2004 Acrylamide in Food Workshop: Update –
Scientific Issues, Uncertainties, and Research Strategies

April 13-15, 2004
Chicago, Illinois

TOXICOLOGY AND METABOLIC CONSEQUENCES WORKING GROUP

Priority Research Needs

Introduction

In October 2002 the food industry in conjunction with JIFSAN and NCFST organized and convened a workshop to discuss the scientific issues related to the occurrence of acrylamide in food and particularly to identify key data gaps and research needs. The Working Group on Toxicology and Metabolic Consequences, organized by ILSI North America, was one of five working groups at the workshop that prepared reports outlining priorities for research.

In April 2004 a food industry coalition, again in collaboration with JIFSAN, held a follow-up workshop to discuss the status and results of research since the October 2002 workshop and to identify remaining critical data gaps. The ILSI North America Technical Committee on Food Toxicology and Safety Assessment assisted JIFSAN with the organization and support of the Working Group on Toxicology and Metabolic Consequences. This comprises the report of the Working Group on Toxicology and Metabolic Consequences from the April 2004 workshop.

The Working Group noted that significant progress has been made towards understanding the toxicology and metabolism of acrylamide. For example, the metabolic fate of acrylamide in rodents and humans is now fairly well defined qualitatively. In addition, the kinetics of acrylamide and its oxidative metabolite glycidamide have been determined in rodents, and good progress has been made on determination of kinetics in humans. A first physiologically-based pharmacokinetic (PBPK) model for acrylamide (in rats) has been published. The hemoglobin adducts of acrylamide and glycidamide have been studied in several labs now as markers of exposure. The important role of glycidamide in the genotoxicity of acrylamide is becoming even clearer, with the identification of new glycidamide-DNA adducts and the linking of glycidamide to the formation of micronuclei and the germ cell mutagenicity of acrylamide. Good progress also is being made in elucidating acrylamide's mechanisms of neurotoxicity. And, importantly, the new NTP two-year carcinogenicity studies of acrylamide and glycidamide in rats and mice are getting underway at NCTR.

In its deliberations the Working Group did not elect to choose the highest among the priority research needs, but some of the major remaining data needs that were discussed include the determination of modes of action and appropriate dose metrics for acrylamide's principal toxic effects, the examination of potential effects from perinatal exposures, the characterization of the dose-response relationship for germ cell toxicity, and the development of a human PBPK model.

Working Group Process

The Working Group began with the research needs identified at the October 2002 workshop, grouping them into four topical areas. The discussion of each topic was initiated and framed by one or two overview presentations, highlighting what was known in October 2002, what has been learned since then that is relevant to each of the research needs, and what the presenter considered to be the key remaining research needs for reducing the substantial remaining uncertainty regarding the type and level of risk that might be associated with exposure to acrylamide in food. The Working Group expresses its sincere appreciation to the overview presenters:

Topic 1: Metabolism, kinetics (incl. PBPK), adducts
Presentation: *Tim Fennell (RTI International)*

Topic 2: Germ cell effects, repro/developmental effects, genotoxicity
Presentations: *Jack Bishop (NIEHS), Rochelle Tyl (RTI International)*

Topic 3: Carcinogenicity (incl. cancer epidemiology)
Presentations: *Dan Doerge (NCTR/FDA), Kathleen Koehler (CFSAN/FDA)*

Topic 4: Neurotoxicity
Presentation: *Richard LoPachin (Albert Einstein College of Medicine)*

The questions that the Working Group was asked to address were:

- For each research need identified in October 2002 –
    - Has it been addressed? Has the question been answered satisfactorily?
    - If not, is it still considered (by this Working Group) to be a critical question/research need for assessing human risk from exposure to acrylamide in foods? What specific studies are recommended?
    - To our knowledge, are such studies in progress or planned? By whom? Schedule?
- Are there toxicology questions/research needs **not** identified in the October 2002 Workshop that are now considered to be critical to the assessment of human risk from exposure to acrylamide in foods? If so –
    - What are they? Why are they critical research needs? What specific studies are recommended?
    - To our knowledge, are such studies in progress or planned? By whom? Schedule?
- Within a topic and overall, which research needs are highest priority?

The results of the Working Group's deliberations are presented in the following paragraphs. Within each of the four topical areas, the research needs considered most critical (Priority Research Needs) are distinguished from those considered to be important but less critical (Other Research Needs) for characterizing the human risk from exposure to acrylamide in food.

Metabolism, Kinetics and Adducts

The 2004 workshop reviewed new data on the metabolic fate and bioavailability of acrylamide (AA) that would support extrapolating dose-response from animal to human and from high to low dose exposures. Kinetic data in rats and mice have been generated using high resolution liquid chromatography/tandem mass spectroscopy in conjunction with stable isotope labeling. Sumner et al. (Toxicol Sci. 75(2):260-70, 2003) provided data on AA distribution, metabolism and formation of hemoglobin adducts in mice and rats following dermal, intraperitoneal, oral, or inhalation exposure. Hemoglobin (Hb) adducts for AA and its oxidative metabolite, glycidamide (GA) have been measured at femtomole per milligram globin levels, and the ratio of AA-Hb to GA-Hb compared

among routes and species. Good correlation between AA exposure and hemoglobin adduct levels indicates that Hb adducts may provide a useful biomarker of acrylamide exposure. Studies on formation of AA- and GA-Hb adducts, metabolism (including the role of CYP2E1 using knockout mice), and GA-DNA adducts following different routes of exposure in rats and mice indicate that GA-DNA adducts are distributed throughout a variety of tissues and correlate to AA tissue levels. First pass effects and differences in species metabolic rates are significant, and there is little evidence of the formation of AA-DNA adducts (FDA and NIEHS studies to be published in 2004). Human urinary metabolites and hemoglobin adduct levels have been measured in adult male (sterile) volunteers following an oral (single dose) or dermal (24 hour) exposure to acrylamide (industry sponsored study to be published in 2004). These rodent and human data will be used to calibrate PBPK models, the development of which are underway within FDA and the private sector. A PBPK model for AA and GA in rats calibrated against older time course data (14C radiolabled AA) was published in 2003 by Kirman et al.(J Tox Env Health Part A 66:253-274). The newer, more highly resolved data should considerably improve PBPK model parameter estimates and predictive capability.

Priority Research Needs

- Further characterize the mode(s) of action (MOA) for acrylamide and glycidamide toxicity to:
    - differentiate between dose metrics for effects and dose metrics for exposure
    - identify the most appropriate PBPK model internal dose metrics for quantitative risk assessment
- Determine bioavailability in food versus drinking water [studies in rodents underway at NCTR]
- Improve the robustness of the PBPK model(s) for rat, mouse, and human based upon:
    - kinetics of AA and GA in humans [basic metabolic parameters have been captured in controlled studies with limited numbers]
    - kinetics of AA across different developmental stages, as well as identification of factors that increase susceptibility [studies planned at NCTR in rats and mice]
    - additional data sets for calibration and testing of PBPK model structure and parameter estimates

Other Research Needs

- Molecular and kinetic characterization of binding to sulfhydryls in target and non-target sites (e.g., measure rate constants for binding to critical target vs. glutathione)
- Development of a biologically based dose response (BBDR) model to simulate the events from the internal dose (i.e., the PBPK model output) to the adverse effect (heritable mutagenicity, repro/developmental, neurotoxicity, and carcinogenicity).

Reproductive and Developmental Effects, Germ Cell Effects, and Genotoxicity

Acrylamide can cause adverse reproductive outcomes in rodents. Dominant lethality studies at high, single doses have suggested that the effects are the result of toxicity to the male germ cell. However, the significance of these observations for the

assessment of human risks from long-term exposure at much lower levels (at least 10^4x) still remains to be determined, leading the workgroup to note again the need for dose-response data for germ cell toxicity at dose levels relevant to acrylamide in food. A proposal, coupling the NIEHS PAINT/DAPI assay methodology with accelerator mass spectrometry (AMS), was discussed. Information on sperm chromosomal abnormalities in highly exposed human populations, if available, also could be helpful in addressing this data gap.

Important new studies in CYP2E1 knockout mice (lacking the enzyme that converts acrylamide to glycidamide), conducted since the October 2002 Acrylamide in Food Workshop, provide strong evidence that acrylamide's dominant lethal effects in male germ cells require the prior formation of glycidamide. Some dose-response data are being generated in these studies, and the workgroup suggested that linking exposure dose to adduct levels (DNA or nuclear proteins) in the germ cells would be useful.

Progress has also been made since the October 2002 Workshop in understanding the genotoxicity of acrylamide. Several DNA adducts of glycidamide have been identified in mice treated with acrylamide or glycidamide, and induction of micronuclei (from chromosomal damage) appears to be due to glycidamide formation in mice treated with acrylamide. The specific roles of DNA adducts and/or other adducts in acrylamide's toxic and carcinogenic effects remain to be elucidated, and the workgroup encouraged continued investigation of adducts with DNA and significant nuclear proteins in potential target tissues, linked to investigation of possible modes of action.

Priority Research Needs

- Investigate formation of adducts of DNA and significant nuclear proteins (protamine, chromosomal motor proteins) at critical target sites such as somatic cells, germ cells, and sites of tumor formation
- Develop dose-response data for germ cell toxicity that addresses dose levels from acrylamide in food (PAINT/DAPI & AMS?)
- Evaluate sperm chromosomal abnormalities (morphology and quality) in highly exposed human populations, if available

Other Research Needs

- Use of specifically genetically modified rodent strains to assess mode of genotoxic damage *in vivo* [studies in progress at NCTR on Big Blue rat and Tk(+/-) mouse]
- Dominant lethal study in CYP2E1 knockout mice to assess role of glycidamide in germ cell toxicity [studies in progress at NIEHS]
    - Determination of dose-response data for adduct levels in germ cells would be useful
- Evaluate variation of human hemoglobin adduct levels (or other marker of exposure/effect) with sister chromatid exchange, micronuclei, or other markers of chromosomal effects [HEATOX project in Europe will look for correlation of hemoglobin adduct levels with micronuclei in relation to intake of certain foods]
- Developmental toxicity study in a non-rodent species (probably rabbit), including toxicokinetics

Carcinogenicity

The evidence for carcinogenic potential of acrylamide is based primarily on two studies in rats. In the previous JIFSAN Acrylamide in Food Workshop, the need for

follow-up carcinogenicity studies of both acrylamide and glycidamide was recognized. Fortunately, this task has been undertaken by the National Toxicology Program, and preliminary dose range-finding studies for both compounds in rats and mice are underway at NCTR. The workgroup noted the paucity of data on the effects of perinatal exposures and suggested consideration of inclusion of a transplacental or neonatal exposure group in the design of the two-year studies, if possible. NCTR plans also include a shorter-term assay in a neonatal mouse model that is sensitive to direct-acting genotoxic carcinogens, and the workgroup wondered if a transplacentally exposed group could be included. Concurrent investigation of the possible carcinogenic MOAs (genotoxic, endocrine-mediated) in these new bioassays was strongly encouraged.

Recognizing that the NTP/NCTR carcinogenicity studies will take several years to complete, the workgroup reiterated recommendations from the October 2002 Workshop to examine some aspects of the existing carcinogenicity data in more depth. For example, an expert pathology working group (PWG) might be convened to review the combined slides from the two previous rat studies to develop a consensus on key tumor diagnoses. Further investigation of the possible MOAs leading to the various tumor types observed in the previous rat studies also was suggested. The workgroup also recognized the value of ongoing or planned mechanistic studies on the role of glycidamide using CYP2E1 knockout mice, including the examination of hormonal effects or adduct (DNA or hemoglobin) formation.

Since the October 2002 Workshop, a few epidemiology studies of existing cohorts have been reported, looking at acrylamide exposures estimated from food intake questionnaires. No positive association with acrylamide exposure has been observed. The workgroup encouraged consideration of the feasibility and utility of additional studies using existing available cohorts.

Priority Research Needs

- Evaluate carcinogenicity of acrylamide in food in new, well-designed studies [planned/underway at NCTR]
- Assess genotoxic and endocrine-mediated mechanisms [some mechanisms under study by NCTR and SNF; other mechanisms may be studied separately]

Other Research Needs

- Evaluate role of glycidamide using CYP2E1 knockout mice
- Assess feasibility of further epidemiology studies of non-occupationally exposed populations using large existing cohorts in which acrylamide exposure (possibly assessed with hemoglobin adducts) could be evaluated
- Establish PWG to review combined tumor slides from previous acrylamide rat carcinogenicity studies

Neurotoxicity

While neurotoxicity is the only toxic response to acrylamide exposure known to occur in humans, substantial uncertainties remain that are relevant to risk characterization for acrylamide at dose ranges of interest from food exposures.

Key among these is the relevance of rodent models for developing appropriate dose-response information in humans. As such, and as with other potential health effects for acrylamide, further information on mode of action is needed in both animals and humans. In addition, PBPK and BBDR models have the potential to greatly reduce the uncertainty in extrapolation of acrylamide dose-response data for neurotoxicity in

animals to doses received by humans from food. Study of human exposures to acrylamide in occupational settings, even in "sub-clinical" dose ranges for acrylamide effects, may also provide information relevant to risk characterization – if such populations can be found.

The neurotoxic properties of acrylamide in humans are known principally from occupational studies of adults at exposure levels that are high relative to dietary exposures. Limited studies of neurodevelopmental effects are available, and these studies in general provide crude measures of central nervous system function. Therefore, as recognized previously, more sensitive indicators of developmental neurotoxicity are needed.

Similarly, on the other side of the age range, given the well demonstrated cumulative nature of acrylamide's neurotoxic properties over intermediate durations, the potential for exacerbation of neurodegenerative disease should be explored.

Based on presentations made to the workgroup and review of the literature and of the Acrylamide Infonet, substantial progress has been made in the planning and conduct of neurotoxicity studies responsive to data needs recognized in the prior JIFSAN workshop. Of particular note for risk characterization in the regulatory context, detailed study of developmental and adult neurotoxicity in rodents is planned by the FDA's National Center for Toxicological Research, with dose range-finding studies completed or in progress. Part of this work is in conjunction with long term cancer bioassays in rats and mice and will benefit from corollary information developed through those detailed studies, including information on bioavailability from food and on toxicokinetics in general.

Priority Research Needs

- Evaluate mechanism [mode] of action in conjunction with dose, duration, and effect-levels and onset of neurotoxicity
    - Reversibility
    - Target site (nerve terminal, axon, other)
    - Protein adduct formation/clearance kinetics for presumed proximate toxic effects (i.e., related to evaluation of dose-duration effects)
    - CYP2E1 studies to distinguish glycidamide versus acrylamide effects
- Improve weight-of-evidence regarding neurodevelopmental effects at doses relevant to food intake; establish NOAEL
    - Neurobehavioral/cognitive [studies planned at NCTR]
    - Mechanistic (cell adhesion, glial interaction, neurite outgrowth)
    - Consider reversibility

Other Research Needs

- Include neurotoxicity evaluations in long-term bioassay [studies planned at NCTR]
- Evaluate existing surveillance studies (e.g. medical monitoring data) in occupational cohorts for additional data on exposure levels that do and do not cause neurotoxicity.
- In animal models or prospective epidemiological analyses, assess potential additive effects to other pre-existing neurological disease such as multiple sclerosis, Parkinson's, and amyotrophic lateral sclerosis.

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