analysis of adducts in human and rodent tissues must be developed and tested collaboratively. In addition, the effect of cumulative exposure on adduct formation should be tested.

Bioavailability studies:

These studies should include effects of storage, various cooking temperatures and conditions, etc. on levels and bioavailability of AC and GC in rodent and human diets and drinking water.

Germ cell mutations:

- Incorporate germ cell toxicity observations into NIOSH epidemiology study. Do defects accumulate over time from repeated exposures to AC?
- Current mouse germ cell data (dominant lethal, heritable translocation, morphological specific locus and PaintDapi) define the type of damage induced by AC and GC at high doses. However, low dose and multiple dose exposure effects should be assessed, and a dose response should be established. Outcomes should be compared when the doses are administered in food or in drinking water as opposed to i.p. administration and/or skin painting.

The remaining research projects will require significantly more time and effort and, to some extent, depend on successful completion of the above three areas.

Metabolism and disposition of AC:

- Examine AC/GC Hb adduct formation/elimination kinetics when doses have been administered from the diet.
- Determine rodent GC-DNA and AC/GC Hb adducts.
- Determine polymorphism of P450 2E1 and its significance in response to AC.
- Synthesize and characterize GC-DNA nucleoside adducts, develop and validate an analytical method (LC-ES/MS/MS) for these adducts. Use leukocytes and target tissues in rodents to determine DNA adduct levels from short-term exposure.

In vivo mutagenicity:

Use transgenic mice (Big Blue, Tk+/- mice) with short-term drinking water exposures to AC and GC. Identify target tissues and correlate with GC-DNA adducts.

Biomarkers in humans:

- Measure “background” GC-DNA and AC/GC Hb adducts in “normal” and smoking volunteers. Compare these levels with rodent dose-responses to estimate exposure. Investigate effect of diet, such as high AC foods, on adduct levels. There are urinary markers of AC exposure in humans. Recent human exposure has been evaluated on levels of

mercapturic acids (AC metabolites with an estimated 8 hour half-life) in mid- to late-workweek, and post-shift urine samples in AC-exposed workers.

Carcinogenicity bioassay:

Drinking water exposure to AC and GC in male and female mice and rats. Correlate GC-DNA adduct levels in target tissues with tumor incidences.

Question 3. Identify any areas of overlap and potential coordination between the agencies or with outside parties (where such research efforts are known to exist) for planned research.

Results of the recent Joint Institute for Food Safety and Applied Nutrition (JIFSAN – consortium between University of Maryland and FDA/CFSAN) conference in Chicago should help in elucidating what areas industry is pursuing in AC research. Results of the meeting will be available in the near future. An acrylamide working group including members of the food industry, academia, and the federal government has been formed. Data are to be shared through the CFSAN Risk Assessment Consortium. It is important that industry research results be made available to federal researchers so that duplication of effort may be avoided and industry results may be evaluated in the light of federal efforts. It would be helpful if industry and federal research groups would use a similar method for analyzing foods for AC so that results would be comparable. Similarly, if industry is analyzing samples of blood or other tissue from humans for either the Hb adduct or the DNA adduct, the methods used by industry researchers should be compatible with those used by federal agency laboratories.

Research on DNA adducts should continue. Dr. Beland of NCTR indicated that an analytical method should be available in about one year.

Ongoing communication should be maintained between federal agencies (and with industry) to prevent overlap and to efficiently utilize resources. One agency may be expected to benefit in the conduct of its AC/GC research by knowing the progress and findings of other federal agencies.

NIOSH is conducting an assessment of both neurotoxicity and male reproductive health. The neurobehavioral assessment will include measurements of tactile sensitivity, postural stability, manual dexterity, and simple reaction time. The male reproductive assessment will include semen, hormone, and prostate specific antigen analysis, as well as reported reproductive health history, of both exposed and unexposed workers. Exposure will focus on biomarkers of exposure and effects. Project officer is William Moorman, NIOSH/CDC.

There is a need to establish a federal interagency workgroup as a means of facilitating communication and, where possible, of enabling coordination (through an IAG or other means) of research across federal agencies.

BREAKOUT GROUP II – Measuring and evaluating exposures to acrylamide through biomarkers (exposure or effect)

Question 1. What research is needed to improve the risk characterization of acrylamide relevant to food exposures?

The group identified the following research needs:

- Measuring chronic exposure to AC and its association to health outcomes and germ mutations

- Establishing correlation between animal studies and human health
- Epidemiological studies (cross-sectional and longitudinal).

The first area of research for developing biomonitoring markers should focus on protein adducts for AC and GC, since this methodology is fairly well developed to date. A key problem is variability; that is, multiple analyses of the same sample may produce widely varying levels of AC. Detection limits need to be established and adducts should be characterized across species, laboratory animals vs. humans. Analytical standards need to be acquired and Round Robin analysis trials will be necessary.

After the methods have been validated, samples from NHANES and other appropriate populations should be sampled for AC and GC. The NHANES sampling will not only permit the establishment of a reference range for humans, but also provide opportunities to look at exposure pathways, such as smoking and possible dietary intake. It may also be possible to segment out more susceptible populations of exposure for further study.

Research on DNA adducts should also continue as work on protein adducts for AC proceeds. Leukocytes and hair were mentioned as possible matrices for DNA adduct research.

FDA/CFSAN may need to address the issue of cumulative exposure of the consumer to AC, *e.g.*, from smoking, from consuming fried foods, from food contact packaging materials, etc.

Question 2. What are the priority needs; what sequencing of research is needed?

Priority should be given to development of a reliable method for measuring AC Hb adducts. CDC should put together a meeting as described above including federal, European, academic and industry researchers by either December 2002 or January 2003. Coordinator is Dr. Gary Myers of CDC.

At the same time, development of the DNA adduct analysis method should proceed, as well as the nomination of AC and GC for the NTP bioassay program.

Communication between federal agencies, industry and academia involved in AC research is a priority.

Question 2. Identify any areas of overlap and potential coordination between the agencies or with outside parties (where such research efforts are known to exist) for planned research.

Possibilities exist for collaboration between CDC and NIOSH on a cohort study and with NCTR on methodological issues. It was suggested that a more permanent interagency working group on AC exposure research be established to increase efficiency in governmental research efforts.

It was agreed that CDC should convene a select working group of laboratory experts to look at measurement issues needed to improve and standardize AC measurement results in humans. This meeting should include representatives of industry, European countries, academia and federal agencies to get the maximum benefit from available laboratory resources. Due to the urgency, CDC will attempt to convene such a meeting in Dec. 2002. Coordinator is Dr. Gary Myers of CDC.

Acrylamide in Food Workshop: Scientific Issues, Uncertainties, and Research Strategies

- Working Group 3: Exposure and Biomarkers
- Working Group 4: Toxicology and Metabolic Consequences
- Working Group 5: Risk Communication
- Priority Research Needs Identified by the Working Groups [pdf]
- Planning Committee's List of Short-Term Action Items [pdf]
- Participant List [pdf]

HOME

Acrylamide in Food Workshop:

Scientific Issues, Uncertainties, and Research Strategies

O'Hare Ramada Plaza Hotel

Rosemont, Illinois

USA

October 28-30, 2002

Introduction

Background

In April 2002, Sweden's National Food Administration announced, at a press conference, the finding of a wide range of amounts (less than 30 ppb to approximately 2,300 ppb) of acrylamide in a select sample of foods. According to scientists at Stockholm University, acrylamide appeared to be formed during the heating (preparation) of several different foods. It had not previously been identified in foods at the levels reported. These findings were released prior to publication in order to alert the world that acrylamide could be an issue in food products.

The toxicological effects of acrylamide have been studied in animals where it has been observed to be carcinogenic. Carcinogenicity in humans has not been demonstrated in epidemiological studies, although it cannot be excluded. Acrylamide has been classified by the International Agency for Research on Cancer (IARC) as "probably carcinogenic to humans" (Group 2A). It is a neurotoxicant whose effects have been observed in humans in cases of occupational exposure.

A Joint FAO/WHO Expert Consultation was rapidly convened in June to undertake a preliminary review of new and existing data and research on acrylamide. The findings of that consultation call for further study of the levels and extent of acrylamide in food products, mechanisms of formation, bioavailability, exposure, and toxicological implications.

Workshop

An ad hoc Acrylamide Working Group composed of food industry, trade association, academic and government representatives has been monitoring and discussing the issue of acrylamide in food since shortly after the first announcement in Sweden. It became apparent that a workshop, concentrating on science, was needed to openly discuss the issues, to identify apparent knowledge gaps, and to identify short- and long-term approaches to generating the required information/knowledge.

As a result, the workshop "Acrylamide in Food: Scientific Issues, Uncertainties, and Research Strategies" was held at the O'Hare Ramada Plaza, Chicago, October 28-30, 2002. The meeting was organized by the Joint Institute for Food Safety and Applied Nutrition (JIFSAN), University of Maryland, College Park and the National Center for Food Safety and Technology (NCFST), Illinois Institute of Technology, Argo-Summit. Funding for the workshop was provided by registration fees from its 170 participatnts from around the world.

Five Working Groups were established to lead the discussions of the experts: Mechanisms of formation of acrylamide in food; Analytical methodology; Exposure and

biomarkers; Toxicology and metabolic consequences; and Risk communication. Each was led by an organization involved in planning the meeting. A position paper was prepared for each of the Working Groups and was used as the basis to initiate discussions of that group during the workshop. Participants and observers were invited for each of the five Working Groups, as well as several "general" observers who circulated among the Working Groups. The workshop was not a consensus-building activity, but provided expert information to the Planning Committee for its further deliberations and distribution for others to use.

A list of seven questions was addressed by each Working Group. An additional list of questions was developed for each Working Group to guide the discussions in their particular subject matter area. Discussions occurred over three half-day sessions. Finally, a limited number of conclusions representing high priority research needs and data gaps were presented by each Working Group in a final plenary session for open discussion among all participants.

Following adjournment of the workshop, the Planning Committee met to review the high priority conclusions with a focus on identifying those that could be addressed through short-term research projects. Specific action projects, coordinated by the ad hoc Acrylamide Working Group, will be developed and initiated.

For each Working Group, the position papers, audiovisuals presented during the meeting, and summary presented at the closing plenary session emphasizing their high priority conclusions are presented. Also included are a listing of the general questions, high-priority conclusions from each Working Group, and the Planning Committee's List of Short-term Action Items.

Questions Addressed by Each Working Group

1. What are the primary areas concerning the occurrence of acrylamide in food in which research is needed?

2. Are methods currently available to accomplish this research?

3. What is the time frame for getting results for the research identified?

4. What missing information is needed to enable the proposed research to be initiated or accomplished?

5. What questions will be answered by each research area proposed?

6. Rank the research areas/projects identified in order of priority for accomplishment.

7. Where is your Working Group linked to others, i.e., from what other Working Groups do you need assistance?

Overview of Acrylamide Toxicity and Metabolism

Prepared for

JIFSAN/NCFST Workshop on Acrylamide in Food

Toxicology and Metabolic Consequences Working Group

October 2002

This background document was developed prior to the workshop and was used by the working group in its discussion of the current state of knowledge regarding the toxicity and metabolic consequences of acrylamide in food and in identifying data gaps and conclusions regarding what research would fill those gaps and what research should occur first. Through the course of the working group's discussion of the state of knowledge, various hypotheses were raised to help formulate specific research projects that might fill the identified data gaps. Some modifications were made to the background document as a result of the working group's discussion and input, but the document should not be considered to necessarily represent the consensus views of the working group or of any individual participant in the working group.

1.0 Background

Recently, it has been reported that acrylamide monomer may form in certain foods cooked at high temperatures. The highest concentrations of acrylamide have been identified in potato and grain-based foods that are cooked at very high temperatures (e.g., frying, grilling or baking) (Tareke et al. 2002). Acrylamide levels as high as 3500 μg/kg have been reported in potato chips and French fries. Acrylamide is thought to form in food principally from the interaction of the amino acid asparagine with glucose or other carbohydrates.

Acrylamide has been extensively investigated and has a large database of very complex toxicity, pharmacokinetic and mode of action studies. The results of the animal toxicity studies indicate that acrylamide is carcinogenic in rodents and produces toxic effects on the reproductive and nervous systems. However, to date, only neurotoxicity has been demonstrated in humans. The purpose of this paper is to provide the reader with a broad overview of the toxicity and toxicokinetics of acrylamide and related issues rather than provide detailed information on each acrylamide study published in the literature. The studies cited in this report were considered the most appropriate for the characterization of the toxicity and issues related to the potential for acrylamide toxicity from the ingestion of acrylamide in food. It should be noted that biomarkers of exposure are discussed in another paper and will not be discussed here.

2.0 Physical Chemical Properties

Acrylamide is an odorless, white crystalline solid at room temperature, with a molecular formula of C_3H_5NO and weight of 71.08. Acrylamide is readily soluble in water (2155 g/l at 30°C) and polar solvents (e.g., acetone, methanol, and ethanol), but not in non-polar solvents (e.g. carbon tetrachloride). Acrylamide has a density of 1.27 g/l (25°C), a boiling point of 136°C at 3.3 kPa and a melting point of 84-85°C. Acrylamide contains an α,β-unsaturated amide system that reacts with nucleophilic compounds via a Michael addition. The major site of reaction is sulfhydryl groups contained on proteins and amino acids.

3.0 Toxicokinetics

A limited number of toxicokinetic studies with acrylamide were available. These studies were conducted with radiolabeled acrylamide, and the results reported are for total radioactivity, unless otherwise noted.

3.1 Absorption

Numerous studies, both *in vivo* and *in vitro*, have been conducted to evaluate the potential dermal absorption of acrylamide; however, no studies attempted to directly quantify absorption of acrylamide following oral or inhalation exposure. Results from distribution and excretion studies indicated that following oral administration in rats, acrylamide was readily absorbed (Miller et al. 1982; Marlowe et al. 1986; Ikeda et al. 1987). In a study conducted by Barber et al. (2001), rats received a single dose of acrylamide by gavage (20 mg/kg/day) or by intraperitoneal injection (50 mg/kg/day). Although no quantitative estimate of oral absorption can be made based on the results of this study, a comparison of the dose-adjusted area under the plasma concentration time curves (AUCs) between the two routes of exposure can provide

a qualitative estimate of oral absorption. Comparison of the dose-adjusted AUCs indicated a difference in absorption between the two routes that could not be explained by differences in dose (20 versus 50 mg/kg/day). This comparison indicated that systemic absorption following oral exposure appeared to be slightly less than observed following intraperitoneal administration of acrylamide, with greater conversion to glycidamide following oral exposure.

Results from *in vivo* studies conducted in rats indicated that dermal absorption ranged from approximately 14% to 61% of the applied dose (Ramsey et al. 1984; Frantz et al. 1995; Sumner et al. 2001). *In vitro* results obtained from rat, pig and human skin samples indicated 42 to 93%, 94%, and 27 to 33% of the applied acrylamide dose was absorbed for each species, respectively (Frantz et al. 1995; Diembeck et al. 1998; Marty 1998).

3.2 Distribution

Regardless of route of exposure, acrylamide appears to be rapidly distributed to tissues (Miller et al. 1982; Ramsey et al. 1984; Marlowe et al. 1986; Sumner et al. 2001). Following oral administration of 1 mg acrylamide/kg body weight, distribution of acrylamide in dogs and pigs was greatest to the muscle tissue, ranging from approximately 30 to 50% of the administered dose (Ikeda et al. 1987). In dogs, the greatest radioactivity after muscle tissue was observed in the liver (~14%), while in the pig it was observed in the gastrointestinal tract (~20%). The authors concluded that this may reflect slower absorption in the pig, compared to the dog. When fasted and pregnant female (days 13.5 and 17.5 days of gestation) Swiss Webster mice were administered 120 mg [2,3-^{14}C]-acrylamide/kg by gavage, fetuses examined at 13.5 days were uniformly labeled with the exception of slightly increased uptake in the fetal brain, which is typical of distribution of many compounds into fetuses at this stage of gestation (Marlowe et al. 1986). At 17.5 days, the distribution pattern resembled that in the maternal tissues, with the exception of high levels in the fetal skin.

At 24 hours following a 6-hour inhalation exposure to 3 ppm acrylamide, the majority of the absorbed dose in rats was found in the blood, followed by the skin, spleen and lung. In mice administered the same concentration via the same protocol, a different pattern of distribution was observed, with the highest fraction of absorbed dose reported in the skin, followed by the subcutaneous fat, testes, and blood (Sumner et al. 2001).

Studies in rats (Sumner et al. 2001) and mice (Carlson and Weaver 1985) evaluated the distribution of acrylamide following dermal application of 100 to 160 mg acrylamide/kg body weight. At 24 hours post-exposure, acrylamide levels were highest in the skin of both species. In the rat acrylamide concentrations of approximately 1 µmol/g tissue were reported in red blood cells. The only other location where the concentrations were higher was at the site of application (e.g., the skin: 4 µmol/g tissue) (Sumner et al. 2001). A similar pattern was observed in the mouse following dermal application of acrylamide although no measurement of acrylamide in the blood was conducted (Carlson and Weaver 1985).

Following intravenous administration of 10, 50, or 100 mg/kg acrylamide to rats, the majority of the administered acrylamide was found in the red blood cells at most time points (Hashimoto and Aldridge 1970; Miller et al. 1982; Ramsey et al. 1984). The distribution of administered acrylamide to the blood is related to the ability of both acrylamide and its metabolite glycidamide to bind to hemoglobin to form adducts (Calleman et al. 1990).

3.3 Metabolism

The major metabolite formed via the cytochrome P450 pathway is glycidamide (Miller et al. 1982). Species differences in the formation of this metabolite have been observed, with acrylamide converted to glycidamide to a greater extent in the mouse than in the rat, based on urinary metabolites (Sumner et al. 1997).

Once absorbed, acrylamide may be conjugated by glutathione-S-transferase (GST) to N-acetyl-S-(3-amino-3-oxopropyl) cysteine or it reacts with cytochrome P450 (CYP450) to produce glycidamide (Figure 1) (Dixit et al. 1980; Dixit et al. 1981c; Dixit et al. 1982; Miller et al. 1982; Calleman et al. 1990; Sumner et al. 1992). The major metabolite formed in both rat and mouse is N-acetyl-S-(3-amino-3-oxopropyl)cysteine, accounting for approximately 70% of the urinary metabolites observed in the rat and 40% of those observed in the mouse (Sumner et al. 1997). Several metabolic studies have been conducted that focused on the interaction of acrylamide with CYP450 and glutathione (GST) in the rat and the mouse (Dixit et al. 1980; Dixit et al. 1981c; Mukhtar et al. 1981; Das et al. 1982; Dixit et al. 1982). Results of these studies indicated that liver, kidney, brain and erythrocyte GST have significant binding capacity with acrylamide, with the liver GST being 3 times more efficient in conjugating acrylamide as compared to brain GST in the rat (Dixit et al. 1984). Additional studies in the rat indicate that acrylamide may inhibit GST, resulting in increased metabolism to glycidamide by the CYP450 pathway (Dixit et al. 1981b; Dixit et al. 1984).

3.4 Excretion

Excretion of acrylamide as the parent compound in rats is low (<2%) and occurs predominantly in the urine (Miller et al. 1982). In studies where radiolabeled acrylamide was administered via oral, dermal, or inhalation routes, approximately 40 to 70% of absorbed radiolabel was excreted in the urine, 5 to 6% in the feces, 6% in exhaled air, and 15% in the bile (Hashimoto and Aldridge 1970; Miller et al. 1982; Sumner et al. 1992; Sumner et al. 2001). The estimated plasma half-life for acrylamide and glycidamide following oral (20 mg/kg) or intraperitoneal (50 mg/kg) administration in male Sprague Dawley rats was approximately 2 hours for both acrylamide and glycidamide following single or repeated exposures (Barber et al. 2001).

3.5 DNA and Protein Adducts

3.5.1 Protein Adducts

Both acrylamide and glycidamide are electrophilic and can form adducts with sulfhydryl groups on hemoglobin and other proteins (Hashimoto and Aldridge 1970; Calleman et al. 1990; Bergmark et al. 1991; Barber et al. 2001). Acrylamide contains an α,β-unsaturated system that reacts with nucleophilic compounds via a Michael addition. The major site of reaction is cysteine with sulfhydryl groups, although acrylamide may also react with amino groups.

Hemoglobin adducts have been used as biomarkers of exposure and to estimate internal dose in occupationally exposed populations (Calleman et al. 1994; Hagmar et al. 2001). In a study of 41 workers employed at an acrylamide production plant, a neurotoxicity index specifically designed for acrylamide peripheral neuropathy correlated with acrylamide hemoglobin adduct levels (Calleman et al. 1994). In a study of 210 workers exposed to grouts containing acrylamide for approximately 2 months, there was a significant correlation between

hemoglobin adduct levels and exposure categories estimated by self-reporting (Hagmar et al. 2001). Hemoglobin adduct levels ranged from 0.02 nmol/g globin to 17.7 nmol/g globin.

When acrylamide was administered to rats, the conversion to glycidamide was 51% following the administration of 5 mg/kg and decreased to 13% after 100 mg/kg, based on the measurement of hemoglobin adducts (Bergmark et al. 1991). The conversion of acrylamide to glycidamide was higher following subchronic dosing as well. Acrylamide hemoglobin adduct formation was approximately 2- or 4.5-fold greater for oral (20 mg/kg/day for 15, 21, 34 or 47 days) or intraperitoneal (50 mg/kg/day for 11 days) dosing, respectively, than glycidamide adduct formation (Barber et al. 2001). Oral administration resulted in approximately 30% fewer acrylamide adducts than intraperitoneal dosing; however, more glycidamide adducts were formed after oral dosing. The authors suggested that the reason for these differences was due to a higher conversion of acrylamide to glycidamide following oral dosing, when compared with intraperitoneal dosing.

Dixit et al. (1986) conducted an *in vitro* study to evaluate the binding of acrylamide to bovine serum albumin (BSA), since acrylamide has shown reactivity with human serum proteins. The binding of acrylamide was measured using equilibrium dialysis, fluorescence studies, and ultraviolet spectroscopy. The results of the equilibrium dialysis indicated an increase in the amount of unbound acrylamide between 6 and 8 hours of dialysis. At this equilibrium point, more than 25% of the total acrylamide remained bound to protein. A concentration-dependent decrease in fluorescence of BSA was observed, as well as the fluorescence of sulfhydryl groups present on BSA, indicating a role of sulfhydryl groups in the binding of acrylamide.

3.5.2 DNA Adducts

There are limited data regarding the potential for acrylamide to form DNA adducts. When isolated nucleosides were incubated with acrylamide *in vitro*, the adduct yield and the rate of formation was low (Solomon et al. 1985). I*n vivo* studies in mice following an oral exposure to 100 mg/kg [^{14}C]-acrylamide, radiolabeled DNA was found in the tissues examined (stomach, liver, lung, skin and testes) (Carlson and Weaver 1985; Carlson et al. 1986). However, DNA adducts were not characterized so these results could be due to nonspecific binding, rather than the formation of adducts. Sega et al. (1990) reported that in mice that received a single intraperitoneal injection of 46 mg/kg of acrylamide, that alkylation increased in the testis and liver (the only tissues evaluated); however, as in the studies cited above, specific DNA adducts were not characterized and the authors attributed the observed results to glycidamide, rather than acrylamide. Segerback and co-workers (1995) identified N-7-(2-carbamoyl-2-hydroxyethyl)guanine adducts in the tissues examined (liver, lung, kidney, spleen, brain and testis) in rats and mice following a single intraperitoneal injection of acrylamide (46 mg/kg or 53 mg/kg, respectively). The formation of adducts in mice were generally higher than in rats.

3.6 Pharmacokinetic Models

An initial attempt at developing a physiologically-based pharmacokinetic (PBPK) model in the rat was conducted by (Kirman 2002). This model was developed using existing data from six published studies and provides a good description of the kinetics for both acrylamide and glycidamide. However, the authors noted that because of the uncertainty surrounding the mode of action by which acrylamide produces carcinogenic effects, a Monte

Carlo analysis was conducted to perform sensitivity analyses for each of four internal dose measures: genotoxicity, sulfhydryl reactivity, dopamine agonist, and glutathione depletion. The results indicated that the most important contributors to variation in dose metrics were the model parameters characterizing metabolism via cytochrome P450 and glutathione-S-transferase and tissue binding.

Calleman et al. (1992) developed a pharmacokinetic model in order to estimate tissue doses of acrylamide and glycidamide, specifically area under the blood concentration curve. Both a linear and a nonlinear model were developed, with the nonlinear model including saturable metabolism of acrylamide to glycidamide. The metabolic parameters (V_{max} and K_m) associated with the conversion of acrylamide to glycidamide were estimated by applying the models to hemoglobin adduct levels measured in rats following single injections of acrylamide ranging from 0.5 to 100 mg/kg body weight. In the linear model, first order elimination rates of 0.50 and 0.48 $(hr)^{-1}$ were estimated for acrylamide and glycidamide, respectively. For the nonlinear model, a V_{max} of 19.1 $(hr)^{-1}$ and a K_m of 66 µM was estimated for metabolic conversion of acrylamide to glycidamide, with first order rates of elimination of 0.21 $(hr)^{-1}$ and 0.48 $(hr)^{-1}$ estimated for acrylamide and glycidamide, respectively. The use of the models for high-to-low dose extrapolation could not be conducted due to a lack of information as to which of the two electrophilic agents, acrylamide or glycidamide, is primarily responsible for the observed toxicity.

4.0 Acute Toxicity

Based on the results of acute toxicity studies, the oral LD50 for acrylamide is approximately 100-150 mg/kg in mice, rabbits, guinea pigs and rats (McCollister et al. 1964; Paulet 1975; Hashimoto et al. 1981).

5.0 Genotoxicity

5.1 Mutagenicity

The genetic toxicity of acrylamide has been evaluated in a variety of *in vitro* and *in vivo* assays. The results of genotoxicity studies conducted in bacterial cell systems consistently demonstrated that acrylamide was not mutagenic in these systems, with or without metabolic activation (Zeiger et al. 1987; Knaap et al. 1988; Tsuda et al. 1993). Similarly, when acrylamide was incubated with mammalian cells *in vitro*, mutation frequency was not increased at non-cytotoxic concentrations (Knaap et al. 1988; Tsuda et al. 1993). For a discussion of the potential mutagenicity in germ cells, see Section 8.3.

5.2 Chromosomal Aberrations

In vitro addition of acrylamide to Chinese hamster V79 cells induced chromosomal aberrations at a concentration of ≥ 0.142 mg/mL after 20 hours of treatment, but not at lower concentrations (= 0.71 mg/mL) (Tsuda et al. 1993). In Chinese hamster lung cell lines DON:Wg3h and LUC2 p5, acrylamide induced alterations in cell divisions at concentrations of ≥ 0.5 mg/mL (Warr et al. 1990). Significant increases in the frequency of tetraploid cells were also observed in LUC2 p5 incubated with acrylamide at concentrations of 0.5 mg/mL, but not at lower concentrations (= 0.25 mg/mL). Polyploidy and spindle disturbances were observed in Chinese hamster V79 cells at concentrations of 0.071 mg/mL or 0.01 mg/mL, or greater, respectively (Adler et al. 1993; Tsuda et al. 1993).

An increase in the incidence of chromosomal aberrations in bone marrow cells was noted in mice exposed to acrylamide via intraperitoneal injection at doses of ≥ 50 mg/kg (Adler et al. 1988; Chiak and Vontorkova 1988). However, there were no significant increases in the frequency of chromosomal aberrations in lymphocytes of mice exposed intraperitoneally to acrylamide at doses up to 125 mg/kg (Backer et al. 1989) or in splenocytes from mice that received 100 mg/kg (Kligerman et al. 1991).

Acrylamide increased the micronucleus frequency in bone marrow cells in mice following doses of ≥ 25 mg/kg (two doses) or a single dose of 50 mg/kg (Adler et al. 1988; Chiak and Vontorkova 1988; Knaap et al. 1988; Chihak and Vontorkova 1990). Significant increases in micronucleus frequency were also reported in reticulocytes and splenic lymphocytes from mice that were exposed to acrylamide at doses of 50 mg/kg or more via intraperitoneal injection (Backer et al. 1989; Kligerman et al. 1991; Russo et al. 1994). However, the micronucleus frequency in reticulocytes collected from rats that received a single dose of 100 mg/kg was not significantly different from the controls (Paulsson et al. 2002a).

Moore et al.(1987)reported an *in vitro* dose-response evaluation of the clastogenicity of acrylamide in L5178Y mouse lymphoma cells. In that study, acrylamide treatment promoted the formation of small-colony mutants, which represent chromosomal alterations to the chromosome carrying the tk (thymidine kinase) locus. In contrast, production of large-colony mutants, representing single-gene mutations, was not significantly increased. The dose-response curve for the small-colony mutants has a very shallow slope at low doses, with a significant point of inflection at which the dose-response curve becomes steep. This suggests the clastogenicity of acrylamide may be nonlinear, or have a very shallow slope that at low doses may be indistinguishable from the background response in this cell line. Moreover, the significant increases in small colony mutants occurred only at cytotoxic doses.

5.3 Sister Chromatid Exchange (SCE)

In *in vitro* studies, acrylamide increased the frequency of SCEs in Chinese hamster V79 cells at concentrations of ≥ 0.3 mg/mL without metabolic activation and ≥ 1 mg/mL in the presence of S9 metabolic activation (Knaap et al. 1988; Tsuda et al. 1993). *In vivo* SCE assays have indicated that acrylamide increased the frequency of SCE in splenocytes and splenic lymphocytes collected from mice that received a single intraperitoneal injection of ≥ 50 mg/kg (Backer et al. 1989; Kligerman et al. 1991). In a study by Knaap et al. (1988), it was reported that when incubated with S9 metabolic activation, a higher concentration of acrylamide was required to induce SCEs, than when acrylamide was incubated without S9 activation.

5.4 DNA Repair and Unscheduled DNA Synthesis

Acrylamide induced a dose-related increase in the percentage of cells in repair in a rat hepatocyte DNA repair test at a concentration of = 355.5 µg/ml (Barfknecht et al. 1988). However, Butterworth et al. (1992) reported that acrylamide did not yield a DNA repair response at higher concentrations (= 710.9 µg/ml), following *in vitr*o evaluation using primary rat hepatocytes. Acrylamide produced a marginal unscheduled DNA synthesis response at a concentration of 71.09 µg/ml in five different samples of human mammary epithelial cells (HMEC), but not in the HMEC strain 184 cell line (passage 9 cells). Acrylamide did not

induce DNA repair in rats that received a single dose of 100 mg/kg or repeated doses of 30 mg/kg for 5 days (Butterworth et al. 1992).

5.5 Cell Transformation

Acrylamide induced cell transformation in various cell lines; however, the requisite concentrations varied with each cell line. SHE cells and mouse BALB/c3T3 cells were transformed at higher concentrations (0.35 mg/mL and 0.71 mg/mL, respectively) than mouse C3H/10T1/2 clone 8 cells (0.05 mg/mL) or mouse NIH/3T3 cells (0.013 mg/mL) (Banerjee and Segal 1986; Tsuda et al. 1993; Park et al. 2002). Park et al. (2002) reported that co-treatment with 1-aminobenzotriazole (ABT), a nonspecific P450 inhibitor, had no effect on the transformation frequency of 0.5 or 0.7 mM acrylamide. Co-treatment with DL-buthionone-[S,R]-sulfoxime (BSO), a selective inhibitor of ?-glutamylcysteine synthetase, the rate-limiting step in GSH synthesis, resulted in a significant increase in the transformation frequency in acrylamide-treated cells. Both the parent and the metabolite are conjugated and eliminated by GSH. Acrylamide-induced cell transformation frequency was significantly decreased when co-treated with N-acetyl-L-cysteine, a thiol donor. Co-treatment with 17-β-estradiol resulted in a significant increase in transformation frequency. The authors noted that this increase was higher than additive. Based on these results, the authors concluded that acrylamide, rather than an oxidative metabolite, was responsible for SHE cell transformation observed. The authors suggested that the cell transformation may have been achieved following GSH depletion.

5.6 Genetic Toxicity of Glycidamide

A very limited number of studies that evaluated the potential genetic toxicity of glycidamide were available. Glycidamide was mutagenic in Salmonella strains TA100 and TA1535 ± S9 activation at 5000 μg/plate (Hashimoto and Tanii 1985). In an *in vitro* gene mutation assay conducted with mouse lymphoma L5178Y TK $^{+/-}$ cells, glycidamide was positive at a concentration of 2.5 mM without metabolic activation (Barfknecht et al. 1988). When tested in unscheduled DNA synthesis assays, glycidamide was negative in primary rat hepatocytes at a concentration of 4 mM (Barfknecht et al. 1988), but was positive in primary hepatocytes isolated from male Fischer 344 rats (1 mM) and in human mammary epithelial cells (Butterworth et al. 1992). In a recent study by Paulsson et al. (Paulsson et al. 2002b), the administration of glycidamide to mice resulted in dose-dependent increases in micronucleus frequency in CBA mice; however no response was observed in Sprague-Dawley rats.

5.7 Summary of Genotoxicity of Acrylamide

The results of the genetic toxicity studies with acrylamide provide evidence that acrylamide is not a direct acting mutagen in bacterial or mammalian cell assay systems. Acrylamide does, however, have weak clastogenic effects. The clastogenic effects of acrylamide are likely mediated by binding with sulfhydryl groups on proteins rather than effects on DNA (see discussion on germ cells below).

6.0 Carcinogenicity

6.1 Epidemiological Studies

Exposures to acrylamide may occur via the inhalation, oral or dermal routes. In the manufacture of polyacrylamides, workers may be exposed via inhalation. However,

exposures via the oral (food and water) and dermal (use of polyacrylamide-containing products) routes may also occur. Currently, OSHA PEL for acrylamide is 0.3 mg/m^3. The NIOSH REL and the ACGIH TLV for acrylamide are 0.03 mg/m^3.

There have been epidemiological studies that consist of cohort mortality studies in workers exposed to acrylamide. A mortality study of a cohort of 8,854 male acrylamide factory workers, 2293 of whom were exposed to acrylamide, was reported by Collins et al. (1989) and updated by (Marsh et al. 1999). Four factories were evaluated – three in the United States and one in the Netherlands. The cohort consisted of workers that were hired between January 1, 1925 and January 31, 1973. Smoking habits were unknown for most of the cohort. Exposure was estimated from available monitoring data and by recall of plant workers with knowledge of past processes. Exposure to acrylamide was defined as a cumulative exposure of greater than 0.001 mg/m^3-years. Mortality rates were compared to the expected rates for the United States and the Netherlands. The follow-up period extended through 1994, at which time the total number of person-years of follow-up was greater than 287,000 (Marsh et al. 1999). There were no statistically significant excesses of mortality due to cancer of any specific site in the study cohort when compared with the expected rates. The increase in respiratory tract cancer at one of the plants reported by Collins et al. (1989) remained increased in the follow-up (Marsh et al. 1999). However, this finding was again confined primarily to the workers exposed to muriatic acid. The results of an exposure-response analysis indicated an excess of deaths due to pancreatic cancer in workers with exposures to acrylamide at ambient concentrations greater than 0.30 mg/m^3-year, but not at the lower exposures. However, the authors noted that smoking histories were not available for each member of the cohort and that smoking is a major risk factor for the development of pancreatic cancer. The exposure-response analysis indicated no correlation between acrylamide exposure and mortality from cancer of the esophagus, rectum or kidney. Marsh et al. (1999) concluded that the results of this study provided little evidence of a relationship between acrylamide exposure and mortality due to cancer.

6.2 Carcinogenicity Bioassays

6.2.1 Studies in Rats

Two chronic bioassays have been conducted in which male and female Fischer 344 (F344) rats were administered acrylamide in drinking water for two years (Johnson et al. 1986; Friedman et al. 1995). In the bioassay conducted by Johnson et al. (1986), groups of Fischer 344 (F344) rats (60/sex/group) received acrylamide-treated drinking water that provided doses of 0, 0.01, 0.1, 0.5 or 2 mg acrylamide/kg/day for two years. Additional groups of 10 rats/sex/group were sacrificed after 6, 12 or 18 months of exposure. Food and water consumption and body weights were evaluated weekly for the initial 3 months of the study. Thereafter, body weights were collected monthly and food and water consumption were collected weekly. Blood was collected for hematological and clinical chemistry evaluation at 3, 6, 12, 18 and 24 months. At necropsy, organ weights were collected and the major organs were preserved for microscopic examination.

The study of Friedman and co-workers (1995) was similar with a few exceptions: fewer low-dose groups, a higher dose group for females, a larger number of animals per dose group, and inclusion of sentinel animals to screen for specific pathogens were used. In the Friedman et al. (1995) study, acrylamide was administered to groups of male F344 rats via the drinking water to provide doses of 0 (204 animals), 0.1 (204 animals), 0.5 (102 animals), or 2

(75 animals) mg/kg/day. Female rats received doses of 0 (100 animals), 1 (100 animals) or 3 (100 animals) mg/kg/day.

The primary observations of these studies were significant increases in the incidence of tumors primarily in hormonally-responsive organs, including tunica vaginalis mesotheliomas in male rats, mammary gland fibroadenomas in female rats and thyroid follicular cell adenomas in male and female rats (Tables 1 and 2). There was also a suggestion of an increase in the incidence of uterine adenocarcinomas, mammary gland adenocarcinomas, and oral papillomas in female rats and pheochromocytomas in male rats in the Johnson et al. study (1986), but not in the Friedman et al. study (1995). The incidence of glial cell tumors was increased in the Johnson et al. study (1986) study when the incidences of all glial tumors and glial cell proliferation were combined. This was not observed in the Friedman et al. study (1995) study.

Tunica vaginalis mesotheliomas. In the Johnson et al. study (1986), a statistically significant increase in the incidence of tunica vaginalis mesotheliomas was observed in male F344 rats that received 0.5 or 2 mg/kg/day. However, Friedman and co-workers (1995) only observed an increase in the incidence of tunica vaginalis mesotheliomas in male rats exposed to 2 mg/kg/day (Table 1). In the lower-dose groups in both studies, the incidence of tunica vaginalis mesotheliomas was comparable to the incidence reported in the respective control groups.

Mammary gland tumors. The incidence of mammary gland adenocarcinomas was not significantly increased in any acrylamide dose group in either study compared with respective controls (Table 2). There was a suggestion of a dose-response for mammary gland adenocarcinomas by Johnson and co-workers based on the results of a trend test (Table 2). However, the dose-response for mammary gland adenocarcinomas was relatively flat and incidence rates for each dose group were comparable to historical control rates, which range from about 2% in two-year bioassays (Goodman et al. 1979; Maekawa et al. 1983; Solleveld et al. 1984; Haseman et al. 1990) to 11% in full life-span studies (e.g., without a planned terminal sacrifice time) (Solleveld et al. 1984).

Dose-response curves for the incidence of mammary gland adenomas and mammary gland fibromas were also flat (Table 2). The incidence of adenomas noted in the Johnson et al. study at 0.5 and 2.0 mg/kg/day of acrylamide was not significantly increased compared to controls, nor was there a dose-related trend. Historical rates for adenomas in female F344 rats ranged from 1% to 4% (Goodman et al. 1979; Maekawa et al. 1983; Haseman et al. 1990). No adenomas were observed in the Friedman et al. study in the 1.0 or 3.0 mg/kg/day dose groups. There was no apparent dose-related increase in fibromas; however, the incidence of fibromas was significantly increased only in the 2.0 mg/kg/day dose group (from (Johnson et al. 1986)), when compared to the control group. This was not observed in the Friedman et al. study in the 3.0 mg/kg/day dose group. Consequently, it is likely that the observed incidence of adenomas and fibromas is representative of background events unrelated to treatment with acrylamide.

The incidence of fibroadenomas in the acrylamide-exposed groups from the Johnson et al. study was not statistically different from the response rate observed in the control. However, the incidence rates in the 1.0 and 3.0 mg/kg/day dose groups in the Friedman et al. study were significantly increased compared to their control groups. Although the incidence of fibroadenomas in all dose groups was within the historical control range reported for this response in female F344 rats [16% to 29% in two-year bioassays (Goodman et al. 1979;

Maekawa et al. 1983; Solleveld et al. 1984; Boorman et al. 1990) and up to 57% in full life-span studies (Solleveld et al. 1984)], the increase in the incidence of fibroadenomas may be related to acrylamide exposure.

Glial cell tumors. Johnson et al. (1986) reported an increase in the incidence of glial cell tumors in female rats that received 2 mg/kg/day of acrylamide, when the incidence of all glial cell tumors and glial cell proliferation were combined. The incidences of glial cell tumors are often combined, i.e., the incidence of astrocytoma may be combined with the incidence of oligodendrogliomas. However, oligodendrocytes are functionally distinguishable from astrocytes and the combination of the tumor incidence of these different cell types may be inappropriate. The incidence of glial cell proliferation was combined with the incidence of glial cell tumors in the Johnson et al. study. Use of a hyperplastic change in combination with tumor incidence should be evaluated very carefully, and it has been argued recently(Aschner 2002) that glial cell proliferation is not a preneoplastic change. Therefore, the incidence of glial cell proliferation was not combined with the tumor incidence data presented in Table 1 and 2. Because the incidence of oligodendroglioma was not related to dose, these tumors were not combined with the incidence of astrocytomas reported in Tables 1 and 2. However, these findings were not observed in the Friedman et al. study in a larger number of female rats. There was no statistically significantly increased incidence of glial cell tumors in male rats observed in the Johnson et al. or Friedman et al. studies.

Astrocytomas were observed in the brain and spinal cord of control and treated animals. However, the incidence of astrocytoma in the brain or the spinal cord was not statistically significantly increased in any treated group in either bioassay when compared to the respective controls. Only when both studies were considered was there a suggestion of a dose-response, although the response was at most a very weak one (Tables 1 and 2). The incidence of astrocytomas in the Friedman et al. and Johnson et al. studies was for the most part similar to the incidences reported in other chronic two-year bioassays in F344 rats in which the incidence rates ranged from approximately 0.1% to 4% (Goodman et al. 1979; Maekawa et al. 1983; Solleveld et al. 1984; Haseman et al. 1990; Haseman et al. 1998). Therefore, it is likely that the incidence of astrocytomas was not related to acrylamide treatment.

Thyroid tumors. In male rats, the incidence of thyroid follicular cell adenomas in both bioassays was significantly increased in the high-dose (2 mg/kg/day) groups (Table 1). However, when combined, the incidence of follicular cell adenoma/adenocarcinoma was significantly increased in the high-dose males of both bioassays.

In female rats, the incidence of follicular cell adenoma was significantly increased in the 1 and 3 mg/kg/day dose groups of the Friedman et al. bioassay (Table 2); however, the incidence of follicular cell adenoma was not significantly increased in female rats that received 2 mg/kg/day (Johnson et al. 1986). The incidence of follicular cell adenocarcinoma was not significantly increased in female rats in either bioassay. As observed in male rats, the combined incidence of adenoma/adenocarcinoma was significantly increased in the 1 and 3 mg/kg/day groups, but not in the 2 mg/kg/day group.

Other tissues. In the Johnson et al. study, significant increases in the incidence of papillomas of the oral cavity and uterine adenocarcinomas were reported in the high-dose (2 mg/kg/day) females. However, only one uterine adenocarcinoma was reported by Friedman et al., and there were no statistically significant increases in the incidence of papillomas of the oral cavity in any treated group (male or female). Because acrylamide is irritating, it is

possible that the oral cavity papillomas were the result of continued irritation. An increase in the incidence of pheochromocytoma was reported in the high-dose males in the Johnson et al. study; however, the incidence of these tumors was not increased in the Friedman et al. study or in female rats in either study. Given that the incidence of these tumors were observed in only one study and/or in one sex, the incidence of these tumors may have been biological variation and not related to acrylamide treatment.

6.2.2 Studies in Mice

In addition to the carcinogenicity bioassays conducted in rats (Johnson et al. 1986; Friedman et al. 1995), nonstandard carcinogenicity assays (e.g., mouse skin painting initiation/promotion studies) have been have been conducted in mice (Bull et al. 1984a; Bull et al. 1984b; Robinson et al. 1986). Increases in lung tumors were reported in the Bull et al. (1984a; 1984b) studies. However, the results of the studies by Bull et al. (Bull et al. 1984a; Bull et al. 1984b) are limited. In one study, the diagnosis of lung tumor was based on gross necropsy observations and was not confirmed microscopically (Bull et al. 1984a). In the second Bull et al. study (1984b), lung tumor yield was provided; however, the incidence of lung tumors was not included. In the study by Robinson et al. (1986), lung tumor incidence was increased only in SENCAR mice, a strain of mice genetically predisposed to the formation of tumors, and not in the BALB/c, A/J or ICR strains. However, the doses used in this study were lower than the doses used by Bull et al. In all studies, skin tumor yields were increased only following the application of a promoting agent, such as 12-O-tetradecanoylphorbol-13-acetate (TPA).

7.0 Neurotoxicity

7.1 Epidemiological Studies

Multiple studies have been conducted to assess neurotoxic effects of acrylamide in occupationally-exposed workers in small factories manufacturing acrylamide or using acrylamide in production in China (He et al. 1989; Deng et al. 1993) and South Africa (Myers and Macun 1991; Bachmann et al. 1992). He et al. (1989) examined 71 workers (45 men and 26 women) between the ages of 17 and 41 exposed to acrylamide by industrial production facilities and had been employed from 1 to 18 months. Fifty-one unexposed workers (33 men and 18 women) served as the reference group. Approximately 73% of the workers exhibited symptoms of acrylamide poisoning. Early symptoms of acrylamide exposure were skin peeling from the hands followed by weak legs and numb hands and feet, and impairment of the vibration sensation in the toes and loss of ankle reflexes. Electroneuromyographic changes included a decrease in the sensory action potential amplitude, neurogenic abnormalities in electromyography and prolongation of the ankle tendon reflex latency. Most of the workers diagnosed with signs or symptoms had handled a 27 to 30% aqueous solution of acrylamide monomer.

A second group of Chinese workers were examined who had been exposed to acrylamide in a chemical factory at concentrations ranging from 0.20 to 1.58 mg/m^3 for 0.5 to 8 years (Deng et al. 1993). Vibration thresholds were significantly higher in acrylamide-exposed workers than those of the 105 healthy unexposed adults in the same age group.

In 1985 in a South African factory in which polyacrylamide flocculants were manufactured, five cases of peripheral neuropathy were diagnosed. These cases led to an industrial hygiene and neurologic evaluation of the remaining 66 exposed and unexposed

workers in that factory (Myers and Macun 1991). Acrylamide monomer concentrations in the factory ranged from 0.02 to 0.75 mg/m^3. The mean duration of exposure was 2 years. The overall prevalence of acrylamide-related abnormalities among the exposed group was approximately 67%, compared to approximately 14% in the unexposed group. Most of the subjects with abnormalities came from the two areas where the air concentration of acrylamide monomers was highest.

In a follow-up to the Myers et al. (1991)study by Bachmann et al. (1992), 75 workers from the same South African factory were examined. Significantly higher prevalences of numbness, limb pain, and peeling and sweating of hands were observed in exposed workers, compared to unexposed. No gross neurological abnormalities were reported and no association was found between vibration thresholds and exposure.

7.2 Neurotoxicity Studies in Animals

The neurotoxicity of acrylamide has been extensively studied in various animal models, including rats, mice, monkeys, dogs and cats, and by numerous dosing regimens and durations of dosing. Overt signs of neurotoxicity were consistent across species. However, while some studies evaluated only overt neurotoxicity (grip strength or leg splay), other studies examined only morphological or biochemical changes in nerve tissues. In some studies, overt signs of neurotoxicity were not accompanied by signs of morphological changes in key peripheral nerves. Few if any of studies examined the time course of acrylamide toxicity as manifested by both central and peripheral changes in biochemical function and morphology along with overt signs of peripheral neuropathy. For the purposes of this report, studies by the oral route of exposure are summarized below.

In general, cumulative dose is important when overt neurotoxicity is evaluated. In rats exposed via the oral route, single doses of ≥ 100 mg/kg were reported to result in alterations in grip strength and motor function (Fullerton and Barnes 1966; Tilson and Cabe 1979). With short-term multiple exposures, clinical signs of toxicity and deceased motor function were reported in rats following the administration of 25 mg/kg/day for 21 days (Dixit et al. 1981a; Aldous et al. 1983). In a subchronic toxicity studies, effects on rotorod performance were observed at doses ≥ 14.5 mg/kg (Tanii and Hashimoto 1981) and hindlimb splay was increased in rats that received ≥ 9 mg/kg (McCollister et al. 1964; Burek et al. 1980). Burek et al. (Burek et al. 1980) also reported increases in morphological changes following electron microscopic examinations of peripheral nerves of rats that received ≥ 1 mg/kg/day for 90 days (it should be noted that nerves of only 3 rats/dose group were examined with electron microscopy). These effects were reversible after 144 days of recovery, with the exception of the group that received 20 mg/kg/day of acrylamide. There were no changes observed following electron microscopic examination in rats that received 0.05 or 0.2 mg/kg/day. In two year chronic toxicity/carcinogenicity bioassays, no signs of overt neurotoxicity based on cage side observations were reported, but significant increases in the incidence of peripheral nerve degeneration was observed in rats that received ≥ 2 mg/kg/day for two years (Johnson et al. 1986; Friedman et al. 1995).

In mice, decreases in rotorod performance were reported following repeated administration of ≥ 9 mg/kg (Hashimoto et al. 1981; Gilbert and Maurissen 1982). The administration of 10 mg/kg/day to monkeys for approximately 45 to 60 days resulted in

evidence of loss of motor function (Maurissen et al. 1983; Eskin et al. 1985; Maurissen et al. 1990). Overt evidence of neurotoxicity and decreased nerve conduction velocity was reported in cats that received doses of 15 mg/kg daily for 4-16 weeks (Post and McLeod 1977). In dogs that were administered 5.7 or 7 mg/kg/day for 42 days, clinical signs of neurotoxicity (muscle weakness, hind limb ataxia) were reported (Satchell and McLeod 1981; Hersch et al. 1989).

Recent studies (LoPachin 2000) measuring multiple neurological parameters (gait, foot splay, grip strength and extensor thrust) across two intoxication schedules (21 and 50 mg/kg) have indicated that acrylamide produces cumulative neurotoxicity. Specifically, dose-rate did not determine final magnitude of neurological deficit (i.e., both dose-rates caused statistically similar maximal changes in each neurological parameter measured), instead dose-rate determined the time of onset and development of neurotoxicity (i.e., maximal neurological deficits were observed on day 11 of the 50 mg/kg dose-rate, whereas a similar level of neurotoxicity was achieved on day 40 of the 21 mg/kg exposure rate38. Research from other laboratories suggests that this cumulative phenomenon applies to dose-rates significantly lower than those used in the aforementioned study (Edwards 1977; Moser 1992; Shell 1992; Crofton et al. 1996).

Morphologic examinations (Leswing 1969; Prineas 1969; Hopkins 1970; Hopkins 1971; Suzuki 1973; Schaumburg 1974; Spencer 1974; Sumner 1974; Sumner 1975; Gold 1988) have revealed that low-dose (dose-rates reviewed in Barber et al. (2001)) subchronic induction of acrylamide neurotoxicity was associated with nerve damage in both the central and peripheral nervous systems. The morphologic hallmark of this toxic neuropathy was considered to be distal nerve terminal and preterminal axon swellings of the longest myelinated fibers (Prineas 1969; Suzuki 1973; Schaumburg 1974). As exposure continued, progressive retrograde degeneration of these distal axon regions ensued with preservation of more proximal segments (reviewed in Spencer and Schaumberg (1974)). Early morphologic and electrophysiologic research (Schaumburg 1974; Sumner 1974; Sumner 1975) suggested that sensory axons and their receptors (i.e., Pacinian corpuscle, annulospiral terminal, Golgi tendon) developed neuropathic changes before motor neurons. However, other studies indicated that both sensory and motor systems were equally vulnerable to acrylamide-induced damage (Fullerton and Barnes 1966; Prineas 1969; Hopkins 1971; Tsujihata 1974; Lowndes 1976).

Although the relative vulnerabilities of the sensory vs. motor systems were debated, the pattern of neuropathological expression induced by acrylamide (i.e., initial nerve terminal damage and subsequent retrograde axon degeneration) was consistent with the theory of toxic "dying-back" neuropathies proposed by Cavanagh (Cavanagh 1964; Cavanagh 1979; Calleman 1996). However, other evidence implied that the dying-back label did not adequately describe the neuropathy caused by acrylamide. For example, Spencer and Schaumburg (Schaumburg 1974; Spencer 1977b; Spencer 1977a) reported that degeneration did not start at the nerve terminal and move rostrally in a seriatim fashion as stipulated by the dying-back hypothesis. Instead, degeneration "bloomed" simultaneously at multifocal sites along distal preterminal axons. Work by Jennekins et al., (1979) indicated that nerve terminals of long axons were not preferentially damaged by acrylamide intoxication as was predicted by the dying-back theory. Considerable other evidence indicated that the "dying back" was not a primary effect (Fullerton and Barnes 1966; Morgan-Hughes 1974; Griffin

1977; Cavanagh 1982; Sterman and Sposito 1985; DeGrandchamp 1990a; DeGrandchamp 1990b; Myall et al. 1990; Gold 1991).

Spencer and Schaumburg (1976) formulated a hypothesis that emphasized direct axonal injury. They proposed that large diameter axons in the CNS and PNS were most sensitive to development of simultaneous, multifocal paranodal axon swellings in distal regions and that these swellings served as initiation points for subsequent degeneration. In the PNS, acrylamide preferentially affected axons in tibial nerve branches supplying calf muscles, plantar sensory nerves innervating the digits and plantar nerve branches supplying the flexor digitorum brevis muscle (Spencer 1977a). Axon swelling and degeneration were noted in certain CNS regions; e.g., dorsal spinocerebellar tract, gracile fasciculus, cerebellar white matter (Prineas 1969; Ghetti 1973; Spencer 1977a). The characteristic spatiotemporal pattern of axon damage in the central and peripheral nervous systems lead Spencer and Schaumburg (1976) to classify acrylamide neuropathy as a "central-peripheral distal axonopathy".

Other morphological evidence has indicated that early nerve terminal damage might be importantly involved in the pathophysiological process leading to acrylamide neurotoxicity (Prineas 1969; Tsujihata 1974; Cavanagh 1982; DeGrandchamp 1990a; DeGrandchamp 1990b). Electrophysiological studies by Goldstein and Lowndes (Lowndes 1976; Lowndes 1978b; Lowndes 1978a; Goldstein 1979; Goldstein 1981; Goldstein 1985; DeRojas 1987) showed that neurotransmission was impaired at spinal cord primary afferent nerve terminals as an early consequence of acrylamide intoxication of cats (see also Tsujihata et al. (1974)). Recent quantitative morphometric research showed that peripheral nerve axon degeneration was not linked to the expression of neurological deficits, but rather appeared to be an exclusive product of lower acrylamide dose-rates (< 21 mg/kg/d); i.e., intoxication at a higher dose-rate (50 mg/kg/d) did not produce peripheral axon degeneration (Lehning et al. 1994; Lehning et al. 1998). This suggested that the axonopathic effect of acrylamide was an epiphenomenon and that the neurotoxicologically significant site of action was elsewhere (reviewed in (LoPachin 2000; LoPachin et al. 2002)). Based on evidence of early structural and functional damage, LoPachin et al. (2002) suggested that nerve terminals were the primary site of acrylamide action and that synaptic dysfunction and subsequent degeneration were necessary and sufficient steps for production of acrylamide neurotoxicity. Corroborative research using the de Olmos silver stain method to detect neurodegeneration has shown that higher dose-rate intoxication (50 mg/kg/day) produced a selective terminalopathy characterized by very early, widespread nerve terminal degeneration in rat PNS (NMJ) and CNS (brain and spinal cord) (Lehning 2002c; Lehning 2002a; Lehning 2002b). Intoxication of rats at a lower dose-rate (21 mg/kg/day) caused initial nerve terminal degeneration in PNS and CNS, which was followed by axon degeneration. Together, these data (Lehning et al. 1998; Lehning 2002c; Lehning 2002a; Lehning 2002b) suggest that, regardless of dose-rate, acrylamide causes initial, generalized nerve terminal degeneration. The early appearance of this effect suggests that nerve terminals are a primary site of action. In contrast, axon degeneration in PNS and CNS was a delayed event and occurred only during lower dose-rate intoxication (Lehning et al. 1998; Lehning 2002c; Lehning 2002a; Lehning 2002b). Because full neurotoxicity can develop at the higher and lower dose-rate used in these studies (LoPachin 2001), the conditional, dose-rate dependent expression of axon degeneration indicates that this effect is not a significant neurotoxicological event (LoPachin 2000; LoPachin et al. 2002). The axon orientation of formative morphological investigations (e.g., Spencer and Schaumburg (1977b; 1977a)) is likely due to a focus on subchronic acrylamide

dosing schedules (reviewed in (LoPachin et al. 2002)). Recent quantitative morphometric studies of PNS and silver stain analysis of CNS (see above discussion) have confirmed that axon degeneration is associated with low dose-rate acrylamide intoxication. However, these studies also demonstrate that intoxication at higher dose-rates does not induce axon degeneration. A growing awareness of dose-rate impact on neurotoxicological expression (reviewed in LoPachin 2000) and advances in computer-assisted quantitative morphometrics and histochemical techniques (de Olmos silver stain) have contributed to the changing view of axon and nerve terminal damage in acrylamide neurotoxicity.

An alternative mechanism proposed by Sickles et al. (1996; 2002) is the inhibition of kinesin, the motor protein for anterograde axonal transport, and the resultant decreases in anterograde axonal transport are critical components in the neurotoxicity of acrylamide (reviewed in Sickles et al. (2002)). In a microtubule motility assay, acrylamide (0.1mM) inhibited kinesin. Several studies (see Table 1 in Sickles et al. (2002)), including *in vivo* studies in rats and mice and *in vitro* studies in primary cultured neurons have reported significant reduction in fast axonal transport with acrylamide exposure. The inhibition of axonal transport produces deficient delivery of macromolecules to the distal axons and nodes of Ranvier, which has been associated with decreases in a fast-transported form of acetylcholinesterase into muscle; decreases in GAP-43 within distal neuritis of primary cultured neurons; decreased quantity of synaptic vesicles and mitochondria within neuromuscular junctions; axonal accumulation of fast transported material; progressive decrease in synaptophysin in neuromuscular junctions with high and low dosing rates; decreases in Na and K channels in nodes of Ranvier that are greater in the distal tibial nerve than the more proximal sciatic nerve and occur with either low or high dosing rates. In addition, similarities of acrylamide intoxication and Drosophila models of kinesis mutants have been reported.

This hypothesis has been challenged by the observations that with high dosing rates, acrylamide produced neurological deficits but did not produce changes in axonal elemental composition, Na/K ATPase activity, sensitivity to anoxia and degeneration. Since these functions are supported by fast axonal transport, it is reasonable to expect a reduction. However, it is also reasonable to expect a differential sensitivity to acrylamide intoxication. The changes in total elements (not ions), Na/K ATPase activity, etc., are correlated with degeneration and therefore may be expressed only under severe conditions, just prior to, or during, regeneration. The mechanistic explanation for differential reductions may be based upon differential effects on kinesin superfamily members, turnover rates of proteins, quantities of each protein, thresholds for functional loss, or safety factors in delivery.

8.0 Effects on the Reproductive System

8.1 Epidemiological Studies

No studies were located that evaluated the potential effects of acrylamide on the human reproductive system.

8.2 Reproductive Toxicity Studies in Animals

Acrylamide has been evaluated for reproductive toxicity in multigenerational studies in rats (Tyl et al. 2000a) and mice (Chapin et al. 1995) and in cross-over breeding studies in rats (Zenick et al. 1986; Tyl et al. 2000b) and mice (Sakamoto and Hashimoto 1986; Chapin et al. 1995). The results of all of the above studies are highly consistent. Acrylamide

administered in drinking water or by gavage to rats or mice at doses equal to or greater than 5 mg/kg/day resulted in significant increases in both pre-implantation and post-implantation losses with resulting significant decreases in the number of live pups per litter (Chapin et al. 1995; Tyl et al. 2000a). These effects were seen in both the F_0 and F_1 generations, with the percentage decrease in the number of live pups per litter similar in the F_0 and F_1 generations in rats (approximately 34% versus 23% in F_0 and F_1, respectively). In mice the effects were more pronounced in the F_1 than the F_0 generation (11% compared to 47%). However, doses of acrylamide less than 15 mg/kg/day did not result in significant changes in:

- clinical signs of toxicity (some reduction in body weight in rats);
- overt signs of neurotoxicity (as measured by heat tilt or leg splay);
- indices of reproductive performance (mating, pregnancy, fertility) or the number of corpora lutea;
- organs weights or signs of histological or neuropathology changes;
- sperm parameters (concentration, motility, morphology); or
- estrus cyclicity (only mice evaluated).

At higher doses (15 mg/kg/day or greater), signs of neurotoxicity and changes in copulatory behavior were noted as well as effects on sperm motility and morphology. At these doses in both mice and rats, acrylamide resulted in decreases in fertility, in addition to changes in pre- and post-implantation loses (Zenick et al. 1986; Tyl et al. 2000b).

Cross-over matings of treated males with untreated females demonstrated that pre- and post-implantation losses, with resulting decreases in the number of live pups/fetuses per litter, could be attributed to effects on males (Sakamoto and Hashimoto 1986; Zenick et al. 1986; Chapin et al. 1995). In a continuous dosing/breeding protocol in mice (4 to 5 litters per breeding pair), the mean number of live pups per litter across litters from the first to the last litter were similar, indicating the absence of progressive reproductive toxicity (Chapin et al. 1995).

Some data suggests that exposure of females may contribute to reproductive toxicity. A delay in vaginal patency was noted in offspring of treated females (14 mg/kg/day, 70 days) mated to untreated male (Zenick et al. 1986) suggesting that perinatal exposure of female pups might result in subtle alterations in the estrogen/progesterone balance. Further, a slight, but significantly higher number of resorptions was seen at gestation day 13 in treated females (19 mg/kg/day, 42 days); however, the number of live pups per litter was comparable to controls (Sakamoto and Hashimoto 1986). Lastly, Tyl et al. (2000a) noted that post-implantation loss at the F0 mating (34.3%) was higher than that seen in the dominant lethal mating (24.9%) when administered the same dose (5 mg/kg/day), suggesting the possibility that the female was contributing to this decrease. Any increase in fetal loss from gestation days 13 to 21 likely reflects the continued influence of male dominant lethal effects (see below). Also, the percentage post-implantation loss was similar in the F_1 generation (23.1%).

8.3.1 Germ Cell Mutagenicity Studies

8.3.2 Germ Cell UDS, DNA Strand Breaks, Chromosomal Aberrations, Micronuclei, SCE, and Protamine Alkylation

In germ cells studies, serial recovery of sperm from the caudate epididymides that were at the early spermatocyte (pre-meiotic/meiotic) stage to the spermatozoa (post-meiotic) stage at the time of treatment showed no increase in unscheduled DNA synthesis (UDS); these stages correspond to those associated with dominant lethality (Sega et al. 1990). In contrast,

DNA strand breaks were increased in these sensitive stages. DNA strand breakage in sperm in earlier stages of development at the time of treatment decrease with time, likely due to DNA repair, prior to becoming functional spermatozoa. Slight increases in DNA strand breaks in isolated human testicular cells have been reported; however, there was considerable variation and no dose response even at the highest concentration 1000 μM (Bjorge et al. 1996). Further, chromosomal aberrations and sister chromatid exchanges were not found in spermatogonia or early spermatocytes in contrast to positive results in late spermatids and spermatozoa (Adler et al. 1988; Backer et al. 1989). In a micronucleus test, weak clastogenic activity was noted in rat primary spermatocytes (100 mg/kg) (Xiao and Tates 1994). Further studies of DNA, total sperm head, and sperm protamine alkylations suggest that the stage specificity can be explained by preferential binding to cysteine sulfhydryl groups in sperm protamine (Sega et al. 1989). In mid-to late spermatid stages, chromosomal histones are replaced by protamines that are relatively rich in arginine and cysteine. Alkylation of free sulfhydryl groups of cysteine in the "immature" protamine of late spermatids and early spermatozoa might prevent normal chromatin condensation leading to stress in the chromatin structure and strand breakage (Sega et al. 1989).

8.3.2 Dominant Lethal Mutation Studies

Eight dominant lethal studies have been conducted in male rats (Smith et al. 1986; Working et al.; Sublet et al. 1989; Tyl et al. 2000a) and mice (Shelby et al. 1987; Bishop et al. 1991; Adler et al. 2000) by the oral route of administration and in male mice by the intraperitoneal injection and dermal routes (Gutierrez-Espeleta et al. 1992). All studies report the induction of dominant lethal mutations by acrylamide. In the oral studies, significant decreases in fertility and increases in dominant lethality were observed in the first week post-dosing (15 mg/kg/day) up to 4 weeks post-dosing (60 mg/kg/day)(Sublet et al. 1989). No significant changes in any parameter were noted in dams that were mated with treated males more than 4 weeks post-dosing (Shelby et al. 1986; Working et al. 1987; Sublet et al. 1989). In the dermal study, significant decreases in the numbers of live fetuses per litter were noted for males receiving 5 dermal applications of 50 mg/kg or greater (up to 125 mg/kg). In males given 5 dermal applications of 25 mg/kg, the number of live embryos per female was not elevated but the per cent of dead implants per female was significantly increased. (Gutierrez-Espeleta et al. 1992). These effects consistently show that acrylamide induces dominant lethal mutations in mid-late spermatids and spermatozoa. Further, acrylamide's effects on fertility and sperm parameters following a single intraperitoneal injection of 125 mg/kg acrylamide were not blocked by pre-administration of aminobenzotriazole (ABT – an inhibitor of P-450 isoform 2E1); however, the dominant lethal effects were completely or partially blocked (depending on the ABT post-administration time of evaluation) by ABT (Adler et al. 2000). This suggests that acrylamide's dominant lethal effects are likely due to the major metabolite, glycidamide, but that the effects on fertility and sperm motility are not. This finding, along with the report by Sega et al. (1989) that dominant lethal mutations correlate with the binding of acrylamide to protamines in spermatids and spermatozoa leaves open the question of the mechanism by which dominant lethals are induced. Generoso et al (1996) reported that a single i.p. injection of 125 mg/kg glycidamide in male mice induced dominant lethal mutations in the same germ cell stages and at somewhat higher frequencies as acrylamide.

8.3.3 Heritable Translocations

Because of positive results of dominant lethal studies, the potential for acrylamide to induce heritable translocations in male mouse germ cells has been evaluated in three studies (Shelby et al. 1987; Adler et al. 1994; Marchetti et al. 1997). All three studies reported the induction of translocations. In the study by Shelby et al. (1987), male mice received 5 daily doses of 40 or 50 mg acrylamide/kg by intraperitoneal injection. The frequencies of translocation carriers among F1 males conceived 7-10 days post-treatment was 24% (40 mg) and 39% (50 mg) compared to an historical control frequency of 0.2%. Adler et al. (1994) treated male mice with a single intraperitoneal dose of 50 or 100 mg/kg. The frequencies of translocation carriers among F1 males and females conceived 7-16 days post-treatment were 0.6% (50mg/kg) and 2.7% (100 mg/kg) compared to an historical control frequency of 0.04%. However, heritable translocations were not significantly increased in male rats given up to 8 mg/kg/day for 80 days (Adler 1990). Generoso et al. (1996) reported the induction of translocations with a single i.p. injection of 100 mg/kg glycidamide, a metabolite of acrylamide. Among male offspring conceived 3.5-7.5 days post-treatment, the frequency of translocation carriers was 20.18%, compared to an historical control frequency of 0.06%. As with the dominant lethal studies, this suggests but does not confirm that the genetic damage induced in mouse germ cells following acrylamide exposure may ultimately be the result of glycidamide rather than the parent compound.

8.3.4 Specific-Locus Mutations

Two specific-locus mutation studies have been conducted to determine if acrylamide induces gene mutations in male mouse germ cells. In the first study (Russell et al. 1991), males received intraperitoneal injections of 50 mg/kg on 5 consecutive days and were then mated for up to 182 days. Five mutant offspring were recovered in the first 7 weeks of matings from among 28,971 offspring, a frequency that is significantly above the historical control. No mutant offspring were recovered from spermatogonial stem cells, i.e., from matings occurring 8 or more weeks post-treatment. This finding suggested that there was no concern for long-term genetic risks associated with mutations induced in the spermatogonial stem cell population. However, a subsequent study reported the induction of mutations in mouse spermatogonial stem cells by acrylamide (Ehling and Neuheuser-Klaus 1992). These authors confirmed that, in male mice receiving a single intraperitoneal injection of 100 mg/kg acrylamide, an increase in specific-focus mutations was induced in post-meiotic germ cell stages. But, contrary to the earlier study, they also observed an increase in mutations in spermatogonial stem cells (6 mutants out of 23489 off-spring compared to 22 in 248,413 in the historical control). These 6 mutant animals were conceived 43 days or more post-treatment (one each on mating days 43, 70, 181, 201, 234, and 439). Based on data from these two studies, it is clear that acrylamide induces gene mutations in post-meiotic male germ cells and the latter study reports mutations induced in spermatogonial stem cells.

8.3.5 Developmental Toxicity Studies

Three developmental toxicity studies are reported in rats, including one study of developmental neurotoxicity. No significant treatment-related effects on embryo/fetal viability, growth, or development or dose-related increases in fetal malformations were found

in off-spring of female rats administered up to 15 mg/kg/day on gestational days 6 to 20 (Field et al. 1990) or of female mice administered up to 15 mg/kg/day on gestational days 6 to 17 (Field et al. 1990). In the off-spring of female rats treated from gestational day 6 to lactational day 10, there were no clinical signs of toxicity and pup survival was not affected at birth or up to 21 days at maternal doses of 10 mg/kg/day or lower (Wise et al. 1995). In this study, pup body weight (preweaning) at a maternal dose of 15 mg/kg/day and reduced pup survival at birth and for days 1 to 3 at a maternal dose of 20 mg/kg/day were observed; however, maternal toxicity and signs of neurotoxicity were significant at these doses (Wise et al. 1995). Developmental neurotoxicity has been assessed in rats (Wise et al. 1995). At doses equal to or less than 10 mg/kg/day during gestation, no effects in behavioral tests (open-field motor activity, auditory startle habituation, or passive avoidance) were noted in offspring when tested either within 21 days post-natal or as adults. Decreases in catecholamine (dopamine) levels in cerebellum, pons medulla, and mid-brain but not the hypothalamus or basal ganglia were noted and were more pronounced in younger rats (12, 15, or 21 days old) than older rats (60 days) when exposed directly to acrylamide (25 mg/kg/day for 5 days) at various ages (12, 15, 21, or 60 days post-partum) (Husain et al. 1987).

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Table 1

Bioassay Data - Males

Study	Dose (mg/kg/day)				
	0	0.01	0.1	0.5	2
Tunica vaginalis mesothelioma					
(Johnson et al. 1986)	3/60	0/60	7/60	11/60*	10/60*
(Friedman et al. 1995)	8/204	_	9/204	8/102	13/75*
Astrocytoma					
(Johnson et al. 1986)	4/60	0/60	0/60	2/60	5/60
(Friedman et al. 1995)	1/204	_	1/98	0/50	3/75
Thyroid follicular cell adenoma/adenocarcinoma					
(Johnson et al. 1986)	1/60	0/58	2/59	1/59	7/59*
(Friedman et al. 1995)	6/202	_	12/203	5/101	17/75*
Oral cavity papilloma					
(Johnson et al. 1986)	4/60	7/60	0/60	5/60	4/60
Pheochromocytoma					
(Johnson et al. 1986)	3/60	7/59	7/60	5/60	10/60*

* Statistically different from control using Fischer's Exact Test, a = 0.05

Table 2

Bioassay Data – Females

Study	Dose (mg/kg/day)						
	0.0	0.01	0.1	0.5	1	2	3
Mammary gland adenoma							
(Johnson et al. 1986)	0/60	1/60	0/60	3/58	–	2/61	–
(Friedman et al. 1995)	0/96	–	–	–	0/94	–	0/95
Mammary gland adenocarcinoma							
(Johnson et al. 1986)	2/60	1/60	1/60	2/58	–	6/61#	–
(Friedman et al. 1995)	2/96	–	–	–	2/94	–	4/95
Mammary gland fibroma							
(Johnson et al. 1986)	0/60	0/60	0/60	0/58	–	5/61*	–
(Friedman et al. 1995)	0/96	–	–	–	0/94	–	0/95
Mammary gland fibroadenoma							
(Johnson et al. 1986)	10/60	11/60	9/60	17/58	_	16/61	_
(Friedman	9/96	–	–	–	20/94*	_	26/95*

et al. 1995)							
Astrocytoma							
(Johnson et al. 1986)	1/60	1/60	0/60	0/60	_	6/61	_
(Friedman et al. 1995)	0/100	_	_	_	2/100	_	3/100
Thyroid follicular cell adenoma/adenocarcinoma							
(Johnson et al. 1986)	1/58	0/59	0/59	0/58	-	5/60	_
(Friedman et al. 1995)	2/100	_	_	_	10/100*	_	23/100*
Oral cavity papilloma							
(Johnson et al. 1986)	0/60	3/60	2/60	1/60	_	7/61*	_
Uterine adenocarcinoma							
(Johnson et al. 1986)	1/60	2/60	1/60	0/59	_	5/60*	

* Statistically different from control using Fisher's Exact Test, a = 0.05

Linear trend by Mantel Hanszel extension of Cochran-Armitage test, $\alpha = 0.05$.

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PRIORITY RESEARCH NEEDS
Toxicology and Metabolic Consequences Working Group

Introduction

The Toxicology and Metabolic Consequences Working Group was one of five working groups convened during the JIFSAN/NCFST-organized Workshop on Acrylamide in Food, held in Chicago on October 28-30, 2002. TheWorking Group reached conclusions regarding data gaps and consequent research needs in each of six focus areas: metabolism and kinetics, genetic toxicity, reproductive/developmental toxicity, carcinogenicity, neurotoxicity, and epidemiology. Data gaps were characterized as areas where additional data (new, improved, etc.) would be most likely to significantly improve our ability to evaluate the potential adverse health effects from exposure to acrylamide in food.

Data gaps/research needs in each of the six focus areas were culled and prioritized to identify the nine most critical research needs, highlighted under the focus areas below. It was the opinion of the Working Group that these top-priority research initiatives would not require extensive method development and that most could be accomplished in a relatively short time (6-12 months from commencement).

One theme of the prioritized research is *to assess the significance of adverse effects observed at high doses for low-level human exposure in foods*. For example, neurotoxicity studies at dose levels orders of magnitude higher than likely human exposures in foods have suggested the possibility that cumulative neural damage may result from long-term exposures at lower levels, so chronic rodent studies designed to investigate this possibility were given a higher priority. In order to link the results of rodent studies to human risks, kinetics and metabolism studies in humans and the identification of appropriate dose metrics for the major toxicity endpoints were identified as critical research needs.

Another theme is *the assessment of the significance for humans of effects observed in vitro or in vivo in rodents.* For example, in rats thyroid follicular cell tumors were increased in both acrylamide carcinogenicity studies. For some chemicals these tumors have been shown to be produced by a mechanism that does not occur in humans and the tumors have therefore been considered irrelevant in assessing human cancer risk. This possibility needs to be investigated for acrylamide. Also, germ cell mutations have been observed experimentally *in vitro* and *in vivo* but have not been reported in humans, so a prioritized research need is to search for these effects in the sperm of highly exposed workers in known cohorts.

Metabolism and Kinetics

Information on the metabolic fate of acrylamide is important to informing extrapolation of health effects from animal data to human health risk. These data are also central to predicting toxic responses at low doses on the basis of those observed at high doses. In this regard data on the metabolic fate of acrylamide from animal test species, particularly the rat, are fairly well established. However, there is a need for **further information on the critical events and dose metrics related to the mode(s) of action at relevant doses for the key toxicities of acrylamide.**

In contrast to the animal data, there exists relatively little, if any, data on metabolic fate and kinetics of acrylamide in humans. Therefore the work group recommended as a top priority **the collection of metabolic fate and kinetic data in humans, including bioavailability from foods.** Methodologies using high sensitivity and resolution bioanalytical instrumentation, coupled with stable isotope techniques should facilitate the collection of these data. It is anticipated that these results could be collected within 6-12 months from the time the project begins.

Other specific elements of research programs aimed at improving understanding of metabolism and kinetics in humans include:

- Assessing the kinetic determinants of potentially susceptible subpopulation
- Improve existing physiologically-based pharmacokinetic models (e.g. quantify rate constants for use in kinetic modeling)
- Assessing kinetic determinants across developmental stages
- Molecular and kinetic characterization of binding to sulfhydryls in target and non-target sites (e.g. measure rate constants for binding to critical targets vs. glutathione)
- Compare and contrast endpoints of acrylamide toxicity to other other –SH active agents

Genetic Toxicity

Data from traditional genetic toxicity studies are well described for acrylamide. The data suggest several possible mechanisms by which genetic damage could affect two of the more important adverse health effects suspected from acrylamide exposure (i.e. cancer and heritable genetic damage). Further elucidation of which of the several possible mechanisms for inducing genetic damage is operating will guide approaches to assessing health risks to humans from dietary exposure to acrylamide. In this regard the working group supported **investigations of the formation of adducts of acrylamide or glycidamide with DNA and significant nuclear proteins (for example, protamine or chromosomal motor proteins), especially at critical target sites such as sites of tumor formation and male germ cells.** Research regarding classification, biological relevance, and the kinetics and activity of adducts, if they occur, is needed to inform potential mode of action development. Species differences, *in vivo* vs. *in vitro* formation, and formation at relevant dose ranges for such adducts are particularly important areas for research. Improvements in current bioanalytical methods for detecting DNA and protein adducts may be necessary to address this line of research at dose levels relevant to those used in animal bioassays and those found in foods.

Other important research needs in this area include:

- Use of specific genetically modified mouse strains (e.g. thymidine kinase knockout and Big Blue (*LacI/lacZ*) mouse models) to assess the mode of genotoxic damage *in vivo*.

Reproductive and Developmental Toxicity

Significant decreases in the number of live pups were observed when acrylamide was administered to parental rats and mice. Dominant lethality studies show this finding

of decreased litter size to be likely related to germ cell toxicity. While a NOAEL for pre- and post-implantation loss can be determined from the multigenerational studies, it is expected that effects on the predominant precursor event, germ cell toxicity, would occur at lower doses. At present, germ cell toxicity studies are single, high dose studies. Therefore, to assess low dose reproductive toxicity and to establish a NOAEL for germ cell effects, a priority research need is **to develop dose-response data for germ cell toxicity that includes consideration of the relevant doses for acrylamide ingestion in food.**

Acrylamide is a neurotoxicant in several species, including man, and signs of neurotoxicity (e.g., grip strength) have been noted in offspring of mice treated with acrylamide. The potential for developmental neurotoxicity in offspring following oral acrylamide exposure of dams during gestation has been evaluated in two rat studies using a limited battery of gross behavioral tests (open field activity, auditory startle habituation, passive avoidance), which did not show effects in offspring. However, other tests and more sensitive indicators of developmental toxicity are needed **to improve the weight-of-evidence regarding neurodevelopmental effects at doses relevant to those expected in food and to establish a NOAEL for those effects.**

Other important research needs in this area include:

- Dominant lethal study in CYP2E1 knockout mice to assess the role of glycidamide in germ cell toxicity and to further refine relevant dose-response relationships
- Developmental toxicity study in a non-rodent species, which should include acquiring toxicokinetic data.

Carcinogenicity

The weight of evidence for carcinogenic potential of acrylamide in humans is based primarily on animal data with limited evaluation in human populations. Although several studies of acrylamide carcinogenicity exist, several questions remain that pertain to the interpretation of observations of tumors from these studies. The working group felt that additional research activities could improve the utility of these studies and reduce uncertainties related to carcinogenic potential. The first activity would be **to conduct a pathology working group meeting in which the histology slides from the animal bioassays would be reviewed by pathology experts using updated diagnostic criteria.** The objective would be to develop a consensus view on diagnoses related to key neoplastic lesions.

The second activity would be **to investigate the mechanism of thyroid tumor induction by acrylamide.** Thyroid tumors were among the most prominent of tumor types diagnosed in the rat cancer bioassays. However, more recent research and guidelines from IARC and the USEPA suggest that certain mechanisms by which thyroid tumors are induced in rats are of questionable relevance to humans. The objective of this research would be to determine the operative mechanism for acrylamide and, thereby, clarify the relevance of these tumors to humans. It is expected that this research could be completed within 6 months.

Other important research needs in this area include, if additional long-term bioassays are considered:

- Evaluation of carcinogenicity under conditions including perinatal exposure

- Evaluation of the role of glycidamide using the CYP2E1 knockout mouse in mechanistic studies supplementing the long-term bioassay, for example by examination of hormone effects or adduct formation in subchronic experiments
- Assessment of other mechanistic issues and inclusion of neurotoxicity evaluations in the long-term bioassay, if feasible.

Neurotoxicity

The working group discussed the various known neurotoxic effects of acrylamide at length. Notably, neurotoxicity is the only toxic response to acrylamide exposure known to occur in humans. Issues include the relevance of rodent models for studying these effects and the dose levels over which mechanisms of neurotoxicity that are relevant to humans may be expressed. Since the neurotoxic properties of acrylamide in humans are known principally from occupational studies of adults at exposure levels that are high relative to our current understanding of dietary exposures, it was felt that the possibility of a similar effect in children warranted research first in animals and possibly subsequent epidemiological follow-up. The animal research proposed was **evaluation of the relationship between dose, duration, and effect-levels and onset of neurotoxicity.** This would include light microscopic (silver staining as well as routine histopathology) and ultrastructural analysis of synaptopathy and axonopathy, linked to nerve function, behavior, and reversibility of effects. Evaluation of exposure durations longer than 3 months is particularly needed. Studies of this type could yield informative data within six months but full completion of the program would likely require at least two years from commencement.

Other important research needs in this area include:

- Evaluation of existing ultrastructure pathology results for evidence of peripheral neuropathy (e.g. Johnson et al. study)
- Assessing potential additive effects to other pre-existing neurological disease using methods such as *in vitro* neurite extension and animal models for multiple sclerosis and amyotrophic lateral sclerosis diseases.

Epidemiology

Peripheral neuropathy has been investigated in workers exposed to acrylamide and this is the one clear effect in humans of high dose exposures to acrylamide. The potential carcinogenicity of acrylamide in humans has been examined in worker cohorts with generally negative results, but the power of the studies was limited. Designing studies of cancer and acrylamide exposures in food will be very difficult, given the relatively low level of exposures and the many potential confounders that will be hard to quantify. However, the potential for reproductive toxicity in human populations has not yet been studied, and this endpoint may be easier to evaluate with respect to confounding. A defined research need is **to design and conduct new epidemiology studies to evaluate sperm chromosomal abnormalities (morphology and quality, if practical) in previously evaluated worker cohorts or other highly exposed populations.** Sperm chromosomal abnormalities would serve as a biomarker for potential reproductive toxicity, and, if found, may require further study. Understanding the relationship between hemoglobin adducts (or other markers of exposure) to chromosomal aberrations would also be useful.

Other important research needs in this area include:

- Evaluation of variation in hemoglobin adduct levels (or other markers of exposure/effect) with sister chromatid exchange (and other markers of chromosomal effects)
- Investigation of the onset and development of neurotoxicity and the potential for cumulative effects and/or long-term effects
- Evaluation of existing surveillance studies (e.g. medical monitoring data) in occupational cohorts for additional data on exposure levels that do and do not cause neurotoxicity
- As a longer-term research need, development of a prospective study using large population surveys, e.g. NHANES or EPIC, in which acrylamide exposure (possibly assessed through hemoglobin adducts) in non-occupational populations would be studied.

Priority Research Needs Identified by the Working Groups

1. Working Group 1: Formation of acrylamide in foods

    - Need database on the quantity of free asparagine on a dried-weight basis for various foods (database - variety, crop conditions, storage) and data on the quantity of glucose, fructose (and other sugars) and amino acids other than asparagine for various foods.
    - Time/temperature/pH/moisture/surface area-mass mapping and kinetics of asparagine/carbonyls reaction in various matrices. May include mathematical modeling. Process investigations and study of the kinetics/pathways of acrylamide formation versus browning and flavor forming reactions.
    - Define the direct correlation of asparagine to acrylamide production in foods.
    - What are the kinetics of acrylamide inhibition/destruction/scavenging under various reaction/process conditions?
        a. Mixed amino acids competitive reactions or scavenging.
        b. Ammonium ion as a possible competitive agent.
        c. Glutathione/cysteine to promote sulfhydryls-disulfide interchange to provide scavengers.
        d. Irradiation
        e. Pressure processing
        f. Fermentation (e.g. yeast)
        g. Hydrolyzed nucleic acids
        h. Asparaginase conversion of asparagine to aspartic acid.

2. Working Group 2: Analytical methodology

    - Establish proficiency testing program and materials
    - Need data on acrylamide content for more foods
    - Is there a need to investigate analytical methods for asparagine, and other possible precursors in food?
    - Is bound acrylamide bioactive and, if so, do current methods extract all acrylamide (including "bound" acrylamide)?

3. Working Group 3: Exposure and Biomarkers

    - Expand database of acrylamide levels in U.S. foods through a clearinghouse for data and through collection of additional data to fill gaps.
    - Establish relationship between biomarker (Hb adducts) and acrylamide in foodstuffs including completion of proposed CDC study of both acrylamide and glycidamide in an on-going human study assessing the relationship between oral administration of acrylamide and the levels of hemoglobin (Hb) adducts.

- Determine the bioavailability of acrylamide and glycidamide found in selected foods in an animal study (availability may be different in different foods).
- Determine glycidamide levels in foods that also contain high concentrations of acrylamide.

4. Working Group 4: Toxicology and Metabolic Consequences

- Metabolism and Kinetics
  - Collect metabolic fate and kinetic data in humans, including bioavailability from foods
  - Develop further information on the critical events and dose metrics related to the mode(s) of action at relevant doses for the key toxicities of acrylamide
- Genetic Toxicity
  - Investigate the formation of adducts of acrylamide or glycidamide with DNA and significant nuclear proteins (for example, protamine or chromosomal motor proteins), especially at critical target sites such as sites of tumor formation and male germ cells
- Reproductive and Developmental Toxicity
  - Develop dose-response data for germ cell toxicity that includes consideration of the relevant doses for acrylamide ingestion in food
  - Improve the weight-of-evidence regarding neurodevelopmental effects at doses relevant to those expected in food and establish a NOAEL for those effects
- Carcinogenicity
  - Convene an expert pathology working group to review the histology slides from existing carcinogenicity studies using updated diagnostic criteria
  - Investigate the mechanism of thyroid tumor induction by acrylamide
- Neurotoxicity
  - Evaluate the relationship between dose, duration, and effect-levels and onset of neurotoxicity in animal studies
- Epidemiology
  - Evaluate sperm chromosomal abnormalities (morphology and quality, if practical) in previously evaluated worker cohorts or other highly exposed populations

5. Working Group 5: Risk Communication

- Attitudinal research
- Information clearinghouse and evidence review
- Communication programs

Planning Committee's List of Short-term Action Items

The Workshop Planning Committee met after the Acrylamide in Food Workshop to discuss the research needs priority conclusions of each of the five Working Groups. While all of the priorities represent important areas for the generation of additional knowledge concerning acrylamide in food, focus was on identification of those high priority needs that are amenable to action in the short term. Projects will be developed for each of the high priority areas identified. The Planning Committee will determine potential funding mechanisms for each of the projects.

Priority areas and projects:

1. Analytical Methods

- Establish proficiency testing program and materials

2. Methods of Formation

- Develop data for various foods on quantity of free asparagine for various foods and data on the quantity of glucose, fructose (and other sugars) and other amino acids.
- Develop data on time/temperature/pH/moisture/surface area-mass mapping and the kinetics of asparagine/carbonyls reactions in various matrices.
- Define the direct relation of asparagine to acrylamide production in foods.
- Develop data on the kinetics of acrylamide inhibition/destruction/scavenging under various reaction/process conditions.

3. Exposure

- Expand the database of acrylamide levels in U.S. foods through collection of additional data and establish mechanisms for information sharing, such as the WHO/FAO Acrylamide in Food Network operated by JIFSAN through its Food Safety Risk Analysis Clearinghouse.
- Develop and conduct appropriate animal and/or human studies on the bioavailability of acrylamide in foods.

4. Toxicology

- Develop data on the absorption/distribution/metabolism/excretion of acrylamide.
- Develop and conduct studies on DNAprotein glycidamide and acrylamide adducts to determine metabolic consequences of acrylamide.

5. Risk Communication

- Conduct qualitative attitudinal research on consumer awareness and knowledge of acrylamide in food. Triggers for behavioral change may be identified for acrylamide and other potential health hazards.
- Formally document the unique process used to develop and accomplish the Acrylamide in Food Workshop.
- Establish an information clearing house and evidence review process with full participation by a broad group of stakeholders. The WHO/FAO Acrylamide in Food Network provides the starting foundation for this.

BRIEF INTRODUCTION TO
THE ENVIRONMENTAL TOXICOLOGY OF ACRYLAMIDE

Acrylamide (AA) is a high-production volume chemical (>200 metric tons/yr worldwide) whose polymeric forms are widely used in international commerce (IARC, 1995). Major uses for polyacrylamide include water treatment, crude oil processing, pulp-paper processing, concrete, and grouts (IARC, 1982). Monomeric AA is used extensively in biochemical applications for PAGE analysis of proteins and is a component of cigarette smoke (1-2 μg/cigarette, Smith et al., 2000). More recently, AA has been measured in baked and fried starchy foods, notably french fries (0.4 ppm), potato chips (1.5 ppm), and bread (0.04-1.7 ppm, Rosen and Hellenas, 2002; Tareke et al., 2002).

Following oral administration to rodents, absorption and elimination (plasma $t_{1/2}$ □2 hr) of AA is rapid and distribution to tissues is extensive, as predicted for a small water-soluble compound (Miller et al., 1982; Barber et al., 2001). In the mouse, AA is converted to a reactive epoxide metabolite, glycidamide (GA), primarily through the action of CYP 2E1 (Sumner et al., 1999). In the rat, the elimination half-time for GA (plasma $t_{1/2}$ □1.5 hr) is slightly shorter than that for AA, and the GA/AA AUC ratio following an oral dose is approximately 0.3 (Barber et al., 2001). The formation of GA in the rat is linear at low doses of AA, but saturation of enzymatic oxidation is apparent at high doses (Bergman et al., 1991). As a result, the fraction of AA converted to GA increases with decreasing dose such that > 50% conversion to GA is observed at low doses in rats (Bergman et al., 1991). AA and GA react with nucleophilic amino acids in hemoglobin, notably the N-terminal valine and cysteine residues, reactions that have been used for assessing internal exposures in rodents and humans (Bergmark et al., 1993). In addition, AA and GA form glutathione conjugates that are excreted in the urine as mercapturic acid derivatives in rodents (Sumner et al., 1992) and humans (Calleman et al., 1994). The mouse produces more GA than the rat from similar oral dosing of AA based on the relative amounts of excreted metabolites (Sumner et al., 1992). The observation of GA and AA hemoglobin adducts in humans at ratios similar to those observed in mice and rats suggests that rodents are appropriate models for assessing exposures to GA derived from AA metabolism (Paulsson et al., 2002).

AA is a well-described neurotoxicant that causes peripheral neuropathy of central and peripheral origin in experimental animals (Spencer and Schaumburg, 1975) and clinical evidence for adverse neurological effects was reported in a cohort of AA workers (Callemen et al., 1994). Based on respective levels of hemoglobin adducts, the doses of AA that cause human neurotoxicity from occupational exposures are two-three orders of magnitude above those relevant to dietary exposure (Callemen et al., 1994; Tareke et al., 2000). There is also evidence for reproductive toxicity of AA in rodents, particularly in the male (IARC 1995).

Consistent evidence for genotoxicity of AA has been reported. Although AA reacts with DNA very slowly (Solomon et al., 1985), GA is more reactive and forms several DNA adducts, including an N7-guanine derivative resulting from depurination *in vitro* and *in vivo* (Segerback et al., 1995) and two putative exocyclic hydroxypropano-derivatives of deoxyadenosine and deoxythymidine (Solomon, 1999). Although AA is not mutagenic in *Salmonella* tester systems, GA is (IARC, 1995). AA also causes chromosomal damage in and transformation of mammalian cells *in vitro* (IARC, 1995). There is evidence for initiation of carcinogenicity in mice from topically and orally administered AA when followed by a tumor promoter (IARC, 1995). Finally, there are two 2-year carcinogenicity studies of AA administered chronically in drinking water both of which reported elevated tumor incidences in multiple organs in male and females rats (Johnson et al., 1986; Friedman et al., 1995).

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