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## 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 1$^{st}$-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.