DRAFT

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.
   - o Assess low dose and multiple dose exposure effects;
   - o Establish a dose response;
   - o Assess the results from acute, subchronic and chronic exposures, and
   - o 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.
   - o Use Big Blue Mouse, Big Blue Rat and Tk+/- animal models.
   - o 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.