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EFSA Scientific Colloquium
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ACRYLAMIDE CARCINOGENICITY

NEW EVIDENCE IN RELATION TO DIETARY EXPOSURE

22-23 May 2008, Tabiano (PR), Italy

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About EFSA

The European Food Safety Authority (EFSA) was established and funded by the European Community as an independent agency in 2002 following a series of food scares that caused the European public to voice concerns about food safety and the ability of regulatory authorities to fully protect consumers.

In close collaboration with national authorities and in open consultation with its stakeholders, EFSA provides objective scientific advice on all matters with a direct or indirect impact on food and feed safety, including animal health and welfare and plant protection. EFSA is also consulted on nutrition in relation to Community legislation.

EFSA's work falls into two areas: risk assessment and risk communication. In particular, EFSA's risk assessments provide risk managers (EU institutions with political accountability, i.e. the European Commission, European Parliament and Council) with a sound scientific basis for defining policy-driven legislative or regulatory measures required to ensure a high level of consumer protection with regards to food and feed safety.

EFSA communicates to the public in an open and transparent way on all matters within its remit. Collection and analysis of scientific data, identification of emerging risks and scientific support to the Commission, particularly in case of a food crisis, are also part of EFSA's mandate, as laid down in the founding Regulation (EC) No 178/2002 of 28 January 2002.

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I INTRODUCTION

The eleventh meeting in the EFSA Scientific Colloquium series was held to consider recent information on the carcinogenicity of acrylamide in relation to dietary exposure. The formation of acrylamide in food and the possible health effects of consumption of acrylamide-containing foods have been the subject of intense research since the finding by Swedish scientists in 2002 of significant amounts of acrylamide in foodstuffs heated to high temperatures (Tareke et al., 2002).

Acrylamide is a contaminant that may be formed in foods, particularly plant-based foods rich in carbohydrate during cooking, frying, baking or roasting, at temperatures of 120oC or higher. The critical effects of acrylamide are its neurotoxicity and carcinogenicity. The compound has been identified as genotoxic and carcinogenic in laboratory animals.

In 2005, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) carried out a risk assessment of acrylamide in food. The JECFA applied the margin of exposure (MOE) approach to the data on various tumour sites identified in the animal carcinogenicity studies and concluded that the MOEs were low and that this may indicate human health concern at current estimated dietary exposure levels. However, the JECFA cautioned that there were a number of uncertainties in its conclusions because the toxicological database was incomplete and the committee recommended a re-evaluation of acrylamide when further relevant data became available (FAO/WHO, 2005). EFSA's Scientific Panel on Contaminants in the Food Chain (CONTAM) agreed with the principal conclusions and recommendations of the JECFA, that there may be human health concerns associated with dietary exposure to acrylamide and that there should be a re-evaluation once new data on carcinogenicity or on human biomarkers of acrylamide exposure became available (EFSA, 2005).

The objective of this EFSA colloquium was to stimulate an open exchange of views and expertise on the new information relevant to the carcinogenicity of acrylamide that has become available since 2005. At the colloquium, the participants explored whether the new evidence on epidemiology, human biomarkers, carcinogenicity and dietary exposure was such that a revision of the previous risk assessment of acrylamide in food was warranted at this time.

Following an introductory plenary session, in which keynote speakers summarised recent evidence on the epidemiology, toxicology, mode of action of acrylamide as a carcinogen, and European dietary exposure data, the participants broke up into four groups to discuss the following topics in more detail:

- Epidemiological evidence relating acrylamide exposure to cancer risk in humans, including discussions on uncertainties.
- The applications of biomarkers for acrylamide and models in relation to the exposure, metabolism and elimination (toxicokinetics) and the mode of action of acrylamide in experimental animals and humans (toxicodynamics).
- The state of the art on the genotoxic and non-genotoxic mechanisms of carcinogenicity of acrylamide.
- The current knowledge on dietary exposure to acrylamide across Europe and the exploration of any new potential food source contributing to dietary exposure.

The outcomes of the discussion groups were presented and discussed and some conclusions and recommendations to EFSA were drawn up in a final plenary session.

Dr. Josef Schlatter (Federal Office of Public Health, Switzerland) and Dr. Ada Knaap (EFSA Scientific Committee) acted as overall chairs. Professor Alan Boobis (Imperial College London, United Kingdom) and Dr. Susan Barlow (EFSA Scientific Committee) were the overall rapporteurs. Professor Rolaf van Leeuwen (National Institute for Public Health and the Environment, The Netherlands), Professor Peter Farmer (University of Leicester, United Kingdom), Dr. Diane Benford (Food Standards Agency, United Kingdom) and Dr. Detlef Müller (Foodrisk, Germany) offered to be discussion group chairs. Dr. Kathryn Wilson (Harvard School of Public Health, USA), Dr. Jean-Lou Dorne (EFSA), Dr. Wolfram Parzefall (University of Vienna, Austria) and Dr. Leif Busk (National Food Administration, Sweden) were the corresponding discussion group rapporteurs.

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II REPORTS FROM DISCUSSION GROUPS

DG1: EPIDEMIOLOGICAL STUDIES – EVALUATING EVIDENCE AND ADDRESSING UNCERTAINTIES

Over the last few years, a number of research groups from around the world have published data from epidemiological studies in relation to dietary exposure to acrylamide and human cancer risk in different target organs (kidney, bladder, endometrium, ovaries, breast). Key considerations in exploring such relationships are:

- the power of the studies to detect effects;
- how the exposure assessment was carried out;
- how well can food frequency questionnaires, and other diet assessment methods, capture dietary acrylamide exposure (several studies comparing FFQ-assessed acrylamide exposure with biomarkers of acrylamide exposure are now available);
- what kind of statistical tools have been applied and what are the confounding variables.

It is important to review all of the available evidence and to identify whether the methodologies applied in various epidemiological studies are comparable, and if uncertainties have been identified and taken into account, where possible.

1. Review the epidemiological evidence relating dietary acrylamide exposure and cancer risk.

The group began with a discussion of prospective versus retrospective study designs. Retrospective studies were the first to be published following the discovery of acrylamide in foods; these studies found no association between dietary acrylamide exposure and risk of a variety of cancers (Mucci et al., 2003; Mucci et al., 2004; Pelucchi et al., 2003; Pelucchi et al., 2006; Pelucchi et al., 2007). However, prospective studies are preferred, particularly in nutritional epidemiology, because the information on diet and hence on acrylamide exposure, is collected from participants prior to any disease diagnosis.

It was also discussed that studies in different populations may yield different results due to differences in acrylamide exposure and other differences in dietary and non-dietary factors. It is also possible that the sources of acrylamide are more easily measured in some populations than others. For these reasons, associations between acrylamide and cancer risk need to be studied in a variety of populations.

Several reports using existing prospective cohorts have been published in the past several years. All but one of these studies assessed dietary acrylamide exposure using food frequency questionnaires, in which respondents select their frequency of consumption over the previous year, of each in a list of food items from several possible responses (often ranging from "never" to "six or more servings per day").

One study in a cohort of Swedish women found no association between dietary acrylamide exposure and risk of colon cancer (Mucci et al, 2006). Two studies have found no association between dietary acrylamide exposure and breast cancer risk in mostly premenopausal Swedish or postmenopausal Dutch women (Mucci et al., 2005; Hogervorst et al., 2007). Unpublished results from two U.S. cohorts, the Nurses' Health Study and the Nurses' Health Study II seem to confirm this lack of association between acrylamide exposure and breast cancer. In the Netherlands Cohort Study, no association was found between dietary acrylamide exposure and risk of bladder cancer in men and women or prostate cancer in men (Hogervorst et al., 2008).

The Netherlands Cohort Study found a statistically significantly increased risk of ovarian cancer among postmenopausal women and a significantly increased risk of endometrial cancer among never smoking women in association with dietary exposure to acrylamide (Hogervorst et al., 2007). In the same cohort, there was also a suggestion of an increased risk of renal cell cancer among men and women in association with dietary acrylamide exposure (Hogervorst et al., 2008). These recently published data suggest that there are associations between dietary acrylamide exposure and risk of cancer at certain sites. Given the widespread and continuous exposure to acrylamide, even small increases in relative risks may be important from a public health viewpoint.

The findings are suggestive at this point, but more prospective studies are needed to confirm or reject the current findings and to analyse probable additional cancer sites.

2. Review the methodology used for exposure assessment and whether there is comparability between studies. Review evidence on the validity of questionnaire-based acrylamide exposure assessments.

Two methods have been used to assess dietary exposure to acrylamide: food frequency questionnaires (FFQs) and haemoglobin (Hb) adducts of acrylamide and glycidamide, biomarkers of acrylamide exposure over the previous three to four months.

Food Frequency Questionnaire assessment of dietary acrylamide exposure

Assessment of dietary acrylamide exposure using FFQs is difficult because exposure depends on the amounts generated by heating of foods as well as the types of food eaten; FFQs used in ongoing cohort studies have not generally been designed to measure cooking methods or possible cooking carcinogens. The ability of a FFQ to assess acrylamide exposure will depend on which foods contribute to acrylamide exposure in that population, the accuracy with which people report consumption of those foods, and the variability of the acrylamide content of these foods.

These factors will vary according to the specific population that is being assessed as well as the specific FFQ used. Therefore, even though most studies have used the same method, *i.e.* a FFQ, to assess exposure, the validity of this method will vary across studies. In particular, the comparability of the earliest epidemiological studies of acrylamide exposure with more recent studies is questionable because the initial studies often used limited or preliminary data on acrylamide concentrations in foods.

Another limitation in assessing acrylamide exposure is that existing data on the acrylamide contents of foods were not collected for the purpose of assessing exposure; sampling is targeted to specific types of foods often for regulatory or monitoring purposes.

Misclassification of exposure may occur as a result of FFQ being an imperfect measure of dietary history. The presence of measurement errors reduces precision of risk estimates and power of significance tests. When not dependent on outcome (nondifferential error), such misclassification usually attenuates relative risk estimates (shifts them toward one). However, errors in the measurement of exposure can also distort relative risk estimates in any direction. Therefore the variability in risk estimates across studies, regarding acrylamide and cancer, could be partially explained by measurement errors.

Biomarkers of acrylamide such as acrylamide and glycidamide adducts to hemoglobin exposure have been suggested as methods to quantify acrylamide exposure. Those type of measurements could be used as an additional correction factor, in future epidemiological studies for adjusting risk estimates for measurement errors in acrylamide, assuming that errors in the two measurements (food frequency and adducts) being compared are independent.

Biomarkers of acrylamide exposure

Because of the limitations of existing FFQs for measuring dietary acrylamide exposure, it can be useful to combine biomarker data with FFQ data in existing cohorts. One published study has used blood samples collected prospectively to study the association between acrylamide and glycidamide adducts to haemoglobin and risk of breast cancer (Olesen, 2007). This study found an association between acrylamide adduct levels and breast cancer risk. The risk was higher for smoking women although with wide confidence intervals, indicating that more data are needed before final conclusions can be drawn. Because of the strong association between tobacco use and the level of haemoglobin adduct formation by acrylamide and glycidamide, strict control for smoking behaviour will be necessary when studying the effects of dietary acrylamide exposure, using such adducts as biomarkers of exposure. At this point the group agreed that studies, in which exposure is assessed using such adducts, will be most informative when restricted to non-smokers.

The group discussed several other limitations of haemoglobin adducts of acrylamide and glycidamide as biomarkers of dietary acrylamide exposure. Adducts reflect a fairly recent period of exposure (3-4 months), whereas FFQs typically ask respondents about their diet over the past year. Given that long-term dietary exposure is more relevant with respect to development of cancer, it is not clear how well single measures of blood adduct levels will reflect this. On the other hand, adducts reflect all sources of acrylamide exposure, not just the diet, and they reflect individual differences in absorption and metabolism. It is not clear at this point how much passive smoking may affect adduct levels; more research in this area is needed. In addition, the importance of metabolic differences amongst individuals is not clear at this time.

3. Establish whether statistical approaches are consistent between studies and review the sources of uncertainty, particularly confounding variables.

The group agreed that proper adjustment for, or stratification by, smoking status is critical given the importance of smoking as a source of acrylamide. Limiting analyses only to never smokers will help to isolate the effect, if any, of dietary acrylamide. This will require larger studies in which never smokers comprise a sufficiently large group to be studied separately. To date this has been done only in the reports from the Netherlands Cohort Study (Hogervorst et al., 2007; Hogervorst et al., 2008).

Adjustment for dietary factors which may confound the association between acrylamide exposure and cancer risk is also important. Confounders will vary by study population and cancer site studied. Clear criteria for selection of confounders in multivariable models are critical.

There should be adjustment for total energy intake in all multivariable analyses, in order to reduce measurement error in the FFQ. Validation studies of FFQ consistently show that validity of dietary acrylamide exposure assessment improves with adjustment for total energy intake. Ideally, energy-adjusted acrylamide exposure should be used to create quintiles of acrylamide exposure to reduce misclassification in quintile assignments.

Finally, the group discussed the fact that dietary acrylamide exposure may serve as a marker for exposure to a wide variety of Maillard reaction products. Given that acrylamide formation is specific to the asparagine content of foods, it is not clear how good a marker acrylamide is of any broader set of cooking-related compounds.

4. Discuss the power of the studies to detect effects.

The power of a study is the probability that the study will be able to observe a significant association between exposure and outcome given that there truly is an association. Power depends on several characteristics of the study: the size of the study population, the number of cases, the range of exposures in the population, and measurement error in the exposure and confounders.

Based on animal studies, it would be expected that the overall relative risk for cancer would be so low (~1.05) that it would not be detectable in epidemiological studies. However, the relative risks may be higher for some cancer sites and low or null for others. The most recent findings from the Netherlands Cohort Study suggest this may be the case.

The power of future epidemiological studies on acrylamide and cancer could be increased by improving the tools used to measure acrylamide exposure and by increasing sample size to allow stratification for smoking and/ or genetic factors or to test for interaction. Given the availability of existing cohort studies in which dietary exposure has already been assessed with established FFQs, it seems that this possibility is more promising in the shorter term. The European Prospective Investigation into Cancer and Diet (EPIC) is a collection of cohort studies across Europe specifically designed to follow people with very diverse diets. The association between acrylamide exposure and risk of different cancers in these cohorts will be of interest.

5. Discuss whether from the body of evidence conclusions can be drawn on the relationship between dietary acrylamide exposure and increased cancer risk in humans.

Some studies have found associations between dietary acrylamide exposure and cancer risk while others have not. More prospective studies will be critical in reaching conclusions about the relationship between dietary acrylamide exposure and cancer risk.

Of the studies that have reported associations, these were weak, both in terms of the relative risks (generally less than 2 for highest versus lowest quintile of consumption) and in that there were some inconsistencies in cancer sites (*i.e.* renal cell cancer [Mucci et al., 2004; Pelucchi et al., 2007; Hogervorst et al., 2008] and ovarian cancer [Pelucchi et al., 2006; Hogervorst et al., 2007]). However, the magnitude of the relative risks observed is potentially important for two reasons. First, the measurement error present in the exposure assessment is likely non-differential with respect to cancer outcomes; this will tend to dilute the observed associations. Therefore, the true association between dietary acrylamide exposure and risk of ovarian, endometrial, and renal cell cancer may be greater than the relative risks estimated in the studies. Second, the ubiquity of dietary acrylamide exposure means that even small relative risks are potentially important at a population level.

In addition, if there truly were no association between dietary acrylamide exposure and risk of any cancers, it would be expected that studies would show less of an association over time, as exposure assessment has improved through the use of more complete food composition databases for acrylamide. Therefore, the recent prospective studies are suggestive; however, there is very little evidence at this point from which to draw firm conclusions.

The fact that some epidemiological studies have found relative risks much larger than those predicted from animal studies reinforces the possibility that acrylamide may be an important public health issue. At this point, more prospective studies need to examine the possible association between acrylamide and cancer risk in humans.

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DG2: BIOMARKERS – NEW INSIGHTS IN EXPOSURE AND MODE OF ACTION

The characterisation of acrylamide (AA) metabolism has been the basis for the development of biomarkers of exposure to AA. AA metabolism follows two basic routes:

1. CYP2E1- mediated epoxidation to glycidamide (GA) which is then a) either conjugated with glutathione to form N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)cysteine [also known as N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)cysteine] and N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)cysteine or b) enzymatically hydrolysed to glyceramide by epoxide hydrolase;
2. Direct conjugation of AA with glutathione to form the urinary metabolite N-acetyl-S-(3-amino-3-oxypropyl) cysteine. Free unchanged glycidamide is thought to account for AA 's genotoxicity through DNA adduct formation. Longer term exposure to AA has been monitored using haemoglobin adducts since the life-time of erythrocytes is 120 days. More recently, mercapturic acid metabolites of AA and GA have been quantified in human urine as biomarkers of short-term exposure (half-lives range from hours up to a few days). In addition, physiologically based toxicokinetic models (PB-TK) have been developed for AA, GA, and the glutathione conjugates of AA. Liver GA-DNA adducts and haemoglobin adducts have been included as toxicodynamic (TD) components in a toxicokinetic/toxicidynamic (TK/TD) model. Four main discussion points were addressed.

1. Discuss new insights into species differences in the kinetics of acrylamide

The discussion started with a review of the excretion pattern of AA and AA metabolites in test species (rats, mice…) and their relevance for human exposure levels. The AA excretion pattern is well characterised in rats, mice and humans (Fennell et al., 2006; Bjellaas et al., 2007) and the metabolism involves the cytochrome P450 (CYP) enzyme CYP2E1 in animal species studied so far. For example, the involvement of CYP2E1 in the bioactivation of AA has been demonstrated by studies using CYP2E1 knockout (CYP2E1 -/-) mice. New data from swine on the excretion pattern of metabolites that may be more relevant to humans have been reported (Aureli et al., 2007). The amounts of excreted AA and AA metabolites do not account for the complete dose of administered AA (100% of the dose range, currently publications cover only >50% of an administered dose).

A recently published physiologically-based, toxicokinetic/toxicodynamic (PB-TK/TD) model for AA and its metabolites is available for mice, rats, and humans and integrates AA metabolism in these different species on a species-specific basis (Young et al., 2007).

Two main points for further research needs came out during the discussion:

1. The need for methods to quantify AA metabolites in urine in test species in order to provide a quantitative understanding of AA excretion patterns in different species and improve extrapolation to humans.
2. The use of *In vitro* species comparisons (*e.g.* by using isolated hepatocytes, liver microsomes or other *in vitro* systems in order to quantify and better characterise probable species of AA metabolism, including also probable species-specific minor metabolites.

2. Review the current state of the art on the knowledge on acrylamide biomarkers in relation to exposure and effects and whether some biomarkers provide better estimates than others

The discussion group reviewed the use of haemoglobin adducts of AA and GA as biomarkers of longer term exposure to AA. Such Hb-adducts provide useful insights into longer term AA exposure because people's diets are fairly constant. However, inter-laboratory comparisons are difficult since different techniques are used in different laboratories and there is a need to harmonise these techniques.

Another major point of the discussion was the low correlation between dietary exposure and AA- and GA- haemoglobin adduct formation (AA, GA) in the studies published so far. To investigate this further it would be helpful if the analyses of epidemiological studies were based on internal dose and diet information in order to be more quantitative and also included measurement of Hb adducts as a biomarker. To do so, suitable blood samples (also from repeated sampling) should be conserved in a bio-bank in order to determine internal AA dose. Stability of the adducts should be determined, although there is some evidence that they are stable for several years in stored blood samples. Another possibility for future investigation is determination of the presence of other hepatic adducts (*i.e.* adducts on -SH groups in proteins).

DNA adducts as a biomarker of AA exposure were considered to reflect the biologically active dose of AA and could be extrapolated to humans using data from test species (rats, mice) and the PB-TK/TD model developed by Young (Young et al., 2007). The fact that DNA-adducts have not been detected in humans is probably because of the small amounts produced and is consistent with the predicted low levels of the predominant adduct (N-7-guanine adduct). An open question was whether such low levels of adducts would affect cancer rate and whether a relationship could be established between adduct levels and cancer rate in the ongoing study by the National Center for Toxicological Research/ National Toxicology Program (NCTR/NTP study).

Urinary biomarkers could be useful to validate dietary estimates of AA but would not reflect long-term exposure and would therefore not be adequate biomarkers for the correlation between AA exposure and cancer.

Finally, a monitoring study was suggested to explore workers' exposure using biomarkers, *i.e.* samples taken on Friday p.m. and Monday a.m. so that dietary exposure and work exposure can be deduced. Long-term monitoring could also be performed.

3. Review the available physiologically-based toxicokinetic models

The discussion group highlighted three main PB-TK models that are available for AA. These models were discussed in the context of their historical development.

The first model developed by Kirman (Kirman et al., 2003) is a PB-TK/ toxicodynamic model using Hb adducts in a 2-compartment model and has limited use compared to the more recent multi-compartment models.

Walker et al. (2007) developed their model to predict differences in internal dose of acrylamide and some of its metabolites between children, neonates and adults and took into account population variabilities of CYP2E1, glutathione-S-transferases (GSTM1) and epoxide hydrolase (EH), as well as data on the ontogeny of each enzyme for the neonates. However, the relationship between the internal dose and the toxicodynamic aspect of AA (*i.e.* adduct formation) was not taken into account.

In a recent publication Young presents a PB-TK/ toxicodynamic multi-compartment model for AA and its metabolites glycidamide (GA), the glutathione conjugates of acrylamide and glycidamide (Young et al., 2007). Liver GA-DNA adducts and Hb-adducts with AA and GA were included in the toxicodynamic component of the model. Serum AA and GA concentrations combined with urinary elimination levels for all four components were simulated and adduct formation and decay rates were determined from data from test species and extrapolated to a human model. The discussion group concluded that this was the most up-to-date and useful model available.

Several recommendations were also formulated with regards to improving the model:

- DNA-adducts in humans are needed to test the correctness and accuracy of prediction;
- Information is required on the activities of the different metabolic enzymes (CYP2E1, GSTM1, EH) in neonates, infants, and other relevant population subgroups (*e.g.* elderly, sick persons) in order to better understand differences in AA susceptibility within humans.
- The effects of ethanol and other factors and compounds that may potentially interact with AA (*e.g.* through modulation of CYP2E1 activity by solvent exposure, medication, diabetic state, obesity) should be included.

4. Impact of these biomarkers on the risk assessment (both for exposure and the mode of action)

Overall, biomarkers can be useful to:

- provide a more precise estimate of exposure to AA for epidemiological studies based on internal dose;
- extrapolate between test species and humans (metabolism, formation of adducts);
- characterise metabolic polymorphisms (CYP2E1, GSTM1, EH) at the population level.

Suggestions for further research that were suggested included:

- The use of an acrylonitrile Hb-adducts to discriminate the origin of the acrylamide (diet *vs*. smoking);
- Investigation of the possibility of endogenous production of AA;
- Immunological approaches to assess biomarkers of AA (*e.g*. neo-epitope-based antibodies as a high throughput and cheap alternative to "conventional" biomarker analysis, serum antibodies to AA, monoclonal or antimonoclonal antibodies against DNA-adducts);
- The use of '-omics': Such technologies may reveal genes (genomics), mRNA, proteins (proteomics) and metabolic (metabolomics) biomarkers which could be valuable in underpinning species differences in TK and TD of acrylamide and ultimately may improve its risk assessment.

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DG3: MECHANISMS OF CARCINOGENICITY

Acrylamide exposure has been shown to increase incidences of tumours in the thyroid, adrenal medulla, and testicular mesothelium in male rats, and in the thyroid, adrenal medulla, and mammary gland in female rats. The rat thyroid follicular cell tumours and the mammary tumours from two studies were considered of possible relevance for human health and benchmark doses and benchmark dose lower confidence limits have been determined (Shipp et al., 2006). Both genotoxic and non-genotoxic modes of action of acrylamide and its metabolites have been proposed (Klaunig and Kamendulis, 2005; Besaratinia and Pfeifer, 2007).

Although acrylamide is negative in most tests for mutagenicity in prokaryotic cells, it increases chromosomal aberrations, sister chromatid exchanges, unscheduled DNA synthesis, DNA breaks and deletions, cell transformation, and mitotic disruption in mammalian cells. GA has been shown to be mutagenic and genotoxic in various *In vitro* and in vivo test systems. Conjugation of acrylamide with glutathione can result in depletion of cellular glutathione stores, thereby changing the redox status of the cell which can increase cellular oxidative stress and potentially affect gene expression directly or through regulating various redox-dependent transcription factors (Lamy et al., 2008).

Consequently, cell transformation or proliferation and apoptosis might occur independent of acrylamide-induced genotoxicity. Another non-genotoxic mode of action of acrylamide is its hormonal effect in rat endocrine (thyroid) and mammary glands. Another recent report described the first evidence of acrylamide and GA inhibition of a mitotic/meiotic motor protein and speculated that this could be an alternative mechanism to DNA adduction in the production of cell division defects and potential carcinogenicity (Sickles et al., 2007; Chatzizacharias et al., 2008).

1. Review the recent evidence for the mutagenicity and genotoxicity of acrylamide (AA) and glycidamide (GA).

As starting point to their deliberations on the mechanisms of carcinogenicity, the discussion group reviewed the recent evidence of genotoxicity and mutagenicity of AA and GA. AA is activated by CYP2E1 to the reactive epoxide GA. This metabolic step is important because studies have shown that the toxicity of AA is strongly attenuated in CYP2E1 knockout mice (Sumner et al., 1999; Ghanayem et al., 2005).

The parent compound AA was genotoxic *in vitro* as measured in several mammalian cell systems (V79-CHA, L5178Y/TK+/-, human lymphoblastoid TK6) with the following endpoints: induction of chromosome aberrations (CA), micronuclei (MN), sister chromatid exchanges (SCE), and gene mutations at the thymidine kinase gene. The latter was analysed in more detail and was reported to occur by loss of heterozygosity. The results were generally obtained only at relatively high concentrations (in the 10 mM range) and appeared not to require metabolic activation (Koyama et al., 2006; Martins et al., 2007; Mei et al., 2008).

The epoxide metabolite GA showed higher genotoxic potency than AA because the effects were mostly seen at relatively low concentrations starting from 1 µM GA and showing concentration-dependent increases. The genotoxic endpoints were measured *in vitro* in several mammalian cell systems (V79-CHA, L5178Y/TK+/-, human lymphoblastoid TK6) and comprised formation of DNA adducts, DNA damage as determined by Comet assay, CA, MN, SCE, and point mutations at the thymidine kinase gene (Koyama et al., 2006; Martins et al., 2007; Mei et al., 2008).

The group also noted earlier findings of the induction of germ cell mutations and of positive results in cell transformation assays (reviewed by Carrere 2007). The evidence for the genotoxicity of AA and GA in vivo reported since 2005 was discussed and summarised as follows:

AA and GA produced increased mutant frequencies in the Big Blue mouse model at the lymphocyte Hprt and liver cII genes. Similarly, GA increased the mutant frequency in the Hprt gene of Big Blue rats. It was also noted that GA was genotoxic in neonatal TK+/- mice, increasing the mutant frequencies of the Hprt and the TK+/- genes (Besaratinia and Pfeifer, 2007).

The availability of CYP2E1-null mice enabled the unequivocal demonstration that AA is genotoxic through its metabolite GA. Thus, wild type mice, but not knockout mice, showed DNA damage in leukocytes, liver, and lungs and increased erythrocyte MN frequencies on exposure to AA. Dose-related dominant lethality was also observed in AA treated wild-type mice.

AA and GA are distributed throughout the body and DNA adducts were also found in all organs examined. The group also noted the earlier findings that AA and GA showed dominant lethality when administered to males prior to mating with females. The group pointed to the fact that a broad spectrum of target organs is affected by genotoxicity and that this finding is at disparity with the known limited number of tumour target organs.

Another important observation was the finding that carcinogenicity of GA in neonatal mice suggests an increased sensitivity during early life exposure. Metabolic activation of AA to GA is clearly dependent on expression of CYP2E1. This enzyme is expressed early in human infants meaning that GA may be formed when solid food containing AA is introduced at weaning. Thus infants can be exposed to GA and may be at increased carcinogenic risk compared to adults.

Thus far, no thresholds for DNA binding of AA and GA have been identified, inasmuch as even control feed contains low levels of AA which results in a background dose of ~1 µg/kg per day, yielding 1/108 DNA adducts.

2. Review the recent evidence for the non-genotoxic modes of action of acrylamide (and glycidamide)

The discussion group noted the existing tumour data published from two life-time carcinogenicity studies in F344 rats. It was noted that the tumour sites were concordant in both studies and comprised fibroadenoma/carcinoma of the mammary gland, tunica vaginalis mesotheliomas of the male scrotum, and thyroid follicular adenomas (Johnson et al., 1986; Friedman et al., 1995). In contrast, the target organs in mice were liver, lung and Harderian gland (interim results of the NCTR/NTP studies).

Proposals have been made for possible modes of action (MOA) of AA carcinogenicity in the different target organs in the rat, and their relevance to humans (Shipp et al., 2006).

a. Mammary fibroadenomas are the most common spontaneously occurring tumours in aged female F344 rats. It has been proposed that AA treatment enhances the age-related disruption of the hormonal status in female rats.

b. The tunica vaginalis mesotheliomas of the male testes were considered to be related to the spontaneous occurrence of Leydig cell tumours which are prevalent in the male F344 rat. High-dose chronic administration of AA most likely exacerbates hormonal dysfunction. Because both tumour types appear as specific malignancies of ageing F344 rats it was suggested that the MOAs are not relevant to humans.

c. No plausible explanation was found for the formation of thyroid follicular-cell tumours in the rat. The relevance for humans could not be assessed.

d. CNS tumours were reported in one of the rat carcinogenicity studies but not in the other and the members of the discussion group suggested it would be best to wait for new data from ongoing experiments which might help to confirm or discount this finding.

The discussion group discussed other possible non-genotoxic modes of action, such as induction of oxidative stress and cell proliferation or inhibition of apoptosis, but could not identify convincing evidence for such a MOA prevailing at relevant doses. Because the carcinogenicity studies with mice are not yet complete, the tumour spectrum (liver, lung, Harderian gland) reported to date was considered provisional, and may eventually prove to be more extensive. Nevertheless, the tumour sites reported so far clearly differ from those in the rat. No plausible modes of action that might explain these tumour sites in mice have been proposed.

3. Weigh the evidence as to whether AA acts via a non-genotoxic or genotoxic mechanism in contrast to its genotoxic metabolite.

The discussion group stated that the margins of exposure at dietary levels of AA are such that genotoxic mechanisms are likely to be relevant and that at present no evidence exists for the operation of non-genotoxic mechanisms at relevant doses. As a general point, it was agreed that only in exceptional circumstances it would be possible to discount the human relevance of a genotoxic MOA.

4. Exploration of the consequences of changed conclusions about genotoxic versus non-genotoxic mechanism of carcinogenesis for human risk assessment.

The discussion group unanimously agreed that it did not anticipate any changes in these conclusions, based on the currently available information. Instead, a revised question was formulated: **How do we improve the risk assessment?**

The conclusions and recommendations arising from this question were as follows:

- New results, *e.g.* tumour dose-response data and mechanistic data, should increase confidence leading to improved Quantitative Risk Assessment (including interpretation of the Margin of Exposure).
- Information on species sensitivity, DNA and/or protein adduct levels, other biomarkers, and PB-PK modelling should reduce the uncertainty in extrapolation to humans.
- No concordance should be expected of tumour site profiles in different species.
- Different MOA are possible for different tumour types in experimental animals.
- There is a need for a systematic review of the MOA and human relevance for each tumour type.

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DG4: DIETARY EXPOSURE ACROSS EUROPE – CURRENT SITUATION

Since 2003, European Union Member States have been submitting occurrence data for acrylamide in food commodities to the Joint Research Centre (JRC) of the European Commission. The submission of the data to the JRC from Member States, was done through official food control laboratories directly or via their Competent Authorities, and from the food industry on a voluntary basis. The database and the reliability of the data were discussed with special regards to the analytical techniques, their sensitivity and specificity used to report acrylamide concentrations in food commodities and whether Member States report the data consistently. In 2007, the Commission made recommendations to monitor levels of acrylamide in certain food categories. These data will be reported to EFSA by Member States on a yearly basis for the next three years (EC, 2007). The content of acrylamide in different food commodities was also discussed to establish those food sources that contribute most to dietary acrylamide exposure.

1. Data reliability with regards to sensitivity of the analytical techniques and consistency of the data reported by the Member States, including new analytical techniques.

After some discussion, it was concluded that the chemical analyses used to detect and quantify acrylamide are well validated. The principle methods are based on gas chromatography-mass spectrometry (GC/MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS). These techniques provide reliable data both with respect to sensitivity and specificity in all relevant matrices. However, some concern was expressed over the reliability of available analysis of coffee products. This could be of importance since, at least in some populations, coffee products could contribute up to a third of the total dietary exposure. It was pointed out that there is a Nordic Committee on Food Analysis (NMKL) standard method available for cereals and potato products. The NMKL method can probably also be adjusted to other relevant matrices.

It was noted that a number of proficiency programmes are available from different organisations. In addition there are also two certified reference materials on the market, one for toasted bread and one for crisp bread. It was discussed whether there is a need for further certified reference materials. The group concluded that it might be valuable to have other matrices, although the two materials available represent the major sources of dietary exposure, potato and cereal products.

The group addressed the question - what are the main contributors to the uncertainty in exposure? It was concluded that it is probably not the chemical analysis but rather the consumption data, although there are no studies that have addressed this question specifically. Strong indications come from studies trying to relate external and internal exposure. Dutch experience shows that well validated consumption studies decrease the uncertainty considerably. It was concluded that the level of precision needed depends on the ensuing action: *i.e.* regulating or not, direct mitigation efforts, or informing consumers about the risks. These questions are partly value-based and not the focus of the Colloquium. However, it illustrates the importance of involving risk managers and other stakeholders in the planning of further scientific studies.

It was stressed that there is a need to reduce the costs for acrylamide analysis and, in particular, acrylamide adduct analysis in order to facilitate epidemiological studies with internal dose measurements. The group concluded that sampling schemes should be focused according to the objective of the study and that the sampling methods are probably more of a limiting factor than the analytical techniques themselves.

2. Review the occurrence data for acrylamide in food commodities available in Europe. Is there a need to revisit the exposure assessment?

The group concluded that we probably know the main sources of dietary exposure to acrylamide for the "average" consumer. From available data it can be estimated that roughly a third of the acrylamide exposure via foods comes from potato products, cereal products and coffee, respectively. However, it is still important to identify specific risk groups with high exposure, *e.g.* children or "exceptional" consumption of specific food commodities or specific ethnic foods where the levels of acrylamide have not been determined. To cope with this we need better data on both consumption and occurrence.

There are probably differences between countries when it comes to home cooking and catering although there are few solid data available. This hampers the possibilities for mitigation and intervention measures. Generally speaking there is, as pointed out earlier, a need to validate food frequency questionnaires to reduce the uncertainty in the exposure assessments.

The Commission has recommended Member States provide occurrence data for ten different food commodities during three consecutive years. This will allow for a time-trend analysis and the possibility to explore whether dietary exposure decreases as a result of voluntary industry actions.

It was noted that much of the occurrence data is not readily accessible. Some organisations have data that, unfortunately, is often in different formats and not truly compatible with, for example, the one used by the JRC. The group concluded that it would be valuable if EFSA could take the lead in improving the situation. It was noted that EFSA is establishing occurrence databases, in close collaboration with the Member States.

As to the question of whether present exposure assessments should be revised, the group concluded that the Margin of Exposure for the average consumer will not be affected in a substantial way by a revision. However, as said earlier, there is a need to assess the exposure of special groups. It was also noted that, depending on preferred future management options, there might be a need for revised and more precise exposure assessments.

3. Which food commodities contribute most to acrylamide exposure – possibility and efficacy of mitigation measures?

National differences in levels of acrylamide in foods, and more importantly, different consumption patterns make it difficult to give meaningful values for the relative contribution from different dietary sources of exposure at the pan-European level. However, a very rough estimate points to similar contributions from potato products, cereal based products and coffee.

The group concluded when modelling reduction of acrylamide, based on data from laboratory experiments, at maximum a 40% reduction might be possible, through measures taken by food producing industries. Some participants argued that this was a clear overestimation of the potential for reduction. It was agreed that measures have to be taken in home cooking, by catering companies and, perhaps most importantly, by consumers by changing consumption patterns, in order to achieve a substantial decrease in exposure.

4. Recommendations to improve data collection and data assessment in the future.

- Make all relevant data accessible from industry and Member States.
- Assess the exposure of specific risk groups.
- Depending on the preferred management option there might be a need for more precise exposure calculations.
- Consider the side-effects of reduction measures.
- Harmonise and utilise the probabilistic exposure assessment to improve the result of the assessment.
- Consider the relative importance of acrylamide *vs.* other food process contaminants.

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III FINAL DISCUSSION

EPIDEMIOLOGY

The discussion group on epidemiology concluded that:

- Some recent epidemiological studies have found positive associations between dietary acrylamide exposure and increased cancer risk, and some have not. Few reached statistical significance. It was noted that the studies in which positive associations have been found were prospective studies.
- Of the studies that did find associations, the associations seem weak in terms of the size of the relative risks (RRs) and there were inconsistencies between studies with respect to cancer sites reported to be affected.
- However, the RRs reported in studies showing positive associations are potentially important for public health given the likely measurement error in the estimates of exposure and the ubiquity of exposure.
- The fact that some epidemiological studies have found RRs greater than those predicted from the animal carcinogenicity studies reinforces the possibility that acrylamide in food may be an important public health concern.
- More prospective studies will be critical for reaching conclusions on the risks from dietary exposure to acrylamide.

In the subsequent discussion, it was emphasised that in those studies finding an association between dietary exposure to acrylamide and increased risk of certain cancers (endometrial, ovarian, kidney), the RRs were in the range 1.6-2.2 with the lower 95% confidence intervals above 1.10, meaning that these were statistically significant associations. It was noted that results of the US Nurses' Health Study for breast, endometrial, and ovarian cancers (unpublished results) are consistent with those from the recently reported study in The Netherlands (Hogervorst et al., 2007), even though the dietary habits of the two populations are very different. This gives some confidence that the currently used Food Frequency Questionnaires do provide useful information that can be used to stratify dietary acrylamide exposure. It would be important to determine whether other cohort studies that have yet to report (*e.g.* EPIC) reveal similar findings with respect to the cancer sites identified in some studies as having positive associations with dietary acrylamide exposure.

In addition to smoking as a known source of acrylamide and significant confounder in dietary acrylamide studies, the possible role of alcohol was discussed. CYP2E1, the enzyme that converts acrylamide to glycidamide, which forms DNA adducts, is known to be induced by various factors, including alcohol consumption. It was noted that the epidemiological studies that had adjusted for, or stratified the data for alcohol intake (*e.g.* The Netherlands Cohort study, the US Nurses' Health Study) did not find an effect attributable to alcohol intake. However, it was also commented that in EPIC cohorts, the levels of glycidamide-haemoglobin adducts were lower in those who consumed alcohol than in those who did not.

It was acknowledged that other potentially suspect compounds resulting from the heating of food have been identified (*e.g. inter alia* by the EU-funded HEATOX project) and that co-exposure to these together with acrylamide might be an explanation for the higher than expected RRs found in some of the epidemiological studies. However, it was agreed that acrylamide should remain the current focus for epidemiological studies since the laboratory animal evidence indicated a clear potential for a genotoxic and carcinogenic risk from this compound.

Future needs for epidemiological studies that could improve the risk assessment were considered. It was noted that the studies that would likely appear in the near future (such as EPIC) were not specifically designed to assess the possible risks from consumption of acrylamide-containing foods. With the knowledge now available, the design of new prospective studies should enable the issue of accuracy of estimation of acrylamide exposure to be better addressed. Focusing on divergent diets that would give a wider range of acrylamide exposure might yield useful results. The uncertainty generated by the wide range of acrylamide occurrence data within some food categories was also acknowledged. While this indicates that current estimates of exposure may not accurately inform about total exposure to acrylamide, this did not mean the use of such data to estimate dietary exposure in epidemiological studies was not valuable.

The inclusion of measurements of biomarkers of acrylamide exposure in epidemiological studies, in addition to dietary questionnaires, would also be helpful. However, because of the considerably increased levels of glycidamide-haemoglobin adducts in smokers, it was noted that biomarker measurements would only be informative with respect to dietary exposure in non-smokers.

The question was raised of whether there were any human polymorphisms, such as those related to glutathione activity, that might affect acrylamide activation, since focus on such persons might also yield useful data. None were known at present.

BIOMARKERS

The discussion group on biomarkers concluded that:

- The use of biomarkers is helpful in the estimation of dietary exposure to acrylamide in epidemiological studies, in the extrapolation of results from animals to humans, in validating PB-TK models and in the investigation of metabolic polymorphisms at the population level.
- The simultaneous measurement of acrylonitrile adducts could help to identify possible confounding by smoking and might offer the possibility to quantify the amount of acrylamide of smoking origin.
- It is unclear whether there may be endogenous sources of acrylamide, for example from dietary precursors.
- The development of immunological approaches such as neo-epitope-based antibodies, might allow cheaper, higher throughput of biological samples. The state of the art on the development of serum antibodies to high levels of acrylamide and monoclonal antibodies against DNA adducts was unclear.
- The application of 'omics' techniques may reveal new mRNA, protein and metabolic biomarkers.

In the discussion that followed, the value of haemoglobin adducts as a biomarker of chronic exposure to acrylamide was considered. Although such adducts are relatively short-lived, there is evidence that people are fairly consistent in their food habits, in which case even a single measurement on a particular day may be indicative of chronic exposure. It was noted that it was possible to measure adducts in blood samples that had been stored for 10 years. While data on haemoglobin-acrylamide adducts may be a useful measure of acrylamide exposure via the diet, their relevance to any subsequent toxicity needs to be carefully considered. Biomarker data therefore needed to be interpreted with caution. Although measurement of DNA adducts would be preferable, it presents greater technical problems than measurement of haemoglobin adducts.

The question was raised of whether any general conclusions can be drawn about the information from biomarkers that would help in the extrapolation of results from animals to humans. It was agreed, that while toxicokinetic comparisons between rodents, swine and humans, using metabolic biomarkers, have shown the relevance of these species for human risk assessment, more research was needed to establish full comparative metabolic profiles in urine, since not all major metabolites have yet been determined. The practical problem in utilisation of biomarker data was to decide when and how often samples need to be collected and how the results should be integrated into the risk assessment, temporal issues clearly being important. The physiologically-based, toxicokinetic/toxicodynamic (PB-TK/TD) model proposed by Young (Young et al., 2007) had been validated using human urinary data from Germany, but there was also a need to validate the 24h urinary metabolites and the acrylamide and glycidamide adduct data. This did not mean that the model should not be applied now to new datasets, but there might be a need to make refinements to the model as new results appear.

In view of the importance of smoking as a source of acrylamide and a confounder in dietary studies, it would be useful to know whether adduct levels have been measured in humans exposed to passive smoking. Other potential environmental sources of acrylamide were wood burning, which is known to produce amounts of acrylamide in the mg/m^3 range. This should result in seasonal variation if it does contribute to exposure. It was also questioned whether consumption of raw meat might be a source of exposure, since it might contain acrylamide bound covalently to haemoglobin. However, it was considered unlikely that such adducts would be cleaved and then absorbed in significant amounts in a consumer of raw meat.

The potential value of exploiting data on the variation in adduct levels in those exposed occupationally to acrylamide was discussed. Comparison of biomarker levels in workers on Fridays (end of shift) compared with Monday mornings (start of shift), coupled with food frequency and lifestyle questionnaires could generate useful results.

In response to the question raised by the discussion group on biomarkers regarding endogenous formation, it was noted during a study on dietary restriction of food items containing acrylamide that the urinary biomarkers fell almost to zero, suggesting that there is no significant endogenous formation of acrylamide. However, the possibility of dietary precursors of acrylamide was raised as a possibility.

Concerning the possibilities to apply immunological methods for the study of biomarkers, there were divergent views on whether such approaches would improve throughput and on whether or not they would generate additional problems with respect to sensitivity and specificity. One advantage in the use of monoclonal antibodies to haemoglobin adducts is that it allows measurements to be made on very small samples of blood, such as from a finger-prick. It was agreed that the development and use of immunological methods should not be ruled out.

MECHANISMS OF CARCINOGENICITY

The discussion group on mechanisms of carcinogenicity concluded that:

- Glycidamide, and in some systems acrylamide, is genotoxic in a range of assays.
- There is a need for a systematic review of the mode of action of acrylamide and the human relevance for each tumour type identified in experimental animals.
- Different mechanisms are possible for different tumour types in experimental animals.
- The margins of exposure at dietary levels of exposure to acrylamide are such that only genotoxic mechanisms are likely to be relevant to human health.
- The risk assessment could be improved quantitatively by better information on tumour dose-response and mode of action.
- The uncertainty in the risk assessment could be reduced by better information on species sensitivity, biomarkers, and PB-TK modelling.

Questions were raised about the animal carcinogenicity bioassays and modes of action.

Was there a need for studies on ageing rats to see if hormonal influences were important? Tumour rates were very low when rats were treated with a peroxisome proliferator starting at the age of 13-weeks, but were high when treated from 57-weeks of age on (Kraupp-Grasl et al., 1991), suggesting there might be a need for further testing in older rats. However, it was noted that lifetime exposure and late-appearing tumours are characteristic of all carcinogens, irrespective of their mode of action.

Although non-genotoxic mechanisms have been proposed, including hormonally mediated modes of action because of the observations of cancer in endocrine-responsive tissues in rats, there is no good evidence to date for non-genotoxic mechanisms (*e.g.* see Bowyer et al., 2008). The information presented at the Colloquium by Dr. Doerge, including the results from the recently completed NCTR/NTP bioassays, show that acrylamide is a carcinogen at multiple anatomic sites in both rats and mice, although tumour sites differ between the two species, and that tumours appear early following neonatal exposure in the mouse. These findings suggest a genotoxic mode of action.

It was noted that much of the work on genotoxic mechanisms has been conducted in the mouse, in which the formation of glycidamide from acrylamide is higher than in the rat, and that knockout mice can also be used to investigate mechanistic issues. Irrespective of the results in the mouse, it is still not possible to conclude that all acrylamide-induced tumours in all species are attributable to a genotoxic mode of action. However, since glycidamide is known to be genotoxic and is present in humans following dietary exposure to acrylamide, genotoxicity is the relevant mode of action for human risk assessment. The discussion on modes of action underlined the need for a systematic review of this aspect and of the relevance of the different tumour types for human risk assessment, as recommended by discussion group 3.

The question of the relevance of DNA adduct measurements for human risk assessment was raised, given the possibility of DNA repair. It was noted that in rodents repair mechanisms are not thought to be important for the glycidamide-DNA adducts, as these are removed by chemical hydrolysis.

It was noted that the WHO limit for acrylamide in drinking water and the maximum recommended EU limit for acrylamide in cosmetics were both much lower than the levels found in certain foods and some participants questioned why there appeared to be more caution about acrylamide levels in drinking water than in food. In reply, it was pointed out that the WHO limit for acrylamide in drinking water is not based on risk but on a practical lowest level that is technically feasible. Thus there is no inconsistency between the various regulatory sectors on the nature of the possible risks from acrylamide and current differences simply reflect different risk management options and decisions.

DIETARY EXPOSURE ACROSS EUROPE

The discussion group on dietary exposure concluded that:

- Analytical techniques for measuring acrylamide concentrations in food and for adduct measurement in biological samples are well validated, but there is a need to reduce the cost of adduct analysis.
- There is a need to ensure all relevant occurrence data generated both by industry and the Member States is accessible.
- Data should allow the assessment of risks in specific population groups.
- Depending on the preferred risk management option, there might be a need for more precise exposure calculations.
- Probabilistic modelling of exposure could improve the risk assessment, for example, the consequences of various risk management options could be modelled.
- It was important to consider the possible 'side effects' of measures to reduce acrylamide formation in foods, such as an increase in other potentially undesirable Maillard reaction products.
- It was important to consider the relative importance of acrylamide versus other food processing contaminants.

The key message concerning the use of mitigation measures by industry to reduce acrylamide formation in foods was that modelling has shown that if all currently possible measures were utilized, it could halve dietary levels of acrylamide. There would be a similar impact from altering people's dietary habits away from high acrylamide-containing foods by following general dietary recommendations for a balanced diet.

A number of Member States have conducted campaigns and issued advice to consumers about home cooking practices and dietary change, emphasising the role of a healthy diet in reducing acrylamide exposure. However, consumers have, in general, not responded to these messages. For example, a study in Sweden showed that consumption patterns remained unchanged after the advice on acrylamide was issued. It was pointed out that the national differences across the EU in what foods contributed most to acrylamide exposure showed that advice needs to be targeted to the subgroups most at risk. It would be useful to validate urinary biomarkers in relation to the types of cooking methods used by consumers.

Changes in acrylamide exposure of consumers over time, due to industry mitigation measures, have been investigated by the Swedish National Food Administration over the last 3 years. Annual variations in acrylamide levels in foods were found but no overall downward trend in acrylamide exposure has been detected, despite the significant reduction of acrylamide levels in products such as potato crisps. This might be because average and not extreme consumers have been investigated, or because industry made the easiest and most significant changes before the baseline study was started. The Swedish authorities have received very little information from industry on what mitigation measures have actually been implemented.

The German authorities have been looking at 'signal values' for acrylamide in foods over time. A signal value is the lowest measured value of the 10% of products in a food category known to have the highest levels of acrylamide. For some products, such as potato crisps, the signal value has continued to decline, but for other products reductions in acrylamide are not possible and, in recent years, some levels in food that initially went down have since increased. The achievement of an overall reduction in acrylamide levels is hampered by the large variations seen in some product categories.

In some specific foods, such as honey-cake in The Netherlands, reductions in acrylamide levels of about 25% were seen between 2002 and 2006, due to the replacement of ammonium bicarbonate as a raising agent.

The levels of asparagine are critical to acrylamide formation during food processing. In the case of cereals, there is considerable year to year variation in asparagine content. Thus initiatives are also needed at the agricultural end of the food chain to develop cultivars with low asparagine content, as this will have the potential to benefit the whole food chain for cereal-based products. It was noted that the annual variation in levels of asparagine in crops is greater than any reductions that could be achieved with current mitigation measures. Improvements could also be achieved by control of the sugar content of potatoes, accompanied by labelling for consumers. It was noted that in the CIAA (Confederation of Food and Drink Industries of the EEC)

Acrylamide Toolbox database, which contains a large amount of information from analytical data on processed foods, there is no input from the agricultural community, as yet.

In response to a question about whether foods other than those currently known to be sources of acrylamide have been adequately studied, it was noted that while exposure seems to be explained mainly by formation of acrylamide in starch-based foods, there was a need to look at other foods to make sure all potential sources were understood.

It was noted that foods are complex and that some constituents or contaminants in foods may have a protective effect, for example by causing an increase in glutathione or increases in other phase II enzymes in consumers.

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Bowyer JF, Latendresse JR, Delongchamp RR, Muskhelishvili L, Warbritton AR, Thomas M, Tareke E, McDaniel LP and Doerge DR, 2008. The effects of subchronic acrylamide exposure on gene expression, neurochemistry, hormones, and histopathology in the hypothalamus-pituitary-thyroid axis of male Fischer 344 rats. Toxicol. Appl. Pharmacol. March 18, 2008. Epub ahead of print.

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Young JF, Luecke RH and Doerge DR, 2007. Physiologically based pharmacokinetic/ pharmacodynamic model for acrylamide and its metabolites in mice, rats and humans. Chem. Res. Toxicol. 20, 388-399.

IV SUMMARY AND RECOMMENDATIONS

Concerning the **epidemiological data**, there was some evidence for an association between dietary exposure to acrylamide and some types of cancer. However, the relative risks were low and the totality of all the epidemiological evidence is not consistent. Some more recent epidemiological studies indicate there may be greater risks for certain cancer sites but it should be noted that not many of the RRs reached statistical significance. It was cautioned not to expect tumour site concordance between animals and humans. There was a need in future epidemiological studies to develop food frequency questionnaires that would focus on food processing, including cooking in the home. FFQs should also be supplemented by biomarker measurements. It is important to understand and control confounders such as smoking.

Important needs in the area of **biomarkers** were highlighted. These include better understanding of the overall fate of acrylamide in humans as this would help in interspecies extrapolation. The measurement of glycidamide DNA adducts in humans would be a useful advance but at present was not technically feasible. It should be recognised that biomarkers are indicators only of relatively short-term exposure and that they can be confounded by factors other than the diet.

The evidence that genotoxicity is an important **mode of action** is increasing, but there may be other, non-genotoxic mechanisms for certain of the tumour types observed in animals. Accurate dose-response analysis and the derivation of benchmark doses for each tumour site, together with information on mode of action, will continue to be helpful in assessing which tumours are the most important for human risk assessment. Animal data may also indicate some intermediate biomarkers of effect that could be useful in the future.

For **dietary exposure**, the analytical methods for establishing occurrence data and estimating human exposure appear adequate and, even with better data, it seems unlikely that the currently estimated margins of exposure will change dramatically. A tiered approach to exposure assessment is useful and enables such assessments to be tailored to the needs of the risk manager. There is a need to refine the valuable tool of FFQs to enable studies to focus on subpopulations considered to be more at risk, for example, because of dietary or genetic risk factors. The difficulties of persuading the subpopulations most at risk to take up risk management messages should not be underestimated. By analogy with the experience for example on folic acid, mitigation measures may prove to be more important than advice.

The chairs of the colloquium posed the key question of whether the new evidence now available from epidemiological, toxicological and exposure studies since 2005 would warrant a re-evaluation of the existing risk assessments for acrylamide in food.

The participants were aware of a number of important ongoing studies and considered that there was a need to await their outcome. Thus it would be premature to reassess the risk of exposure to acrylamide at this point. It was noted that the outcomes of the new NCTR/NTP carcinogenicity studies and new epidemiological reports are anticipated in the near future and that acrylamide may be scheduled for re-evaluation by JECFA in autumn 2009.

It was commented that the new data published since 2005 have reduced some of the uncertainties mentioned in the JECFA evaluation of 2005 and endorsed by the EFSA Panel on contaminants in the food chain (CONTAM).

On the question of whether the level of concern about the possible risks of acrylamide in food has changed, the vast majority of participants felt that the level of concern was the same as before.

Overall, it was concluded that it is not possible at the present time to improve the existing risk assessments but data anticipated to be available in the near future will be valuable in adding weight to the current risk assessments and in reducing the attendant uncertainties. The new data may enable the formulation of advice that is likely to be quantitatively similar to that given at present but with greater certainty. There are no obvious solutions to the question of how to further reduce exposure to acrylamide, but additional mitigation measures earlier in the food chain may be possible.

ANNEXES

V ANNEXES

Annex 1: Programme of the Colloquium

Annex 2: Participants of the Colloquium

Annex 3: Presentations made at the Colloquium (plenary session)

Annex 4: Slides of the Discussion Groups

ANNEX 1: PROGRAMME OF THE EFSA COLLOQUIUM

Acrylamide carcinogenicity – new evidence in relation to dietary exposure

22-23 May 2008, Tabiano, Italy

PROGRAMME

Chairs:	Josef Schlatter Federal Office of Public Health, CH
	Ada Knaap Scientific Committee – EFSA
Rapporteurs:	Alan Boobis Imperial College London, UK
	Sue Barlow Scientific Committee – EFSA

Day 1

09.00-13.00	**Session 1: INTRODUCTORY PLENARY SESSION**	
09.00-09.10	Welcome and introduction to EFSA	Riitta Maijala Director of Risk Assessment, EFSA
09.10-09.30	Objectives of the Colloquium	Josef Schlatter Federal Office of Public Health, CH

09.30-09.50	Dietary exposure to acrylamide and cancer risk: a summary of recent epidemiological evidence	Jenny Barrett University of Leeds, UK
09.50- 10.00	*Questions*	
10.00-10.20	Biomarkers of acrylamide	Jan Alexander Institute of Public Health, NO
10.20- 10.30	*Questions*	
11.00-11.20	Genotoxic and non-genotoxic mechanisms for acrylamide carcinogenicity	Daniel Doerge FDA, U.S.A
11.20-11.30	*Questions*	
11.30-11.50	Acrylamide Level Monitoring Database - Overview of 5 years of data collection in Europe	Thomas Wenzl Joint Research Centre Geel, BE
11.50-12.00	*Questions*	
12.00-12.20	General discussion	Chairs
12.20-12.30	Introduction to discussion groups	

14.00-18.00 **Session 2:**
DISCUSSION GROUPS (DG)
Four parallel discussion groups to address:

DG 1	Epidemiological studies – evaluating evidence and addressing uncertainties	Chair:	Rolaf van Leeuwen RIVM, NL
		Rapporteur:	Kathryn Wilson Harvard School of Public Health, U.S.A
DG 2	Biomarkers – new insights in exposure and mode of action	Chair:	Peter Farmer University of Leicester,
		Rapporteur:	Jean-Lou Dorne CONTAM Unit, EFSA
DG 3	Mechanism of carcinogenicity	Chair:	Diane Benford FSA, UK
		Rapporteur:	Wolfram Parzefall Medical University of Vienna, AT
DG 4	Dietary exposure across Europe - current situation	Chair:	Detlef Müller Foodrisk, DE
		Rapporteur:	Leif Busk National Food Administration, SE

Day 2

09.00-10.00	**Session 3:** **CONTINUATION OF DISCUSSION GROUPS** Including discussion on the outcomes of the discussion groups and the production of reports to the plenary session	
14.00-18.00	**Session 4:** **FINAL PLENARY SESSION**	
10:30-10:50	Report back from discussion group 1	Kathryn Wilson Harvard School of Public Health, U.S.A
10:50-11:05	*Discussion*	
11:05-11:25	Report back from discussion group 2	Jean-Lou Dorne CONTAM Unit, EFSA
10:50-11:05	*Discussion*	
11:40-12:00	Report back from discussion group 3	Wolfram Parzefall Medical University of Vienna, AT
12:00-12:15	*Discussion*	
12:15-12:35	Report back from discussion group 4	Leif Busk National Food Administration, SE
12:35-12:50	*Discussion*	
12:50-13:30	General discussion Conclusions and take-home message	Chairs
14.30	COLLOQUIUM ADJOURNS	

ANNEX 2: PARTICIPANTS OF THE COLLOQUIUM

Name	Affiliation	Country	Discussion Group (DG)
Prof. Lilianne Abramsson-Zetterberg	Food Administration	SE	3
Prof. Jan Alexander	Norwegian Institute of Public Health	NO	2
Dr Adriana Arisseto	State University of Campinas	BR	4
Dr Peter Ashby	Confederation of Food and Drink Industries of the EEC (CIAA)	GB	1
Prof. Dr Herman Autrup	University of Århus	DK	2
Dr Sue Barlow	EFSA Scientific Committee	UK	1
Dr Jenny Barrett	University of Leeds	UK	1
Dr Matthias Baum	Bund für Lebensmittelrecht und Lebensmittelkunde (BLL), University of Kaiserslautern	DE	2
Mrs Sandra Bašić	Croatian Food Agency	HR	4
Dr David Bell	University of Nottingham	UK	3
Dr Diane Benford	Food Standards Agency (FSA)	UK	3
Dr Ulrike Bernauer	Federal Institute for Risk Assessment (BfR)	DE	2
Dr Alan Boobis	Imperial College London	UK	3
Dr Timo Buetler	Nestlé Research Center	CH	3
Dr Leif Busk	National Food Administration	SE	4
Prof. Angelo Carere	National Institute (ISS)	IT	3
Dr Jaroslav Cepl	Potato Research Institute	CZ	2
Dr Zuzana Ciesarova	Food Research Institute (VUP)	SK	1
Dr Daniel R. Doerge	U.S. Food & Drug Administration (FDA)	US	3
Dr Laura Fabrizi	National Institute (ISS)	IT	2

Name	Affiliation	Country	Discussion Group (DG)
Prof. Peter Farmer	University of Leicester	UK	2
Dr Cristina Fortes	Istituto Dermopatico dell'Immacolata (IDI)	IT	1
Dr Henrik Frandsen	National Food Institute (DTU)	DK	2
Dr Marvin A. Friedman	SNF S.A.S.	FR	3
Ms Barbara Gallani	Food and Drink Federation (FDF)	UK	4
Ms Kit Granby	National Food Institute (DTU)	DK	4
Dr Anja Hallikainen	Food Safety Authority	FI	2
Dr Colin Hamlet	Premier Foods	UK	2
Dr Gabrielle Hawksworth	University of Aberdeen	UK	3
Mrs Judith Heikoop	DSM Food Specialties	NL	1
Dr Michaela Heinemann	Givaudan AG	CH	3
Ms Sirkku Heinimaa	European Commission	BE	4
Dr Karl-Erik Hellenäs	National Food Administration	SE	1
Ms Janneke Hogervorst	University of Maastricht	NL	1
Ms Carmina Ionescu	National Food Administration	SE	4
Dr George Kass	University of Surrey	UK	2
Dr Ada Knaap	EFSA - Scientific Committee	NL	Overall chair
Dr Erik J. M. Konings	Dutch Food and Consumer Product Safety Authority(VWA)	NL	4
Dr Rind Kursat Aktas	Ministry of Agriculture and Rural Affairs	TR	2
Dr John Christian Larsen	National Food Institute	DK	3
Ms Ana López-Santacruz	Spanish Agency of Food Safety and Nutrition (AESAN)	ES	4
Dr Matilde Marques	Centro de Química Estrutural (CQE)	PT	3
Dr Dennis Marroni	Acrylamide Monomer Producers Association, Inc. (AMPA)	FR	2

Name	Affiliation	Country	Discussion Group (DG)
Dr David Mason	Food Standards Agency (FSA)	UK	1
Prof. Dr Reinhard Matissek	Food Chemistry Institute of the Federal Association of the German Confectionery Industries	DE	4
Dr Francisco Morales	Spanish National Research Council (CSIC)	ES	4
Dr Detlef Mueller	FoodRiskConsulting	DE	4
Dr Wolfram Parzefall	Medical University Vienna	AT	3
Dr Jan Erik Paulsen	Norwegian Scientific Committee for Food Safety	NO	3
Dr Gloria Pellegrino	L. LAVAZZA S.p.A.	IT	1
Dr Marino Petracco	illycaffè S.p.A.	IT	4
Dr Mari Reinik	Health Protection Inspectorate	EE	4
Dr Florian Riedel	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL)	DE	2
Dr Jiri Ruprich	National Institute of Public Health	CZ	4
Dr Josef Schlatter	Federal Office of Public Health	CH	2
Dr Katrin Schutte	Procter & Gamble (European Snack Association)	BE	3
Dr Daniel Skrypec	Kraft Foods	US	1
Mr Martin Slayne	European Snacks Association (ESA)	US	4
Ms Laura Smillie	European Food Information Council (EUFIC)	BE	1
Mrs Alexandra Tard	French Food Safety Agency (AFSSA)	FR	4
Ms Christina Tlustos	Food Safety Authority of Ireland (FSAI)	IE	4
Prof. Piet Van Den Brandt	University of Maastricht	NL	1
Prof. F.X. Rolaf Van Leeuwen	National Institute for Public Health and the Environment (RIVM)	NL	1

Name	Affiliation	Country	Discussion Group (DG)
Dr Christine Vinkx	Federal Public Service for Health, Food Chain Safety and Environment	BE	2
Dr Thomas Wenzl	European Commission	BE	4
Ms Kathryn Wilson	Harvard School of Public Health	US	1
Dr Elisabet Wirfält	University of Lund	SE	1
Dr Otmar Zoller	Federal Office of Public Health	CH	4

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Dr Ulla Bertelsen	Risk Assessment, Contaminants Unit (CONTAM)
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Ms Vanessa Descy	Communications, Public Information & Events Unit
Dr Jean-Lou Dorne	Risk Assessment, Contaminants Unit (CONTAM)
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Dr Riitta Liisa Maijala	Director of Risk Assessment
Dr Daniela Maurici	Scientific Committee & Advisory Forum Unit
Ms Francesca Piombini	Communications, Public Information & Events Unit
Dr Francesco Vernazza	Scientific Cooperation and Assistance, Data Collection Exposure Unit (DATEX)

ANNEX 3: PRESENTATIONS MADE AT THE COLLOQUIUM

Introduction to EFSA

RIITTA MAIJALA
Director of Risk Assessment, EFSA

EFSA's Mission

- Provide **science based** risk assessments supporting Risk Management related to food/feed safety
- Provide **scientific** and technical **advice** on all matters within these fields
- **Communicate** all findings publicly (communication task is shared with EC/MS)

EFSA Panels - Generic opinions

- Panel on Plant Health (PLH)
- Panel on Plant protection products and their residues (PPR)
- Panel on Animal Health and Welfare (AHAW)
- Panel on contaminants in the food chain (CONTAM)
- Panel on biological hazards (BIOHAZ)

- Scientific Committee (SC)

EFSA Panels - mainly opinions on applications

- Panel on Genetically Modified Organisms (GMO)
- Panel on additives and products or substances used in animal feed (FEEDAP)
- Panel on additives, flavourings, processing aids and materials in contact with food (AFC):
  - Panel on food additives and nutrients sources added to food (ANS)
  - Panel on food contact materials, enzymes, flavourings and processing aids (CEF)
- Panel on dietetic products, nutrition and allergies (NDA)

Directorate of Cooperation and Assistance

Comprising:

- Data collection and exposure Unit (DATEX)
- Zoonosis Unit
- Assessment methodology Unit (AMU)
- Pesticides risk assessment Unit (PRAPeR)
- Scientific cooperation Unit (SCO)
- Emerging risks Unit (EMRISK)

Activities of the CONTAM Panel

- To deliver scientific opinions and advice on contaminants in food and feed, associated areas and undesirable substances such as natural toxicants, mycotoxins and residues of non -authorised substances not covered by another Panel
- To respond to emerging issues in the area of contaminants, if needed in a fast manner
- To apply current risk assessment methods such as benchmark dose modelling, margin of exposure calculations and risk-benefit analysis, if needed, it its risk assessments

An EFSA Colloquium is

- An interactive event rather than a passive listening to lectures
- A platform for scientists to have in-depth discussions on scientific approaches and methods available and tools and data needed for conducting a scientific assessments
- An event to explore opportunities and limitations for defining a common understanding of the current state-of-the-art in scientific progress and limitations and
- An opportunity to define further (research) needs

An EFSA Colloquium is not

- An attempt to agree on the details of a preferred strategy or approach, if any
- An attempt to finalise a blue print for the work ahead of us
- A "who is right and who is wrong" discussion

Issues

- The outcome of epidemiological studies relating acrylamide dietary exposure and human cancer risk can vary
- Impact of biomarkers on the risk assessment of acrylamide from an exposure and a mode of action point of view
- Is there any recent evidence related to the genotoxic and non-genotoxic modes of action of acrylamide and its metabolites?
- What is the current situation on dietary exposure to acrylamide in Europe?

A free and open debate should be the basis to discuss the state-of-the-art on acrylamide carcinogenicity

Thank you for sharing your views with EFSA.

Thank you for being frank, open and constructive.

Objectives of the Colloquium

JOSEF SCHLATTER
Federal Office of Public Health,
Switzerland

- Short History
- Neurotoxicity / Reproductive Toxicity
- Mutagenicity / Carcinogenicity
- Assessment by the 64 JECFA: MOE Calculation → BMDL, Intake

Acrylamide: History - 1997

Leakage of acrylamides from a tunnel construction work:
http://ean.cepn.asso.fr/pdf/program4/An-TORNQVIST.pdf

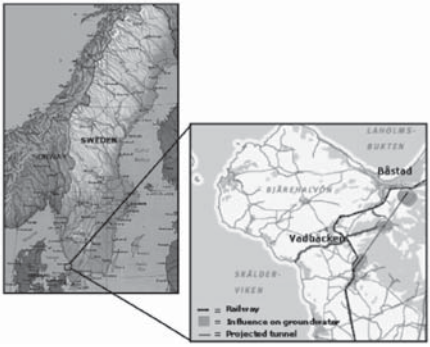
5. August–30. Sept 1997 Hallandsås Tunnel construction.
Application of Rhoca Gil due to water inrush

→ Fish kill

→ 3 cows with paralysis of the hindlimbs

→ High conc. of Rhoca Gil monomer in water

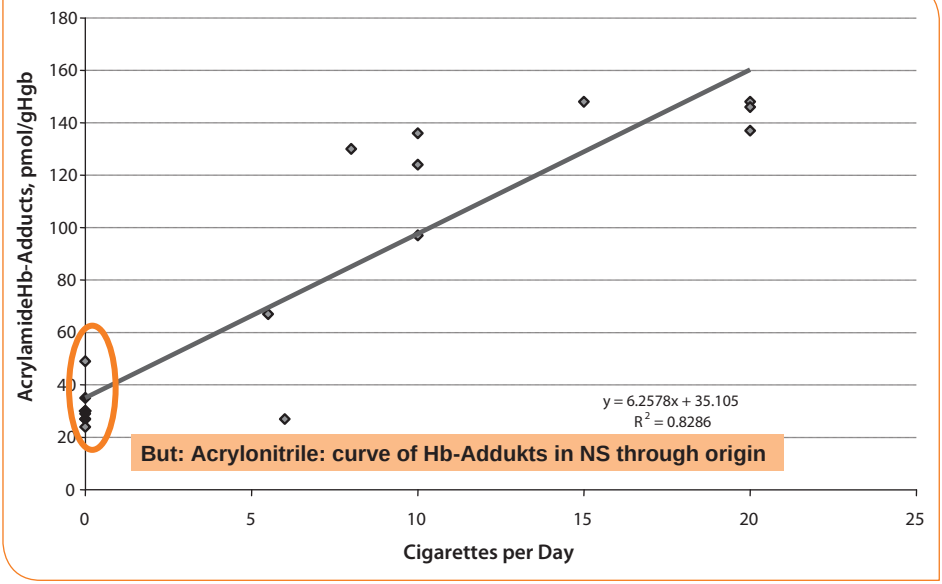
→ Stopp of Rhoca Gil use on 30.9.1997



7. October: Tunnel construction stopped

10. October: identification of Acrylamide-Hb-Adducts in cows
Investigation of the tunnel-workers for Hb-Adducts
[Median 250, maximum 3000 pMol/g Hb]
Adducts also found in non-smoking control grup **[40 pMol/g Hb]**

Acrylamide: Hb Adducts in Smokers (Bergmark, 1997)



Acrylamide: History

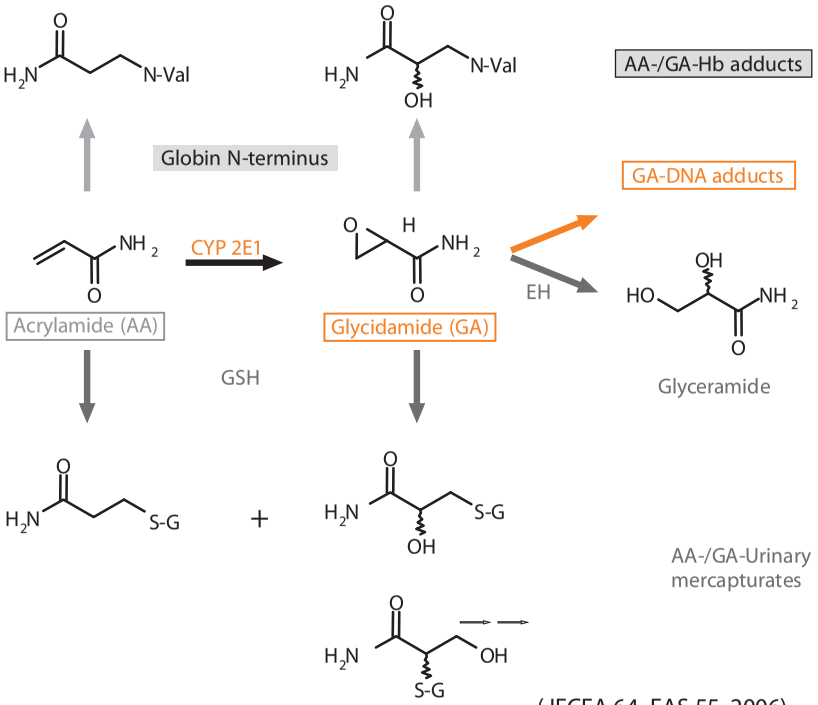
- 2000: Acrylamide Hb-Adducts in rats after feeding on fried feed
- Investigation of foodstuffs
- **24. April 2002:**
  Press conference of the Swedish Authority

LIVSMEDELS VERKET
NATIONAL FOOD ADMINISTRATION

Elevated levels of acrylamide found in starch-containing and heated foodstuffs

- 25.-27. June 2002 Joint FAO/WHO Consultation, Geneva
- JIFSAN Workshop Chicago October 2002, April 2004

- Readily soluble in water
- Rapid and uniform distribution in the body including
  - breast milk
  - fetus
- Metabolism to the epoxide Glycidamide
  - issaturable
  - dose dependent
  - species differences (mice > rat)
- rel. fast elimination (hrs)



Acrylamide: Neurotoxicity

- is neurotoxic upon repeated "high" doses:
- Peripheral neuropathies (NOEL 0.5 mg/kg bw)
- Morphological nerve changes (EM): NOEL 0.2 mg/kg bw
- **Mode of action: likely due to direct covalent binding to proteins**

  ["Motor-proteins" important for membrane fusions → functionning of synapses, growth of neurons]
- Single neurotox. Dose:
  ≥100 mg/kg bw (convulsions)
- Reduced fertility
  & effects on reproductive organs:

  repeated 10-15 mg/kg bw
  NOEL 2 mg/kg KG

Acrylamide: Genotoxicity / Carcinogenicity

- AA mainly **negative** in prokaryotic *in vitro* test systems but predominantly **positive** in mammalian and in in vivo tests
- Most of the genotoxicity of AA mediated by GA
- causes gene mutations in vivo & *in vitro* (somatic & germ cells)
- causes chromosomal aberrations (breaks) in vivo & *in vitro*
- (induction of micronuclei)
- **Is genotoxic**
- **Increases tumour incidence in rats at doses of 1-2 mg/kg bw**
- IARC: Group 2A: *Probably carcinogenic to humans*

DNA Adduct Summary-Rodents

- Wide tissue distribution of DNA adducts
- Reactivity with DNA bases GA > AA
- GA produces higher levels of DNA adducts in rodents than AA
- DNA adducts proportional to GA AUC for rats and mice
- DNA adducts accumulate - repeated dosing
- DNA adduct removal in rats and mice - spontaneous depurination (N7 & N3)
- Species differences in DNA adducts apparent at high dose of AA – not at low dose (rats less sensitive than mice) → Link to metabolism

Acrylamide: carcinogenicity (JECFA 64, FAS 55, 2006)

Table 3. Numbers of Fischer 344 rats with tumours at various organ sites after receiving drinking-water containing acrylamide for 2 years

Type of tumour	Sex	Dose[a] (mg/kg bw per day)				
		0	0.01	0.1	0.5	2.0
Thyroid gland, follicular adenomas	M	1/60	0/58	2/59	1/59	7/59*
Peritesticular mesotheliomas	M	3/60	0/60	7/60	11/60*	10/60*
Adrenal gland,[b] pheochromocytomas	M	3/60	7/59	7/60	5/60	10/60*
Mammary tumours	F	10/60	11/60	9/60	19/58	23/61*
Central nervous system, glial tumours	F	1/60	2/59	1/60	1/60	9/61*
Thyroid gland, follicular adenomas or adenocarcinomas	F	1/58	0/59	1/59	1/58	5/60*
Oral cavity, squamous papillomas	F	0/60	3/60	2/60	1/60	7/61*
Uterus, adenocarcinomas	F	1/60	2/60	1/60	0/59	5/60*
Clitoral gland, adenomas[c]	F	0/2	1/3	3/4	2/4	5/5*
Pituitary adenomas[b]	F	25/59	30/60	32/60	27/60	32/60*

Data from Johnson et al. (1986), as compiled by Rice (2005)

F, female; M, male

[a] Asterisk (*) indicates $P = 0.05$; pair-wise Mantel-Haenszel comparison with the control group adjusted for mortality.

[b] The historical incidence of adrenal gland pheochromocytomas in males was 8.7% (range, 1.2–14.0%); that of pituitary adenomas in females was 38.1% (range, 28.2–46.9%).

[c] Only clitoral glands with gross lesions were examined histologically.

Table 4. Numbers of Fischer 344 rats with tumours at various organ sites after receiving drinking-water containing acrylamide for 2 years[a]

Type of tumour	Sex	Dose[b] (mg/kg bw per day)						
		0	0	0.1	0.5	1.0	2.0	3.0
Peritesticular mesotheliomas	M	4/102	4/102	9/204	8/102	–	13/75*	–
Brain and spinal cord, glial neoplasms[c]	M	1/102[d]	1/102[d]	2/204[e]	1.102[f]	–	3/75[d]	–
	F	0/50[g]	0/50[g]	–	–	2/100[g]	–	2/100[g]
Thyroid gland, follicular adenomas	M	2/100	1/102	9/203	5/101	–	15/75*[h]	–
	F	0/50	0/50	–	–	7/100	–	16/100*[h]
Thyroid gland, follicular cell carcinomas	M	1/100	2/102	3/203	0/101	–	3/75	–
	F	1/50	1/50	–	–	3/100	–	7/100
All follicular cell neoplasms	M	3/100	3/100	12/203	5/101	–	17/75	–
	F	1/50	1/50	–	–	10/100	–	23/100*
Mammary gland, fibroadenomas and adenocarcinomas	F	7/46	4/50	–	–	21/94*	–	30/95*

Data from Friedman et al. (1995), as compiled by Rice (2005)

[a] Certain tumours that occurred at increased incidence in treated rats in the previous study (Johnson et al., 1986) were not reported as occurring at increased incidences in this study. These included papillomas of the oral cavity in females, adenomas of the clitoral gland and uterine adenocarcinomas. Numbers of these neoplasms were not given.

[b] Asterisk (*) indicates statistical significance, $P < 0.001$.

[c] Does not include seven rats with "malignant reticulosis" of the brain, including five dosed females, one dosed male and one control male.

[d] All brains of high-dose rats and all control brains (both subgroups) were examined, but only 82/102 and 90/102 control spinal cords and 51–75 high-dose spinal cords were examined.

[e] Only 98/204 brains and 68/204 spinal cords were examined.

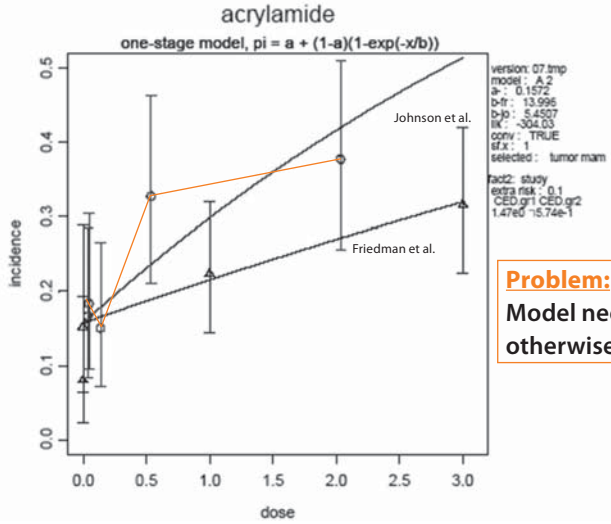
[f] Only 50/102 brains and 37/102 spinal cords were examined.

[g] All brains were examined, but only 45/50, 44/50, 21/100 and 90/100 spinal cords in control, control, low- and high-dose females, respectively, were examined. The study used two groups of control animals in an effort to increase the statistical power of the study and to obtain a better description of the dose–response curve.

[h] Includes three male rats and one female rat with multiple tumours in the highest dose groups.

64 JECFA BMDL-calculations mammary tumours

Figure 8. Incidences of total mammary tumours, with fitted one-stage model. Circles: Johnson et al. (1986); triangles: Friedman et al. (1995). Dose is expressed in mg/kg bw per day.



Problem: **Dose-response-curve: Model needs to be restricted, otherwise infinite slope at 0 !**

(JECFA 64, FAS 55, 2006)

Acrylamide: 64 JECFA BMDL-calculations

Table 17. Summary of the results of dose–response modelling for induction of selected tumours in rats given drinking-water containing acrylamide

Tumour	Study			
	Johnson et al. (1986)		Friedman et al. (1985)	
	Range of BMD (mg/kg bw per day)	Range of BMDL (mg/kg bw per day)	Range of BMD (mg/kg bw per day)	Range of BMDL (mg/kg bw per day)
Total mammary tumours	0.48–0.57	0.30–0.46	1.4–1.5	0.89–1.1
Peritesticular mesothelioma	0.97	0.63–0.97	NA	NA
Thyroid follicular adenoma	NA	NA	0.88–1.2	0.63–0.93
Central nervous system tumours of glial origin	1.9–2.0	1.3–1.6	NA	NA

BMD, benchmark dose for 10% extra risk of tumours; BMDL, 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls; NA, not applicable

(JECFA 64, FAS 55, 2006)

Acrylamide: 64 JECFA intake estimates: Summary

Major contributing foods to total human exposure: (most countries)

Potato chips (french fries)	16-30%
Potato crisps (chips)	6-46%
Coffee	13-39%
Pastry and sweets biscuits (cookies)	10-20%
Bread, rolls, toast	10-30%
Others	<10%

- Average **national intake** 0.3 - 2.0 µg/kg bw per day
- 90. - 97.5 percentile: 0.6 - 3.5 µg/kg bw per day
- 99. Percentile: up to 5.1 µg/kg bw per day
- children: about 2–3x higher than adults on bw basis
- **International average intake** 3.0–4.3 µg/kg bw per day (5 GEMS/Food regional diets, bw 60 kg).

JECFA: concluded that based on national estimates, an intake of acrylamide of 1 µg/kg bw per day could be taken to represent the average for the general population and that an intake of 4 µg/kg bw per day could be taken to represent consumers with a high intake. Children are also included in these estimates for average to high intake.

Acrylamide: resulting MOEs

- **MOE 200 and 50** for morphologic nerve changes (NOEL 0.2 mg/kg bw)
- **MOE 2000 and 500** for reproductive, developmental and other non-neoplastic effects (NOEL 2 mg/kg bw)

JECFA concluded that adverse effects were unlikely at the estimated average intakes, but that morphological changes in nerves could not be excluded for some individuals with a very high intake

- **MOE 300 and 75** for carcinogenic effects (breast tumours) (BMDL 0.3 mg/kg bw)
- **MOE 750 and 200 at intakes of 0.4 and 1.5 µg/kg bw**

JECFA considered **these MOEs to be low** for a compound that is genotoxic and carcinogenic and that **this may indicate a human health concern**.

Therefore, appropriate efforts to **reduce concentrations** of acrylamide in food and beverage should be continued.

EFSA- CONTAM Statement April 2005

- JECFA cautioned that there are **uncertainties** in its conclusion as the **toxicological database** is incomplete and recommended that:
- acrylamide be re-evaluated when results of ongoing **carcinogenicity** and long-term **neurotoxicity** studies become available.
- work should be continued on using **PBPK-modelling** to better link **human biomarker data** with exposure assessments and toxicological effects in experimental animals.
- appropriate efforts to **reduce acrylamide concentrations in food** should continue.

The CONTAM Panel noted the use of the MOE approach that incorporated data from European countries, including information gathered under collaborative initiatives between the Commission and EFSA. The Panel agrees with the principal conclusions and recommendations of the JECFA and concludes that at present an additional evaluation by EFSA is not necessary.

What-If Scenarios (M. Di Novi, US FDA)

Based on food consumption survey CSFII, 1994-96, 98, 2+ Population

	mean	90th Percentile
Estimated exposure	0.43	0.92 µg/kgbw-d
Remove AA from French Fries	→ 0.37	0.78 µg/kgbw-d
Remove AA from Snack Foods	→ 0.38	0.85 µg/kgbw-d
Remove AA from Breakfast Cereal	→ 0.38	0.84 µg/kgbw-d
Remove AA from Coffee	→ 0.40	0.88 µg/kgbw-d

http://www.jifsan.umd.edu/presentations/acry2004/acry_2004_dinovihoward.pdf

Risk-Benefit Considerations of Mitigation Measures on Acrylamide Content of Foods – A Case Study on Potatoes, Cereals and Coffee

C. J. Seal[1], A. de Mul[2], G. Eisenbrand[3], A. J. Haverkort[4], K. Franke[5], S. P. D. Lalljie[6], H. Mykkänen[7], E. Reimerdes[5], G. Scholz[8], V. Somoza[9], S. Tuijtelaars[10], M. van Boekel[11], J. van Klaveren[2], S. J. Wilcockson[1], L. Wilms[12]

Table 11. Exposure to acrylamide for different mitigation scenarios, based on labscale experiments

	Mitigation measure	Acrylamide reduction	Acrylamide exposure (µg/kg bw per day) P50	P95	P99
Original scenario			0·44	1·15	1·58
Mitigation scenarios					
Wheat bread	2 h yeast fermentation	80 %	0·40	1·07	1·52
Crisp bread	Asparaginase	80 %	0·43	1·12	1·55
Biscuits	Different measures	69 %	0·38	1·03	1·47
Ginger bread	Sugar → sucrose	90 %	0·41	1·09	1·51
Potato crisps	Combination of measures	50 %	0·41	1·03	1·40
Coffee	Storage	20 %*	0·42	1·12	1·57
Total		All scenarios	0·27	0·74	1·11

*It has to be stressed that reaction mechanisms leading to storage loss are not an option to date to reduce acrylamide concentration in coffee, since is it directly linked to quality and organoleptic properties and, consequently, consumer acceptability. However, for the purpose of this paper, an estimated degree of potentially achievable decrease is used in the modelling approach in order to assess the impact of acrylamide mitigation in coffee on human exposure and MOE.

The objectives of the Colloquium are:

- To discuss the **epidemiological evidence** relating acrylamide exposure to cancer risk in humans including discussions on uncertainties.
- To discuss the applications of **biomarkers** for acrylamide and PBPK models in relation to exposure, metabolism and elimination (toxicokinetics) and the mode of action (toxicodynamics) of acrylamide in experimental animals and humans.
- To discuss the state of the art on genotoxic and non-genotoxic **mechanisms for the carcinogenicity** of acrylamide including new *in vitro/in vivo* evidence in experimental animals and humans.
- To discuss the current knowledge on **dietary exposure** to acrylamide across Europe and to explore if there are possibly new potential food sources contributing to the dietary intake.

→ To explore whether the **additional information** that has become available since the 64 JECFA in 2005 in epidemiology, carcinogenicity and exposure would **call for a revision** of the previous risk assessment of acrylamide in food.

Dietary exposure to acrylamide and cancer risk:

a summary of recent epidemiological evidence

JENNY BARRETT
Leeds Institute of Molecular Medicine
University of Leeds, UK

Outline

- Concerns about acrylamide
  - Animal studies, occupational studies, acrylamide in diet
- Epidemiological studies of dietary exposure
  - Characteristics of studies to date
  - Main results by cancer site
- Summary and discussion points

Concerns about acrylamide

- Acrylamide (AA) was classified by IARC as a probable human carcinogen in 1994 mainly on the basis of animal studies
- Exposure to humans thought at that time to be mainly from occupational exposure and smoking
- In 2002 presence of AA was discovered in carbohydrate-rich food cooked at high temperatures
- JECFA monograph summarising evidence in 2005, but more epidemiological data since then

Occupational studies

- Sobel (1986)
  - 371 male US AA workers, updated and extended by Swaen (2007) to now include 696 employees with longer follow-up
  - More deaths than expected from pancreatic cancer (SMR 222), but not significant and not related to estimated AA exposure
- Collins (1994)
  - Mainly male workers from US and Dutch plants, updated by Marsh (2007); based on nearly 9000 workers
  - Potential overall excess risk of various cancers from earlier analysis now less strong
  - However non-significant raised SMRs (141 for pancreatic, 140 for rectal, 127 for renal cancer) when restricted to "exposed" workers

Epidemiological studies of diet

Study	Design	Dietary assessment	Cancer sites	Sample Size	References
Italian and Swiss hospital-based case-control studies 1991-2000	C-C	2 questions on fried/ baked potatoes and estimated daily AA intake from FFQ	Oral Oesophageal Laryngeal Colorectal Breast Ovarian Prostate Renal	749/1772 395/1066 527/1297 2280/4675 2900/3122 1031/2411 1294/1451 767/1534	Pelucchi (IJC, 2003, 2004, 2006, 2007)
Swedish	C-C	FFQ used to estimate daily AA intake, plus specific foods	Colorectal Bladder Renal	591/538 263/538 133/538	Mucci (BJC, 2003)
Swedish	C-C	FFQ used to estimate daily AA intake, plus specific foods	Renal	379/353	Mucci (IJC, 2004)
Swedish Women's Lifestyle and Health Cohort	Cohort	FFQ used to estimate daily AA intake, plus specific foods	Breast	667 40k cohort	Mucci (JAMA, 2005)
Swedish Mammography Cohort	Cohort	FFQ used to estimate daily AA intake, plus specific foods	Colorectal	741 60k cohort	Mucci (IJC, 2006)

More recent studies

Study	Design	Dietary measure	Cancer sites	Sample Size	References
Nurses' Mothers' Study	C-C	Childhood French fries from FFQ asked of mothers	Breast	582/1569	Michels (IJC, 2006)
Netherlands Cohort Study on Diet and Cancer	Case-cohort	FFQ used to estimate daily AA intake, plus specific foods	Endometrial Ovarian Breast Renal Bladder Prostate	221 195 1350 339 1210 2246 1.5 to 4k subcohort	Hogervorst (CEBP, 2007, AJ Clin Nutr 2008)
Danish Diet Cancer and Health Study	Case-cohort	Biomarkers AA	Breast	374/374	Olesen (IJC, 2008)

Breast cancer

Relative risk	Dietary measure	Design	Sample size	Reference
0.9 (0.8-1.1) >1/week vs 0	Consumption of fried/ baked potatoes	Case-control	2569/2588	Pelucchi, 2003
1.19 (0.91-1.55) highest vs lowest quintile	AA intake estimated from FFQ	Cohort	667/43404	Mucci, 2005
1.27 (1.12-1.44) per additional serving/ week	Pre-school 30-item FFQ obtained from mothers: French fries	Case-control	582/1569	Michels, 2006
1.06 (0.88-1.28) highest vs lowest quintile	AA intake estimated from FFQ	Case-control	2900/3122	Pelucchi, 2006
0.90 (0.73-1.13) highest vs lowest quintile	AA intake estimated from FFQ	Case-cohort	1350/1796	Hogervorst, 2007
1.5 (0.8-3.0) per 10-fold increase in adduct concentration	AA haemoglobin adduct levels	Nested case-control	374/374	Olesen, 2007

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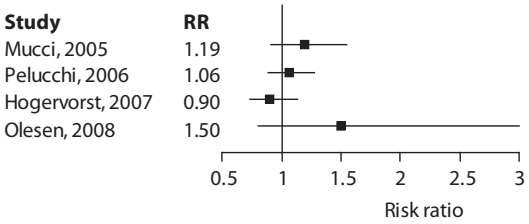
Breast cancer



Olesen observed an even stronger effect on risk for ER +ve breast cancer after adjusting for smoking RR 2.7 (1.1-6.6)

Ovarian and endometrial cancer

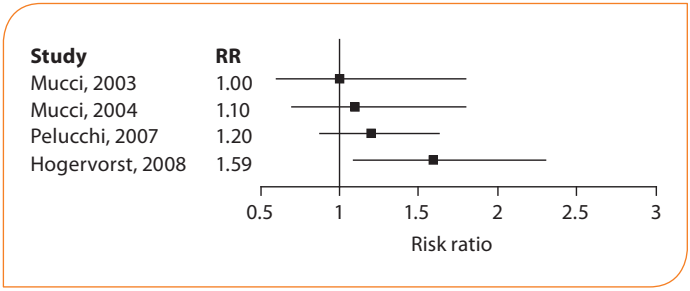
Relative risk	Dietary measure	Design	Sample size	Reference
Ovarian				
0.97(0.73-1.31) highest vs lowest quintile	AA intake estimated from FFQ	Case-control	1031/2411	Pelucchi, 2006
1.77 (1.11-2.82) highest vs lowest quintile	AA intake estimated from FFQ	Case-cohort	195/1778	Hogervorst, 2007
Endometrial				
1.17 (0.76-1.79) highest vs lowest quintile	AA intake estimated from FFQ	Case-cohort	221/1481	Hogervorst, 2007

Hogervorst observed stronger effect for endometrial cancer when restricting analysis to non-smokers: RR 1.99 (1.12-3.52) after adjustment for other covariates

Renal cancer

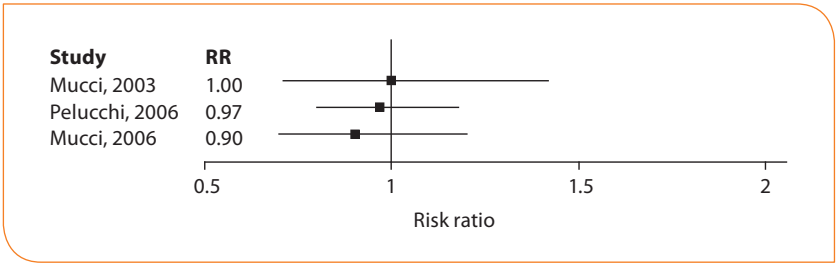
Relative risk	Dietary measure	Design	Sample size	Reference
1.0 (0.6-1.8) highest vs lowest quartile	AA intake estimated from FFQ	Case-control	133/538	Mucci, 2003
1.1(0.7-1.8) highest vs lowest quartile	AA intake estimated from FFQ	Case-control	379/353	Mucci 2004
1.20 (0.88-1.63) highest vs lowest quartile	AA intake estimated from FFQ	Case-control	767/1534	Pelucchi 2007
1.59 (1.09-2.30) highest vs lowest quintile	AA intake estimated from FFQ	Case-cohort	339/4095	Hogervorst 2008

Renal cancer estimates



Colorectal cancer

Relative risk	Dietary measure	Design	Sample size	Reference
1.0 (0.71-1.42) highest vs lowest quartile	AA intake estimated from FFQ	Case-control	591/538	Mucci, 2003
0.97 (0.80-1.18) highest vs lowest quintile	AA intake estimated from FFQ	Case-control	2280/4675	Pelucchi, 2006
0.9 (0.7-1.2) highest vs lowest quintile	AA intake estimated from FFQ	Cohort	741/60k	Mucci, 2006



Discussion points from studies

- How well is dietary intake of AA measured?
- Study design
  - Retrospective vs prospective studies
  - Could anything be gained by pooling data?
- Sample size and power
- Adjustment for confounders (especially smoking)

Measures of exposure: FFQ

- Apart from some early studies that only examined specific food items, all but one study (Olesen, 2008) estimate AA intake by applying estimates of average AA content of food items to FFQ responses on key foods
- Two potential sources of uncertainty and heterogeneity:
  - FFQ as measure of usual diet
  - Conversion of FFQ data into AA intake. (How much) has this improved over time?
- Additionally it is uncertain what is the most relevant exposure (total lifetime exposure, early exposure?)

Measures of exposure: biomarkers

- Several studies have used biomarkers (AA adduct levels in blood) to assess validity of estimates based on reported diet
- Olesen (2008) used these biomarkers (and glycidamide adduct levels) as measures of exposure in study of breast cancer
- Biomarkers provide a more direct measure, but
  - Inevitable small sample size
  - Only a measure of short-term exposure
  - Cannot distinguish between sources (e.g diet vs smoking)

Study design

- Two categories of design
  - Case-control studies where diet measured retrospectively
  - Cohort based studies (cohort, case-cohort, nested case-control) where diet measured prospectively
- Cohort studies avoid recall bias, but generally at expense of sample size
- Pooling data would increase sample size/power but studies are probably too heterogeneous

Sample size and power

- Some studies have – on the face of it – good sample sizes (>1000 cases and controls)
- However what effect size are we expecting to see?
- Few studies conducted to date can rule out ~ 20% increase in risk from high to low exposure
- It may be unlikely risk is greater than this (extrapolating from animal studies and based on epidemiological evidence to date), but this size of effect would represent an important public health issue

Adjustment for confounders

- Smoking is a major potential confounder since it is a risk factor for numerous cancers and also a major source of AA exposure. This is a particular issue when using biomarkers
- Some studies have stratified by smoking status and found differences in results. Some studies are too small for this or have incomplete smoking data
- Should future studies by matched/stratified for smoking status?
- What about other potential confounders?

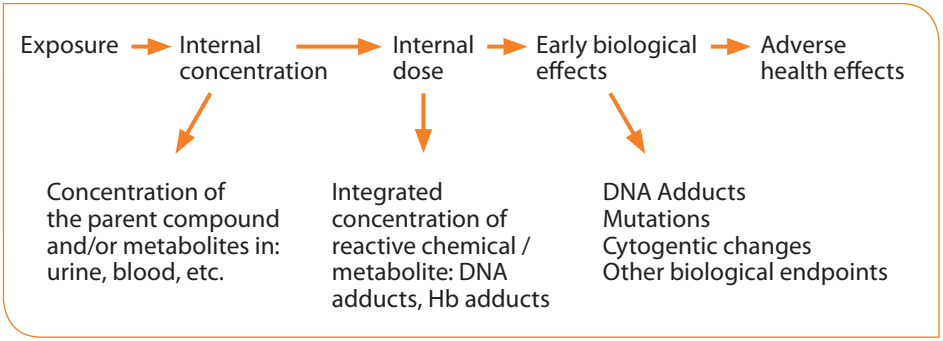
Summary

- The epidemiological evidence to date probably rules out a very strong effect on risk of most cancers from dietary intake
- Nonetheless the evidence is certainly consistent with an important increase in risk in public health terms, for some cancers especially
- Need to come up with creative study designs, perhaps combining FFQ with biomarker data, so preserving advantages of sample size, and adjusting adequately for confounders
- It may take some time to accumulate sufficient evidence to rule AA out or in as a cancer risk factor

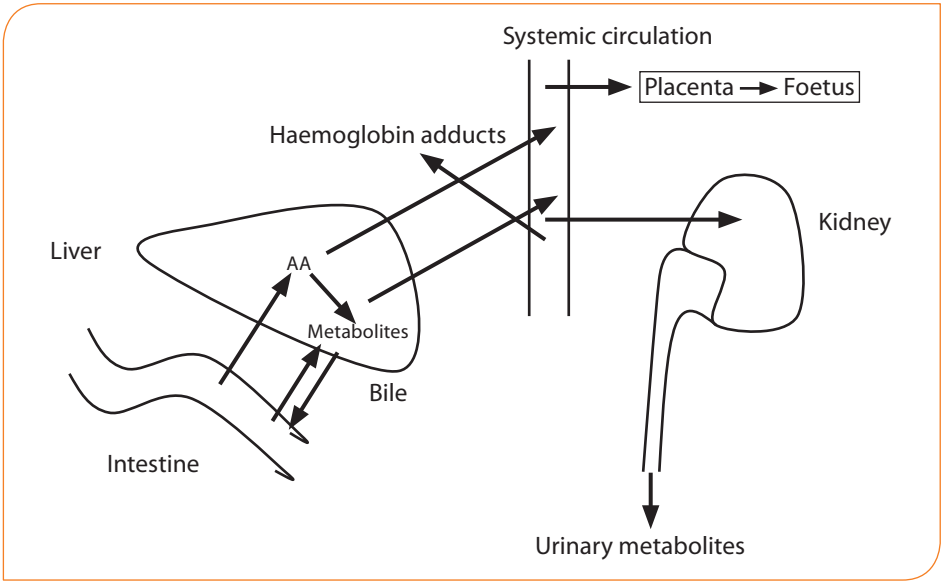
Biomarkers of acrylamide

JAN ALEXANDER
Department of Food Safety and Nutrition
Norwegian Institute of Public Health

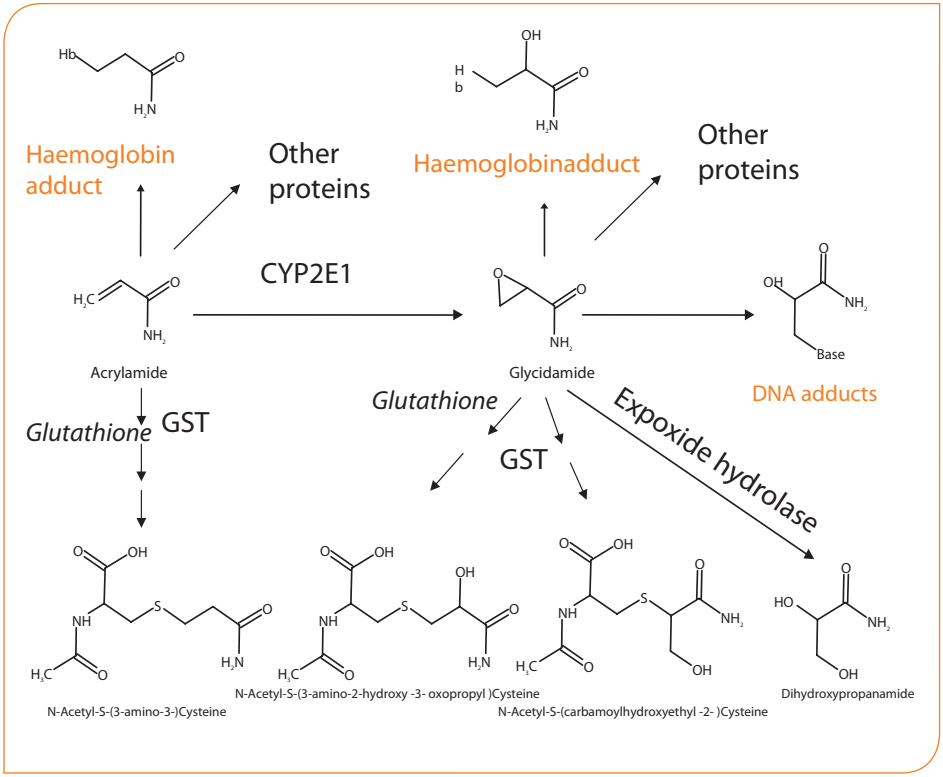
Scheme of biomarkers



Absorption, distribution, metabolism and excretion



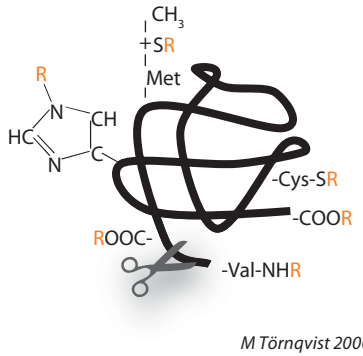
Biotransformation



Acrylamide – chemical biomarkers

- AA and GA are both reactive compounds, T1/2: 4.6 and 1.9 hrs in humans
- Urinary mercapturic acid, T1/2 ~ 3.5 hrs
- DNA adduct, major T1/2 ~ 4 days
- Hb-adducts – stable, life time that of red blood cells,
  - ~ 120 days humans,
  - rat 60 days, mouse 40 days

N-terminal valine adduct determined



M Törnqvist 2006

Transplacental transfer

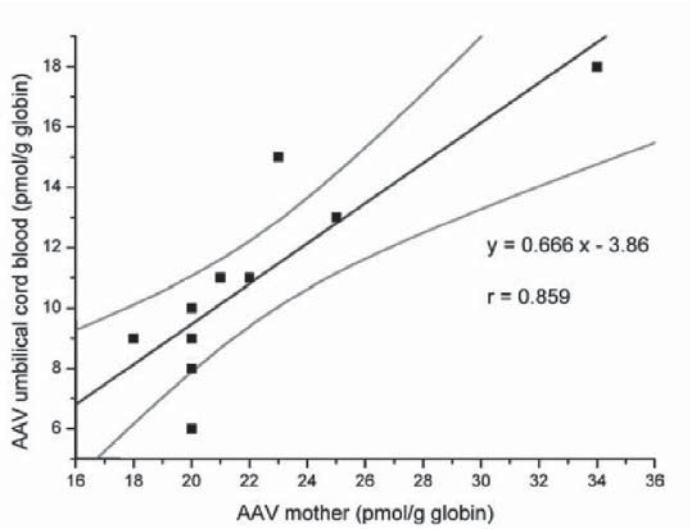


Fig. 1 Correlation between haemoglobin adducts of acrylamide (AAV) in mothers' blood and umbilical cord blood. Note that the results for the smoking woman (person no. 1) have been excluded in this correlation

Schettgen et al 2004

Determinants of biomarkers

- External exposure
- Modifiers:
  - Other sources of exposure than food, *e.g.* smoking
  - Metabolic capacity of enzymes
    - Developmental stage
      - newborn *vs.* children *vs.* adults
    - Genetic – polymorphisms
      - GST enzymes, Cyp 2E1, EPxH
    - Inducers, ketones, ethanol
    - Inhibitors, ethanol

Purpose of using biomarker

- Validate external dose estimate
- Determine food source
- Determine bioavailability
- Assess conversion rate from AA to GA
- Compare dose parameters with early biomarkers of effect
- Assess internal dose for use in epidemiological studies
- Extrapolation between species in risk assessment

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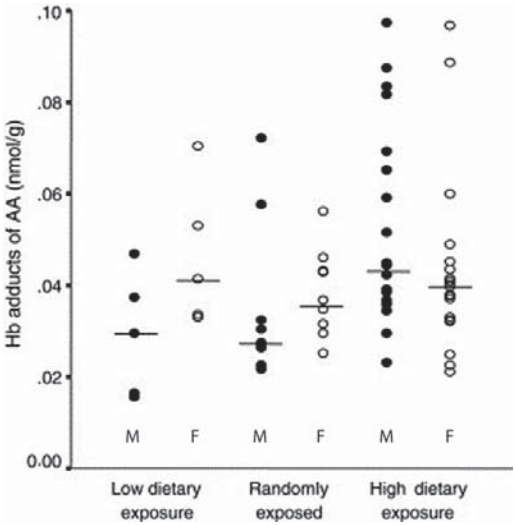
Biomarkers of acrylamide

- Haemoglobin adducts of AA and GA
- Urinary metabolites
  - Mercapturic acids
- DNA adducts
  - Only used in animal studies
- Chromosomal abberations
  - Studies on workers exposed to acrylamide
- Micronucleus assay,
  - Studies following dietary exposure
- Sister chromatide exchange and mutations
  - Only used in *in vitro* or animal studies

External exposure vs. biomarkers

Intake estimates by food frequency questionnaire vs haemoglobin adducts

Hagmar et al. 2005



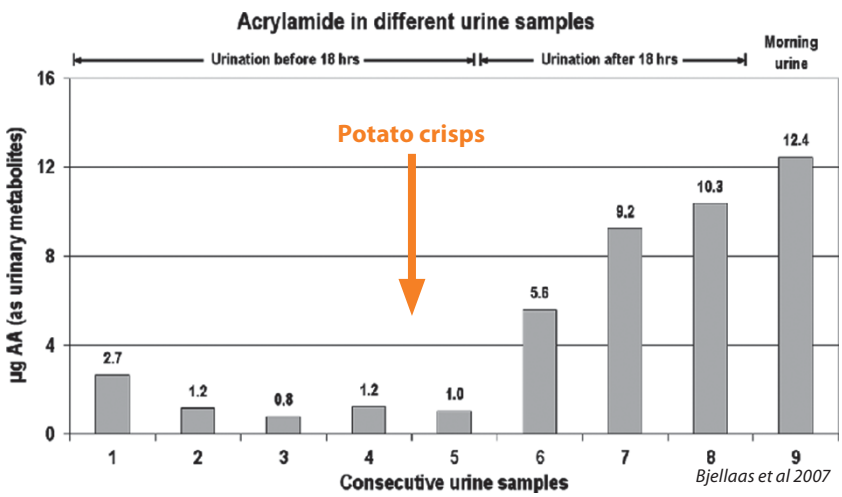
External exposure vs. biomarkers

- Bjellaas et al 2007: Hb adducts did not correlate with estimated dietary intake, (n=50)
- Wirfält et al 2008: Hb adducts: Strong correlation with smoking, weaker correlation with estimated dietary acrylamide in non-smokers (n=40)
- Kütting et al 2008: Hb adducts: Strong correlation with smoking, weak but significant correlation with estimated dietary exposure in non-smokers, r_{sp} = 0.178 and 0.168 in females and males, respectively (n=828)
- Non- or weak correlations between dietary intake estimates and AA-Hb adducts. Correlation dependent on accuracy of intake estimate.
- Strong influence of smoking

External exposure at population level fits well with biomarkers:
Background Acrylamide Hb adducts:
0.01 – 0.05 nmole/g, average: 0.03 nmole/g
Corresponds to an intake of about:
80 µg/ day or 1.1 µg/kg bw/day

Taken from Törnqvist

External exposure and urinary biomarkers



Bjellaas et al 2007

- In a study of 47 non-smoking persons estimated acylamide intake did not correlate with urinary biomarkers.
  - 24 hrs AA derived urinary metabolites: 16 (7-47) µg
  - dietary intake 24 hr recall: 21 (13-178) µg

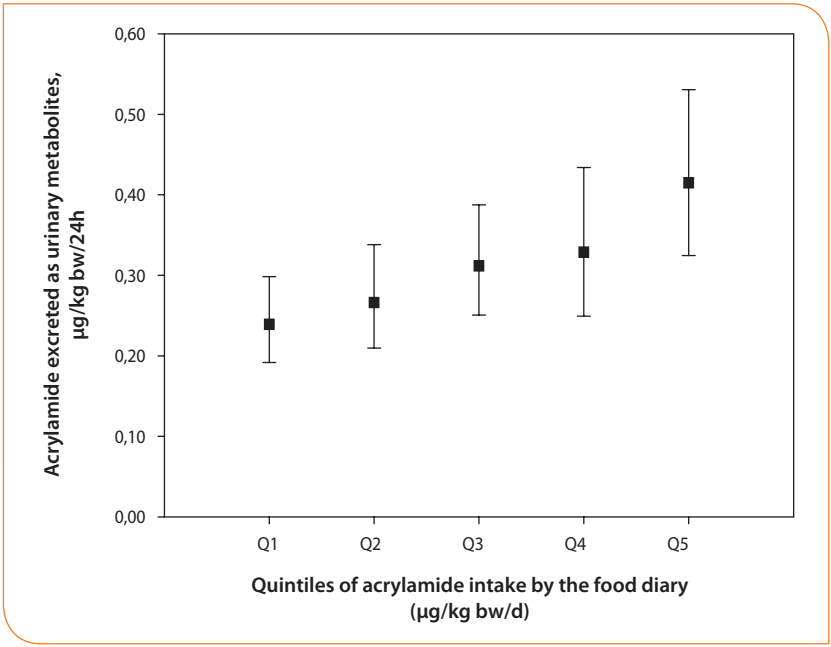
Bjellaas et al 2007

- In a study of 119 pregnant non-smoking women the dietary median and (95 perc.) intake of acrylamide was:
  - 0.48 (0.92) µg/kg bw per day as estimated by FFQ
  - 0.41 (0.82) µg/kg bw per day as estimated by Food diary
  - 0.42 (0.70) µg/kg bw per day as estimated by probabilistic approach
- total 24 hrs AA-derived urinary metabolites was:
  - 0.16 (0.50) µg/kg bw per day
  - Assuming 55 % recovery: 0.30 (0.91) µg/kg bw per day
    Significant correlation between biomarker and estimated dietary intake

Brantsæter et al. in press

- **Reason for discrepancy: New data on acrylamide in Norwegian food items in last study? In Bjellaas et al 2007, For some food items EU acrylamide date for food used. Better basis for more accurate intake estimates improves the correlation.**

Ranking of dietary intake vs. urine biomarkers

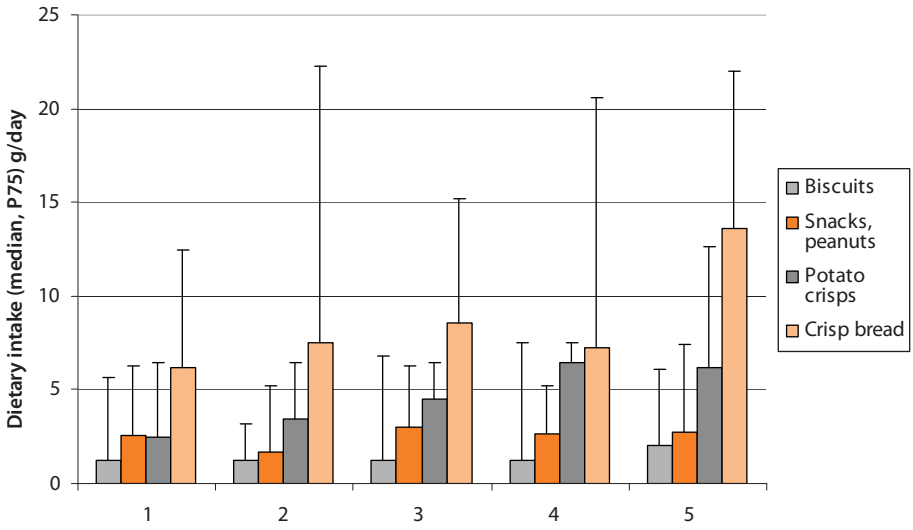


Classification into quintiles:

- 65 % classified in the same or adjacent quintile
- less than 10 % classified into opposing quintile

Brantsæter et al. in press

Contributing foods in various quintiles of urinary biomarker levels



Brantsæter et al. in press

Urinary biomarkers

Urinary biomarkers can be used for validation of dietary intake estimate (FFQ, FD Probabalistic) and ranking of of persons with regard to exposure

Brantsæter et al in press

Disadvantage with urinary biomarkers:

- Short term exposure only
- Not all major metabolites (*e.g.* glyceramide) determined

Foods contributing to Hb adducts or urinary biomarkers in Norway

- AA-Hb adducts: crisp bread, potato chips/snacks
- No correlations with GA-Hb-adducts (n=50)

Bjellaas et al 2007

- Total AA-derived urinary metabolites correlated with intake of aspartic acid, protein, starch and coffee (n=53)

Bjellaas et al 2007

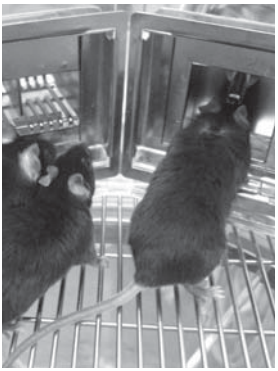
- Total AA-derived urinary metabolites correlated with intake of crisp bread, potato crisps, cooking oil and garlic. (n= 119 pregnant women)

Bratsæter et al, in press

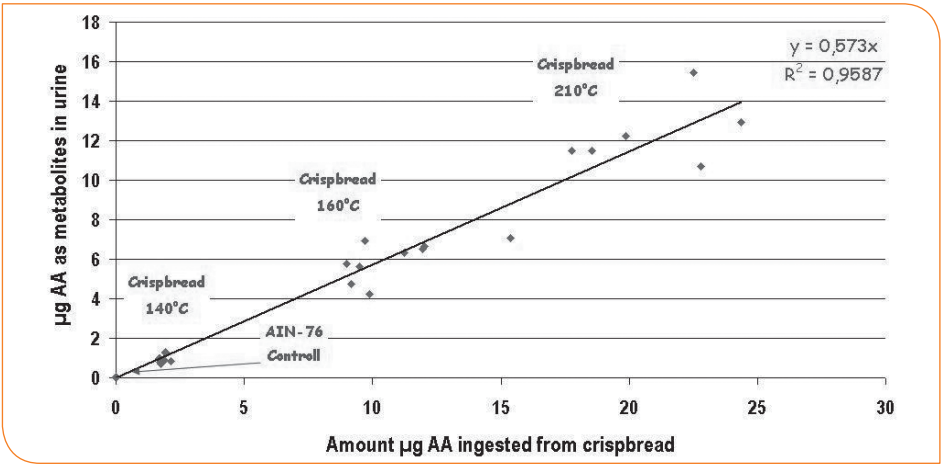
Biomarkers and bioavailability

Bioavailability of crisp bread in mice

- Crisp bread with different content of AA
  - 191 µg/kg,1020 µg/kg, 2650 µg/kg
- Steady-state feeding: 24 h intake crisp bread (4x)
- Steady-state excretion: 24 h urinary metabolite



Intake versus excretion



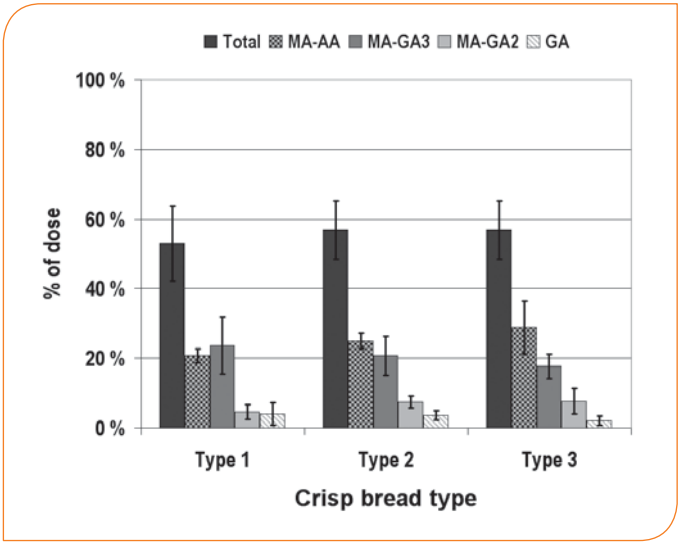
Recovery of urinary AA metabolites from crisp bread feeding = 55%

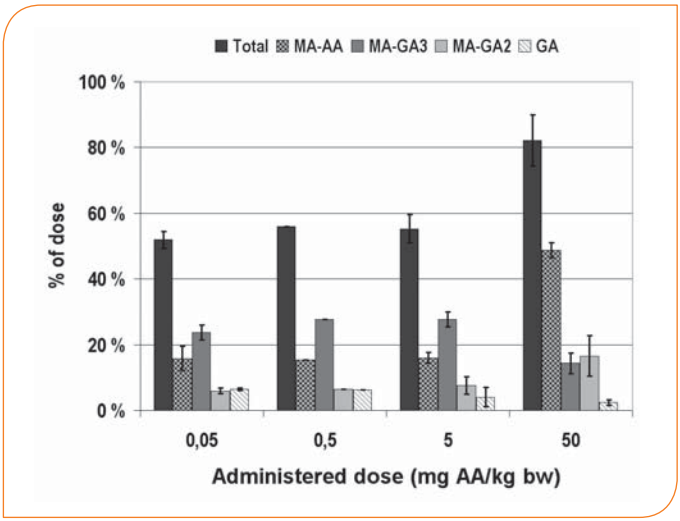
Recovery of urinary AA metabolites from sc AA injection (0.05 – 5 mg/kg bw) = 54%

Bioavailability ~ 100%

Bjellaas et al 2007

Recovery of AA as urine metabolites





Enzymic polymorphism

- Hb adduct levels were studied *in vitro* with fresh blood from *GSTT1-/+* and *GSTM1-/+* donors followed by incubation with acrylamide.
- No effect of genotype or GST/EPXH inhibitor (effect with pos. control: ethylene oxide)

Paulsson et al 2005

- Tendency of influence on Hb adduct level of *GSTT1-/+* and *GSTM1-/+* status of tunnel workers exposed to acrylamide

Kjuus et al 2005

Internal dose and epidemiology

- Significant positive association between AA-Hb adduct level and oestrogen receptor positive breast cancer after adjustment for possible confounders
  - RR: 2.7 (CI 1.1-6.6) per 10 fold increase in adduct level.
- Danish Diet, Cancer and Health Study: case control study, cases 374, controls 374

Olesen PT et al 2008)

Intake of food containing high level of acrylamide

- In a study from Sweden individuals were assigned to two groups
  - group 1: eating food, which were low in acrylamide
  - group 2: eating the same food as group 1, but fried,
- in addition potato crisps
- The eating were done for 4 days
- The acrylamide intake was about 15 times higher in group 2.
- Micronuclei (MN) were determined before and after.
  - The change was significantly higher in group 2
  - Incrased acrylamide in group 2 was confirmed by AA-Hb adducts
- But, according to author, compared with data on acrylamide doses inducing MN in mice, it is unlikely that the particular amount of acrylamide could have caused this increase

Zetterberg Abramsson et al, in press

Conclusion

- A lot of progress has taken place in the use of biomarkers for various purposes in studies on acrylamide
- Biomarkers can probably be very useful in various aspects of epidemiological studies
  - particularly as internal dose parameter
  - Validation of dietary intake calculations
  - For dietary acrylamide and cancer studies smoking is a confounder – influences biomarker level and influences outcomes because of high content of other carcinogens
- The use of biomarkers for extrapolation of risk from animals to humans is still limited as:
  - studies in animals of adverse outcomes have not used biomarkers
  - studies with early endpoints are still limited
  - PBPK modelling could facilitate the inter species comparison
- Analytical quality should be assured

Genotoxic and non-genotoxic mechanisms for acrylamide carcinogenicity

DANIEL R. DOERGE
National Center for Toxicological Research
U.S. Food and Drug Administration
Jefferson, AR 72079

Outline

- History
- Summaries of evidence for:
  genotoxicity, pre- and post-2005;
  endocrine disruption, post-2005;
  in vitro effects post-2005.
- Carcinogenic mechanism for acrylamide
- Chronic NCTR/NTP cancer bioassays
- PBPK modeling & extrapolation to dietary risks
- Conclusions & remaining issues

History of acrylamide research

- Occupational exposures – peripheral neuropathy
- Discovery of ppm levels of AA in many baked and fried starchy foods (2002 M. Törnqvist et al./Swedish Food Authority)
- Two 2 y carcinogenicity bioassays for AA in F344 rats
- Short-term studies in mice
- 1993 – EPA; 1994 – IARC; NTP – 2002; JECFA - 2005: probably carcinogenic to humans
- 2003 FDA priority nomination to NTP – mechanistic studies and chronic bioassays (cancer - AA and GA in mice and rats; neurotoxicity - rats)

Evidence for GA as the Genotoxic Metabolite of AA (pre-2005)

- Structural similarity to ethylene oxide, glycidol
- GA reactivity with DNA bases >> AA
- GA-DNA adducts (N7-Gua & N3-Ade) in all tissues tested
- DNA adducts accumulate with repeated dosing
- GA more mutagenic than AA *in vitro* (*Salmonella*, Big Blue mouse embryonic fibroblasts - *cII* mutations predominately G:C → T:A transversions
- Overlapping F344 rat tumor sites from ethylene oxide, glycidol, acrylonitrile & AA (CNS, peri-testicular mesothelium, thyroid, mammary)
- N-Methylolacrylamide carcinogenic in $B6C3F_1$ mice (not F344 rats)
- CNS tumors only from DNA-damaging agents

Evidence for GA as the Genotoxic Metabolite of AA in vivo (2005-present)

- GA causes DNA adducts, micronucleus & DNA damage (comet assay), germ cell mutations & dominant lethality in *wt*, but not CYP2E1 ko mice (2005)
- AA is a genotoxic mutagen for adult Big Blue mice via metabolism to GA (↑ MF at *Hprt* (spleen) & cII (liver) – major mutation G:C → T:A transversions) (2006)
- GA is a genotoxic mutagen (↑ MF at Tk and Hprt - spleen) in neonatal $Tk^{+/-}$ mice (2008)
- GA is a genotoxic carcinogen in liver from neonatal mice (2008)

Proposed Endocrine Mechanisms for AA Carcinogenesis in F344 Rats

Type of Cancer & Gender Affected	Hormonal Aberration Contributing to Increase
Thyroid follicular adenoma/ adenocarcinoma (M/F)	Prolonged TSH stimulus DA dysregulation
Mesothelioma – tunica vaginalis (scrotum)	Prolonged stimulation by PRL, LH, FSH DA dysregulation
Fibroadenoma/carcinoma mammary gland (F)*	Prolonged stimulation by progesterone, PRL (F) DA dysregulation
CNS tumors (M/F)	Not Discussed

Shipp et al., Crit. Rev. Toxicol. 36, 481 (2006)

The Effects of Sub-chronic Acrylamide Exposure on Gene Expression, Neurochemistry, Hormones, and Histopathology in the Hypothalamus-pituitary-thyroid Axis of Male Fischer 344 Rats

Toxicol. Appl. Pharmacol. (2008)
Bowyer, J.F. et al.

Neuroendocrine mechanisms for aa carcinogenicity in F344 rats

- AA in drinking water : 0, 2.5, 10, 50 mg/kg in male F344 rats
- 2.5 mg/kg = cancer bioassay dose; 10 mg/kg intermediate;
- 50 mg/kg neurotoxic but not lethal
- 14 day exposure (minimize accommodation)
- Eliminate estrus cycle considerations (males only)
- Brain neurotransmitters (dopamine & 5HT) and metabolites (LC/MS/MS)
- Serum PRL, FSH, LH, TSH, T3/T4, sex steroids
- Genomic Analysis-Macroarrays (ca. 2,400 cDNA probes): expression of hormone releasing factors; neurotransmitter receptors in hypothalamus, striatum, pituitary; thyroid;
- oxidative stress; apoptosis; cell proliferation
- Histology – thyroid, pituitary, brain, testes; cell proliferation

Hormonal Mechanisms for AA Carcinogenesis - Summary

- F344 rat -14 d study : ≤ 50 mg/kg bw/d => steady state [AA, GA] ≤ 25 µM
- Some evidence for hormonal changes from direct effects on thyroid and testes
- No evidence for mRNA changes in HPT-related genes; oxidative stress; etc.
- No evidence for increased cell proliferation in target tissues of male F344 rat
- No evidence for disruption of HPT axis by demonstrably toxic doses of AA

Effects of AA in vitro (2005-present)

- Oxidative stress through GSH depletion only at [AA, GA] ~ mM levels (2005-7)
- Effects on kinesin-related microtubular proteins (mitotic spindle) at [AA, GA] ≥ 100 µM (2007)
- Effects on rat thyroid cells – comet assay at [AA, GA] ≥ 10 µM (2006)
- Effects on human lymphocytes – comet assay at [AA] ≥ 0.5 µM (2006)
- mRNA expression – MCF7, CaCo-2 cells; minor changes in fold change ≤10 µM AA or GA (2007)
- Cancer bioassay doses
  F344 rat: ≤ 3 mg/kg bw/d => [AA, GA] ≤ 2 µM

Effects of Acrylamide in vitro - Summary

- No evidence for oxidative stress effects at AA concentrations relevant to cancer bioassay doses
- No evidence for effects on microtubular-associated proteins at [AA or GA] relevant to cancer bioassays (Neurotoxicity?)
- Consistent evidence for DNA damage in target tissues (comet assay) at [AA] relevant to cancer bioassay
- Minimal evidence for mRNA expression changes at [AA or GA] relevant to cancer bioassays

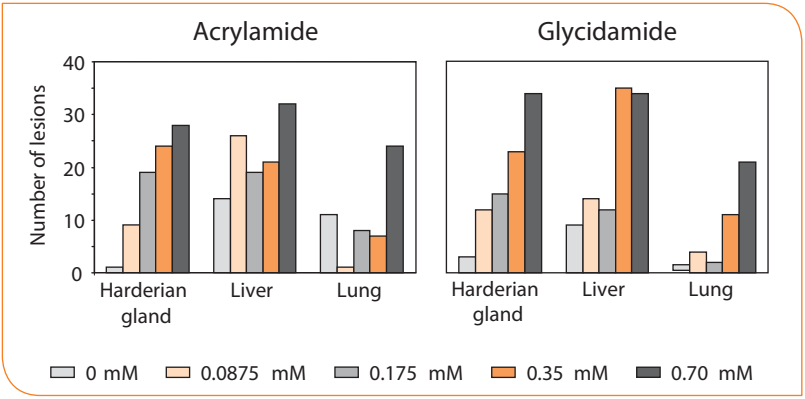
Chronic carcinogenicity study B6C3F$_1$ mice – water (f. Beland)

Test agent	Sex	Dosed water (mM)	Target daily intake (mg/kg bw)
AA & GA	Male & female	0.70 0.35 0.175 0.0875 0	14 7 3.5 1.75 0

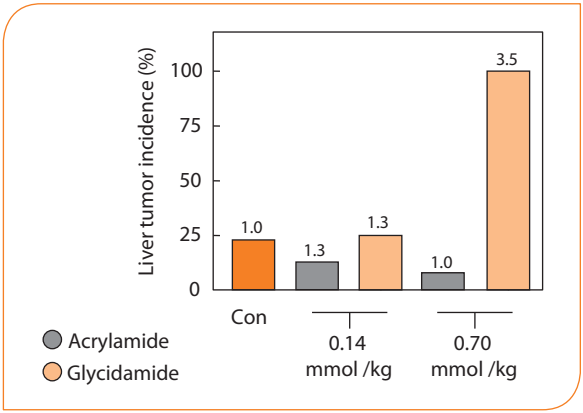
Chronic carcinogenicity study F344 rats – water (f. Beland)

Test agent	Sex	Dosed water (mM)	Target daily intake (mg/kg bw)
AA & GA	Male & female	0.70 0.35 0.175 0.0875 0	5 2.5 1.25 0.625 0

Preliminary gross pathology: lesions in male B6C3F$_1$ mice treated chronically



Lesions in male B6C3F$_1$ mice treated neonatally



NCTR carcinogenicity bioassays AA & GA : projected schedule

- In-life complete 8-07
- Pathology complete 07-08
- PWG 01-09
- Tumor data available on NTP website
- Statistics & QA complete 03-09
- BSI report preparation 08-09
- NTP Technical Report Subcommittee 11-09

Carcinogenic Mechanism(s) for Acrylamide

- A compelling body of evidence for a DNA-reactive mechanism for AA carcinogenicity via metabolism to GA
- Reproducibly carcinogenic at multiple anatomic sites in both rats and mice
- Tumor sites in mice are indicative of small epoxide carcinogens
- The only agents known to induce tumors of the CNS and peritesticular mesothelium in rats are all DNA-reactive (mutagenic)
- The hormonal disruption & oxidative stress mechanisms proposed for AA as tissue-specific alternatives to a DNA-reactive mechanism are highly speculative, unsupported in vivo
- Other mechanisms possible at higher doses; likelihood decreases as level of exposure decreases => less relevant for risk assessment of human dietary exposures

PBPK/PD Model for Acrylamide and Its Metabolites In Mice, Rats, and Humans

***Chem. Res. Toxicol.* (Mar. 2007)**
J.F. Young, R.H. Luecke, D.R. Doerge

Prediction of steady state human dna adducts from dietary exposures using pbpk/pd modeling

- PBPK/PD (urinary metabolites & Hb adducts from non-smokers – 0.4 µg/kg bw/d) 0.26 N7-GA-Gua/108 nucleotides
- Empirical relationship between rodent GA-Hb and DNA adducts (diet and 1 mg/kg bw/d) 0.2-0.3 N7-GA-Gua/108 nucleotides
- Range of estimated human cancer risks from dietary AA using rat tumor data
  1×10^{-4} (F thyroid) to 4×10^{-4} (F mammary)

Conclusions

- Hypothesis: AA = genotoxic carcinogen via metabolism to GA
- Tumor incidences from rodent bioassays ("external" dose)
- GA-Hb adduct measurements in humans exposed through diet only ("internal" dose)
- PBPK predictions of steady state DNA adduct levels in human tissues ("effective" dose) - cancer risk assessment

Remaining questions

- What to do about a genotoxic carcinogen that is pervasive throughout the diet?
- Significant proportion of total caloric content of global agriculture in cereals and tubers
- Diet-Cancer linkage robust
- Sufficiently powered epidemiological studies to relate AA in diet with cancers at specific sites unlikely?
- What about exposure to other known cooking carcinogens? (B[a]P (4 ng/kg bw/d), HAAs (15), furan (300), AA (400-1000)
- Unknown compounds?
- Holistic risk assessment for all "cooking carcinogens"?

Acrylamide Level Monitoring Database

Overview of 5 years of data collection in Europe

THOMAS WENZL
IRMM -Institute for Reference Materials and Measurements
Geel -Belgium

Overview of data

Number of data submitted to data base: ~9373

- Within last 12 months: ~100
- Majority of data came from Germany (~6270)

Rest from official bodies of Austria, Belgium, Finland, Greece, Ireland, Italy, The Netherlands, Spain, UK and the food and beverage industry (represented by CIAA)

Data assessment

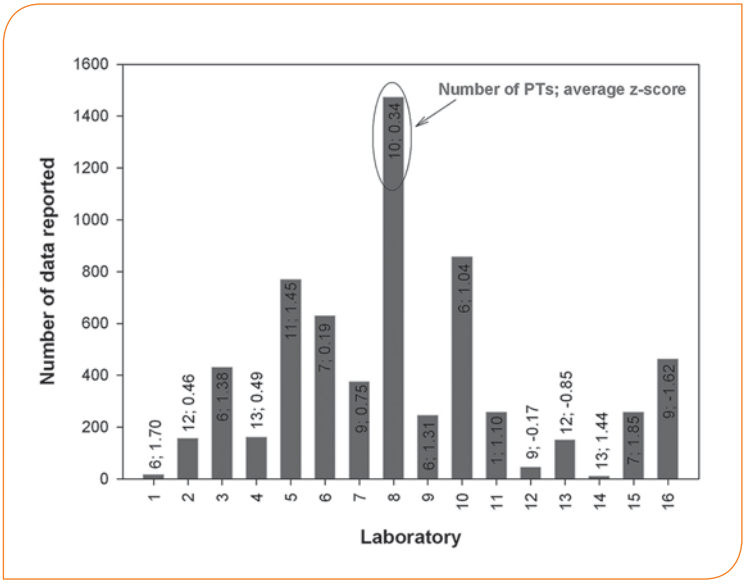
Criteria for data exclusion:

1. AA content (numeric value) below the reported LOQ.
2. AA content "below LOQ", with an LOQ ≥ 100 µg/kg.
3. LOQ was not reported.
4. Values for LOD and LOQ were identical.
5. LOQ was missing.
6. Summary z-score (SZ) was equal or greater than |2|.
7. Value greater than the LOQ, but not exactly specified (*e.g.* < 100 µg/kg with a LOQ of 30 µg/kg)

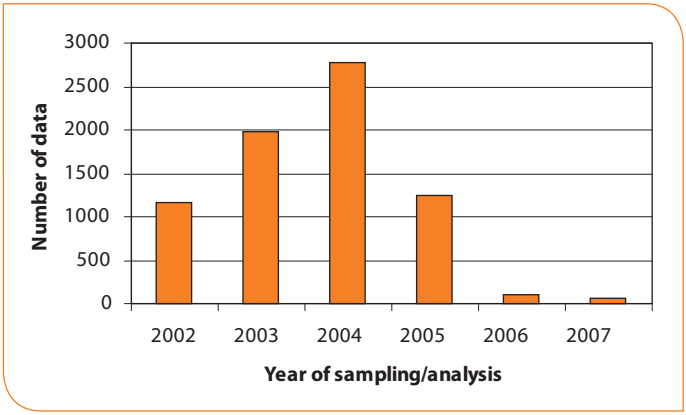
Overview on data suppliers

Number of laboratories reporting data: **34**

Number of data supplied as well as participation and performance of data supplier in method performance tests



All data reported – independent of food commodity



Results of data assessment

- Number of data that passed assessment: **7357**
- Number of data from laboratories with satisfactory performance in PTs (reported to JRC): **6300**
- Good representation of important food matrices (potato crisps, potato chips, crispbread, breakfast cereals, fine bakery ware (different biscuits), coffee)

Recent PT results

What can be expected now?

- FAPAS 3015 (2006): AA in crispbread; 1179 µg/kg;
  49 participants; 88 % satisfactory performance
- JRC PT 4 (2007): Acrylamide in potato crisps:
  344 µg/kg; 36 participants; 67 % satisfactory performance
- FAPAS 3019 (2008): AA in biscuits: 1256 µg/kg; 37 participants; 95% satisfactory

(list is not exhaustive)

Data evaluation

- Characteristics of data distribution determined
  (min, max, quartiles, median)
- Plot of acrylamide (AA) levels against production/ expiry date of product
  - Production/expiry date information was not reported for all samples

Content of EU database

Potato products

	Potato chips (French fries)	Potato crisps (Potato chips)	Potato pancake
Total number	1399	836	121
	µg/kg	µg/kg	µg/kg
Min	5	5	10
25%	85	315	172
Median	186	532	352
75%	362	933	713
95%	868	1800	2067
Max	4653	4215	3072

Bakery ware

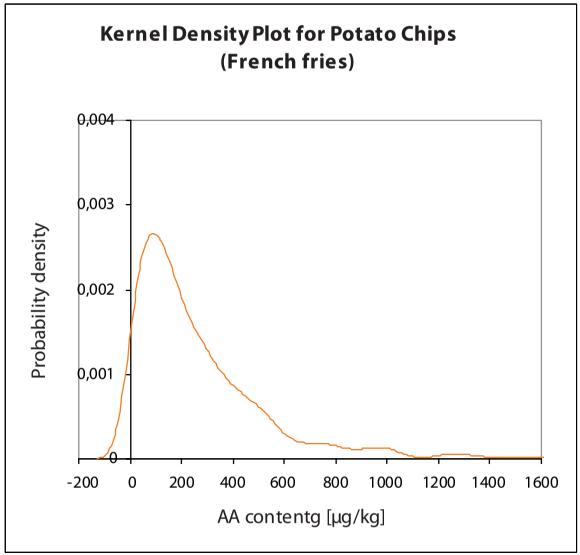
	Biscuits	Crispbread	Gingerbread	Bread	Other bakery ware
Total number	899	413	1009	192	192
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
Min	5	5	5	5	5
25%	67,5	79,5	136,7	10	15
Median	165	244	301	30	40
75%	381	507	669	110	163
95%	920	1389	1805	322	740
Max	3324	2838	7834	1987	1300

Other food products

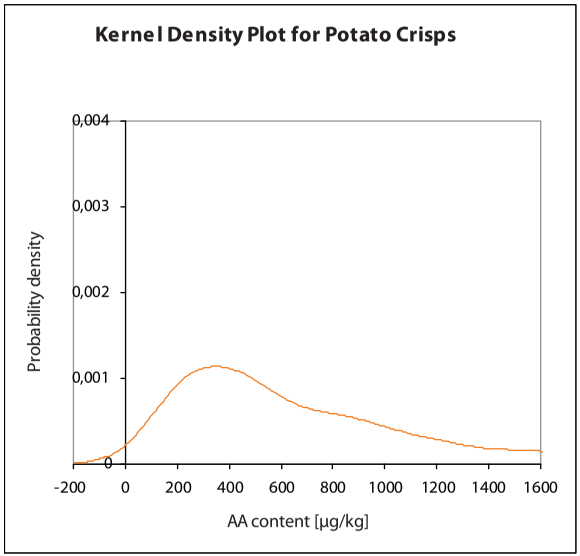
	Cereals	Muesli	Coffee	Coffee substitutes	Infant food	Food for diabetics
Total number	269	69	235	108	275	402
	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg	µg/kg
Min	5	5	79	116	5	5
25%	30	10	221	497	35	102
Median	70	30	286	773	79	230
75%	150	88	373	1226	183	620
	355	209	574	2325	384	1752
Max	1649	946	975	2955	910	3044

Frequency distributions

Variability of AA contents



235 samples



836 samples

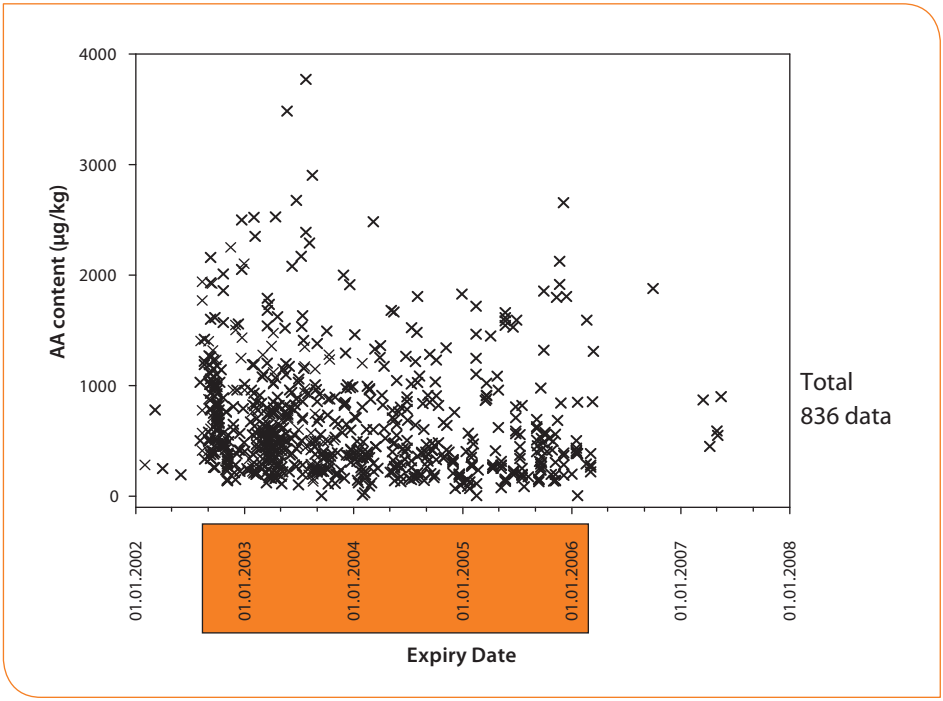
Graphical presentation of data

- Food categories with sufficient number of data with given production/expiry date information selected
- Contents reported as below LOD resp. LOQ replaced by LOD/2 resp. LOQ/2
- Information on production/expiry date available only for a fraction of data
- Inhomogeneous data distributions with respect to production/expiry date found

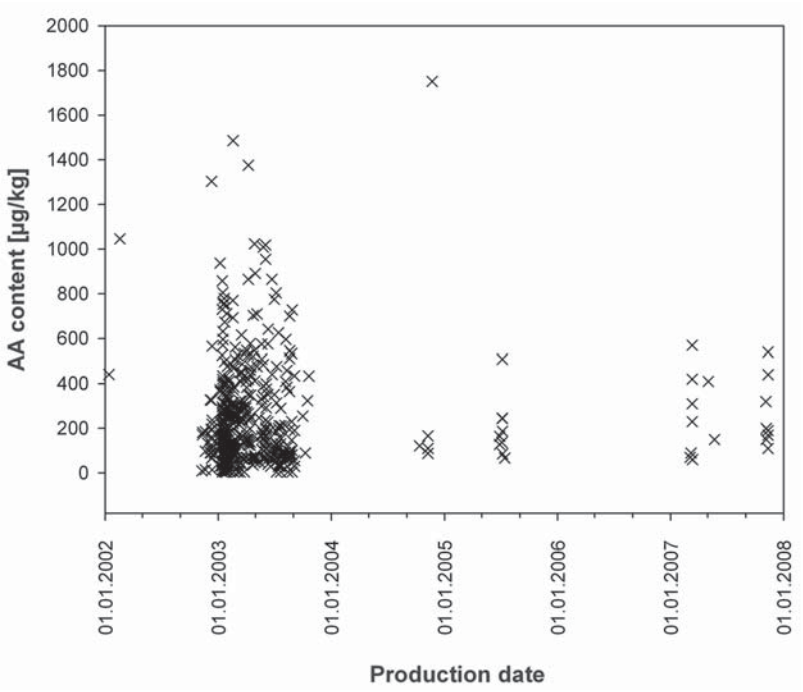
Time dependency of AA content

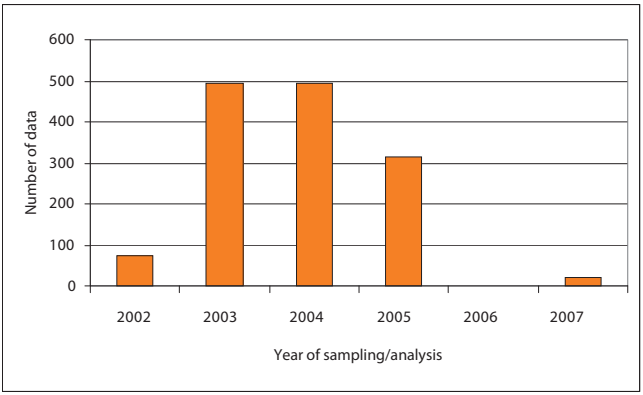
Potato crisps: 743 data

~3 yearsof observation



Potato chips (French fries): 479 data

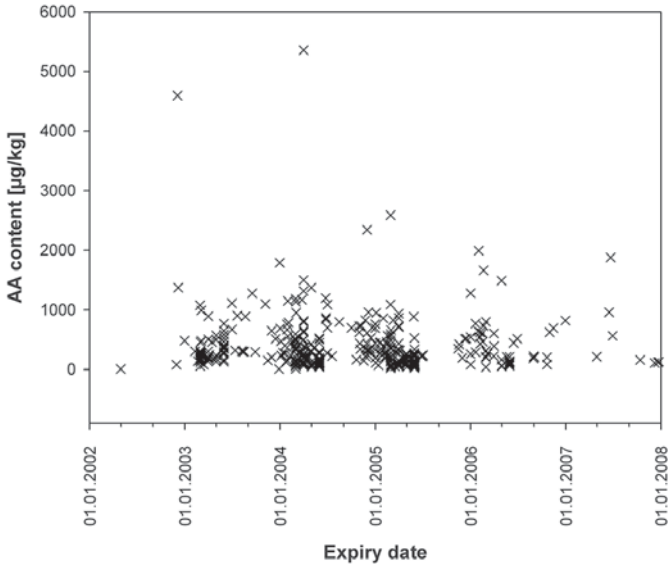




Total: 1399 data

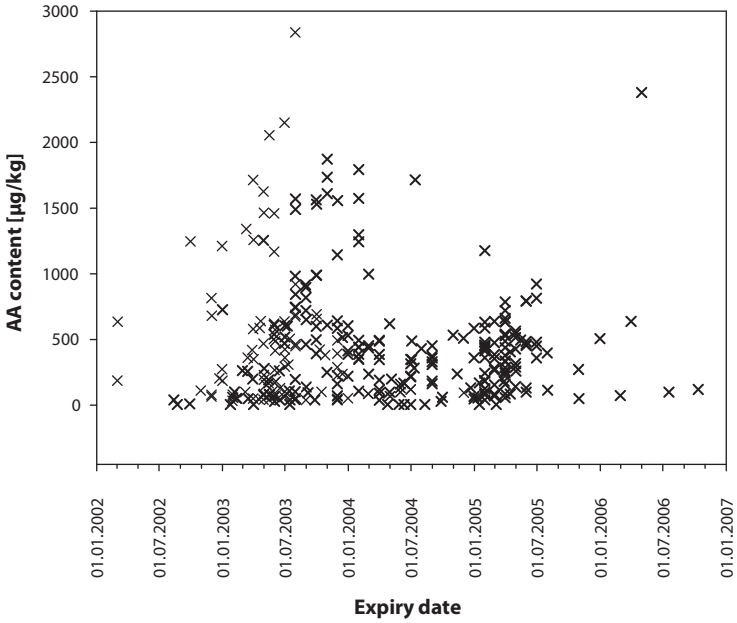
Gingerbread: 458 data

Data of 4 production seasons



Total
1009 data

Crispbread: 318 data



Trend analysis

Difficult to estimate trends

Potential reasons:

- Number of data per food commodity too low
- Variability of data due to influence of
- Different countries of production
  e.g.: different preferences in roasting degrees of coffee
  - Different production sites / processes
  - Different raw materials
  - Different laboratories

Summary

- Data collecting: successful
- Nearly 7400 data considered reliable
- Good representation of important food matrices
- Updated database will be soon on the Web

http://irmm.jrc.ec.europa.eu/html/activities/acrylamide/database.htm

Future

- New data will be submitted
  - From 2006
  - Will be integrated in database
- Database handed over to EFSA

Instructions for Discussion Groups

DR. STEF BRONZWAER
EFSA

Organisation

- 4 discussion groups
- Looking for input from all
- Free scientific debate
- You received briefing notes
- Discussion points: to allow efficient reporting
  - Report back to plenary
  - Summary Report

Programme

- Today: 14.00 – 18.00 Discussion groups
- Tomorrow: 09.00 – 10.00 Discussion groups
  - Finalise outcome of discussion
- Tomorrow: 10.30 – 13.00 Plenary Discussion
  - Expose groups' work to plenary
  - Provide input to other groups
  - Conclude with overall discussion

Summary report

- Draft summary report
- 1st review by DG chairs and rapporteurs
- Advanced draft to all participants for comments (August 2008)
- Publication of summary report and on EFSA website
- and later in EFSA Science Colloquium Report Series

Group number	1	2	3	4
Discussion Group	Epidemiological studies	Biomarkers	Mechanism of carcino-genicity	Dietary exposure
Room	Rossini	Bellini	Puccini	Vivaldi
Chair	Rolaf van Leeuwen	Peter Farmer	Diane Benford	Detlef Müller
Rapporteur	Kathryn Wilson	Jürgen Angerer	Wolfram Parzefall	Leif Busk
	Peter Ashby	Jan Alexander	Lilianne Abramsson-Zetterberg	Sandra Bašić
	Susan Barlow	Herman Autrup	David Bell	Adriana Arisseto
	Jenny Barrett	Matthias Baum	Alan Boobis	Pedro Burdaspal
	Maia Beruashvili	Ulrike Bernauer	Paul Brantom	Kit Granby
	Zuzana Ciesarova	JaroslavCepl	TimoBuetler	Carmina Ionescu
	Cristina Fortes	Henrik Frandsen	Angelo Carere	E.J.M. Konings
	Barbara Gallani	Anja Hallikainen	Daniel Doerge	Ana López-Santacruz
	Judith Heikoop	George Kass	Marvin Friedman	Reinhard Matissek
	Karl-Erik Hellenäs	Sam Lalljie	Gabrielle Hawksworth	Francisco J. Morales
	Janneke Hogervorst	Dennis Marroni	Michaela Heinemann	Marino Petracco
	David Mason	Josef Schlatter	John Christian Larsen	Mari Reinik
	Gloria Pellegrino	Christina Tlustos	Matilde Marques	Juri Ruprich
	Daniel Skrypec	Christine Vinkx	Jan Erik Paulsen	Martin Slayne
	Piet van den Brandt	Rind Kursat Aktas	Katrin Schutte	Alexandra Tard
	Elisabet Wirfält	Jean-Lou Dorne	Claudia Heppner	Thomas Wenzl
	Ulla Bertelsen		Daniela Maurici	Otmar Zoller
	Stef Bronzwaer			Stefan Fabiansson
	Pietro Ferrari			Mari Eskola

ANNEX 4: SLIDES OF THE DISCUSSION GROUPS

Discussion group 1

Epidemiological studies – evaluating evidence and addressing uncertainties

1. Review the epidemiological evidence relating acrylamide dietary exposure and cancer risk.

- New data from prospective studies suggest there may be associations for some cancer sites.
- The data do not show a strong association between acrylamide (AA) and cancer risk.
- Given the widespread exposure to AA, low relative risks may be important.
- More prospective studies are needed to confirm or reject current results and to analyze additional cancer sites.

2. Review the methodology used for the exposure assessment and if there is comparability between studies.

3. Review evidence on the validity of questionnaire-based acrylamide intake assessments.

Two methods:
Food frequency questionnaires
Biomarkers of exposure

Food Frequency Questionnaires:

- All but one study used an FFQ to measure AA intake. However, they may not be comparable (particularly earlier studies with cruder acrylamide data).
- None of the FFQs was designed specifically for acrylamide. AA poses important challenges to FFQs because foods are grouped by nutrient content rather than preparation conditions. One item (*e.g.* biscuits) on an FFQ may represent foods with quite different AA concentrations. AA is also complicated by how food is prepared at home (*e.g.* fried potatoes) → Measurement error is a major problem for epi studies of AA.
- Epidemiologists should work together with food analysts to address these questions.
- Another issue in exposure assessment is uncertainty in occurrence data. Occurrence data typically not collected for exposure assessment, rather for monitoring and control purposes.
- The problem of measurement error depends partly on what foods contribute AA in a population.
- Result of this random measurement error is likely to dilute associations, so actual relative risks may in fact be greater.

Biomarkers of exposure

- One study used AA and GA adducts of hemoglobin to measure exposure and its association with breast cancer risk.
- Important approach because it deals with some of the problems of FFQs and differences in diets between populations.
- However, smoking is such an important determinant of adduct levels that handling of smoking is critical to the validity of such studies. Adduct information is more informative in nonsmokers.
- Issue of passive smoking and AA adducts needs to be explored, as well.
- Another limitation of adducts is that they represent a very short-term period of exposure, when long-term exposure is of interest for carcinogenesis.

4. Establish whether the statistical approaches are consistent between studies and review the sources of uncertainty (particularly confounding variables).

- Proper adjustment or stratification for smoking is critical, this requires larger studies.
- Adjustment for dietary factors that may confound the association between AA and cancer risk is also critical. (These will vary by study population.) Clear criteria for selection of confounders in multivariable models is critical.
- Total energy intake should be adjusted for to reduce measurement error in FFQ.
- Is AA a marker for Maillard products, the tip of the iceberg of cooking carcinogens???

5. Discuss the power of the studies to detect effects.

- Depends on the study (specifically on size of cohort, number of cases, range of exposures, measurement error in exposure and confounders).
- Based on extrapolations from animal studies we would expect an overall relative risk for cancer so low (~1.05) that it would never be detectable in epidemiology studies. However, relative risks may be higher for some cancer sites and low or null for others → most recent findings suggest this might be the case.
- Power could be improved by
  1. improving instruments used to measure AA intake,
  2. studying populations with diverse diets and wide range of intakes (EPIC?),
  3. making more international comparisons.

6. Discuss whether from the body of evidence conclusions can be drawn on the direct relationship between acrylamide dietary exposure and increased cancer risk in humans.

- "Direct" relationship is complicated, goes beyond epi studies

Conclusions about current epi studies

- Some studies have found associations between AA intake and cancer risk, and some have not.
- More prospective studies will be critical for reaching conclusions.
- Of studies that do find associations, the association seems weak (in terms of relative risk and inconsistencies in cancer sites).
- However, the RRs seen are potentially important given measurement error in exposure assessment and ubiquity of exposure.
- The fact that epi studies have found RRs much larger than those predicted from animal studies reinforces the possibility that AA may be important in public health.

Discussion group 2

Biomarkers – new insights in exposure and mode of action

1. New insights into species differences in the kinetics of acrylamide

- Excretion pattern in rat, mice and humans known (Doerge et al; Fennell et al., 2006), Knock out mice (CYP2E1 -/-)
  New data on swine relevant to man in excretion pattern of metabolites (Aureli et al., 2007)
- Relevance of species at doses relevant to human exposure
- PB-PK PD model for Acrylamide and Its Metabolites in Mice, Rats, and Humans (Young et al., 2007).

Research needs

Methods to detect all metabolites in urine (cover whole dose range)
In vitro comparisons between species (isolated hepatocytes)

2. Acrylamide's biomarkers-effects in relation to exposure some better than others.

Haemoglobin adducts

- AA Hb adduct peaks at 2, GA at 16 h longer term 120 days
  Inter-laboratory comparison difficult different techniques in different labs
- Low correlation between Dietary intake (FFQ) and adduct formation (AA, GA)
  Basing epidemio studies on internal dose and diet information-
  more precise approach

Research needs

- Bio-banking material/ repeated sampling for internal dose determination
- For epidemio studies one should include Hb adducts as marker
- Stability of the adduct ?
- Other hepatic adducts (SH groups) ?

DNA adducts

- Extrapolated in Young et al., 2007
  Reflect the biological active dose
- Not measured in human- why ?
- Small amount–Would it affect cancer rate at such predicted low levels?
  Low levels of predicted N7 (predominant adduct)
- Relationship between adducts and cancer (new NTP study ?) ?

3. Use of urinary metabolites (acrylamide and glycidamide) as biomarker

Research needs

- Intake correlation with urinary biomarkers better with more accurate intake estimates but,
  1. Specificity of metabolites-confounding factors
     (other sources of metabolites, medication, age, disease)
  2. Short term biomarker only because half life short
- Depends on purpose of study, for validation of dietary estimates useful
  but for cancer not reflecting long-term
- Chemical Industry
  Explore worker exposure: sample Friday pm- Monday am deduce work exposure
  from food questionnaire / also more long term monitoring

4. Physiologically-based pharmacokinetic models

3 models

- From **Kirman et al., 2003** PB-TK TD Hb adducts- 2 compartment model
- **Walker et al, 2007** Children only TK multi-compartmental model adjusted in neonates and children for CYP2E1 ontogeny- no Effects
  Population variability CYP2E1, GSTM1 AND EH
  EPA code not released , comments available scientific advisory board website (US)
- **Young et al al, 2007**- PB-TK TD multi-compartmental models

Suggestions for future research

- Inconsistencies ?
  Data gap GST, EH ontogeny
- **Young et al., 2007**
  Critical data TK GA serum and Hb-adduct formation curve
- Data on DNA-adducts in humans needed to test the correctness and accuracy of prediction
  Lacking data on population variability
  Refinement of the young model would be to include neonate and infants
- Ethanol and interaction, ethyl carbamate and solvents (eg)

5. Impact of biomarkers on the risk assessment (both for exposure and the mode of action)

Conclusions and Research needs

- Biomarkers help estimate intake, extrapolation animal-man, epidemio, metabolic polymorphism population level.
  Acrylonitrile adducts to discriminate origin of AA (diet/smoking)
  Endogenous production of acrylamide ?
- Immunological approaches
  Neo-epitope-based adducts high throughput and cheap
  Serum antibodies to high levels of AA ?
  Monoclonal Anti-bodies against DNA-adducts ?
- Type of biomarker OMICs: gene, proteins, metabolites

Discussion group 3

Mechanism of carcinogenicity

1. Review the recent evidence for the mutagenicity and genotoxicity of acrylamide and glycidamide

- Recent in vivo evidence includes:
  a. GA is formed by CYP2E1 from AA (not in CYP2E1 ko)
     Genotox endpoints:
     DNA damage (Comet assay), adducts, micronuclei, germ cell mutations, dominant lethality.
  b. Mutations in vivo: hprt, cII (AA+GA), TK+/-(GA)
  c. Cannot identify thresholds for DNA binding:
     (lowest dose from control feed ~1 µg/kg*d, corresponding to 1/108 DNA adducts)
- GA and DNA adducts are distributed through the whole body.
- Genotox alone does not explain the target sites for tumors.
- Tumorigenicity of GA in neonates suggests increased sensitivity during early life exposure.
- Human infants express CYP2E1 by the time of weaning and presumably form GA.

2. Review the recent evidences for the non-genotoxic mechanism of acrylamide (and glycidamide)

- Proposals for the mode of action (MOA):
- Mammary tumors
  (disruption of hormonal status in aged female rats)
- Testes
  (related to Leydig cell tumors, prevalent in the male F344 rat)
- Question if both are relevant to humans
- Thyroid tumors: No plausible explanation.
- CNS tumors are disputed
  (new data will confirm or discount)
- Tumor spectrum im mice still incomplete but so far differing from the rat tumor sites.
- No theories for the mouse tumors (liver, lung, Harderian gland)
- No convincing evidence of non-genotoxic mechanisms (*e.g.* oxidative stress, cell proliferation, apoptosis) at relevant doses.
- Overall conclusion to this point.
- Need for a systematic review of the MOA and human relevance for each tumor type.
- Different mechanisms are possible for different tumor types in experimental animals.

3. Weight the evidence as to whether AA acts via a non-genotoxic or genotoxic mechanism in contrast to its genotoxic metabolite.

- The margin of exposure (MOE) at dietary levels are such that only genotoxic mechanisms are likely to be relevant.
- We do not have evidence for non-genotoxic mechanisms at relevant doses.
- Only in exceptional circumstances could you discount the relevance of a genotoxic MOA.

4. Exploration of the consequences of changed conclusions about genotoxic versus non-genotoxic mechanism of carcinogenesis for human risk assessment.

- No changes anticipated.

Revised question:
How do we improve the risk assessment?

- New results, *e.g.* tumor dose-response data and mechanistic data should increase confidence leading to improved Quantitative Risk Assessment (QRA)
- Information of species sensitivity, DNA and/or protein adduct levels, other biomarkers, and PBPK modelling should reduce the uncertainty in human extrapolation.
- We should not expect concordance in tumor site profiles in different species.

Discussion group 4

Dietary exposure across Europe - current situation

1. *Data reliability with regards to sensitivity of the analytical techniques and consistency of the data reported by the member states, including new analytical techniques*

- We have the necessary analytical methods
  - GC/MS, LC/MS/MS
- New techniques - no added value
- Two certified reference materials are available
  - Toasted bread and crisp bread. Other matrices?
- Proficiency programs are running
- Standardised NMKL method available
  - Can be adjusted to new matrices
- Need to reduce costs for AA and AA adduct analysis
- Ensure objective focused sampling schemes
- Sampling and consumption data probably more limiting than analytical techniques

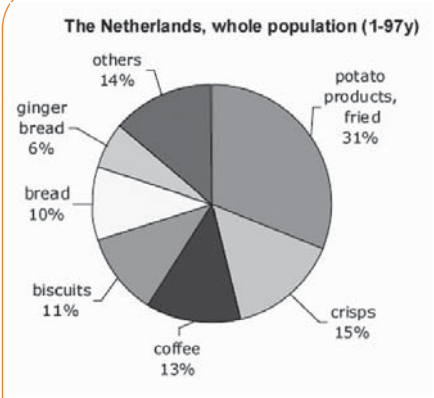
2. Review the occurrence data for Acrylamide in food commodities available in Europe. Is there a need to revisit the exposure assessment?

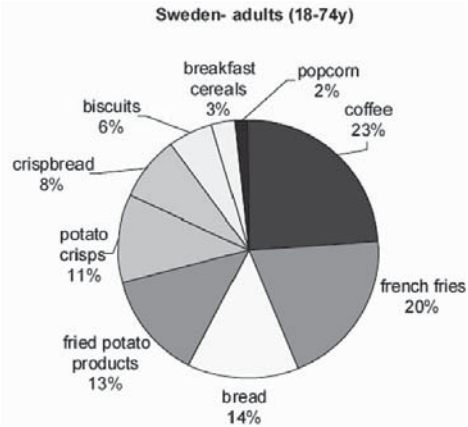
Need for revision – need for precision?

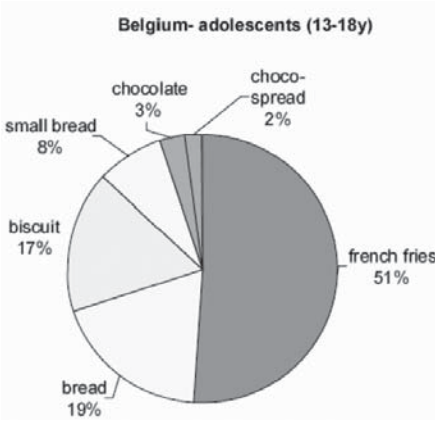
- MOE will not be affected in a substantial way by a revised current exposure assessment
- Different needs depending on whether you should
  - Consider a decision on whether specific managing activities should be introduced
  - Direct mitigation efforts
  - Inform consumers on the risks or issue dietary advice
- Depending on the preferred management option there might be a need for more precise exposure calculations
- We **probably** know the main sources of exposure **for the "average" consumer**
  - Roughly 1/3 each from potato products, cereal products, coffee
  - It is still important to identify specific risk groups with
    - High exposure, *e.g.* children
    - "Exceptional" consumption of specific food commodities or specific ethnic foods, where the levels of Acrylamide are not known
    - Requires better data on both consumption and occurrence
- Differences between countries on home cooking and catering
  - few solid data available
  - few handles for intervention available
- Need to validate FFQ
  - *E.g.* by duplicate diet studies

3. Which food commodities contribute most to Acrylamide exposure – possibility and efficacy of mitigation measures?

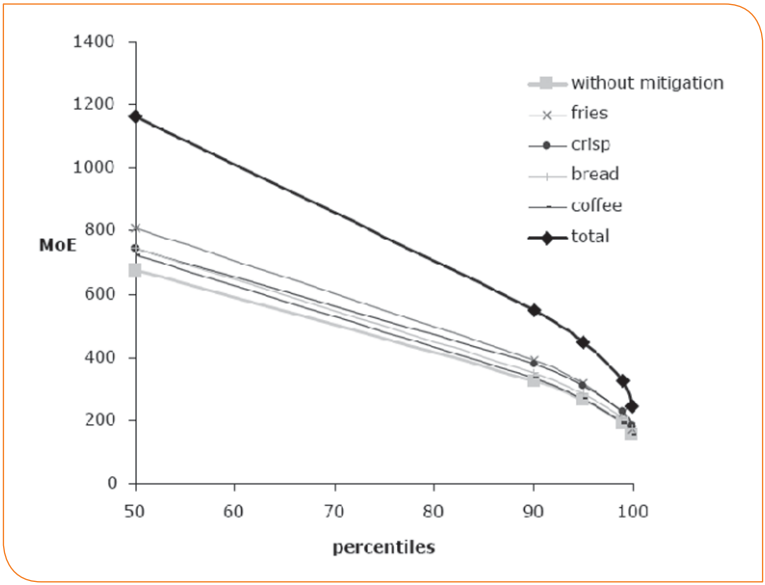
Contribution of different food groups to the acrylamide exposure.







Modeled distribution of Margin of Exposure (MoE) of the acrylamide reduction scenarios over the percentiles.



- Mitigation measures are of great importance and should be pursued.
- Many Companies have implemented some mitigation measures
- Desirable to consider further measures to reduce exposure, *e.g.* home cooking, catering, dietary changes

4. Recommendations to improve data collection and data assessment in the future.

- Analytical techniques are well validated
- Make all relevant data accessible, industry and MS
- Assess specific risk groups
- Depending on the preferred management option there might be a need for more precise exposure calculations
- Consider the side effects of reduction measures
- Harmonise and utilise the probabilistic exposure assessment to improve the result of the assessment
- Consider the relative importance of acrylamide vs other food process contaminants.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