

SUMMARY REPORT

EFSA SCIENTIFIC COLLOQUIUM NO. 11

ACRYLAMIDE CARCINOGENICITY NEW EVIDENCE IN RELATION TO DIETARY EXPOSURE

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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 120°C 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-oxopropyl) 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 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 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 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 glutathionamide (GA).

As starting point to their deliberations on the mechanisms of carcinogenicity, the discussion group (DG) 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 (Besaratnia 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 $1/10^8$ DNA adducts.

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

The DG 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 DG suggested it would be best to wait for new data from ongoing experiments which might help to confirm or discount this finding.

The DG 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 DG 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 DG 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 that modelling the experiences from laboratory experiments suggests a 40% maximum reduction might be possible, based on 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 DG 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 DG 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/m³ 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 DG 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 DG 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 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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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 that have been published since 2005 have reduced some of the uncertainties mentioned in the JECFA evaluation of 2005 and endorsed by the EFSA CONTAM Panel.

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.