codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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DISCUSSION PAPER ON ACRYLAMIDE

Governments and international organizations wishing to submit comments on the following subject matter are invited to do so **no later than 16 February 2004** as follows: Netherlands Codex Contact Point, Ministry of Agriculture, Nature and Fod Quality, P.O. Box 20401, 2500 E.K., The Hague, The Netherlands (Telefax: +31.70.378.6141; E-mail: info@codexalimentarius.nl, with a copy to the Secretary, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy (Telefax: +39.06.5705.4593; E-mail: Codex@fao.org).

BACKGROUND

1. The 35th session of the Codex Committee on Food Additives and Contaminants (CCFAC) agreed that a drafting group led by the United Kingdom and the United States of America, with the assistance of Australia, Ireland, Japan, Switzerland, European Commission, International Nut Council and World Health Organization, would prepare a Discussion Paper on Acrylamide for circulation, comments and further consideration at its 36th Session.

2. The Committee noted the difficulties experienced by countries in identifying and measuring acrylamide and other contaminants. Special consideration should be given to children, taking into account that this is part of the population subjected to higher consumption of some of the foods related to acrylamide exposure. The Committee also noted that assistance from FAO and WHO, was needed to enhance capacity building in this area, particularly in developing countries.

3. The 35th CCFAC also placed acrylamide on its priority list of substances for risk assessments by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Acrylamide is scheduled by the joint Secretariats for evaluation at the 65th JECFA meeting in February 2005.

INTRODUCTION

4. In April 2002, researchers from the Swedish National Food Administration (SNFA) and the University of Stockholm announced that acrylamide is formed in a variety of baked and fried foods cooked at high temperature[58]. Since the Swedish report, similar findings that acrylamide is formed primarily in starch-rich food prepared or cooked at high temperatures have been reported by numerous other countries, including, Norway, Switzerland, the United Kingdom, and the United States of America [28,30,45,60].

5. In 2002, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) held a consultation on the "Health implication of acrylamide in food" and made a number of recommendations [65].

6. The discovery that acrylamide is unintentionally formed in some cooked foods is a concern because acrylamide is a potential human carcinogen and genotoxicant based on high-dose animal studies, and is a known human neurotoxicant[7,8]. Acrylamide is carcinogenic in animals, producing increased incidences in a number of benign and malignant tumours identified in a variety of organs (for example thyroid, adrenals)[21]. The potential carcinogenicity of acrylamide has not been thoroughly investigated in humans [21], particulary after chronic dietary exposure to humans.

7. Studies conducted to date suggest that acrylamide formation is particularly likely in carbohydrate-rich foods cooked (i.e baked or fried) at temperature of above approximately 120ºC [9,34,50]. The underlying mechanism or mechanisms leading to the formation of acrylamide is still not fully understood. One potential pathway that has been identified involves a chemical reaction between the amino acid asparagine and certain reducing sugars, both of which are found naturally in foods [6,41,51,56]. Acrylamide has not been detected in boiled foodstuffs[62,63].

8. Acrylamide is a chemical intermediate (monomer) commercially produced for the production and synthesis of polyacrylamide. This monomer occurs in white flowing crystalline form and in aqueous solution, it is soluble in water, ethanol, methanol, dimethyl ether and acetone. It readily polymerises on reaching melting point or exposure to UV light. Solid acrylamide is stable at room temperature, but may polymerise violently when melted or exposed to oxidating agents. Acrylamide polymerisation is used *in situ* in the formulation of grout agents, for construction and repairing of sewers and tunnels and in the preparation of polyacrylamide gels used in laboratories [54]. Acrylamide polymers have a range of uses, including water treatment, soil treatment and as a cosmetic additive. Acrylamide is also a component of tobacco smoke[8].

TOXICOLOGY

9. Acrylamide has been shown to induce tumors in experimental animals and has been classified by the International Agency for Research into Cancer (IARC) as "probably carcinogenic for humans"[36]. Acrylamide induces genetic mutations and chromosomal abnormalities in cultured cells *in vitro* [36]. It has also produced positive results in *in vivo* genotoxicity studies [54]. It is known to metabolized to glycidamide, a chemically reactive epoxide that forms DNA adducts and may be the proximate genotoxin. Long-term studies in rats produced a dose related increase in the incidence of tumours of the mammary gland, thyroid, oral cavity, and reproductive tract with some evidence of tumours in the brain [33,37]. Screening assays in mice have also produced evidence of carcinogenicity[14]. The FAO/WHO consultation also looked in detail at the toxicological properties of acrylamide and concluded that more studies need to be conducted on different aspect of toxicity of acrylamide. See Annex 1 for a summary of information on the toxicity of acrylamide.

10. In 1991, the European Union (EU) Scientific Committee on Food (SCF) evaluated acrylamide as a monomer in food contact materials and concluded that acrylamide was a genotoxic carcinogen. Following the 2002 findings of acrylamide in food, the SCF re-evaluated acrylamide and maintained its conclusion that acrylamide is a genotoxic carcinogen, but that it was not possible at that time to determine the actual risk from acrylamide in food [54]. The SCF concluded that exposure should be reduced to as low as reasonably achievable, although more data were needed in several areas to help towards reducing levels and to help clarify the safety implications. In addition, the SCF noted that acrylamide is neurotoxic in experimental animals and in humans exposed to acrylamide in the workplace. Studies in rats and mice have shown that acrylamide impairs male fertility, although it is not clear whether this effect was secondary to neurotoxicity[54]. However, the SCF indicated from available data that the levels of dietary intake of acrylamide were likely to be considerably lower than those levels with neurotoxic implications.

ANALYTICAL METHODS

11. Several analytical methods have been used to quantify acrylamide in food. The most commonly used methods are: gas chromatography/mass spectrometry (derivatized), (GC-MS (derivatized)); gas chromatography-mass spectrometry (underivatized), (GC-MS (underivatized)); liquid chromatography-mass spectrometry, (LC-MS) and high performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) [44,64]. See Annex 2 for more information on these analytical methods and on the various proficiency tests conducted so far.

EXPOSURE AND RISK (HUMAN)

Dietary exposure

12. Since the first reporting of the levels of acrylamide in food, a range of levels have been reported in different food and food products [2,17,49,61]. Table 1 gives a summary of the typical results of the analysis of acrylamide in food to date. There are limited numbers of analyses conducted for some of these food groups. Significant variability of acrylamide levels among foods within particular categories and within batches of products processed under the same conditions have been observed.

Table1: Reported levels of Acrylamide in Food

Food / Product Group	Acrylamide levels (µg/kg)	
	Minimum	**Maximum**
Potato crisp	170[65]	2510[28]
Potatoes (raw)	<10[2]	<50[2]
Potatoes (boiled)	<4[35]	<50[2]
Potato chips/ French fries	59[35]	12800[2]
Corn crisps	120[58]	220[28]
Bakery products	24[28]	364[28]
Bread	<10[28]	130[28]
Bread (toast)	25[2]	1430[39]
Biscuit and crackers	18[46]	650[58]
Breakfast cereals	22[46]	1400[58]
Crispbread	<30[58]	1900[58]
Noddles	11[46]	581[46]
Coffee (roasted)	45[28]	374[28]
Tea	142[61]	567[61]
Roasted barley grains	210[46]	578[46]
Chocolate products	<2[35]	909[28]
Nuts	28[28]	339[28]
Infant formulas and baby foods	<10[28]	130[28]
Fish and seafood products, crumbed, battered	<2[46]	39[65]
Poultry or game , crumbed, battered	<10[28]	64[65]
Beer	<6[35]	<30[39]

13. In a recent study, dietary exposure to acrylamide of different age groups of the Dutch population were analyzed and the results were used to estimate the acrylamide exposure of consumers who participated in the National Food Consumption Survey (NFCS) in 1998. Daily exposure to acrylamide was estimated using a probabilistic approach. It was concluded that risk of neurotoxicity is negligible, but the cancer risk might not be negligible[39]. In another recent study, the estimated dietary intake of acrylamide for the Swedish population was 9.1, 27 and 62 mg/person/day for the 5th, 50th and 95th percentile respectively, (mean 31 mg/day) from a range of food/product groups with low to high levels of acrylamide (<30–2300 mg/kg), such as processed potato products, bread, breakfast cereals, biscuits, cookies, snacks and coffee[59].

14. The June 2002 FAO/WHO consultation[65] reported that short-term intake estimates for USA and the Netherlands populations, ranged from 0.8 μg/kg bw day for the average consumer to 3 μg/kg bw day for the 95th percentile consumer. Due to the sparse unrepresentative data on acrylamide occurrence in foods available at that time, the FAO/WHO consultation was not able to consider estimates over long periods of time including chronic and lifetime exposures. A call for data on levels of acrylamide in food and the total diet has been placed on the FAO/WHO acrylamide food network[26]. These data will be provided to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for its safety evaluation of acrylamide in food.

15. To estimate daily intake of acrylamide for an individual in the U.S.A, the U.S. Food and Drug Administration (FDA) have used probabilistic modeling with data from three food consumption surveys: 2-day consumption records from the Continuing Survey of Food Intakes by Individuals (CSFII) 1994-96 and 1998 CFSII Supplemental Children's Survey; 3-day consumption records from CSFII 1989-92; and 14-day consumption records from Marketing Research Corporation of America. Results of this initial exposure assessment showed that the mean population intake of acrylamide is consistent with previous exposure estimates[65] and that 8 food categories (regular french-fries, oven baked french-fries, potato chips, breakfast cereals, toast, cookies, soft bread, coffee) contribute to more than 80 percent of mean population acrylamide intake and that no one food accounts for the majority of the mean population acrylamide intake[29].

Occupational exposure

16. In 2002, the UK prepared a report on behalf of the European Union, on the risk assessment of acrylamide under the EU framework, for control of the risks of "existing" substances. Occupational exposure assessment was based on information supplied by industry. The studies looked at different sectors (manufacture of acrylamide/polyacrylamide preparation, use of electrophoresis gels and use of acrylamide grout) where occupational exposure to workers may occur. It was concluded that existing risk reduction measures should be considered by workers, in view of the carcinogenic and genotoxic nature of acrylamide and in view of the low margin of safety values obtained for neurotoxicity and reproductive toxicity in some exposure scenarios[21].

Other exposures

17. Studies have shown that smoking tobacco is an important source of exposure[8,53].

18. Exposure to low levels of acrylamide might result from the presence of residual acrylamide in polyacrylamide used in cosmetics, water treatment and soil conditioners etc[21]. Exposure to low levels of residual acrylamide may occur through drinking water from the use of polyacrylamide as flocculents or coagulants to condition sludge, to clarify raw water and to treat effluent streams from sewage plants[21]. The U.S. Environmental Protection Agency established a limit of 0.5 μg/L of residual acrylamide allowed in drinking water. The European Union has set a limit of 0.1 μg/L residual acrylamide in drinking water[22], this was based on the WHO guidelines for drinking water quality and the opinion of the European Commission's Scientific Committee on Toxicology and Ecotoxicology.

19. The European Commission's Scientific Committee on Cosmetic Products and Non-Food Products considered the levels of acrylamide in cosmetic products in 1999. It recommended that a tolerable content of acrylamide in polyacrylamide should be <0.1 parts per million (ppm) in bodycare products and <0.5ppm in other cosmetic products[52].

OCCURRENCE IN FOOD

20. Acrylamide has been detected in foods prepared by commercial processing and domestic cooking. The broad range of foods susceptible to acrylamide formation represents important nutritional components in the diet.

21. The announcement that acrylamide is found in human foods was followed swiftly by research into the mechanisms of acrylamide formation. A number of theoretical mechanism have been proposed, including pathways from amino acids only, from acrolein intermediates, from acrylic acid intermediates and from Maillard browning precursors[38].

22. Current studies demonstrate that in food, acrylamide is mainly produced in high temperature induced reactions between the amino group of the free amino acid, asparagine, and the carbonyl group of reducing sugars, such as glucose, during baking and frying[5,41,51,56,67,68]. Foods rich in both of these precursors are largely derived from plant sources such as potatoes and cereal grains[32].

23. Based on mechanistic studies, three additional formation pathways for acrylamide from food have been hypothesized[66]: (a) heating at 180 °C of either asparagine or glutamine forms acrylamide from thermal degradation, (b) ammonia produced from alpha-amino acids such as asparagine via Strecker degradation reacts with acrylic acid, formed from acrolein, in turn formed from lipid breakdown, and (c) an acrylic acid radical from high-temperature heating of acrolein reacts with an amine radical formed from high-temperature heating of an amino acid. The authors hypothesize that acrylamide can form from many different food constituents in addition to amino acids.

24. A survey conducted in the United Kingdom (2002), tested whether residual acrylamide was present in samples of paper board that may be in contact with food and drinks and if so whether there was any detectable migration of this substance into food and drinks. Acrylamide was detected in a small minority of paper and board packaging samples (12 out of 140). The survey concluded that it was very unlikely that paper and board packaging was a source of acrylamide in food[31].

25. Due to the agricultural usage of polyacrylamide, several studies have been conducted to investigate whether residual acrylamide is taken up and bioaccumulates in crops grown using polyacrylamide. In 1991, Castle *et al*[15] analysed tomato fruits from plants grown hydroponically on polyacrylamide gel to determine possible uptake of acrylamide monomer from the nutrient solution to the fruits during cultivation. No acrylamide monomer was detected at a limit of detection of 1µg/kg (ppb) level. The authors concluded that the monomer was not transferred from the growing medium into tomato fruits. Further studies were done in 1993 on mushrooms to also assess the possible uptake of acrylamide[16]. The mushrooms were grown on a casing mixture containing polyacrylamide gel. This later analysis demonstrated that acrylamide does not either translocate from mycelia to the mushrooms or bioaccumulate.

26. In 1999, four different crops (corn, potatoes, sugar beets and beans), that were grown in soil that had been treated with polyacrylamide to stabilize soil erosion were analysed for residual acrylamide. Residual acrylamide was not present in any of the analysed crops at a level above 10 parts per billion (ppb). It was concluded that bioaccumulation of residual acrylamide in plant tissues was highly unlikely[10].

27. Another possible route of formation of free acrylamide was proposed to be from the degradation of polyacrylamides that are used in agriculture[20]. Recently, a study was conducted to investigate whether depolymerization of polyacrylamides used in agriculture may contribute to acrylamide formation in heated foods. No acrylamide was detected following the heating of polyacrylamide at 175^{o}C for 15 and 30mins respectively. It was concluded that even if polyacrylamides were to contaminate agricultural crops or foods derived therefrom (which itself is an unproven suggestion), there was no s evidence that polymers would depolymerize on heating of food to form acrylamide[1].

STRATEGIES FOR REDUCTION OF ACRYLAMIDE LEVELS IN FOOD

28. Different strategies for reducing acrylamide levels in food have been suggested for example[13], (a) remove or reduce reactants (asparagine, reducing sugar) (b) disrupt reaction and (c) remove acrylamide after formation.

Remove or reduce reactants

29. Whenever one reactant (asparagine or glucose) is at a reduced concentration, there is a reduction in the formation of acrylamide. Reducing or eliminating a reactant will alter the amount of acrylamide formed. Researchers have identified two possible ways to reduce the amount of reactant in potatoes. During storage, the amount of sugar increases in potatoes. Using fresh potatoes rather than stored potatoes could result in less acrylamide being formed. Storing potatoes below 8-10ºC can promote the formation of reducing sugars[17,34]. The presence of this reducing sugar together with asparagine may lead to acrylamide formation. The variety of potato also affects the amount of acrylamide formed because of the relative amounts of reducing sugar and asparagine[4].

Disrupt reaction

30. There is a time-temperature relationship to the formation of acrylamide in food. Changing the temperature and duration of cooking will affect the level of acrylamide in a food. It has been suggested that, when the temperature of a food rises above 120ºC, the rate of acrylamide formation increases rapidly with temperature over a limited range [13]. Studies have also demonstrated a decrease in acrylamide levels at 170ºC and above[9,41]. Acrylamide formation in food is also dependent on pH. Suggested optimum pH for acrylamide formation in food is approximately 7. At pH significantly below 7, acrylamide formation is inhibited[13]. Other inhibitors of acrylamide formation are under investigation. In addition to modifications in temperature, cooking time, and pH, the enzyme asparaginase has been used to disrupt the formation of acrylamide in foods[12]. Water activity also seems to be a critical factor, although this has not been fully investigated.

Remove acrylamide after formation

31. To date, attempts to reduce acrylamide levels in food by UV light treatment and supercritical CO_2 extraction have not been successful[13].

32. The effect on the overall diet has to be considered carefully when making potential modification to reduce acrylamide levels in food. For example lowering the frying temperature of french fries may lower the acrylamide content, but may also increase the fat content. Also, the long-term storage of potatoes at temperatures below 8-10ºC may be unavoidable as cooler storage may be required to prevent sprouting. In some cases the uses of sprout-suppressing agents[55] may be inappropriate or a less acceptable alternative.

CO-ORDINATION OF ACTIVITIES

FAO/WHO (Acrylamide Infonet)

33. The June 2002 FAO/WHO consultation recommended that an international network on acrylamide in food should be established inviting all interested parties to share relevant data as well as ongoing investigations. As a result, the FAO/WHO Acrylamide in Food Network[26] was established. As at end December 2003, there are currently 118 research projects listed in the research database in addition to eight listed in the Studies in Development database on the Acrylamide Infonet[26].

Europe

34. The European Commission met with stakeholder in October 2002 and agreed the need for co-ordination of EU activities on acrylamide in food. The European Commission in collaboration with the European Food Safety Authority (EFSA) developed, and is maintaining, a summary of EU research activities. The summary includes details of studies on levels of acrylamide in food, reducing levels, formation, methods of analysis, bioavailability, epidemiology, toxicology etc.[24] (This information was made available for inclusion in the FAO/WHO Infonet.) The European Commission's Joint Research Centre developed a programme to investigate methods of analysis for acrylamide in food [23, 64]. A research topic was introduced into the Commission Research Framework Programme and a large scale international project (HEATOX) including 23 participant organisations began in November 2003[24].

ACTIVITIES ON ACRYLAMIDE

35. The European Commission Experts on Contaminants in Food held a workshop in October 2003 on ways to reduce acrylamide levels in food. An outcome document with details of approaches shown to lower the levels of acrylamide formed in foods will be made available on the EC's website[24].

36. The European Food Safety Authority held a workshop on the 17th of November 2003 on the formation of acrylamide food. The workshop was organised by the UK's Food Standards Agency and the Dutch Food and Consumer Product Safety Authority (VWA) with assistance from Confederation of the Food and Drink industries of the EU (CIAA) and the European Commission. The outcome of which will be available from the EFSA website.

37. In 2004, the American Chemical Society will hold a symposium on the "Chemistry and Safety of Acrylamide in Food". Various areas that will be addressed include Mechanisms of Formation in Food and Safety and Toxicology [3].

38. Following the Food Industry Coalition/JIFSAN "Acrylamide in Food Workshop" held in 2002, a workshop will held on the 13 –15 April 2004.

39. In order to perform the risk assessment of acrylamide in food, a call for data on levels of acrylamide in food and the total diet has been placed on the FAO/WHO acrylamide food network (Acrylamide Infonet). The levels of acrylamide in the total diet are required to provide an exposure assessment for the general population as well as certain vulnerable groups[26].

40. The European Union Joint Research Centre's Institute for Reference Materials and Measurements (IRMM) is compiling a database of acrylamide levels in food in the European Union[25].

41. In Japan, studies have been conducted on analyses of acrylamide in foods and there are research projects ongoing, to reduce the level of acrylamide in food and to prevent the toxic effects of acrylamide.

42. In the U.S. the FDA developed an extensive action plan for acrylamide in food that outlines FDA's goals and planned activities on the following issues: 1) methods development, 2) occurrence in foods, 3) research on formation, 4) measuring exposure, 5) data on toxicology and health effects, 6) epidemiology, 7) risk assessment, and 8) educating the public. This action plan was presented to the FDA's Food Advisory Committee in December 2002 and February 2003 that supported the action plan. Currently, extensive work is underway in the U.S to carry out the various activities outlined in the action plan.

43. As detailed on the Acrylamide Infonet, much research is being conducted in the UK, with the UK's Food Standards Agency contributing to the international research effort with investigations of minimisation of acrylamide in food.

CONCLUSIONS

44. Acrylamide appears to form as a byproduct of high-temperature cooking processes particularly in carbohydrate-rich foods such as potatoes and cereals and also in coffee.

45. One of the suggested mechanisms for formation of acrylamide in carbohydrate-rich foods cooked at high temperatures has been identified, as the reaction between the amino acid asparagine and certain sugars, both of which are found naturally in foods. There is still the possibility that other mechanisms of formation play a role in acrylamide formation in food.

46. Acrylamide causes cancer in laboratory animals in high doses. As a result, acrylamide is considered a potential human carcinogen. However, it is not clear whether acrylamide causes cancer in humans, at the much lower levels of exposure from its presence in food. Limited epidemiological studies of people exposed to acrylamide in the workplace and through the diet have been conducted. The studies did not show increased cancer risk with acrylamide exposure. However, these studies do not rule out the possibility that acrylamide in food can cause cancer because they have limited power to detect this effect. Also, there is not enough information to rule out the possibility that subtle effects can occur on the developing nervous system at acrylamide doses lower than the high exposures that have been associated with effects in animals and humans.

47. There are significant uncertainties about the impact of acrylamide on public health. People have been eating some of the foods now reported to contain acrylamide for decades. To better address the risk of acrylamide, more information is needed regarding which foods acrylamide is formed in, levels of acrylamide in foods, dietary exposure to acrylamide, the bioavailability of acrylamide in food, biomarkers of acrylamide exposure, the potential of acrylamide to cause cancer when consumed in food, acrylamide's potential to cause germ cell mutations and neurotoxic or neurodevelomental effects. Also the overall mechanism /mode of toxic action(s) of acrylamide need to be elucidated. Several projects are being conducted in these areas[24,26].

48. At this time, not enough is known about acrylamide formation to identify safe, effective, and practical modifications to food processing techniques that will clearly prevent or reduce formation. Identifying all mechanisms of formation is an important step in identifying ways to reduce or prevent acrylamide formation during cooking. Many studies on reducing acrylamide formation are underway.

49. The FAO/WHO consultation in June 2002 concluded that the presence of acrylamide in food is a major concern, and recommended more research such as on mechanisms of formation and toxicity. The consultation recommended that people continue to eat a balanced diet rich in fruits and vegetables, and advised that food should not be cooked excessively, i.e., for too long a time or at too high a temperature. It noted however, that it is important to cook all food thoroughly, particularly meat and meat products to destroy foodborne pathogens (bacteria, virus, etc.) that might be present.

RECOMMENDATION

50. It is recommended that CCFAC develop specific requests for JECFA to address in its safety evaluation of acrylamide in food.

Suggested requests for JECFA

i) Comment on the extent to which acrylamide is bioavailable in food and on the safety implications.

ii) Provide a tolerable dietary intake of acrylamide for threshold-based endpoints of concern, such as neurotoxicity and reproductive toxicity.

iii) Provide estimates of dietary exposure for various population groups, including susceptible groups such as young children and regional population and identify and quantify as far as possible the major sources (e.g., food groups/commodities) of dietary exposure.

iv) Provide estimates of margins of exposure safety and/or exposure for various endpoints of concern (non-cancer and cancer). These estimates should include comparisons between the levels of acrylamide exposure shown to produce effects in animal studies and the demonstrated no-effect levels versus estimates of dietary exposure for humans.

v) Provide quantitative estimates of risk for various endpoints, including cancer, for varying degrees of dietary exposure to acrylamide.

vi) Provide comment on the toxicological significance of the main metabolite glycidamide, and whether this may be more genotoxic than the parent compound.

ANNEX 1: TOXICOLOGICAL PROPERTIES OF ACRYLAMIDE

51. The following sections are extracts from both the report of the FAO/WHO consultation on the "health implications of acrylamide in food and from the opinion of the Scientific Committee on Food on the new findings regarding the presence of acrylamide in food[54,65].

52. Acrylamide is absorbed from all routes of exposure. Animal studies have shown that acrylamide and its epoxide metabolite glycidamide are widely distributed in all tissues of the body, including milk. Acrylamide probably accounts for neurotoxicity while glycidamide may be more critical for the carcinogenic and genotoxic properties in animals.

53. The major metabolic pathway for acrylamide is qualitatively similar in humans and laboratory animals, however, quantitative differences must be considered in assessing risk for humans. Because metabolism and elimination involve pathways where there is genetic variability (e.g., conjugation and P450-mediated metabolism), there may be variation in the sensitivity of humans to the effects of ingested acrylamide. The elimination of acrylamide and glycidamide is about 2 hours in rats. Pharmacokinetic data in human are sparse.

Chronic toxic effects--Neurotoxicity

54. Neurotoxicity is the only recognized adverse effect of acrylamide exposure in humans. Evidence of the neuropathological effects comes from case reports and workplace surveys of persons exposed to acrylamide in the workplace. No information is available to establish a dose-response relationship.

55. Neuropathological effects of acrylamide exposure have been shown in animal studies. In rats given 20 mg/kg bw per day, severe lesions developed in peripheral nerves and the rats showed signs of peripheral neuropathy and toxicity at other sites: atrophy of testicles, skeletal muscles, and decreased erythrocyte parameters. In monkeys administered 10mg/kg bw per day for 12 weeks, clinical signs of peripheral neuropathy appeared along with marked visual system effects.

56. Less is known about the effects of acrylamide on the developing nervous system, although major persistent structural or functional perturbations of the brain or behavior resulting from in utero or post-natal exposure have not been found in animal studies.

57. The molecular mechanisms of acrylamide neuropathy, specifically degeneration of nerve fiber axons, is unknown.

58. In sum, rodent studies, primate studies, and human occupational study, support a no observed adverse effect level (NOAEL) for acrylamide neuropathy of 0.5 mg/kg bw per day. Since the estimated average human daily intake for acrylamide in food is of the order of 0.001 mg/kg bw per day, there is a 500-fold margin between exposure and the NOAEL.

Chronic toxic effects--Effects on fertility

59. Male rats exposed to levels of acrylamide of 15 mg/kg bw per day or more for 5 days and mice exposed to levels of acrylamide up to 12 mg/kg bw/day for 4 weeks showed impaired fertility which may have been caused by low sperm counts and lower sperm motility.

60. In rat and mice studies where rats were exposed to15 mg/kg bw/day and mice were exposed to 45 mg/kg bw/day, minor signs of developmental toxicity appeared. There was an increase in skeletal variation and impaired body weight gain appeared. However, these effects were associated with maternal toxicity during a major period of organogenesis and were likely to be secondary to maternal toxicity.

61. There is sufficient evidence to conclude that acrylamide impairs male fertility, and No Observed Adverse Effect Levels (NOAELs) may be identified from some studies: 2 mg/kg bw per day from a two-generation rat study (10-11 weeks of treatment), and 9 mg/kg bw per day from a 27-week mouse study.

Single dose toxicity

62. Toxic effects on the nervous system of humans and animals and on the male reproductive organs of rats are known to occur after single oral doses of acrylamide that are equal to or greater than four to five orders of magnitude higher than the estimated daily intake (EDI) of acrylamide from food (EDI=1-10 μg/kg bw per day).

Carcinogenicity

63. Acrylamide is carcinogenic in laboratory rats in standard 2-year bioassays, producing increased incidences in the number of benign and malignant tumors in a variety of organs e.g., testicles, mammary gland, thyroid, adrenals. Two separate independent studies have confirmed this phenomenon at a dose of 2mg/kg per day, administered in drinking water. In a series of carcinogenicity bioassays in mice (oral gavage, intra peritoneal, or dermal administration), acrylamide induced lung and skin tumors.

Genotoxicity

64. Acrylamide does not induce gene mutations in bacteria, but the epoxide metabolite glycidamide does induce bacterial gene mutations in the absence of metabolic activation. Acrylamide showed equivocal, negative, or weakly positive results when tested for the induction of gene mutations in mammalian cells. Acrylamide induces chromosomal aberrations, micronuclei, sister chromatid exchanges (SCE), polyploidy, aneuploidy and other mitotic disturbances (e.g., C-mitosis) in mammalian cells in the absence of metabolic activation. Acrylamide was unable to induce unscheduled DNA syntheses (UDA) in rat hepatocytes. Glycidamide induced UDS in human mammary cells, with the same results in rat hepatocytes. For micronuclei induction, a mixed breakage (prevalent)-aneuploidy mechanism was shown.

65. Acrylamide was positive in the mouse spot test, in the bone marrow chromosome aberration assay and in particular in the bone marrow micronucleus assay. In a transgenic mouse model (MutaMouse) acrylamide induced a small increase in mutation frequency.

66. Acrylamide induced somatic mutations as well as sex-linked recessive lethal mutations in *Drosophila.*

67. In germ cells, acrylamide produced several genetic effects such as chromosome aberrations, micronuclei, SCE, UDS, single-strand breaks in DNA, dominant lethal mutations, specific locus mutations and heritable translocations. Glycidamide also induces dominant lethal mutations.

68. Acrylamide can cause germ cell mutation in rodents with the potential to induce heritable genetic damage at gene and chromosome level. Acrylamide impairs fertility in male rats, most likely through a direct toxic effect. Whether acrylamide has an adverse effect on fertility through genetic damage is unclear.

Adduct formation

69. Acrylamide contains an electrophilic α, β-unsaturated system that reacts via a Michael addition with nucleophilic compounds. Within proteins, the sulfhydryl group of cysteine is the major site of reactions, although reaction also occurs to a lesser extent with amino groups, such as those at the N-terminal position of the protein.

70. Hemoglobin adducts are used as a measure of human exposure to electrophilic compounds over the previous 4 months (i.e., the life span of human red blood cells), but are not an indicator of toxicity. Adduct formation at the N-terminal valine of hemoglobin has been used as a marker of *in vivo* exposure to acrylamide. A similar approach is used for measurement of hemoglobin adducts of glycidamide. Detection of the hemoglobin adducts of glycidamide confirm the formation of this metabolite *in vivo.* Binding of acrylamide to other proteins in nervous and testicular tissue may be relevant to the toxic action of acrylamide to these tissues.

71. Adduct formation of acrylamide with DNA also occurs, however, the reaction is slow. Among the products formed *in vitro* are formamidoethyl or carboxethyl adducts with exocyclic amino groups or ring nitrogens in DNA bases. The mutagenic significance and repair capabilities of these adducts are unknown. The only adduct detected in mice and rats exposed to acrylamide has been reported to be an adduct of the epoxide metabolite glycidamide with guanine. Glycidamide is expected, because of its structure, to be of more significance than acrylamide in relation to the gentoxic effects *in vivo.*

Mode of carcinogenic action

72. Acrylamide is genotoxic *in vivo* in somatic cells and germ cells, and is known to be metabolized to glycidamide, a chemically reactive epoxide that forms DNA adducts. The finding that acrylamide induces tumors at a number of different sites in both rats and mice is consistent with a genotoxic mode of action of the chemical. The existence of adducts in experimental systems is supportive of a genotoxic mechanism of carcinogenesis of acrylamide. While suggestions have been made that additional modes of action might contribute to the observed spectrum of tumors seen in acrylamide-treated rats, especially tumors of hormone-responsive tissues, these suggestions are only speculative at this time.

EPIDEMIOLOGICAL STUDIES

73. Epidemiological studies have been conducted on a cohort of 8.854 workers, of whom 2,293 were exposed to acrylamide in monomer and polymer production plants during 1925-1976. In 1983, an evaluation of the studies revealed no statistically significant excess risk of cancer in any organ, and no trend in cancer mortality was seen with increasing cumulative exposures[19].

74. Data for this cohort were subsequently updated for the period 1984-1994, for 8,508 workers, including 2,004 exposed workers, and again no statistically significant excess cancer risks were observed, with the single exception of pancreatic cancer for which a doubling of risk was found in workers most heavily exposed. The statistical power of this study was adequate to have detected a 75% excess incidence of brain cancer, a 40% increase in pancreatic cancer, a 15% increase in lung cancer, or a 9% increase in all cancers combined[40]. All epidemiological studies have limited power to detect small increases in tumor incidence. Therefore, the absence of positive results found in most studies on acrylamide cannot be interpreted as proof that the substance cannot induce cancer in humans.

75. A population-based case-control study in Swedish men and women age 51 to 77 years found no positive association between acrylamide food exposure and risk of cancer of the large bowel (591 cases), bladder (263 cases) or kidney (133 cases) compared with 538 controls. Acrylamide food exposure was estimated from food intake patterns measured previously using a food frequency questionnaire[42]. The exposure estimate omitted certain foods later found to be important acrylamide sources, such as coffee[43].

76. In a hospital-based study of 527 cases and 1297 controls among men and women in Switzerland and Italy, a positive association was found between laryngeal cancer risk and consumption of certain fried foods: beef/veal, fish/shellfish, eggs/omelette, and potatoes[11,12]. However, a positive association of fried potato consumption with laryngeal cancer risk was not found in a later analysis of the same data used by Borsetti et al.[11,12], together with a group of coordinated, hospital-based case-control studies among men and women in Switzerland and Italy[47]. The later analysis found no positive association between intake of fried or baked potatoes and risk of cancer of the oral cavity, esophagus, larynx, large bowel, breast or ovary. The different outcomes of the two analyses of laryngeal cancer may have been due in part to different categorization of potato consumption.

77. The statistical power of these food exposure studies was not specifically stated in the articles. As noted for the occupational epidemiology studies, all epidemiology studies have limited power to detect small increases in tumor incidence. Therefore, the absence of a positive association can not be interpreted as proof that the substance cannot induce cancer in humans. Additionally, for these food exposure case-control studies, the finding of lack of positive association is limited to the particular cancer sites studied, and does not rule out cancer risk at other sites.

ANNEX 2: ANALYTICAL METHODS

78. The most commonly used measurement techniques for acrylamide in foodstuffs alongside other alternative methods have recently been reviewed [64]. Special attention was given to sample preparation. The review covers information on methods from peer-reviewed articles and other sources (for example a survey carried out among official and private laboratories of the Member States of the European Union). On comparing methodologies, there were huge differences in clean-up strategies for both the GC- and LC- based methods. There was a large spread of different sample preparation procedures used in conjunction with the LC analysis methods. Comparability of the GC methods was difficult due to the difference in the sample matrices. The influence of different extraction techniques or extraction solvents/solvent has not yet been fully investigated. It was concluded that to achieve correct monitoring of acrylamide in foodstuffs, for example for exposure assessment, the applied methods should be viewed critically with respect to their performance criteria [64].

79. Relative advantages and disadvantages of these analytical methods were discussed at the European Workshop on the "Analytical Methods for the Determination of Acrylamide in Food Products" that was organised by the European Commission's Joint Research Centre in April 2003[23].

80. Levels of acrylamide content in certain foods are comparable among methods. In 2002, both LC-MS/MS and GC methods were used to analyze acrylamide contents in certain foods[1,46,49]. There was good agreement between the results obtained from both methods. A proficiency test was organized by the German Federal Institute of Risk Assessment in 2002. The test was for German laboratories from the food industry, private and official control laboratories. Six different food samples were analyzed using GC and LC methods. No statistically significant differences appeared between the results obtained[27].

81. Proficiency testing of samples comprising of crispbread [18], potato crisps, breakfast cereals (wholewheat biscuits) and roasted ground coffee have been undertaken by FAPAS®. These rounds assess performance of laboratories in measuring acrylamide contents in the samples. Results obtained using of GC-MS, LC-MS or LC-MS/MS were comparable[18,23].

82. The EU European Commission's Joint Research Centre has recently conducted a proficiency testing exercise on acrylamide determination in crisp bread and butter cookies. The results are available shortly in a special EU Report and will also be published in the scientific literature. A second round on crisp bread is planned in order to gain more information. In addition, another proficiency testing exercise on coffee and cocoa is foreseen for 2004 in collaboration with the BfR (Germany).

83. Although the U.S. is not planning to conduct any proficiency testing of its method, it will participate in a ring trial, sponsored by the U.S. National Food Processor Association (NFPA), that will evaluate method performance using different matrices by various laboratories.

84. The European Commission's Joint Research Centre (JRC) is planning to validate in 2004 two methods (GC and LC) for acrylamide determination in selected food matrices. In addition, the JRC will prepare certified reference materials.

85. Most methods have shown to have limits of detection (LOD) at about 10-30 µg /kg. Musser, S. in a FDA Food Advisory Committee Contaminants and Natural Toxicants subcommittee meeting, compared characteristics of some of the commonly used analytical methods. See table below for details.

Method	Limit of Quantification	Specificity	Percent CV
GC-MS (underivatized)	10 ppb	High	10
GC/MS (derivatized)	50 ppb	High	5-10
LC-MS	20 ppb	Medium	5-10
LC-MS/MS	10 ppb	High	5-10

cv coefficient of variation

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