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Food and Chemical Toxicology 43 (2005) 365–410
www.elsevier.com/locate/foodchemtox

Human exposure and internal dose assessments of acrylamide in food

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Received 2 September 2004; accepted 9 November 2004

Abstract

This review provides a framework contributing to the risk assessment of acrylamide in food. It is based on the outcome of the ILSI Europe FOSIE process, a risk assessment framework for chemicals in foods and adds to the overall framework by focusing especially on exposure assessment and internal dose assessment of acrylamide in food.

Since the finding that acrylamide is formed in food during heat processing and preparation of food, much effort has been (and still is being) put into understanding its mechanism of formation, on developing analytical methods and determination of levels in food, and on evaluation of its toxicity and potential toxicity and potential human health consequences. Although several exposure estimations have been proposed, a systematic review of key information relevant to exposure assessment is currently lacking. The European and North American branches of the International Life Sciences Institute, ILSI, discussed critical aspects of exposure assessment, parameters influencing the outcome of exposure assessment and summarised data relevant to the acrylamide exposure assessment to aid the risk characterisation process. This paper reviews the data on acrylamide levels in food including its formation and analytical methods, the determination of human consumption patterns, dietary intake of the general population, estimation of maximum intake levels and identification of groups of potentially high intakes. Possible options and consequences of mitigation efforts to reduce exposure are discussed. Furthermore the association of intake levels with biomarkers of exposure and internal dose, considering aspects of bioavailability, is reviewed, and a physiologically-based toxicokinetic (PBTK) model is described that provides a good description of the kinetics of acrylamide in the rat. Each of the sections concludes with a summary of remaining gaps and uncertainties.

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doi:10.1016/j.fct.2004.11.004

1. Introduction

On 24 April 2002, the Swedish National Food Agency and Stockholm University presented data, that, in part, showed high concentrations of acrylamide in certain fried, baked and deep-fried foods, and later in coffee (Swedish National Food Agency, 2002). Since acrylamide has been classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC, 1994) and a Category 2 carcinogen and Category 2 mutagen by the European Union (http://ecb.jrc.it/classification-labelling/), this finding caused worldwide concern (WHO, 2002). After the initial reporting generated by research from the Stockholm University (Tareke et al., 2000, 2002), similar findings that acrylamide is formed primarily in carbohydrate-rich food prepared or cooked at high temperatures, have been reported from many European countries and the USA.

An ILSI Europe Task Force on Acrylamide was set up after a brainstorming meeting that was held in December 2002. This Task Force met in March 2003 and recommended to set up an expert group to develop a risk assessment framework for acrylamide based on the outcome of the FOSIE project (Food Safety in Europe—Risk Assessment of Chemicals in Food) (Barlow et al., 2002; Renwick et al., 2003).

The ILSI Europe Expert Group on Acrylamide, which has collaborated with ILSI North America, met for the first time in July 2003. The Expert Group is composed of scientists from universities, public health institutes and industry in Europe and the US. It was recognised that a large quantity of data pertaining to acrylamide exposure have emerged, but that there has been little evaluation of such data with respect to risk assessment. The Expert Group therefore decided that an important contribution to the overall risk assessment framework of acrylamide in food would be to collate and present information relevant for acrylamide exposure assessment, as well as internal dose assessment. The present report therefore describes the following elements: (1) acrylamide formation, analytical methods and levels in food and diet, (2) amounts of acrylamide-containing food consumed, acrylamide intake in individuals and intake in special population groups, (3) biomarkers of acrylamide exposure and internal dose, (4) bioavailability, and (5) acrylamide target doses/physiologically-based toxicokinetic (PBTK) modelling. The uncertainties in the assessments, as well as the data gaps, are also discussed. The food consumption and acrylamide content data reported here is based on a questionnaire survey sent to 31 individuals and institutions reporting on existing acrylamide dietary exposure assessments and databases.

The main purpose of this exercise was not to make an exhaustive inventory, but to give examples of the type of study designs and dietary exposure data available for risk assessors, and to highlight the current methodological strengths and limitations for estimating and interpreting these data.

2. Exposure assessment

2.1. Introduction to the exposure assessment

Estimating consumer exposure to acrylamide is a high priority for governments and industry alike. Acrylamide is now known to be formed during industrial food processing, retail, catering and home food preparation. Extensive research is underway to determine the extent to which acrylamide found in food is bioavailable and to identify methods to reduce levels in order to decrease consumer exposures.

Consumer exposure assessments will need to be conducted in order to gauge the utility of various control options. It is particularly important to be able to assess the impact of proposed changes to the food supply including the impact of modifications in processing and cooking procedures on consumer exposures. It is also important to know whether there are significant differences in exposures in different subgroups of the population.

A framework for conducting exposure assessments is needed so that the results will be transparent. A framework should also provide insight into the strengths and weaknesses of various exposure assessment methods. The compilation of existing knowledge on acrylamide formation, exposure and internal dose assessment proposed in this document was designed specifically for the purpose of ensuring that assessments of consumer exposure to acrylamide will improve the understanding of baseline exposures and relative changes in exposures with modifications to the food supply.

The recommended framework for estimating exposure to acrylamide addresses each of the parameters that are included in the exposure calculation algorithms, as well as providing criteria for assessing the usefulness and quality of existing data. Methods to evaluate the reliability of the exposure assessments address potential sources of acrylamide that have not been identified (foods that have not been identified as potential sources as well as non-dietary sources).

The framework includes the evaluation of impact of assumptions that are used in designing the exposure assessment. Since most assessments of acrylamide exposure will be used for decision-making, it is also important

that the framework allow for "scenario" or hypothesis testing in order to determine the impact of potential changes that result in modifications in the levels of acrylamide in one or more foods.

The framework addresses each phase of the exposure assessments:

- Initial definition of the scope of the assessments including techniques for determining the range of anticipated consumer exposures
- Criteria for selection of the exposure models including the specific algorithms.
- Criteria for selection of data for analyses.

2.1.1. Initial definition of the scope of the assessment

Prior to defining the criteria for detailed assessments, it is critical that ranges of potential exposure be estimated—ideally using different techniques. In the case of acrylamide, the framework should include evaluations of the results of biomonitoring to estimate total exposure. The potential contribution of the diet should be assessed if possible by conducting a total diet survey (either a direct duplicate diet type study or a broad survey of foods available in the market place).

2.1.2. Criteria for selection of the exposures models including the specific algorithms

New exposure models have been recently developed that provide more realistic estimates of consumer exposure and that provide estimates of exposure of typical (average, median) consumers as well as extreme consumers (high consumers), and that allow the analyst to better understand the impact of assumptions made in the model, as well as to evaluate the impact on different consumers of proposed changes to foods. Most of the new models are computer based and permit the use of distributional analyses and provide tools for calculating the source contributions to exposure (Edler et al., 2002; Gibney and van der Voet, 2003).

The criteria for selecting the exposure model are:

- Ability to match exposure scenarios to the toxicity endpoint of concern.
- Time period.
- Range of exposures (e.g. low dose chronic exposure).
- Reflect total diet and if appropriate non-dietary sources of exposure.
- Relative precision should be as good as that for the toxicity data.
- Ability to use the best available data (and conversely where only limited data are available to conduct screening type exposure assessments).
- Ability to provide qualitative and quantitative information about the sources of exposure including impact of industrial processing treatments as well as retail, catering and home food preparation.
- Ability to consider unique exposures scenarios (different subgroups, heavy consumers of certain foods, etc.).
- Ability to conduct "what if" hypothesis testing.

2.1.3. Criteria for selecting computation algorithms

It is recommended that the framework address each of the key components of the exposure calculation algorithms and follow the criteria described below:

(1) Consumption of foods that could contain acrylamide

The criteria for selecting data on consumption should be based on the general principles adopted by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (1987).

- Food consumption estimates

Criteria that are particularly important for assessing acrylamide exposures include:

  - The number of subjects surveyed.
  - Consumption information for the entire diet or at least the foods of interest.
  - Measurements of inter- and intraperson variability in consumption patterns and the ability to evaluate subgroups within the overall population.
  - Inclusion of methods of preparation that have been applied to the food before it is consumed.

(2) Levels of acrylamide in foods

In the short time since acrylamide was reported to be present in foods many studies have been undertaken. Section 2.2 provides an inventory of current knowledge. Exposure assessors will want to conduct additional searches since research is ongoing at many institutions. The most appropriate data will depend upon the purpose of the assessment.

The criteria for selecting data on acrylamide levels in foods include:

- Sample representativeness/appropriate extrapolations/surrogations.
  - Types of foods.
  - Impact of including industrial food processing, retail, catering and home food preparation.
- Quality of the analytical results.
- Precision (measure of variability).
- Statistical considerations such as power/sample numbers.

The analyst should review the available data and select the data that are as representative as possible of the foods being consumed by the population whose

exposure is to be estimated. This includes ensuring that the types of foods that are consumed are included, as well as the forms of those foods. Foods prepared by different methods will be extremely important for reliably estimating exposures to acrylamide. Where the desired data are limited it may be necessary to extrapolate from existing data through a surrogation process that matches the available data to the foods that are consumed. The impact of the assumptions made as part of the surrogation should be tested by conducting analyses with alternative assumptions.

2.1.4. Evaluating the quality of the analytical data

In addition to ensuring that the foods are representative, the best quality data should be selected. Data on samples analysed with appropriate analytical methods will be critical. The data should be evaluated in comparison to recommended methods (for current assessments the reader is referred to Wenzl et al. (2003) and the results of the JIFSAN Workshop on Acrylamide in Food, Working Group 2: Analytical Methodology, 2004).

The analyst should provide estimates of precision and reliability of the estimates, as well as an indication of the impact of the limitations of the data.

- Relevant adjustment factors

Since the consumption data and acrylamide data will be collected as independent samples it may also be necessary to include adjustment factors to allow a reliable exposure assessment to be determined. That is adjustment factors may be used to match consumption data to exposure assessment data. The criteria for selection of adjustment factors will include:

  - Improved matching of consumption data to residue data.
  - Improved relevance to actual consumer practices.

- Relevance of consumer exposure to toxicological concerns

Exposure assessments need to be conducted for the time frame that is relevant to the toxicological issue that is being evaluated. In the case of acrylamide more than one toxicological issue will need to be considered and the most appropriate exposure assessment procedure will need to be selected in conjunction with experts in toxicology. The criteria for selecting the most appropriate exposure procedure include:

  - Matching time frames.
  - Matching dose levels.
  - Selection of appropriate age/sex groups (children, pregnant females, elderly).

The compilation of existing knowledge on acrylamide formation, exposure and internal dose assessment proposed in this document will give advice on tools and procedures needed to conduct assessment of consumer exposure to acrylamide that can be used for a wide variety of decision making purposes. It that will provide the users of those assessments with an understanding of the exposure assessment.

2.2. Current knowledge on formation, content and analysis of acrylamide in food

2.2.1. Determinants of acrylamide formation

Since the announcement in April 2002, significant progress has been made in understanding how and from which precursors acrylamide is formed in various foods. The fact that acrylamide was first described in French fries, potato chips, [1] bread and crispbread triggered immediate research not only on its formation, but also on its reduction, since these foodstuffs are widely consumed by a large number of consumers. Later, coffee was found to contain considerable levels of acrylamide, the consumption of which is especially high in certain countries (see below). These food types also corresponded well with the hypothesis of formation during heat treatment from asparagine and reducing sugars, precursors that are highly abundant in these foods. However, the more recent finding of acrylamide in black olives and prune juice (Roach et al., 2003) and in laboratory animal feed sterilised by autoclaving (Twaddle et al., 2004a) may implicate the involvement of another route of formation during curing or at lower temperatures. Thus different mechanisms of formation should be considered in specialty food as yet unknown to be a 'classical' source of acrylamide formation. Even if the overall acrylamide exposure of the average consumer will probably not be influenced by acrylamide from products such as prune juice or olives, special food types may well be relevant for specific subgroups of consumers, as e.g. certain age groups, consumers with a special diet due to diseases, or cultural subgroups.

It is now generally agreed that acrylamide is formed by the Maillard reaction from the condensation of the amino acid asparagine with reducing sugars such as fructose or glucose upon heating at temperatures above 120 °C (Stadler et al., 2002; Mottram et al., 2002). Acrylamide has not been reported in boiled foods.

Other possible mechanisms for the formation of acrylamide in food have also been identified that may result in acrylamide in some foods (JIFSAN Workshop on Acrylamide in Food, 2004).

The major reaction mechanism under low moisture conditions involves the formation of a Schiff base from

[1] In this report the term "potato chips" denotes products typically based on thinly sliced (usually <2 mm) and subsequently deep fried potatoes consumed predominantly as snacks, whereas the term "French fries" is used for pieces of potato usually 5–10 mm square and having the typical length of the potatoes used, which are deep fried but predominantly consumed as side dishes with meals or considered a small meal by themselves.

asparagine and a reducing sugar that can further react through several proposed mechanisms to form acrylamide (Stadler et al., 2004; Yaylayan et al., 2003; Zyzak et al., 2003). The key step of the reaction is the decarboxylation of the Schiff base leading to Maillard intermediates (imines) that can either directly release acrylamide or indirectly via the precursor 3-aminopropionamide (Zyzak et al., 2003). Yaylayan et al. (2003) proposed the formation of the decarboxylated Heyns product that undergoes facile beta elimination to afford acrylamide (Yaylayan et al., 2003). However, the key intermediates have so far not been fully characterised, and therefore the detailed chemical reactions leading to acrylamide remain largely hypothetical.

Under certain model conditions, significant amounts of acrylamide were reported to be formed from ammonia and acrolein in the absence of asparagine, a pathway that was suggested to play a role in lipid rich foods (Yasuhara et al., 2003). Acrolein can be formed by heat decomposition of triglycerides present in frying oil. This route of acrylamide formation via acrolein does, however, not seem to be involved in the formation of acrylamide in fried potatoes (Becalski et al., 2003). The various pathways of formation have been extensively reviewed recently (Taeymans et al., 2004).

In addition, another pathway was recently proposed from heat decomposition of carnosine present in meat. The levels found, based on pyrolysis gas chromatography (GC) analysis, were, however, relatively low (20–50 μg/kg; unpublished results presented at the JIFSAN Workshop on Acrylamide in Food, 2004).

Model studies have indicated that fructose is more efficient in the formation of acrylamide than glucose or galactose (Biedermann and Grob, 2003). A possible explanation is the melting point, which is lower for fructose under low moisture conditions, increasing the mobility and, therefore, reactivity of the molecule. Although not a reducing sugar, sucrose is a possible reaction partner considering its hydrolysis into the individual monosaccharides upon heat treatment (Becalski et al., 2004).

It is presently clear that the major determinants of acrylamide formation in food are the presence of asparagine and reducing sugars or reactive carbonyls. However, the reaction between the two components is not very efficient. Only a small proportion of the two reactants finally react to form acrylamide. Reaction yields on a molar basis of around 0.1–0.3% of the initial asparagine content were repeatedly found under optimal model reaction conditions (Becalski et al., 2003; Stadler et al., 2002; Yasuhara et al., 2003; Mottram et al., 2002).

In food model systems, conversion rates were similar. Surdyk et al. (2004) reported conversion rates in the range of less than 0.3% in bread crust after spiking the dough with different amounts of asparagine. Conversion rates in another model study on acrylamide formation in potato, wheat flour and corn starch were slightly higher, but generally less than 1% (Biedermann and Grob, 2003). The latter study also demonstrated a strong effect of ammonium hydrogen carbonate, used as rising agent in certain bakery products such as ginger bread, which increased the conversion rate up to 5%.

Estimations of conversion rates are strongly complicated by the important role of formation versus elimination. In coffee, high amounts of acrylamide are formed early in the roasting process (reaching peak levels of 2000 ppb) and brewing of coffee extracts acrylamide from the matrix fairly efficiently (Andrzejewski et al., 2004). During the roasting process, acrylamide formation is outweighed by an unknown elimination reaction, leaving an average residual acrylamide content of 250 ppb at the end of the roasting process (Taeymans et al., 2004). Thus, the time and temperature profiles during processing of the various foods play a fundamental role on the levels of acrylamide in the final product.

Depending on the composition of raw materials with respect to the concentrations of free asparagine and reducing sugars such as glucose or fructose, the rate limiting factor in acrylamide formation will be either the amino acid or the sugar precursor. For example in model bread systems based on wheat flour, asparagine is present in much lower concentrations compared to reducing sugars. Surdyk et al. (2004) showed that increasing the asparagine concentration by spiking the dough with asparagine before baking strongly increased the levels in bread crust, whereas the addition of fructose had no effect (Surdyk et al., 2004; Biedermann and Grob, 2003). In contrast, in potatoes, where asparagine concentration is very high, increases in free reducing sugar content during storage augments the acrylamide concentration after frying (Becalski et al., 2004). Reducing the asparagine content in potatoes through enzymatic digestion with asparaginase dramatically reduces acrylamide formation (Zyzak et al., 2003).

There are indications that controlling the selection of raw materials with respect to their free amino acid profiles and their content of reducing sugars (and e.g. the formation of free reducing sugars upon cold storage of potatoes, Chuda et al., 2003) (EFSA, 2004) could be important means to potentially reduce the levels of acrylamide formed in processed food. Other factors additionally influence the formation of acrylamide, such as the presence of rising agents containing ammonia, the thermodynamics of frying and baking conditions, the kinetics of formation and elimination, moisture content, pH, etc. Some of these aspects have been used in approaches to reduce the formation of acrylamide. The most feasible of these approaches to date have been summarised in a report based on a workshop organised by the European Commission Health and Consumer Protection Directorate-General (EU DG SANCO) in 2003 (EU, 2003) and will be briefly discussed in the

following section for three food groups (potatoes, cereals, coffee).

Potatoes. The content of reducing sugars appears to be a strong determinant for acrylamide formation in potatoes, therefore, selection of cultivars low in reducing sugars and storage at moderate temperatures (not below 8–10 °C due to the increase in sugar content at lower temperatures) might present an option for reduction (Amrein et al., 2003). The impact of farming conditions, fertiliser use, cultivar, time of harvest etc. on the high variability of sugar content remain as yet largely unknown. Pre-blanching of potatoes before frying, lowering the pH and increasing the moisture have been shown to reduce acrylamide levels (Jung et al., 2003; Taubert et al., 2004). To aim for a golden yellow instead of a brown colour after frying at temperatures not higher than 175 °C, has been recommended as an approach also for home cooking (EU, 2003).

It should be noted, though, that not the absolute temperature, but the overall thermal input affects acrylamide formation. If frying at lower temperature necessitates a longer frying time to achieve the desired product quality, the net impact on acrylamide formation may be very limited or even reversed. Frying for a short time at higher temperatures may be a better option if this helps to reduce the thermal input. Changes in process conditions should also be assessed for their effect on other relevant parameters, e.g. a higher fat uptake seen at low frying temperatures.

However, care must be taken not to compromise the quality and the organoleptic properties of the product. A promising approach so far under laboratory conditions has been the degradation of asparagine using the enzyme asparaginase, which seems to be applicable to potato chips (acrylamide reduction of 97%) and French fries (acrylamide reduction of 80%), retaining acceptable flavour and colour of the product (Zyzak et al., 2003 and unpublished results presented at the JIFSAN Workshop 2004). As for all reduction measures, the scaling from a test tube approach to large scale industrial production remains an additional challenge.

Cereals. The only recommendation to date for cereals is the avoidance of excess browning during baking (EU, 2003). Since the limiting factor for acrylamide formation in cereals is the asparagine content, selection of grain varieties with low asparagine content is an option. The use of asparaginase has not yet been tested for applicability to cereal products (EU, 2003).

Coffee. No practical solutions currently exist for reducing acrylamide levels in coffee. Any change of the processing conditions (temperature, duration of the roasting) will considerably change the final product. For roasted coffee beans, a decrease in acrylamide levels over time was observed upon simple storage at room temperature (Andrzejewski et al., 2004), a fact that calls for further investigations.

2.2.2. Analytical methods

The analytical methods developed to date and their applicability to different food matrices, as well as their selectivity and sensitivity, recently have been reviewed (Taeymans et al., 2004; Wenzl et al., 2003). A variety of methods have been employed to measure acrylamide levels in food since 2002, including different extraction and cleanup procedures for different food matrices. These methods are mainly based on a chromatographic step by either liquid chromatography (LC) or gas chromatography (GC) coupled to mass spectrometry (MS). The GC step is based on derivatisation (usually bromination to increase volatility) of the analyte or on direct analysis without derivatisation. Limits of quantification range from 30–50 μg/kg for LC–MS down to 10–30 μg/kg for GC–MS. For the detection of acrylamide levels below 30 μg/kg, GC–MS with bromination is to be favoured over the non-derivatised method (Taeymans et al., 2004). A critical factor for analyte recovery appears to be the extraction and cleanup of acrylamide from the different food matrices. For more details the reader is referred to the reviews mentioned above.

Roach et al. (2003) recently described an analytical method employing LC–MS/MS that reduced the limit of quantification down to 10 μg/kg with a recovery rate of 95%. The precision of the method appeared satisfactory for cereals, bread crumbs, potato chips and coffee with a relative standard deviation of 0.9–6.9%. The method was suggested for use in regulatory settings. The method was applied in several rounds of proficiency testing with a good performance. Furthermore, the authors stressed the importance to use isotope labelled internal standards to assure the quality of the results. Exploratory data gathered using this method were made available on the Internet (http://www.cfsan.fda.com).

An updated analytical LC–MS/MS method for ground and brewed coffee was published recently (Andrzejewski et al., 2004) with limits of detection of 10 μg/kg and 1 ng/ml, respectively.

So far, acrylamide does not seem to be specifically entrapped in the matrix: Jezussek and Schieberle (2003) showed that protease or amylase digestion of bread samples prior to aqueous extraction did not increase acrylamide recovery.

Variability analysis. Various proficiency testing approaches have been initiated world-wide to determine the performance of the various analytical methods employed, the influence of the sample preparation methods and the performance of individual participating laboratories.

The US National Food Processors Association (NFPA) and the Swiss Federal Office of Public Health compared data from different laboratories for "simple" matrices. Proficiency tests were also organised by the UK's Central Science Laboratory (CSL) under its Food Analysis Performance Assessment Scheme (FAPAS).

Table 1
A comparison of the assigned values (best estimate of the true concentration and a consensus value) with the satisfactory range obtained by participating laboratories (within the acceptable z-scores range of +2 to −2) as calculated by the modified Horwitz equation for seven FAPAS rounds from July 2002 to June 2004 with five different food matrices

	R1, Jul 02	R2, Nov 02	R3, Feb 03	R4, Jul 03	R5, Aug 03	R6, Nov 03	R7, Jun 04
Test material	Crispbread	Chips	Cereal	Coffee	Crisp bread	Cereal	Oven chips
Assigned value (ppb)	1213	167	109	312	707	95	1843
Satisfactory range (ppb)	836–1590	97–237	61–158	193–431	468–945	53–137	1306–2381

Data from the FAPAS scheme (Owen et al., 2005) show relatively large variability for a range of matrices from laboratories achieving acceptable z-score (as described by Thompson and Wood, 1993) (Table 1). The interlaboratory scheme organised by the German Bundesinstitut für Risikoforschung (BfR) showed similar variability for similar matrices. For example for crispbread 81–286 mg/kg (CVR = 28%) and mashed potato 3.55–11 mg/kg (CVR = 25.6%) (Fauhl et al., 2002).

The high interlaboratory variability indicates that the acceptable range is too large for the food products tested, even for relatively simple matrices. Hence, a better approach to calculate z-scores is required. The impact of the high interlaboratory variability may not significantly impact exposure assessment as long as the exposure data set is not dominated by data from poorly performing laboratories. Also, in practice the impact of interlaboratory variability on the exposure assessment may be limited, since in many cases the natural and/or process related variability of real acrylamide levels even for one brand in a given product category is much wider than the interlaboratory variabilities reported.

However, improved z-scores will improve the quality of acrylamide measurements and this will be beneficial especially in the monitoring of acrylamide levels during mitigation efforts (this will be further discussed in Section 2.2.4).

The report on an interlaboratory comparison initiated by the EU, on the determination of acrylamide in crispbread and butter cookies in 62 laboratories was published recently (Wenzl et al., 2004). The study used samples of crispbread and butter cookies, and native and spiked bread extract samples, as well as aqueous extracts with defined, but non-disclosed levels of spiked acrylamide. The results were evaluated by statistical methods, and the performance of laboratories was estimated by calculation of z-scores. The basic outcome of the study was that the applied technique had a statistically significant impact on the analytical results, and that especially for the crispbread matrix, a large proportion of the results was outside the satisfactory range. Interestingly, those laboratories that participated in previous proficiency test rounds with satisfactory z-scores also performed well in this study. Significant effects on the analysis results were found for the measurement technique and the composition of the extraction solvent, which was in this study only the case for the crispbread sample, thus the solvent used for the extraction is important with respect to the type of matrix. In general, laboratories using GC/MS without derivatisation overestimated the acrylamide content compared to GC–MS with derivatisation or LC–MS/MS. The quality of the results furthermore decreased when the acrylamide content was close to the limit of detection of 30–50 μg/kg. The authors concluded that additional work needs to be done to identify problems in the analytical procedures. Critical points identified in this study were the need of robust methods for difficult food matrices such as cocoa powder, coffee and high salt flavourings; methods of extraction will probably have to be developed individually for each type of matrix.

An important source of variability appears to be lot-to-lot and bag-to-bag variability, as shown in the study of Roach et al. (2003). The variability of results for replicate testing using the same analytical method in the same laboratory were generally low (relative standard deviation between 0.9 and 6.9%), irrespective if replicate samples were tested concurrently or on different days. Similar variabilities were obtained using different matrices, such as cereals, crumbs, chips or coffee. When different bags of the same lot and different lots of the same potato chip product were tested, the between lot variability was much bigger than within lot variability. The variabilities in the study were in the order: within sample $\ll$ within lot $\ll$ between lot $\ll$ between different products of the 'chips' category. To illustrate this, the difference between the highest and the lowest value was ~125 μg/kg within a lot, ~300 μg/kg between lots and more than 2500 μg/kg between different chips products (the difference between the highest and the lowest value was not available for replicate samples in this study).

Another study performed by Ono et al. (2003) confirmed the high variability between different products of the same category. Concurrently, the values obtained by using two different analytical methods, LC–MS/MS and GC–MS, were compared. Although the number of measurements was small, it was obvious that the differences between results of different samples were much bigger than the differences between the two methods.

Although it seems that between-sample variability is by far the largest source of variability, statistical analysis

must be employed that takes into account the different sources of variability in order to improve the power of such tests. Wenzl et al. (2003) were the first to publish data in the scientific literature on the statistical analysis of proficiency testing, applying robust statistics to calculate *z*-scores, re-sampling statistics and multifactorial analysis of variance (MANOVA) for the elucidation of significant influences on test results. Parameters included in the analysis were parameters of the extraction method, the extraction solvent, and the analytical method employed. Their study revealed a significant influence of the analytical method on the test result depending on the sample matrix. However, this study included testing of few selected samples of several laboratories in a proficiency test approach, and not large numbers of different samples from the market, which would add another, significant source of variability. Similar statistical methods may thus be applicable also to the huge data sets generated to date, provided that additional information on a variety of potentially important parameters as the ones mentioned above are available for these measurements, and that the sample numbers are big enough to perform statistical tests.

2.2.3. Main sources of dietary acrylamide and levels in food

In order to facilitate the exchange of data and to provide an adequate and reliable database for a proper exposure and risk assessment, the EU Joint Research Center and the WHO have set up monitoring databases (Lineback et al., 2005). Calls for data and spreadsheets for submission of data were posted on the Internet (http://www.irmm.jrc.be/). Other than the data published and posted by the FDA (see above), data submitted to the database are from different sources, using different analytical methods (LC–MS/MS, GC–MS, LC–MS, LC–UV), which makes the assessment of the reliability of the data necessary by providing data on quality measures covering general information and confidentiality, method used, product type and acrylamide level found, and product information.

2.2.3.1. Food levels. Extensive amounts of data on levels of acrylamide have been collected by European authorities, the Confederation of the Food and Drink Industries in the EU (CIAA) and the US Food and Drug Administration (FDA). A website has been created to allow the rapid compilation of data. The website is organised by WHO/JIFSAN. In addition national governments maintain data on their websites. For example, the most recent update of data analysed by the US FDA for acrylamide in foods, posted by the FDA/CFSAN (http://www.cfsan.fda.gov/~dms/acrydata.html) contains 451 datapoints covering a wide range of products tested in the US using the method detailed by Roach et al. (2003) (summarised in Table 2 and Fig. 1).

A large proportion of the current total number of ~5200 entries of the European Monitoring Database was contributed by Germany and the CIAA. Smaller data sets were from the Netherlands, Greece, Austria, Ireland and the UK. After a rigorous review of performance criteria, covering criteria such as limit of detection (LOD), limit of quantification (LOQ) and running *z*-scores, 3442 data sets remained that were considered as reliable by the Institute for Reference Materials and Measurements of the European Commission. A recent update of the database is available on the Internet (http://www.irmm.jrc.be/ffu/acrylamidemonitoringdatabase_statusJune04.xls). The most important matrices included in the database are potato chips, French fries,

Table 2
Summary of acrylamide levels in food derived from the most updated FDA data (collected from 2002 through March 2004)

Food commodity	*n*	Min	25‰	Median	75‰	Max	St. dev.
Baby food and infant formula	36	0.0	0.0	10.0	31.8	130.0	36.6
French fries and chips	97	20.0	220.0	318.0	462.0	2762.0	427.9
Protein foods	21	0.0	0.0	10.0	25.0	116.0	27.7
Breads and bakery products[a]	49	0.0	15.0	34.0	96.0	432.0	107.9
Cereals and muesli	23	11.0	49.0	77.0	166.0	1057.0	249.1
Crackers and snack foods	32	12.0	92.5	169.0	302.3	1243.0	331.1
Gravies and seasonings	13	0.0	0.0	0.0	0.0	151.0	43.4
Nuts and butters	13	0.0	28.0	89.0	236.0	457.0	143.0
Chocolate products	14	0.0	2.5	20.5	84.3	909.0	243.6
Canned fruits and vegetables	33	0.0	0.0	10.0	70.0	1925.0	411.7
Coffee, ground	59	37.0	158.0	205.0	299.0	539.0	106.3
Coffee, brewed	20	3.0	6.0	6.5	8.0	13.0	2.4
Miscellaneous[b]	41	0.0	0.0	10.0	43.0	5399.0	1018.8

Data were calculated from the data published by the FDA on the Internet (Exploratory Data on Acrylamide in Food, March 2004 (http://www.cfsan.fda.gov/~dms/acrydata.html)). The database contains data collected from 2002 through October 1, 2003. The categories were used as given by the FDA. For coffee, only data for roasted coffee were used (total sample number $n = 439$).

[a] Including cookies, pies and pastry, bagels.

[b] Hot beverages other than coffee (Postum, caffeine free coffee substitute), frozen vegetables, dried foods, dairy, juice and other miscellaneous.

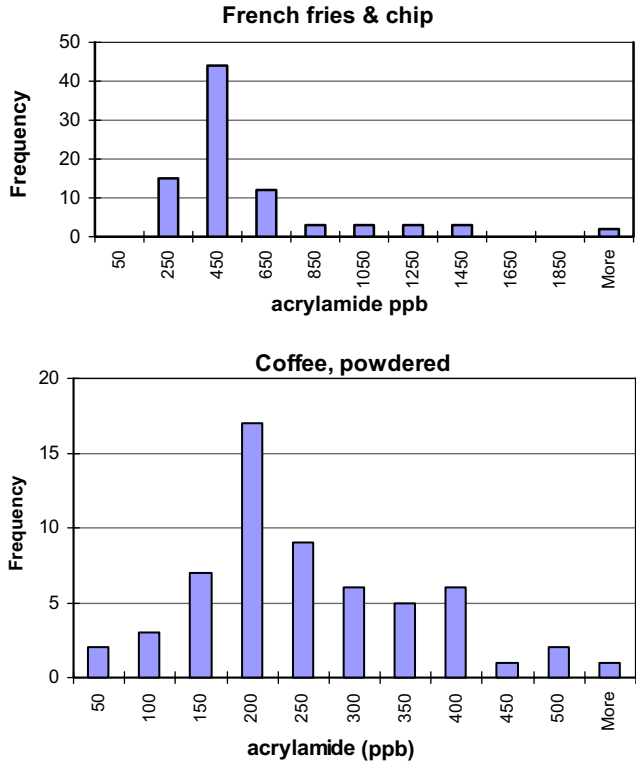


Fig. 1. Distribution of acrylamide levels in potato products (French fries and potato crisps, 85 samples) and coffee (roasted and ground, 59 samples). Data were derived from the FDA results updated on March 2004 (http://www.cfsan.fda.gov/~lrd/pestadd.html#acrylamide).

crispbread, breakfast cereals, fine bakery products and coffee. The database stays open for submission of data (Table 3).

Besides the data on acrylamide levels from European member states and the US, few reports are available on acrylamide in food of Asian or African countries. Leung et al. (2003) analysed ~400 food samples from Asian food in Hong Kong, which, consistent with the known data today vary largely between and within different food categories. Data on human average exposure were not available from this report. Another, smaller study reported levels of acrylamide in food in Japan (Ono et al., 2003). A study on detection, quantification and reduction of acrylamide in African food was launched in Nigeria (Acrylamide Infonet, http://www.acrylamide-food.org/), but results are not yet available.

2.2.3.2. Exposure contribution. After the initial finding of considerable acrylamide levels in various foods, several independent studies have estimated overall exposure levels of the average consumer. Mostly, these estimations were based on acrylamide levels determined by domestic analytical laboratories, and exposure calculations used were based on national food surveys that partially dated several years back and that were therefore not originally designed to assess acrylamide exposure (see Section 2.3).

There are considerable differences even between many of the European countries, due to the differences in food consumption patterns and cooking traditions. Irrespective of the cultural differences in nutritional habits, the overall daily intake is around 0.4 μg/kg bw/day with a 90th percentile of 0.9 μg/kg bw/day according to the most recent FDA estimates, basically confirming earlier estimations from the US and several European authorities (FAO/WHO, European Commission, Swiss Federal Office of Public Health, Swedish National Food Agency, Norwegian Food Control Authority, German Federal Institute for Risk Assessment, Konings et al., 2003; Dybing and Sanner, 2003; Svensson et al., 2003; summarised in Table 4).

The foods that contribute most to acrylamide exposure vary depending upon the population's eating habits and the way the foods are processed and prepared.

Table 3
Acrylamide levels in food as collected by the EU Joint Research Center (updated June 2004)

Food commodity	*n*	Min	25‰	Median	75‰	Max
French fries	741	5.0	90.0	178.0	326.0	2228.0
Chips	569	5.0	378.0	600.0	980.0	3770.0
Potato fritter[a]	75	15.0	215.0	492.0	797.6	2779.0
Fine bakery ware	485	5.0	67.0	160.0	366.0	3324.0
Gingerbread	414	5.0	152.0	298.5	650.7	7834.0
Crispbread	261	5.0	81.0	251.0	602.0	2838.0
Infant biscuits	63	5.0	64.3	90.0	275.1	910.0
Diabetics cakes + biscuits	212	5.0	92.5	291.5	772.3	3044.0
Breakfast cereals	162	5.0	30.0	60.0	152.5	846.0
Coffee roasted	102	79.0	192.0	264.0	337.0	975.0
Coffee substitutes	50	115.6	439.4	739.0	1321.8	2955.0

Data were taken from the monitoring database on acrylamide levels in food (http://www.irmm.jrc.be/) maintained by the IRMM (Institute for Reference Materials and Measurements, together with Health and Consumer Protection DG (DG SANCO). This database comprises 3442 samples of acrylamide levels in food products throughout the EU, including the data collection from the CIAA (Confédération des Industries Agro-Alimentaires de l'UE). The categories were used as given in the data collection.

[a] Grated potatoes fried into a pancake.

Table 4
Exposure estimates from 2002–2004

Exposure assessment	Daily intake μg/kg bw/day		Source
	Mean (age group)	95th percentile; *90th percentile	
FAO/WHO (2000)	0.3–0.8		http://www.who.int/foodsafety/publications/chem/en/acrylamide_full.pdf
EU, SCF (2002)	0.2–0.4		http://europa.eu.int/comm/food/fs/sc/scf/out131_en.pdf
BfR, Germany (2002)	1.1 (15–18)	3.4	http://www.bfr.bund.de/cm/208/abschaetzung_der_acrylamid_aufnahme_durch_hochbelastete_nahrungsmittel_in_deutschland_studie.pdf
BAG, Switzerland (2002)	0.28 (16–57)		http://www.bag.admin.ch/verbrau/aktuell/d/DDS%20acrylamide%20preliminary%20communication.pdf
AFSSA, France (2002)	0.5 (>15) 1.4 (2–14)	1.1 2.9	http://www.afssa.fr/ftp/afssa/basedoc/acrylpoint2sansannex.pdf
FDA (2002)	0.7		http://www.jifsan.umd.edu/presentations/acry2004/acry_2004_dinovihoward_files/frame.htm
FDA (2003)	0.37 (>2) 1.00 (2–5)	0.81* 2.15*	See FDA above
SNFA, Sweden (2002)	0.45 (18–74)	1.03	Svensson et al. (2003)
NFCS, Netherlands	0.48 (1–97) 1.04 (1–6) 0.71 (7–18)	0.60 1.1 0.9	Konings et al. (2003)
SNT, Norway (2003)	0.49 (males) 0.46 (females) 0.36 (9, boys) 0.32 (9, girls) 0.52 (13, boys) 0.49 (13, girls) 0.53 (16–30, males) 0.50 (16–30, females)	1.01* 0.86* 0.72* 0.61* 1.35* 1.2*	Dybing and Sanner (2003)
FDA (2004)	0.43 (>2) 1.06 (2–5)	0.92* 2.31*	See FDA above

Generally, the most important categories of food appear to be: fried potato products such as French fries and chips, ready-to-eat breakfast cereals, baked goods such as cookies, pies and cakes, brewed coffee and breads, according to the most recent results of the FDA/CFSAN (Workshop on Acrylamide in Food, 2004 http://www.cfsan.fda.gov/~dms/acrydino.html).

The main sources of acrylamide in food vary with the national/regional food habits, e.g. French fries and other potato products are consumed at relatively high amounts in the US (35% of the average daily acrylamide intake), whereas coffee and bread contribute relatively little to the average daily US acrylamide intake (7% for coffee, 11% for toast and soft bread). The contribution of potato products is even higher in The Netherlands, with French fries and chips taken together contributing up to 50% (Konings et al., 2003). On the other hand, the contribution of coffee and bread or crispbread is much higher in European countries. According to Dybing and Sanner (2003), potato products contributed ~30% to the daily acrylamide intake in Norway, whereas coffee and bread contributed 28% and 20%, respectively. Similar results for Sweden were reported by Svensson et al. (2003) (coffee 39%, potato products 26%, bread and crispbread 17%).

The overall useful perspective provided in such studies has to be applied with caution, though, when conclusions are to be drawn for specific categories or products. In some cases, the product classifications used in these studies are not aligned with the actual market situation. The total amounts of a category available in the market place may be significantly less than would be assumed from the exposure estimates. The exposure figures reported e.g. for Norway or Holland are equivalent to the per capita consumption of snacks in general, including pretzels, nuts, crackers and many other products with a relatively low acrylamide content. Only about 37% (for The Netherlands) or 47% (for Norway) of the total snack consumption are indeed potato chips and other potato based products.

In several cases the estimated dietary intake of acrylamide by children, adolescents and younger men

were considered to be significantly higher than for adults in general. A study of the German BfR identified a higher consumption of French fries and potato chips by children and young adults (10–24 years of age) as compared to the rest of the population (BfR, 2003). At the same time, the coffee consumption is lower, so that the relative contribution of French fries and potato chips to overall acrylamide exposure becomes higher. Children also have a lower average body weight than adults and a higher average food intake per kg body weight than adults. Thus on a body weight basis the exposure to various substances could be higher for children compared to adults. This was particularly evident from estimates made on the intake of acrylamide in children and adolescents in Norway. According to the Scientific Committee of the Norwegian Food Control Authority (SNT, 2002), potato chips contribute mostly to the total mean intake (24.4% males, 32.6% males (16–30 years), 24.4% females). The largest sources of intake of acrylamide both in the 9- and 13-year olds were potato crisps, but also crackers and biscuits are important sources. Together with other snacks, potato crisps constituted a third of the estimated intake in adults. In younger men, the proportion of acrylamide from potato and other snacks is estimated to over 40%. Also, fried potatoes and French fries contribute relatively much to the intake of acrylamide compared to the amounts consumed of these foods. Bread, with the exception of potato cakes and crispbread, contains low amounts acrylamide. However, since bread is eaten daily in relatively high amounts in Norway, the calculations show that the food groups white bread and bread, other (white bread, medium-grain and grain bread together with crispbread and potato cakes/hot dog roll) contribute with a considerable proportion of the acrylamide intake (males 29.2%, males (16–30 years) 20.9%, females 32.7%).

Uncertainty analysis. Exposure estimates can be uncertain due to the underlying data as well as due to assumptions used in the exposure assessment methods. In the case of acrylamide, analytical methods have evolved rapidly; the results to date may be uncertain due to evolving development of analytical methods as well as differences in the extent of sampling of foodstuff.

Even though the analytical methods to determine the levels in different food matrices have been considerably refined compared to 2002, and although also exposure assessments were conducted using different survey data with different objectives, sample sizes and, therefore, different weaknesses, most of the recent data did not prominently differ from the early estimates performed between 2002 and 2003. This suggests that any risk assessment, at least for the average consumer, will most probably not be influenced by ever more exact analytical methods.

Existing food consumption surveys were designed for purposes other than assessing acrylamide intake and therefore assumptions as to the levels of acrylamide in different foods are required. The assumptions made, regarding what foods are consumed by which consumers and how those foods were prepared contribute to the overall uncertainty.

Thus, the assessment of the risk for subgroups with higher intakes/potential susceptible subgroups will clearly be impacted by the type and quality of food consumption surveys (see Section 2.3). In this respect, a food survey among Berlin 10th grade pupils between 15 and 18 years of age should be highlighted, which discovered an average acrylamide intake of 1.16 μg/kg bw/day and a 95th percentile of 3.24 μg/kg bw/day. Even if the study was not representative for the overall population (restricted to Berlin) and was selective for a period of time with a bias towards a high consumption of specialty food (pre-Christmas, e.g. gingerbread that was found to have very high acrylamide levels), the study demonstrates especially high exposure of a certain subgroup.

Little information is available on the acrylamide levels in food cooked at home and the overall contribution of home cooked food to acrylamide exposure. Estimates of a 50% contribution to overall acrylamide intake have been made. Research on the formation of acrylamide during home cooking are under way in the US (National Center for Food Safety and Technology, NCFST) and in the UK (Food Standards Agency, FSA) (http://www.acrylamide-food.org/), but no quantitative results on exposure contribution are available to date.

A small duplicate diet study was performed in Switzerland (Swiss Federal Office of Public Health, 2002) with 27 participants, who delivered a mirror image of their diet to the analytical laboratory during two days. The contribution of the different meals and beverages to the overall acrylamide intake was as follows: breakfast 8%, lunch 21%, dinner 22%, snacks 13% and coffee 36%. An average daily intake of 0.28 μg/kg bw/day was estimated, however the study seemed to have been biased towards an under-representation of fried, deep fried, baked and roasted potatoes, probably the most critical type of high level acrylamide foods prepared at home. In addition, the study did not discriminate between the food actually prepared (cooked, fried, baked) at home and the food bought as ready for consumption.

2.2.4. Approaches to control and reduce acrylamide food levels

When introducing measures to mitigate acrylamide food and exposure levels, one has to discriminate between the different types and levels of approaches. The following section on the German dynamic minimisation concept describes a tool on the level of risk management. The second example on the Swiss pilot study on

French fries is a very pragmatic approach on the actual transfer of recommendations made by experts to the 'real' world of restaurants and catering services that could potentially be applied also to home cooking. The third example is a purely hypothetical exposure calculation of 'what if' scenarios. Considering the latter example it should be stressed that, based on the available knowledge of acrylamide formation in food, the complete elimination of acrylamide from any food category is not a realistic possibility for the foreseeable future.

2.2.4.1. German dynamic minimisation concept. The minimisation concept was introduced in Germany (Federal Office of Consumer Protection and Food Safety (BVL), the German federal states, industry, and the Federal Ministry of Consumer Protection, Food and Agriculture) as a measure to encourage and monitor the reduction of acrylamide levels in food. Analytical results from official food surveillance laboratories were used to calculate a signal value, which is defined as the lowest value of the upper 10th percentile, determined for different food categories. A signal value higher than 1000 μg/kg is automatically set to 1000 μg/kg. If individual values exceed the signal values, the food control authorities contact food producers and enter into the so called 'minimisation dialogue' with the aim to reduce the levels found via changing ingredients and/or processing conditions. Three signal value calculations were published to date based on approximately 4200 samples obtained in Germany (Table 5). The signal values are updated on a regular basis by the BVL. Separate information collected by the German chip manufacturers during the co-operation with the German authorities on the dynamic minimisation approach have indicated a net decrease of approximately 30% in this category after accounting for the large seasonal fluctuations observed, and after exploitation of the available mitigation opportunities (Raters and Matissek, 2004). This illustrates even for a relatively simple food category both the potential successes and the barriers for reduction efforts.

Table 5
Summary of the signal values established by the German Federal Office of Consumer Protection and Food Safety, BVL[a]

Food group	Signal values acrylamide (μg/kg)		
	17.09.2002	31.01.2003	26.11.2003
Fine bakery products of short pastry	800	660	575
Breakfast cereals	260	260	200
Coffee roasted	370	370	370
Chips	1000	1000	1000
Crispbread	610	610	610
French fries prepared	770	570	570
Potato fritter prepared	1000	1000	1000
Gingerbread and bakery ware containing gingerbread	1000	1000	1000
Thin almond biscuits	1000	710	710
Children's biscuits	nc[b]	nc[b]	360
Diabetics' cakes and biscuits	nc[b]	nc[b]	1000
Coffee extract	nc[b]	nc[b]	1000
Coffee substitute	nc[b]	nc[b]	1000

[a] *Source*: http://www.bvl.bund.de/acrylamid/dl/signalvalues_all.pdf.
[b] Not calculated.

However the difficulties and uncertainties remaining in analytical methods, especially in the light of the different sources of variability, need to be taken into consideration. Small reductions in acrylamide levels will be hidden by the large variability between samples even though the accuracy (low bias) and precision (variability) of the analytical methods are satisfactory to date (repeated testing of the same sample would not improve the result). The high variability between samples, even between different samples of the same production lot, would make the testing of a large number of samples necessary in order to prove statistical significance of a reduction effect. This is even more complicated when analysing data from different laboratories using different methods, which is a weakness of the German minimisation concept.

Due to practical constraints in data collection, the observed reduction of acrylamide levels in specific food categories were not regarded as significant (BfR, 2004). However, the BVL considered the importance of the approach and has taken action with respect to improve the co-ordination of sampling, the traceability of samples and the categorisation of product groups. It should be pointed out that this approach is limited to products as sold, and does not apply to food prepared at home, by catering services or restaurants.

In addition, depending on the amount and frequency of consumption, even a significant reduction of the signal value in one or more categories would not necessarily decrease overall exposure of consumers to acrylamide. Consequently, a direct assumption as to a reduction of exposure due to reduction of signal values cannot be made.

2.2.4.2. Swiss pilot study on the reduction of acrylamide levels in French fries. In 2003, the Swiss Federal Office for Public Health initiated a pilot study involving restaurants, hotels and catering services aiming at the reduction of acrylamide levels in French fries without compromising the quality and organoleptic properties. Participants were advised to maintain the frying oil temperature at 170 °C, to use no more than 100 g of potatoes per liter of oil, and to aim for a golden colour of the fries rather than strong browning. The analysis of more than 150 samples revealed a more than 4-fold reduction compared to previous measurements (http://www.bag.admin.ch). Similar advice with respect to browning, frying and baking temperatures is given to

consumers by various consumer organisations. However, domestic or restaurant preparation of French fries cannot be controlled or standardised as strictly as it is the case for processing conditions applied in the food industry, e.g. in the production of potato chips or the industrial roasting of coffee. In addition, it remains to be established if such measures eventually result in a significant reduction of the average exposure of consumers. Thus, any effort to understand or estimate the level of acrylamide uptake from domestic cooking should be accompanied by the assessment of exposures using biomarkers of exposure that can give a more reliable estimate of the true actual exposure of consumers (see Section 2.5).

2.2.4.3. Theoretical impact of mitigation of acrylamide exposure. An interesting theoretical effort has been made to determine effects of mitigation on overall acrylamide exposure (unpublished data presented on the JIFSAN Workshop on Acrylamide in Food, 2004, summarised in Table 6). 'What if' scenarios were calculated based on the updated exposure assessment of the FDA, entirely eliminating one or the other source of acrylamide exposure, such as e.g. coffee or French fries. Although one of the major sources of acrylamide exposure, the total elimination of acrylamide from French fries in the US would reduce the overall acrylamide exposure by 14%, whereas elimination of acrylamide from coffee would reduce the acrylamide intake by only 7%. On the one hand such calculations have to take into consideration cultural differences in food consumption, on the other hand they demonstrate the difficulties in considerably reducing the overall acrylamide intake. Acrylamide is ingested from many different types of food, with different levels of acrylamide and at different frequencies. A total elimination of acrylamide from the food high in acrylamide today cannot be expected, thus small reduction steps will only marginally influence the overall acrylamide intake, at least on a short term perspective.

Assuming that the 'what if' scenarios by FDA used indeed relevant intake estimates, the actual improvements observed for potato chips in Germany (see above), i.e. a reduction by ca.30%, would leave only a reduction of approximately 4–14% in overall acrylamide intakes. This impact would be even less in markets such as most European countries where chip consumption is much lower.

2.2.5. Exposure calculations based on the measurement of biomarkers

Apart from the calculation of exposures based on levels in food and food consumption data, the exposure to acrylamide has been calculated back from the levels of haemoglobin adducts determined in human blood. Haemoglobin adducts of acrylamide are used as biomarkers of exposure covering 120 days (corresponding to the lifespan of red blood cells). The background level of haemoglobin adducts has been estimated to correspond to a daily intake of approximately 100 μg of acrylamide per day, corresponding to 1.7 μg/kg bw/day for a 60 kg person (Tareke et al., 2002). Exposure levels recently calculated from haemoglobin adducts were 1.44 μg/kg bw/day (unpublished data presented on the JIFSAN Workshop on Acrylamide in Food, 2004), thus being around 3-fold the exposure calculated from acrylamide concentrations found in food (cf. calculation in Section 3.3.2.5). It cannot be ruled out to date if these higher estimates are related to an unknown source of acrylamide exposure. The cohort used for the latter estimation was very small and, therefore, probably not representative for the average population. Ongoing efforts will clarify this, as e.g. a study monitoring acrylamide adducts in blood in the US National Health and Nutrition Examination Survey (NHANES), a national health survey that collects biological samples and health data from individuals throughout the USA or the EPIC study (European Prospective Investigation into Cancer and Nutrition) currently conducted in Europe.

2.2.6. Conclusions

- Analytical studies both on chemical model systems and in food model systems confirmed the Maillard reaction between asparagine and reducing sugars as the main mechanism of acrylamide formation. Several minor pathways have been identified, probably with little contribution to overall levels in most foods.

Table 6
Calculated reduction scenarios based on data presented by FDA/CFSAN[a]

Daily acrylamide intake	Mean (μg/kg bw/day)	90th percentile (μg/kg bw/day)	% reduction	
			Mean	90th
Current average exposure	0.43	0.92	–	–
Elimination of acrylamide from French fries	0.37	0.78	14.0	15.2
Elimination of acrylamide from snack foods	0.38	0.85	11.6	7.6
Elimination of acrylamide from breakfast cereal	0.38	0.84	11.6	8.7
Elimination of acrylamide from coffee	0.40	0.88	7.0	4.3

[a] Workshop on acrylamide in food, 2004; http://www.jifsan.umd.edu/acrylamide2004.htm.

- Analytical methods are available to determine levels of acrylamide in food with sufficient accuracy and precision as shown in several interlaboratory proficiency tests. Extraction methods are available also for difficult matrices such as coffee and cocoa.
- Consequently, the ongoing efforts to refine analytical methods will not markedly influence any risk assessment.
- The high between-sample variability is still a problem in terms of the verification of slight reductions in acrylamide levels in food (mitigation efforts, German minimisation concept) to overall reductions in consumer exposures. (High sample numbers will be necessary to reach adequate statistical significance/power).
- Databases were set up in the EU and the US that collect reliable, quality assured measurements. The EU monitoring database covers more than 3000 reliable datapoints, however largely contributed by Germany, but covers the relevant food categories that contribute the most to overall exposure. An initial evaluation showed stagnation in the reduction of acrylamide levels in food.
- Attempts to reduce acrylamide levels in foods should be positioned against associated changes in the product which may potentially increase the levels of other undesired components (e.g. fat) and/or decrease the levels of substances with desired properties. An appropriate comparison of added risks and/or reduced benefits with the benefit of acrylamide reduction is needed.
- The major food categories contributing to most of the human exposure are similar in North America and Europe, comprising French fries, potato chips, cereals, crispbread, bread, coffee, pies and pastry. Little is known about non-European, non-North American countries. Some food levels are available from Japan, Hong Kong and South Korea, but no exposure levels were determined.
- The major food categories containing acrylamide and exposures estimates of the average consumer appear to be confirmed. Recent research has confirmed that early studies were largely correct, while also providing increased reliability of analytical methods and expanding the underlying databases.
- Exposure estimates are available for North America and some European countries. Several exposure assessments have identified a higher exposure for the younger age groups, which appears to be significantly higher than the average consumer.
- Subgroups who may be particularly susceptible to the effects of acrylamide have not been specifically identified. Pregnant women do not seem to represent a susceptible subgroup, since a recent NTP/CERHR panel report concluded a negligible concern for adverse reproductive and developmental effects for exposures in the general population (NTP CERHR, 2004).
- A significant reduction of acrylamide exposure based on reduced levels of acrylamide in food would clearly have consequences on assessed risks.

2.2.7. Uncertainties/gaps

- A few specialty food types containing acrylamide do not fit into the general picture of foods heated to high temperatures (olives, prune juice, autoclaved laboratory animal feed).
- Other pathways of formation may have to be considered under certain conditions for specific food types, however these are unlikely to make up a large fraction of overall acrylamide intake.
- Some recommendations were made to reduce levels in food (EU guidance), with limited applicability, for example to potato products, or catering services/home cooking. Recommendations to other home cooked foods or industrially produced products (cereals, coffee) are still largely lacking.
- Data on overall exposure appear to be consistent for EU and the US, but the role of different diets (cultural differences) on exposure has not been assessed in detail.
- Information on contribution of home cooked food and the catering sector to the overall acrylamide exposure is lacking.
- Susceptible subgroups have not been identified to date.
- Extrapolation of external exposure (dietary food levels) to internal exposure (biomarkers) or vice versa should be improved. There may be unknown sources of acrylamide (discrepancy between calculations from biomarker and food intake, see Section 2.5).

2.3. Parameters influencing the result of the exposure assessment

It is well recognised that the exposure to acrylamide is related both to food and non-food sources. A substantial non-food source of exposure to acrylamide is during tobacco smoking and possibly also passive smoking (Bergmark, 1997). Dermal exposure to acrylamide in polyacrylamide from cosmetics and indirectly in drinking water from water treatment, seems to contribute at a level well below the intake levels implicated for foods (European Commission, 2002b).

One of the key questions to be raised in order to try to quantify the risk of exposure to acrylamide in food is to assess the correct quantity of this substance ingested by human populations. Therefore many scientists and risk managers are performing exposure assessment using data available for them and, very often, without considering the necessity for a comparison between similar

exercises at a later stage. The aim of this section is to point out the influence of the quality of the initial data set on the result of the assessments, but can be also used as a check list of the parameters to be considered before comparing studies from various origins.

2.3.1. Matching exposure time frames

Since a potential critical endpoint of acrylamide exposure is cancer, the intake for this substance should be assessed over a 'chronic' or 'life-time' period. Moreover, intake assessment for acrylamide combines food consumption data with data on the level of acrylamide in food. Therefore, the resulting output of this assessment should be ideally the magnitude of the exposure and the size of the population exposed during life-time over a certain limit considered as virtually safe by risk assessors.

2.3.1.1. Choice of data on food consumption. Food intake data are generally not obtained from studies designed originally for food safety issues, but more often for economical monitoring or nutritional purpose (Kroes et al., 2002). Available data are derived from two types of surveys:

- Economical surveys for households or population collected during several months and up to 1 year (www.nut.uoa.gr).
- Individual food consumption data collected during 1–7 days by various techniques (EFCOSUM, 2002).

It appears that none of those data are fully adequate to represent the individual long-term exposure. At first, food balance sheet data (population-based method) and household economic surveys and retail sales surveys (both household-based methods), do not account for household wastage, which would tend to overestimate intake. On the other hand, they may underestimate intake for individuals highly exposed (WHO, 1997). That is the case, on the one hand, of consumers of food with a high concentration of acrylamide in a country-based survey or could be the case, on the other hand, of children in a household budget survey.

In contrast, surveys based on individuals can overestimate the magnitude of the exposure for food which is not consumed regularly. For example considering a consumer eating 1 time a month a bag of 140 g of potato chips: if the bag is eaten during a survey period of 7 days, the resulting consumption will be 140/7 = 20 g per day. At the same time if the bag is not eaten during the duration of the survey, the subject will not be included as a consumer; therefore the percentage of consumer will be underestimated. A report on this specific topic was published (Lambe et al., 1998). Such a bias can be avoided using advanced statistical techniques (Slob, 1993).

2.3.1.2. Food categorisation/level of aggregation. Food consumption expressed in terms of foodstuffs consumed is variable from one country to another as a function of national dietary habits even if the intake in nutrients is not fundamentally different between human populations. Each food intake survey is structured with a food categorisation system corresponding to the declaration of survey participants, aggregated or not as a function of the objectives of the study. The number of food items could be from several tens to several hundreds (for example, 880 in the French study on individuals (CREDOC, 1999)). At such an accurate level a consistency between countries would be very unlikely. Moreover, it is not possible to match each of these food items with information on chemical contents.

Therefore, one solution consists of "aggregating" the food consumed in broader food categories in order to get a better fit between national databases. Such an aggregation process is conducted in general with 15–50 food categories common with both chemical concentration data and dietary surveys (Cost 99; Gems Food; Eurofood). However, this method can also overestimate the exposure if a chemical of concern is contained in a very specific food, consumed by a limited part of the population. For example the use of a broad category "cereals and cereal products" allows for a good comparison at the international level, but implies when considering acrylamide that this compound should be contained at a similar level in all "cereals and cereal products" which is usually not the case.

2.3.1.3. Composite foods. Another point which should be raised as influencing the precision of exposure assessment is the fact that a large part of the diet is based on composite food. Therefore, when a chemical is measured in a food component (e.g. flour) the concentration of the chemical in food as consumed will depend of the percentage of the component (e.g. flour in pizza or in a breaded fish). The extrapolation of concentration of chemical from food component to whole food as consumed can results in important overestimation of the exposure.

2.3.1.4. Food intake distribution or mean intake. There is not a unique standard for methodologies to be used and surveys performed at national level are difficult (if not impossible) to combine. Therefore international exposure assessments are often using average values for food consumption data or by summarising the ranges reported by national authorities. A number of comparisons have been done in order to relate this mean intake with a high percentile of the distribution for a considered food item and it is generally accepted that when the food item is consumed regularly by 100% of the population, the 95th percentile is two times the mean and the 97.5th percentile is three times the mean (WHO, 1997).

It is important to note that for certain food categories consumed in large amounts by a relatively small number of consumers, this ratio can be higher. In consequence when the distribution of food consumption is not available, the mean consumption can be considered as a representative value for at least 50% of the population. The extrapolation to high percentiles should be interpreted carefully but can, in most cases, give an indication whether or not a more accurate study is necessary.

2.3.1.5. Choice of data on food concentration. Interpretation of the analytical data produced from the total number of food samples analysed for acrylamide; as for other chemicals in food, is difficult for three reasons. Firstly, some samples effectively do not contain acrylamide. Secondly, some samples do contain acrylamide at a level below the limit of detection (LOD), and lastly some samples contain acrylamide at an amount which is below the limit of quantification (LOQ) of the analytical method. In addition, those limitations are different from one laboratory to another; therefore it is necessary to define a rule for using those data points which could represent 30–50% of the results. Even if there is no perfect solution, it is generally agreed that the solution proposed by WHO (WHO, 1985) can be used. It proposes to replace results for samples below the LOQ by half of the LOQ value if the number of samples below the LOQ is lower than 60% of the total number of samples.

Analytical results available for risk assessment consist in both individual and aggregated results, i.e. one result could correspond to a single sample or to many samples aggregated together. In order to generate a distribution curve, it is necessary to combine those data submitted under various formats and from different sources (FAO/WHO, 2000). The combination of these data should permit a mean contamination level to be calculated, with possible weighting of each result as a function of the number of single samples from which it was composed. In addition, based on the assumption that the distribution of contaminant data follows a log-normal distribution, a log transformation of the data can provide the standard deviation and a simulated distribution including high percentiles.

2.3.1.6. Choice of method to combine data. The consumption of food, and thereby the concentration of chemicals in food is not constant or linear, but distributed following a relationship depending on the food and chemical considered. As a function of the objectives of the assessment, those two distributions can be combined differently therefore providing different levels of accuracy.

The point estimate is a simplistic way consisting of multiplying a single level of consumption by a single level of contamination. Such a method is very useful as a screening tool because in many cases, the intake resulting of a high percentile of food consumed multiplied by a high percentile of contamination level, may be below the toxicological level of concern.

When this is not the case, a more sophisticated approach should be taken as a second step to assess the probability of exceeding a guideline value with respect to exposure duration and amongst the population. The estimation of other 'points' such as mean consumption multiplied by mean contamination or high consumption multiplied by mean or median contamination should give an useful idea of the magnitude of the exposure and the size of the population exposed, but should not be taken as a precise representation of the reality.

Various methods could be performed to assess the probability of exceeding a toxicologically-based limit. The most classical one is the plug-in method also called ''Monte Carlo'' which consists in randomly combining levels of food consumed by level of chemical in food. Another method is to combine the distribution of food consumption with the mean or median concentration level and to measure the empirical probability of exceeding a limit considered to be safe. A third method consists in modelling of the tail of distribution accordingly with mathematical validated methods (Tressou et al., 2004).

Any of these approaches should be carefully considered before being used, and the choice will depend of the state of knowledge and the quality and quantity of data available. In general when the exposure is intended to be compared with real long-term (life-time) effects, the double random (stochastic) approach will be overestimating the risk compared with the empirical probability using the mean or the median concentration value. In addition, the use of the median is in general more realistic than the mean, which will be overestimating the risk when the distribution curve is very skewed (a lot of points in the tail). Finally, when the exposure is low compared with a guideline value, a modelling of the tail is necessary in order to avoid underestimating the risk for rare highly exposed subjects.

2.3.1.7. Body weight. Most of the surveys based on individuals are designed for nutritional purposes and include the collection of body weight. Therefore, it is possible to express the exposure to acrylamide or to any chemical in mg per kg body weight. Compared with the classical approach consisting of using a typical weight of 60 kg, this assessment is more accurate and considering the real range of body weight of the European population, it corresponds to an improvement of the precision which could reach 15–20%.

2.3.2. Conclusion

The aim of this section was to raise the key parameters to be considered in order to assess the exposure to a chemical in general and to acrylamide in particular. It is

clearly apparent that both the data sets considered and the methods chosen to combine the data, are crucial to determine the uncertainty and variability of the assessment, and to know if one assessment is comparable to another. In addition, this section could be used by specialists as a check list of the data submitted.

2.4. Inventory of the existing acrylamide surveys and databases

A questionnaire on published and unpublished studies on acrylamide dietary exposure was circulated by the European branch of the International Life Sciences Institute (ILSI Europe) to relevant national and international institutes and individuals in 31 countries in Europe and elsewhere (USA, Australia, Korea, Japan, WHO). The questionnaires were completed for studies conducted in 10 countries and summarised in Tables 7–10, Fig. 2 and Appendix A. For the other countries, no information was obtained either because no studies were conducted, because the studies were still on-going or because the right contact person or address was not known. The main purpose of this exercise was not to make an exhaustive inventory, but to give examples of the type of study designs and dietary exposure data available for risk assessors and highlight the current methodological strengths and limitations for estimating and interpreting them.

Most of the acrylamide exposure measurements reported in Table 7 were assessed using existing available data from national monitory surveys. These involve representative and large population samples enabling exposure assessments according to gender and different age groups, depending on the studies. However, the number and age group definition vary across studies, particularly among (young) children and adolescents (Table 10). All exposure assessments were performed in the same short time interval (i.e. 2002–2004), but using consumption data collected up to 14–18 years before.

The predominant dietary methods used for acrylamide assessment in the selected studies (Table 8) are open-ended methods such as (repeated) 24-h diet recalls (HDRs) and (weighed) dietary records. The number of repeated dietary measurements per individual varies from 1 to 7 across studies and is collected consecutively, i.e. not evenly distributed among seasons (or weekdays). Although these methods differ (Bingham, 1987; Cameron and van Staveren, 1988; Willett, 1990), they share common features by providing information on current or recent diet (i.e. previous day for 24-HDRs) and a high level of detail on the foods consumed as compared to food frequency questionnaires (FFQs). A FFQ was used only in Norway and Germany (with two consecutive day records). This method consists in collecting information on usual diet over a longer time-period, usually the previous year. The frequency of consumption on a restricted and fixed list of foods (e.g. 180 in Norway) is systematically collected while standard or individual food portions are used for food quantification, depending on the type of questionnaire design used (i.e. non-, semi- or quantitative FFQs) (Bingham, 1987; Cameron and van Staveren, 1988; Willett, 1990).

The food consumption monitoring surveys have the advantage of providing information from a large and representative sample of the general populations. These easily available data also enable first rapid dietary assessments for risk assessors and health authorities, particularly in cases of immediate concern or newly identified dietary exposures. However, they also have some limitations. The time interval between the dietary data collection and the actual acrylamide assessment may be long (up to 14–18 years in the German study using monitoring survey data collected in 1985–1989). The exposure estimates may then not represent the current level of intakes, particularly among populations at risk, with rapid dietary changes such as young population groups and children (Amorim Cruz, 2000; Rolland-Cachera et al., 2000; Samuelson, 2000). Because of the immediate situation concerns raised following the identification of acrylamide in foods, available existing dietary data had to be used. However, these studies and the dietary methods used were not designed to respond specifically to the question addressed. For example, the preparation and cooking methods of industrial and homemade foodstuffs, considered to be one of the main determinants of the formation of acrylamide (see Section 2.2), was not systematically asked for in all studies. One third of them did not collected details on individual cooking methods. This information is crucial during the data handling in order, for example, to separate boiled potato, which does not contain acrylamide, from the other potato products with different levels of acrylamide, depending on their physical state, preparation and cooking method processes (>120 °C and low moisture). Another problem is the initial food aggregation and classification used which may not have the flexibility to be re-considered according to new hypotheses, depending on how the data had been handled and stored.

Twenty four-hour diet recalls and food record methods seem more appropriate for investigating acrylamide exposures than FFQs by providing greater levels of detail about the processing and preparation procedures for the consumed foods. For example in the case of starch-rich foodstuffs, both industrial (brand names, product names) and homemade (preparation and cooking processes) need to be known in order to accurately predict acrylamide levels.

However, the major problem which may be associated with these 24-h diet recalls and record methods is an insufficient number of repeated measurements per study subject in order to measure accurately individual

Table 7
Study design and references

Country	Year of acrylamide assessment	Study (source of dietary data)			References
		Year	Name	Type	
Australia	2002	1995	Acrylamide dietary exposure assessment for Australia (National Nutrition Survey of Australia)	National monitory survey	Results used in the WHO–FAO consultation report (Geneva, June 2002)
	2003	1995	Australian survey of acrylamide in carbohydrate based foods (National Nutrition Survey of Australia)	National monitory survey	Croft et al. (2004)
Belgium	2003	1997	Teenager-study	Local survey in the city of Ghent	Available on: http://www.publichealth.ugent.be Matthys C. et al. (2004)
Czech Republic	2002 and 2003	1997	Dietary exposure monitoring. Study of acrylamide exposure sources in foods	National monitory survey (HHBS)	Available on www.chpr.szu.cz/publications/2004/TDS2004.pdf (Ruprich et al., 2004)
France	2002	1998–1999	INCA survey (Individual and national French food intake survey)	National monitory survey	Volatier, (2000)
Germany	2003 2003	1985–1989 2002 (Oct–Nov)	(1) Nationale Verzehrstudie (NVS 1989) (2) Acrylamide Jugendbefragung, Berlin (2002)	National monitory survey Feasibility study	Adolf et al. (1995) Available on http://www.bfr.bund.de/cm/208/abschaetzung_der_acrylamid_aufnahme_durch_hochbelastete_nahrungsmittel_in_deutschland_studie.pdf
Norway	2002	1997 (adults) 2000 (children)	(1) NORKOST (1997) (2) UNGKOST (2000)	National monitory survey	Johansson and Solvoll (1997) Overby and Andersen (2000) Dybing and Sanner (2003)
Sweden	2002	1997–1998	Riksmaten	National monitory survey	Becker and Pearson (2002) Svensson et al. (2003)
Switzerland	2002 (Sept)	2002 (Sept.)	Assessment of acrylamide intake by duplicate diet study	Duplicate diet study	http://www.bag.admin.ch/verbrau/aktuell/d/DDS%20acrylamide%20preliminary%20communication.pdf
The Netherlands	2002	1997–1998	Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks	National monitory survey	Konings et al. (2003) Rosén and Hellenäs (2002)
USA	2004	1994	USDA Continuing Survey of Food Intake by Individuals	National monitory survey	http://www.usda.gov (dietary survey), http://www.cfsan.fda.gov (presentation of acrylamide analysis—not yet available)

(a): Does not include results on acrylamide.

Table 8
Study population characteristics and dietary methods

Country	Population				Dietary method	Number of foods	Cooking method
	Origin	Sample size	Gender	Age (years)			
Australia	General population	(1) 13,858 (2) 989 (3) 1090 (4) 928	(1) F + M (M = 48%) (2) F + M (M = 48%) (3) F + M (M = 52%) (4) F + M (M = 53%)	(1) ⩾2 (2) 2–6 (3) 7–12 (4) 13–18	A 24-h recall	Open-ended	Yes
Belgium	Adolescents	341	F + M (M = 38%)	13–18	7 consecutive day record (diary)	Open-ended	No
Czech Republic	General population	420 HH with 1030 persons		1–98	1 month household record method. Calculation for average person (64 kg)	Open ended	Yes
France	General population	(1) 1474 (2) 1018	(1) F + M (M = 46%) (2) F + M (M = 52%)	(1) >15 (2) 3–14	7 consecutive day record (diary)	Open-ended	Yes
Germany	(1) General population (2) Adolescents (non-representative sample)	23,209 1085	F + M (M = 47%) F + M (M = 52%)	4–>64 15–18	7 consecutive weighed day records (diary) 2 consecutive day recall + semi-quantitative FFQ for selected food groups	Open-ended 11 food groups	Yes No
Norway	General population	(1) 2672 (2) 1815	(1) F + M (M = 49%) (2) F + M (M = 50%)	(1) 16–79 (2) 9–13	(1) Self-reported Quantitative FFQ (2) 4 consecutive day record	(1) 180 (2) Open-ended	No No
Sweden	General population	1211	F + M (M = 48%)	18–74	7 consecutive day record (diary)	Pre-coded/ open-ended	Yes
Switzerland	General population (non-random sample)	27	F + M (M = 52%)	16–57	Duplicate portions of all solid foods consumed during 2 non-consecutive days. The beverages and their quantities were only listed[a]	Not relevant	Yes
The Netherlands	General population	(1) 6250 (2) 1009 (3) 530	(1) F + M (M = 54%) (2) F + M (M = 51%) (3) F + M (M = 48%)	(1) 1–97 (2) 7–18 (3) 1–6	2 consecutive day record	Open-ended	Yes
USA	General population	(1) 18,081 (2) 5447	F + M (M = 51%) F + M (M = 51%)	(1) >2 (2) 2–5	One day record and one 24-h recall	Open-ended	No

[a] For more details see the protocol available in the website address given in Table 7.

Table 9
Food groups and concentration data used for estimating the dietary acrylamide intakes

Country	Food categories used		Concentration data used	
	Number	Food category	Number	Source, method of analysis used and/or references
Australia	10	Bread/rolls and English muffins, breakfast cereals/muesli, sweet and savoury biscuits, batter based products (donuts, pancakes), fish and seafood products (battered and crumbed), poultry or game meat (battered or crumbed), hot potato chips/fries/potato wedges/battered and fried potato, potato crisps, corn crisps/extruded snacks/pretzels, mineral water/water	(A) 120	Data from the Swedish National Food Administration (2002) and the UK Food Standard Agency. Use the median values from all samples for a given food group
	101	Includes bread/rolls, toasted bread, English muffins, breakfast cereals/muesli, sweet and savoury biscuits, cakes/muffins/slices, batter based products (donuts, pancakes), rice, pasta, flour, hot potato chips/fries/potato wedges/battered and fried potato, potato crisps, corn crisps/extruded snacks/pretzels, popcorn. The full list is available in the published paper	(B) 112	Analysed data from national samples. LC/MS/MS method (Australian Government Analytical Laboratories, AGAL) *Acrylamide concentration data*: available from the authors
Belgium	14	Bread, small bread, crisps, chocolate, choco-spread, french fries, biscuit, coffee, gingerbread, breakfast cereals, popcorn, spiced biscuits, sweet spiced biscuits, rusk	150	Analysed data from national samples (data on rusk from The Netherlands). LC–MS–MS method (department of pharmaco-bromatology of the scientific institute of public health, Brussels, Belgium) *Acrylamide concentration data*: available in the Belgian Food Agency (Federal Agency for the safety of the Food Chain), htpp://www.favv.be
Czech Republic	9[(a)], 20[(b)]	List[(a)]: Bread, Rye bread, wholemeal bread, rolls and frenchloaf, rye rolls, cakes, biscuits, potato products, wafers. *List*[(b)]: List[(a)] + potato, nuts, chocolate, chocolate confectionery, cream cakes, cocoa, flour and yeast, cereals (other), semolina, coffee, tea	19[(a)] or 41[(b)]	Analysed data from national samples (9 or 20 samples representing 19 and 41 individual items, respectively). GC–MS/MS method (lab. Karvina CZ). *Acrylamide concentration data*: available from the authors
France	20	Pasta/semolina, breakfast cereals, rusks/toasted bread/toasts, soft loaves, other breads, crackers (excl. sweet crackers), croissant/brioche/rolls etc., biscuits, pastry, crisps, fried potatoes, coffee, fried meat/poultry, fried fish, chocolate, chocolate drink, malt drink, fermented dairy products and milk-based desserts, sweets, fruit pasta/dried fruit	20	Analysed data from a French industrial database. Seven out of 20 values are from the Scientific Committee on Food (SCF). New analyses are currently on-going in the AFSSA. The acrylamide intakes will be re-calculated soon. *Acrylamide concentration data*: available from the authors
Norway	9	Bread, cakes/biscuits, cereal products (other), potato products (french fries, fried potato), meat, fish, beverages (coffee), snacks (potato chips, etc.), sugar	30	Analysed data from national samples from Norway and Sweden. LC–MS–MS method (AnalyCen Lidköping, Sweden for both Swedish and Norwegian samples) Acrylamide concentration data: available from the authors

Sweden	10	Potato crisps, French fries, Fried potato products, cookies/biscuits/wafers, crisp bread/thin unleavened bread, bread, breakfast cereals, tortilla crisps, popcorn, coffee	130	Analysed data from national samples. LC–MS–MS method (Livsmedelsverket, Swedish National Food Administration Uppsala, Sweden) *Acrylamide concentration data*: Svensson et al. (2003)
Germany	6	French fries, potato chips, bisquits + waffles, Muesli + cornflakes, crispbread, Coffee + surrogates	768	Data collected by German BVL through Jan 2003 (http://www.bvl.bund.de/acrylamid/dl/Ergebnisuebersicht_1.pdf)
	12	Cereal products(Müsli, Cornflakes), special cereal products(Müsli-Riegel), crispbread, prepared toast, biscuits, potato chips, french fries, snacks, fried potato, craker, roasted nuts, coffee (was derived from the "National Consumption Survey")	1137	Analysed data from national samples performed by an Acrylamide inter-laboratory study supervised by the Federal Institute of Risk Assessment (BfR) using different methods of analysis (about 40) (see http://www.bfr.bund.de/cm/245/proficiency_testing_studie.pdf). Acrylamide concentration data: available on http://www.bvl.bund.de/acrylamid/dl/Ergebnisuebersicht_1.pdf
Switzerland	Not relevant	Chemical determination of acrylamide content of pooled samples of the whole meals. The beverages have been analysed separately.		The meals were pooled together and analysed as 4 pooled samples for breakfast, lunch, dinner and snacks (four pooled sample per day × 9 study groups × 2 days); 3 individuals per study group). The samples of the different beverages were also analysed. GC-HRMS and LC_MS/MS method (method description available on the SFOPH website http://www.bag.admin.ch/verbrau/aktuell/d/AA_methode.pdf)
The Netherlands	10	Bread, toasted bread and crispbread, potato chips, potato crisps, breakfast cereals, biscuits/cookies, spiced biscuits, Dutch spiced cake, coffee, peanuts	287	Analysed data from national samples. LC –MS–MS method according to Rosén and Hellenäs (2002)
USA	286(*)	(*)286 core foods in the US were derived from 4 market baskets and analysed for acrylamide concentration. Those included foods below the General Limit of Quantification (LOQ = 10 ppb). Out of the 286 core foods 74 have an acrylamide concentration above the LOQ at least two of marker baskets samples. The full list is available on: http://www.cfsan.fda.gov/~dms/acrydat2.html	>750	Analysed data from national samples (286 foods from 4 marker baskets). LC–MS–MS method (FDA, 2002). Full description of the method available on: http://www.cfsan.fda.gov/~dms/acrylami.html. Concentration data: available on http://www.cfsan.fda.gov/~dms/acrydat2.html and http://www.cfsan.fda.gov/~dms/acrydata.html

(a) and (b): For the study conducted in 2002 and 2003, respectively.

Table 10
Dietary intake of acrylamide in μg/kg body weight/day [μg/day]

Country	Population group(s)	*N*	Mean	Median	5th	90th	95th	97.5th	Min–Max
Belgium									
	Adolescents, all (13–18 y)	341	NA	0.51	0.19		1.09		
	Adolescents, boys	129	NA	0.64	0.23		1.26		
	Adolescents, girls	212	NA	0.46	0.17		0.94		
Czech Republic									
	Total population (>1 y)[a]	1030	0.14	NA	NA		NA	NA	NA
	Total population (>1 y)[b]	1030	0.28	NA	NA		NA	NA	NA
France									
	Adults (>15 y)	1474	0.40 [26.40]				0.87 [55.21]		
	Children (3–14 y)	1018	1.06 [29.51]				2.20 [60.74]		
Germany[c]									
	NVS study, 1989								
	Total population (>4 y)	23209	0.89 [56.83]						
	Children, boys (4–6 y)	450	1.31 [24.94]						
	Children, boys (7–9 y)	441	1.14 [29.51]						
	Children, boys (10–12 y)	358	0.95 [33.41]						
	Children, boys (13–14 y)	275	0.87 [42.75]						
	Adolescents, boys (15–18 y)	592	0.73 [44.97]						
	Adults, men (19–24 y)	1182	0.71 [49.92]						
	Adults, men (25–50 y)	4974	0.58 [43.27]						
	Adults, men (51–64 y)	1699	0.54 [40.66]						
	Adults, men (>64 y)	930	0.46 [34.32]						
	Children, girls (4–6 y)	469	1.26 [23.86]						
	Children, girls (7–9 y)	382	1.12 [29.12]						
	Children, girls (10–12 y)	346	1.04 [36.41]						
	Children, girls (13–14 y)	291	0.71 [34.67]						
	Adolescents, girls (15–18 y)	730	0.64 [35.99]						
	Adults, women (19–24 y)	1504	0.69 [39.92]						
	Adults, women (25–50 y)	5304	0.62 [36.99]						
	Adults, women (51–64 y)	2103	0.59 [35.65]						
	Adults, women (>64 y)	1179	0.63 [37.64]						
	Berlin's study, 2001[c]								
	Adolescents (15–18 y)	1085	1.16 [70.27]	0.83 [51.64]		2.40 [146.59]	3.24 [182.64]	5.1 [250] (99th)	
Norway[d]									
	Adults, men (16–79 y)	1246	0.52 ± 0.95	0.4	0.2		1.18	1.4	0–3.0
	Adults, women (16–79 y)	1336	0.47 ± 0.33	0.4	0.2		1.0	1.2	0–3.5
	Children, girls (9 y)	348	0.68 ± 0.72	0.5	0.1		1.9	2.5	0–7.1
	Children, boys (9 y)	339	0.62 ± 0.56	0.5	0.1		1.5	1.9	0–5.5
	Children, girls (13 y)	443	0.69 ± 0.96	0.4	0.1		2.5	3.8	0–8.1
	Children, boys (13 y)	403	0.66 ± 0.88	0.4	0.1		2.1	2.7	0–7.9
Sweden									
	Adults (18–74 y)	1211	? [31]	? [27]	? [9.1]		? [62]		? [0–138]
Switzerland									
	Adults (16–57)	27	0.28						
The Netherlands									
	Total population (1–97 y)		0.48	0.2			0.6	0.8	
	Children/adolescents (7–18 y)		0.71	0.2			0.9	1.1	
	Young children (1–6 y)		1.04	0.3			1.1	1.3	
USA									
	Total population (>2 y)	18081	0.43	0.29	0.06	0.9	1.3	1.6	0–0.80
	Children (2–5 y)	5447	1.1	0.76	0.15	2.2	3.0	3.8	0–15

NA: Not applicable.
[a] For the study conducted in 2002 and 2003, respectively.
[b] For the study conducted in 2002 and 2003, respectively.
[c] Only consumers.
[d] Only individuals with reported body weight were included.

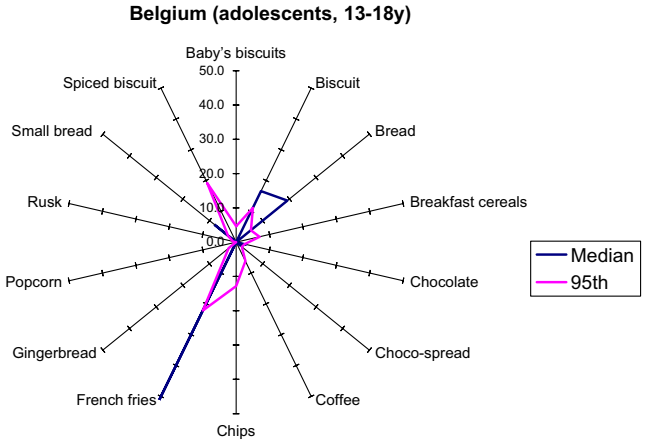


Fig. 2. Comparison of acrylamide food sources between median and 95th intakes.

and mean acrylamide intake and its distribution (Beaton et al., 1979; Nelson et al., 1989; Volatier et al., 2002; Kroes et al., 2002). This requires knowing the actual within- and between-subjects variability in acrylamide intake and its major food sources, which is currently lacking. Although a small number of repeated dietary measurements per individual may still provide good mean intake estimates, provided that the sample size is sufficiently large, accurate estimates of other parameters of the distribution (i.e. standard deviation, median and other percentiles) and subsequently of the populations at risks is more critical. The observed distribution may not then represent subjects at their usual high or low level of exposure, but reflects more likely their levels of exposure on the given day(s) of data collection, depending on the still unknown day-to-day variability of acrylamide intakes. FFQs are less prone to this type of error since the dietary intakes refer to the usual long-term intakes, but other problems are related to these methods. In contrast to 24-h diet recall or (weighed) dietary record methods, FFQs have fixed questions concerning a restricted list of aggregated food categories not necessary defined for acrylamide exposure assessment. Recent methodological studies also suggest that FFQs tend, overall, to underestimate dietary intakes more than repeated dietary recall or record methods (Day et al., 2001; Schatzkin et al., 2003; Bingham et al., 2003). A possible alternative approach is to combine both specifically designed FFQs, particularly for identifying long-term non-consumers, and repeated 24-HDRs or records and take advantage of the two methods. This approach has been adopted in a feasibility study conducted among a non-representative sample of 10th class pupils at the age of 15–18 years in Berlin to test a dietary method developed to estimate acrylamide intakes combining a short semi-quantitative FFQ ($n = 11$ items) and 2-day records (Table 8). However, the difficulties of combining data from dietary methods with different designs, content, levels of detail and time-scale should not be disregarded. Other study designs such as the duplicated diet study conducted in Switzerland provides further insights on the actual level of acrylamide exposure in a strictly controlled study, although these are usually based on small and non-representative samples ($n = 27$).

The type and number of both food categories and concentration data used to estimate acrylamide intakes vary substantially across countries from 6 to 74 and 19 to 1137, respectively (Table 9). Although they reflect different food habits and dietary methods used across studies, they also suggest important methodological differences in the way acrylamide food sources have been defined and aggregated into categories. The foods with the highest acrylamide concentration and rich-starch foods contributing to the total diet are usually considered, but there are greater differences for other food groups (e.g. coffee, breakfast cereals, rusks, biscuits, chocolate and chocolate-spreads) and on the definition and precision of food categories. All concentration data used were obtained from locally analysed samples by authorised laboratories (Table 9). In Australia the same population sample was used to estimate acrylamide intakes first using available Swedish and UK concentration data, then re-analysed using national concentration data (Table 10).

2.4.1. Exposure assessment from databases

Table 10 summarizes the acrylamide dietary exposures estimated in the 13 selected studies, whenever available. Most of the studies provide figures of mean acrylamide exposure in μg/kg body weight/day and/or μg/day but more differences exist for parameters of the population distribution (median, other percentiles and minima/maxima) which are not systematically given, thus making comparison between studies difficult.

When all considered together, the mean total intakes of the entire population (>1, 2 or 4 year old studies) ranges between 0.14 and 0.89 μg/kg bw/day in studies conducted in Europe, USA and Australia. The study conducted in the Czech Republic reported the lowest values (0.14 and 0.28 μg/kg bw/day for the most recent study and using an average subject of 64 kg) whereas the NVS study in Germany reported the highest value (0.89 μg/kg bw/day) using dietary data collected in 1985–1989. The other studies reported narrow ranges of exposure between 0.30 and 0.48 μg/kg bw/day.

For adult population groups in France, Norway, Switzerland and Sweden (only in μg/day), the intake ranges were of the same order between 0.28 (Switzerland, duplicate diet study) and 0.46/0.49 μg/kg bw/day for females/males (Norway). In Germany, the values remain higher as compared to other studies with overall higher values in women (0.59–0.69) than in men (0.46–0.71), when considering four age groups from 19–24 to >64 year old. When available, the median is systematically

lower than the mean, suggesting a highly skewed distribution with 95th or 97.5th percentile values from 2- to more than 5-fold higher than the mean or median ones. The intakes in (young) children and adolescents are systematically higher as compared to adults when expressed in kg of body weight, except in Norway.

The age categories vary across countries and make the strict comparison across countries difficult. However, all together the values reported for young children (i.e. different age categories involving subjects between 1 and 6 year olds) range between 1 and 1.31 μg/kg of body weight/day whereas the average values reported for older children and adolescents are usually lower. The values from adolescents (age categories between 13/15 and 18 year olds) range between ~0.50 and 1.16 (Table 10).

The decreasing of acrylamide intakes according to increasing age categories is consistently shown in several countries (The Netherlands, Australia and the NVS study in Germany) (Table 10). In Germany this trend is particularly clear with an inverse association between acrylamide intakes and age categories of 2–3 year intervals. For boys, for example, the following intakes are reported by age categories in children and adolescents: 1.31 (4–6 years), 1.14 (7–9 years), 0.95 (10–12 years), 0.87 (13–14 years) and 0.73 (15–18 years). The same trend is observed in girls and other studies in The Netherlands and Australia. This observation might be due to relatively higher dietary intakes per kg body weight in children and young populations compared to adults. It is important to note that most of these figures are crude values, without adjustment for age or other possible confounders.

Appendix A provides examples of the contribution of acrylamide food sources to the total intakes in different countries and age categories. Although they are not comparable across countries, these figures suggest different dietary patterns within and between countries which share common features. For example, although their definitions and contributions vary across study populations the foods contributing consistently 80–100% of the total intakes are: potatoes (French fries, chips, fried potatoes), coffee, bread (toasted, wholemeal rye bread, crispbread, soft bread and other breads), biscuits/cakes (incl. wafers, gingerbread, spiced cakes) and to a lesser extent breakfast cereals, crackers, rusks and other chips. Coffee, which has been relatively recently identified as an important acrylamide source, contributes up to 30–40% of the total intakes in adults from Norway and Sweden, respectively (i.e. as much as or more than the contribution from potato products). Not all studies included coffee as an acrylamide food source category and this may underestimate the intakes in adult populations where coffee is highly consumed. Different dietary patterns are also observed across age categories with a relatively higher consumption of French fries, chips and similars, biscuits and chocolate among children and adolescents as compared to adults. In the Netherlands, a higher relative consumption of chips, Dutch spiced cakes and biscuits is observed among children and adolescents, as compared to the whole population, whereas coffee is specific to adult population or consumed in relatively small quantities by adolescents in Germany (5%) and Belgium (6%). Different dietary patterns are also observed between the mean/median and populations potentially more at risk (95th percentile) (Fig. 2). When compared, two phenomena are usually observed. One is an increasing contribution of main acrylamide food sources to the total intakes at 95th/97.5th percentile as compared to the mean or median intakes. One also observes the consumption of foods at the 95th and 97.5th which were not, or at low level, consumed by the population at the mean/median. In the study in Belgium for example, 36% of the total intakes at the 95th percentile is attributed to foods which were not contributing to the median intakes (i.e. chips, coffee, baby foods, gingerbread, breakfast cereals, spiced biscuits and rusks). These figures may reflect different dietary patterns according to the level of exposures, but possibly also some tricky methodological issues due to an insufficient number of repeated measurements per individual to actually cover the day-to-day variability of acrylamide food sources. The dietary patterns observed at the highest percentiles of the distribution may not concern the actual long-term high consumers, but only subjects who reported having consumed these foods on the given recorded or recalled day(s). This reflects the need to understand the difference between day-to-day variability in those consumers who occasionally have a day with high exposures versus consumers who may always be at the high end of exposure as a result of consumption of certain foods that contain high levels of acrylamide. The numbers of individuals in these surveys may be too few to adequately characterise the "tails" of the distributions given the potential sources of variability.

2.4.2. Conclusions

The recent discovery of acrylamide in foods has challenged risk assessors to perform exposure assessments using the available consumption data and incomplete knowledge on this newly identified dietary exposure. The results on acrylamide studies reported in this section should therefore be considered with caution and seen mainly as examples on available data and current methodological limitations for estimating and interpreting them. These results also provide further insights into the order of magnitude of possible acrylamide exposures in different population groups. More data for risk assessors will soon be provided by on-going studies using the latest up-to-date knowledge available on acrylamide.

While acrylamide levels were mostly gathered during the last two years by local, authorised laboratories, most of the dietary surveys used to estimate acrylamide

exposure used national food survey data that partially dated back many years, and were thus not specifically designed for acrylamide exposure assessment. Consequently, they do not necessarily reflect current dietary consumption habits and exhibit limitations concerning e.g. information on preparation and cooking method, or preparation at home versus industrial preparation.

The methods used to record dietary intakes varied considerably, with a large variability in the number of repeated measurements, the number and distribution of groups (age, gender), the number and detail of food items recorded, the aggregation of food categories etc. Different parameters of distribution were not systematically given, with different levels of coverage of the mean, median, standard deviations and percentiles. In addition, the number of concentration data varied considerably. These different methodological issues may introduce an unknown level of imprecision and bias, possibly varying across studies and population groups, and make the interpretation and comparison of results difficult. However, despite these methodological limitations, the overall results remain consistent with the first conclusions of the FAO/WHO consultation and the following meetings. The mean acrylamide intakes are below 1 μg/kg of body weight/day in adults (i.e. between 0.28 and 0.71 μg/kg of body weight/day) and about 1.5-fold higher or more among children and adolescents, depending on the studies and age categories considered. This trend is consistently observed in several countries with an inverse association between acrylamide intakes and age, when body weight is controlled for, i.e. expressing acrylamide intakes per body weight.

2.4.3. Uncertainties/gaps

- Knowledge on the nature and magnitude of dietary acrylamide exposures in various population groups and dietary patterns is currently lacking.
- The current methodological differences in dietary acrylamide assessment across studies do not permit full comparison and interpretation of results, and efforts should be made to harmonize them better. This is particularly important knowing that dietary acrylamide is a universal exposure affecting all dietary patterns and countries.
- The different levels of exposures across age categories and study populations need further investigation in order to identify in particular the different determinants and characteristics of the populations potentially at risk as well as the confounding factors and co-variables to be systematically considered in the statistical analyses such as age and smoking status, for example.
- A better understanding of the within- and between-subject variability of acrylamide and its main sources is warranted in order to determine the number of repeated measurements actually needed to estimate acrylamide intakes, when repeated 24-h dietary recalls or diet (weighed) records are used.
- Information on home cooking methods and industrial processes (e.g. through brand names and/or product names) and sufficient descriptive detail on food sources of acrylamide should be asked systematically for in order to estimate dietary acrylamide intakes.
- The reliability of the external dietary exposures should be evaluated against validated biomarkers of internal exposures (e.g. haemoglobin or DNA adducts) (see section on biomarkers of exposure, 2.5) in order to identify the best cost-effective dietary method(s) to be used for acrylamide exposure assessment and its association with health outcomes.

2.5. Biomarkers of exposure

2.5.1. Introduction

The molecule of acrylamide contains a reactive α,β-unsaturated structure, which makes its double bonds susceptible to nucleophilic attack by Michael addition. Thus, acrylamide covalently interacts in vivo with cellular nucleophiles, predominantly the sulphydryl groups in reduced glutathione and in proteins, and to a lesser extent protein amino groups. Acrylamide is absorbed from all routes of exposure and is widely distributed in tissues, but is also metabolised by the action of cytochrome P450 2E1 to the epoxide glycidamide. Glycidamide is also subject to nucleophilic attack, in this case by ring opening of the epoxide by sulphydryl or amino groups, or by water. As indicated in Section 3.3, interaction of glycidamide also occurs with the nucleophilic nitrogens in DNA, producing DNA adducts, which suggests that glycidamide may be more important for carcinogenic and genotoxic properties than the parent compound acrylamide, whose reaction with DNA constituents is much slower. However, acrylamide is believed to the more responsible component for neurotoxicity than glycidamide because of its potent ability to react with proteins. Details of the metabolism of acrylamide are summarised below in this section on 'Biomarkers of exposure', and covalent adducts of acrylamide and glycidamide are discussed in the later section 'Biomarkers of internal dose' (Section 3.3), although this separation is somewhat arbitrary as there is clearly overlap in the association of 'metabolism' and 'adducts' to these areas.

An outline of the major metabolic routes for acrylamide is shown in Fig. 3. Direct conjugation of acrylamide with glutathione results in the subsequent formation of a mercapturic acid propionamide. Glycidamide, which is produced by oxygenation by cytochrome P450, undergoes further metabolism either by hydrolysis to

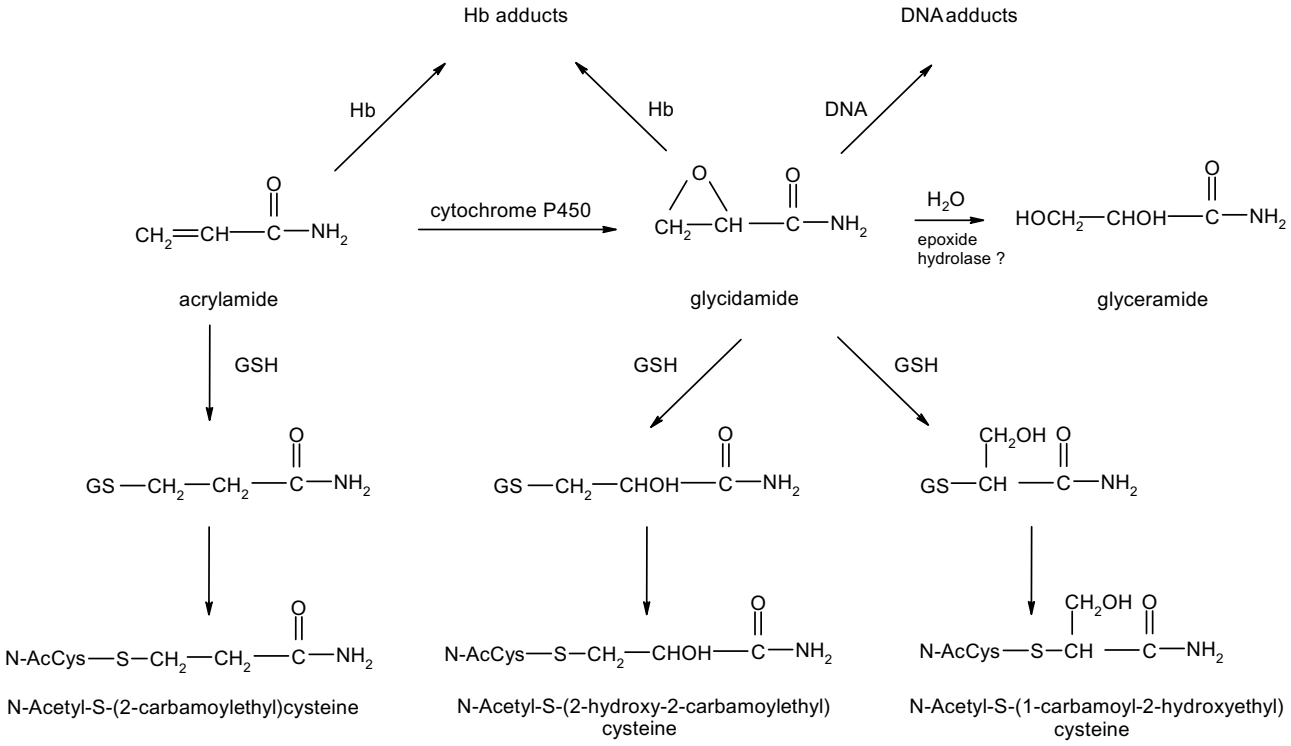


Fig. 3. Major metabolic routes of acrylamide.

glyceramide, or glutathione conjugation, resulting in the subsequent formation of two mercapturic acid products excreted in urine. Biomarkers of exposure to acrylamide have focused on the quantitation in blood, tissues or urine of acrylamide, glycidamide or of their interaction products with glutathione (and their metabolic products).

2.5.2. Biomarkers of exposure—metabolites

In selecting and using different biomarkers for acrylamide exposure, there are various issues that need to be considered.

Mode of action—Effects of acrylamide vs. glycidamide. An issue in the selection of appropriate biomarkers is whether one is interested in the exposure to or internal dose of acrylamide alone, or also in the fraction of glycidamide formed metabolically, and its internal dose. If all toxic effects were mediated by acrylamide, then markers related to acrylamide would be of the most interest. However, since it is expected that the formation of glycidamide and its interaction with DNA may account for most of the mutagenic and other genotoxic effects of acrylamide, then biomarkers of glycidamide formation are also of interest. Of particular relevance in evaluating the potential health effects of exposure to acrylamide is the balance between acrylamide and glycidamide in the body.

Specificity of biomarker. Exposures to chemicals rarely occur as pure single substances, and the specificity for a biomarker is an important consideration. The initial investigation of biomarkers in exposed workers in China comprised exposures to both acrylamide and acrylonitrile (Bergmark et al., 1993; Calleman et al., 1994). Methods that involved acid hydrolysis of proteins to release cysteine adducts derived from acrylamide or acrylonitrile would result in the formation of *S*-2-carboxyethylcysteine from both chemicals. Similarly, *S*-(2-carboxyethyl)cysteine is the product of hydrolysis of mercapturic acids of both acrylamide and acrylonitrile.

Interspecies extrapolation issues. In estimating the potential risk of exposure to toxic chemicals in humans, one is relying primarily on the results of tests in animals, often at high doses, and using a variety of exposure routes and dosing scenarios. The measurement of biomarkers in rodents under the conditions used in evaluating toxicity provides important information that can be used in the interspecies extrapolation component of risk assessment. In relating the effects that are generated in toxicity tests to the likely effects that may be observed in humans, biomarkers can provide alternative dose metrics that can be used to replace default assumptions that are used for interspecies scaling of dose. In relating biomarker measurements to exposure, it is not always possible to have an understanding of the exact relationship between exposure and the biomarker. Investigations in rodent studies can provide important information on dose responsiveness, and the effect of route of exposure.

Species differences—rodents vs. humans. It is important to bear in mind that species difference in the expression of enzymes that metabolise acrylamide could occur, and that the extent of metabolism via different routes may vary among species.

Sources of exposure—diet, smoking, workplace, cosmetics, water. While biomarkers of exposure such as haemoglobin adducts can be investigated in the general population or in targeted populations such as exposed workers, the source of exposure cannot automatically be established and remains often unknown.

Analytical methods. For some biomarkers that have been investigated for acrylamide there may be a single study only. For other biomarkers, measurements may have been conducted in a number of laboratories, using the same or different methods of analysis. It is important to bear in mind the technology used in specific studies, and how it may impact the results obtained.

Some kinds of measurements have only been made in rodents with high levels of exposure to acrylamide. As analytical methodologies improve with the use of sensitive techniques such as LC–MS/MS, it may be feasible to have more comparable measurements made in humans, as well as in defined exposure studies.

Polymorphisms. A concern in using biomarkers to evaluate exposure is that polymorphisms involved in metabolism would influence the rate of metabolism and subsequently could result in the erroneous evaluation of exposure. However, biomarkers provide a tool for evaluating the potential role of polymorphisms in humans exposed to acrylamide. Both polymorphisms and the levels of expression of enzymes could result in alterations in biomarkers.

2.5.3. Metabolism

A number of studies have investigated the metabolism of acrylamide in rodents. Miller et al. (1982) investigated the toxicokinetics and metabolism of acrylamide in male F-344 rats administered [2,3-^{14}C] acrylamide by intravenous (i.v.) or gavage administration at doses of 1, 10 or 100 mg/kg. Five metabolites were detected, and one was characterised as the *N*-acetyl-*S*-(2-carbamoylethyl)cysteine. Radioactivity was excreted extensively in the urine, with little radioactivity excreted in faeces. Approximately 10% of the administered radioactivity was bound to red blood cells.

Dixit et al. (1982) also described the excretion of *N*-acetyl-*S*-(2-carbamoylethyl)cysteine in urine of rats administered acrylamide.

In studies conducted investigating the haemoglobin adducts derived from acrylamide, Calleman et al. (1990) provided evidence for the production of glycidamide as a metabolite of acrylamide.

Sumner et al. (1992) investigated the metabolism in mouse and rat of a 50 mg/kg oral dose of (1,2,3-^{13}C) acrylamide by ^{13}C NMR spectroscopy. Metabolites were detected and quantitated directly in urine without isolation. Unchanged acrylamide was detected in the urine, but was not quantitated. The major metabolite in both rats and mice was the *N*-acetyl-*S*-(2-carbamoylethyl)cysteine, identified by the NMR signals of the three labelled carbon atoms derived from acrylamide. The other metabolites were derived from glycidamide, including glycidamide itself, glyceramide, and *N*-acetyl-*S*-(2-hydroxy-2-carbamoylethyl)cysteine, and *N*-acetyl-*S*-(1-carbamoyl-2-hydroxyethyl)cysteine. The amount of metabolites derived from the metabolism via glycidamide was higher in mice (59%) compared with rats (33%). Approximately 60% of the administered dose was recovered in the urine of the exposed animals.

The role of cytochrome P450 2E1 was investigated by comparing the metabolism of (1,2,3-^{13}C) acrylamide by ^{13}C NMR spectroscopy in the urine from wild type and CYP2E1 null mice (Sumner et al., 1999). While extensive metabolism of acrylamide via oxygenation was evident from the urinary metabolites in the wild type mice, there were no glycidamide-derived metabolites detected in the null mice. This indicates that in the mouse at this dose of acrylamide administered, the major enzyme responsible for the oxidation of acrylamide was CYP 2E1. No alternative oxidative pathways of metabolism appeared to be involved and the entire metabolism of acrylamide in the null mice appeared to be via direct conjugation with glutathione.

The route of exposure can have an impact on the two pathways of metabolism of acrylamide. Sumner et al. (2003) compared exposure to acrylamide by inhalation, orally, dermally and by intraperitoneal (i.p.) injection. With i.p. and gavage administration of a 50 mg/kg dose to rats, there were slight, but not significant differences in the percentage of the dose recovered in the urine (62 ± 12% by i.p. and 53 ± 8% by gavage). The overall metabolism was similar between the two exposure routes, with approximately 30% of the metabolites derived from metabolism via glycidamide. With dermal (150 mg/kg) and inhalation (3 ppm for 6 h) exposures, a higher percentage of the urinary metabolites were derived from metabolism via glycidamide (48% for dermal, and 36% for inhalation). In the mouse exposed to acrylamide by inhalation, 73% of the urinary metabolites were derived from metabolism via glycidamide.

2.5.4. Acrylamide and metabolites as biomarkers of exposure

2.5.4.1. Acrylamide and glycidamide in blood. Toxicokinetic studies have been conducted on acrylamide by a number of investigators. Miller et al. (1982) examined the toxicokinetics of [2,3-^{14}C]acrylamide administered to rats by i.v. injection at a doses of 10 mg/kg. The amount of acrylamide in blood was measured by HPLC coupled with quantitation of radioactivity coeluting with acrylamide. The kinetics of acrylamide elimination could be

described by a one-compartment model, with a half-life of 1.7 h in blood. Barber et al. (2001b) developed an HPLC method with UV detection for quantitation of acrylamide and glycidamide in the plasma of rats administered acrylamide. They also investigated the toxicokinetics of acrylamide and glycidamide following administration of [2,3-^{14}C] acrylamide by oral or i.p. injection, both in naïve rats, and in rats previously administered acrylamide (Barber et al., 2001c). C_{max} from a single oral administration of 20 mg/kg acrylamide was 7.9 μg/ml (111 μmol/l). T_{max} was 1.73 h, and the elimination half-life was 1.63 h The AUC for acrylamide (2236 μg min/ml) was considerably higher than that for glycidamide (486 μg min/ml).

In a recent study, LC–MS/MS has been applied to the detection and toxicokinetic analysis of acrylamide and glycidamide in the serum of mice administered 50 mg/kg acrylamide by gavage (Twaddle et al., 2004b). Maximal blood concentrations for acrylamide were described at the first time point of 0.5 h. The elimination half-life was 0.73 h, and C_{max} was 450 μmol/l. For the glycidamide formed, C_{max} of 190 μmol/l was achieved at 2 h. AUCs for glycidamide (880 nmol h/ml) and acrylamide (920 nmol h/ml) were similar.

In the only study conducted in humans exposed occupationally to acrylamide, Calleman et al. (1994) measured the amount of acrylamide present in plasma at the end of shift. Detectable acrylamide was reported in 3/10 of the control group, and in 17/41 of the workers exposed to acrylamide, the concentrations detected ranged from 0.6 to 3.5 μmol/l in plasma. Interestingly acrylamide was reported in plasma of three of the control subjects without occupational exposure to acrylamide (0.9 μmol/l). The smoking status of the control subjects was not described.

2.5.4.2. Acrylamide in urine. In disposition and toxicokinetic studies conducted with [^{14}C]acrylamide administered to rats, Miller et al. (1982) reported that less than 2% of the dose of acrylamide was excreted in urine as unchanged acrylamide.

In a recent study, Sorgel et al. (2002) described the excretion of acrylamide in urine from humans who consumed up to 500 g of potato chips or crispbread. Up to 5 μg of acrylamide was recovered in urine collected for up to 8 h following consumption of the acrylamide-containing food. The amount of acrylamide ingested in the potato chips was not reported.

2.5.4.3. Acrylamide in tissues. Miller et al. (1982) determined the amount of both ^{14}C and acrylamide in studies with [2,3-^{14}C] acrylamide administered by i.v. injection to rats. The removal of acrylamide from tissues (liver, muscle, skin, blood, kidney) was described as a first-order process with half-lives ranging from 1.4 to 3 h. The kinetics of acrylamide in testis was described with both an absorption and elimination phase, with an elimination half-life of 1.4 h.

Acrylamide in tissues was determined following derivatisation to form 2-bromopropionamide, and analysis by gas chromatography (Crofton et al., 1996). Acrylamide was administered to male Long Evans rats by a single dose (0–150 mg/kg), or 10-day (0–30 mg/kg/day), 30-day (0–20 mg/kg/day), and 90-day (0–10 mg/kg/day) exposures. The concentration of acrylamide was determined in serum and in sciatic nerve at 30 min after the last dose of acrylamide. Concentrations of acrylamide were similar in serum and in sciatic nerve, and increased in a dose dependent manner, with an approximately linear response. The dose–response curves for both serum and sciatic nerve were similar between single and 10-day, 30-day, and 90-day exposures.

2.5.4.4. N-Acetyl-S-(2-carbamoylethyl)cysteine in urine. As noted above, *N*-acetyl-*S*-(2-carbamoylethyl)cysteine is a major metabolite of acrylamide detected in a number of metabolism studies conducted in rodents. To determine *N*-acetyl-*S*-(2-carbamoylethyl)cysteine in the urine of exposed humans, Calleman et al. (1994) treated urine samples with HCl and heat which resulted in the formation of *S*-(2-carboxyethyl)cysteine. This was derivatised with *o*-phthalaldehyde, and quantitated by HPLC. The workers examined in this study, in addition to being exposed to acrylamide, were exposed to acrylonitrile. The major metabolite of acrylonitrile is *N*-acetyl-*S*-(2-cyanoethyl)cysteine, which on treatment with acid will also yield *S*-(2-carboxyethyl)cysteine. Thus, the marker measured was not specific for acrylamide, and is likely to have a significant contribution from acrylonitrile exposure, which was evident from measurement of acrylonitrile haemoglobin adducts. The amount of *S*-(2-carboxyethyl)cysteine detected by this method was expressed as the amount excreted in a 24-h sample. In unexposed humans, levels were low (3.0 μmoles/24 h) and were significantly higher in the exposed workers, ranging from 9 to 318 μ moles/24 h.

2.5.4.5. Glycidamide in urine. Glycidamide is readily detected in urine from rodents administered acrylamide (Sumner et al., 1992, 1997, 1999), but has not yet been reported in human urine. Similarly, direct detection of glycidamide in human serum, plasma or blood has not been reported.

2.5.4.6. Other metabolites. The other urinary metabolites detected in rodent metabolism studies have not yet been described in studies in humans (Sumner et al., 1992, 1997, 1999).

2.5.5. Conclusions

Following administration of acrylamide to rodents, both acrylamide and glycidamide have been detected

in blood, acrylamide being more abundant. The detection of low concentrations of acrylamide in human plasma indicates that measurement of this might be a possible biomarker of human exposure. Urinary excretion of acrylamide in humans has also been reported and has similar potential as a biomarker. Glycidamide has so far not been reported in human urine. The major urinary metabolite, *N*-acetyl-*S*-(2-carbamoylethyl)cysteine, should in theory be a more sensitive biomarker of acrylamide exposure, although the analytical method used to date is not specific for this compound.

2.5.6. Uncertainties/gaps

The major uncertainties/gaps in the use of metabolites as biomarkers of exposure are:

- lack of knowledge on interspecies extrapolation (e.g. effect of metabolism differences),
- effect of polymorphisms in metabolising or detoxifying enzymes,
- improved analytical methods are needed with greater specificity for acrylamide exposure (as opposed to say acrylonitrile),
- lack of extensive validation of some analytical methods.

3. Internal dose assessment

3.1. Bioavailability of acrylamide

In a study where male Fisher-344 rats were administered ^{14}C-acrylamide (10 mg/kg by gavage), total recovery in tissues, body fluids, urine and faeces was 90.2 ± 13.1% (Miller et al., 1982). The only tissue which served as a major depot for the radiolabel was the erythrocyte. By the first day, the binding to erythrocytes had plateaued at 12% of the dose and accounted for essentially all of the ^{14}C remaining in the blood. By seven days after administration, 71% of the dose had been excreted in the urine. An additional 6% of the dose was excreted in the faeces. The half-life of parent acrylamide in the body is extremely short; however, a small percentage of radiolabel remained in tissues for several weeks.

In a study with male beagle dogs, average recovery of the dose (40 mg acrylamide/kg unlabelled compound in feed for 3–4 weeks, then radiolabelled acrylamide in oral capsule corresponding to 1 mg/kg) in all tissues and excreta was 72.2% at 4 days and 72.8% at 14 days (Ikeda et al., 1987). After 14 days 59.1 ± 6.4% of the radiolabel was excreted in urine and 6.9 ± 1.5% in faeces. Thus, approximately 7% of the radiolabel was retained in blood and tissues.

In male Hormel-Hanford miniature pigs, average recovery of the dose (40 mg acrylamide/kg unlabelled compound in feed for 3–4 weeks, then radiolabelled acrylamide in oral capsule corresponding to 1 mg/kg) in all tissues and excreta was 95.0% at 4 days and 98.6% at 14 days (Ikeda et al., 1987). After 14 days 63.0 ± .6% of the radiolabel was excreted in urine and 26.6 ± 2.0% in faeces. Thus, approximately 9% of the radiolabel was retained in blood and tissues.

In male Sprague-Dawley rats (50 mg acrylamide/kg by gavage), the total percentage of radioactivity present in the urine and faeces 6 days after administration was 61.0 ± 2.7 and 3.9 ± 0.7, respectively (Kadry et al., 1999). The percentage radioactivity in the examined tissues was 3.0 ± 0.6 and 0.8 ± 0.1 after 28 and 144 h, respectively. Elimination from blood fitted a two-compartment model with distribution phase half-life of 7.93 h and elimination phase half-life of 374 h. The elimination from blood was 62 times longer than from plasma.

The toxicokinetics including metabolism of acrylamide following subchronic oral or short-term repeated intraperitoneal (i.p.) administration was studied in rats (Barber et al., 2001a). Acrylamide was administered either 50 mg/kg bw i.p. daily for up to 11 days or 2.8 mM in drinking water during 34 days (equal to 20 mg/kg bw/day). On day 11 of the i.p. dosing schedule, animals received a single injection of ^{14}C-acrylamide (50 mg/kg bw). On day 34 of the oral dosing schedule, the animals received a solution containing ^{14}C-acrylamide (20 mg/kg bw) by gavage. Other groups not previously exposed to acrylamide received one similar dose of ^{14}C-acrylamide. Blood was collected at sequential time points and plasma levels of radiolabelled acrylamide and glycidamide were quantified. The results of acrylamide measurements in plasma is given in the Table 11.

Table 11
Toxicokinetic parameters of acrylamide in the rat (Barber et al., 2001a)

Acrylamide administration	C_{max} (μg/ml)	t_{max} (min)	$t_{1/2}$	AUC
Intraperitoneally, single	29.4 + 1.3	60 + 7	136	9849 + 506
Intraperitoneally, repeated	30.1 + 2.7	99 + 17	144	9643 + 475
Orally, single	7.9 + 0.4	104 + 17	98	2236 + 203
Orally, repeated	11.6 + 0.6	87 + 7	118	2828 + 227

C_{max}: peak plasma concentration; t_{max}: time to peak plasma concentration; $t_{1/2}$: plasma half-life; AUC: area under the curve (plasma concentration integrated from 0 to 580 min post ^{14}C-acrylamide administration).

According to the authors, no quantitative estimate or oral bioavailability can be made based on this study (probably because of the lack of plasma data after intravenous administration). However, according to Rompelbeg and Baars (2003) it is possible to obtain some insight into oral bioavailability by calculating the relative bioavailability for the two administration routes. By doing this, the relative bioavailability of a single oral dose of acrylamide was calculated to be approximately 57% of the i.p. dose, whereas it was approximately 73% after subchronic administration.

In male F344 rats administered a single dose of 50 mg/kg [1,2,3-^{13}C] acrylamide by gavage, 53.0 ± 7.6% of the dose appeared in the urine after 24 h (Sumner et al., 2003).

Groups of six male aspermic, non-smoking volunteers were administered ^{13}C-labelled acrylamide orally at doses of 0, 0.5, 1 or 3 mg/kg bw and urine was collected for up to 24 h (Fennell et al., 2004). After the first day of administration, 34.0 + 5.7% of the dose had been excreted in the urine of the group that had received 3 mg acrylamide/kg bw.

3.1.1. Conclusion

The bioavailability of acrylamide after oral administration is high with 68–90% of the dose being absorbed in rats, 73% in dogs and 99% in miniature pigs, respectively. In a human volunteer study, 34% of an oral dose appeared in the urine during the first 24 h.

3.1.2. Uncertainties

- The oral bioavailability of acrylamide in humans is not known with certainty.
- It is unclear whether various food matrices may influence the bioavailability of acrylamide.

3.2. Kinetic studies and modelling with acrylamide

3.2.1. Introduction

A better understanding of the kinetics for acrylamide and its metabolite glycidamide may lead to insight into the mode(s) of action by which acrylamide produces its carcinogenic effects, and should aid in the assessment of risk to humans from acrylamide exposure. Physiologically based toxicokinetic (PBTK) modelling has become an important tool in risk assessment to assist in extrapolations and data integration. General advantages of simulation modelling in physiology, toxicology, and risk assessment have been well established (Yates, 1978; Andersen et al., 1995). Some generic advantages available from the development of PBTK models as a core part of a research strategy are listed below.

- Codification of facts and beliefs (organise available information).
- Expose contradictions in existing data/beliefs.
- Explore implications of hypotheses about the chemical.
- Expose data gaps limiting use of the model.
- Predict response under new conditions.
- Identify essentials of system structure.
- Provide representation of present state of knowledge.
- Suggest and prioritise new experiments.

It is best to conduct model development in concert with data acquisition for any compound. First, the PBTK model can be used to assess the correlation between various measures of tissue dose and toxic response to evaluate possible causal relationships among various measures of tissue exposure and outcome. Second, these models can serve to test the consistency of various studies describing diverse aspects of acrylamide metabolism and disposition. And, thirdly, critical data gaps for model development can be used as part of the design criteria for new studies. Some of these attributes are evident in the work done to date with PBTK modelling for acrylamide/glycidamide.

3.2.2. Acrylamide PBTK model—state of the art

PBTK models are developed to support extrapolation from tested to untested or even untestable situations. The primary extrapolations are from high to low dose, from one dose route to another, from laboratory animals to humans, and from one exposure scenario to another (for instance, a constant exposure rate in controlled animal testing to highly variable rates in a human population). In these models, compartments correspond to particular tissues with volumes, blood flows and concentrations of metabolic enzymes (Leung, 1991). The models are often greatly simplified by lumping various tissues into a smaller number of tissue groupings by maintaining only a metabolising organ, the liver, and a single lumped body compartment for reactive compounds.

Kirman et al. (2003) has published a PBTK acrylamide model, which contains a component for glycidamide, and was developed based on three main data sets from the following experiments with rats: Raymer et al., 1993; Miller et al., 1982; Sumner et al., 1992. Data on macromolecular interactions were taken mainly from Miller et al., 1982; Ramsey et al., 1984 (see details of studies in Sections 2.5, and Section 3.3). It should be noted that in the last few years several acrylamide kinetic and metabolism studies have been published that would have relevance in the development of a refined rodent/human PBTK model (Barber et al., 2001a; Sumner et al., 2003; Fennell et al., 2003, 2004; Twaddle et al., 2004b). At least one additional PBTK modelling effort for acrylamide is currently underway at the US Food

and Drug Administration's National Center for Toxicological Research; results from this work are as yet unpublished.

According to the Kirman et al. (2003) model, acrylamide is distributed within five compartments (arterial blood, venous blood, liver, lung and all other tissues lumped together) and is linked to the glycidamide portion of the model via metabolism in the liver (Fig. 4). Glycidamide is distributed within the same compartments as acrylamide. Although additional compartments corresponding to sites of toxicity and carcinogenicity could be added (peripheral nerves, central nervous system, testes, adrenal gland, thyroid, mammary gland, uterus, and oral cavity), limitations in available data did not justify separating them from the lumped compartments in the initial model. The arterial and venous blood compartments were further divided into serum and blood cell subcompartments to allow for modelling certain data sets (chemical bound to haemoglobin in red blood cells). Exposures to acrylamide via intraperitoneal injection (i.p.) were described with first-order input to the venous blood compartment, and gavage dosing was described with first-order input to the liver. Uptake of chemicals from the blood to tissues was perfusion limited and dependent on tissue:blood partition coefficients (see Ramsey and Andersen, 1984).

According to the model, the metabolism of acrylamide and glycidamide occurred solely in the liver. Hepatic metabolism of acrylamide proceeds via two pathways: (1) a saturable epoxidation by cytochrome P450 to produce glycidamide (quantified assuming Michaelis–Menten kinetics) and (2) a first-order conjugation with glutathione (GSH) via glutathione-*S*-transferase (GST) to ultimately yield *N*-acetyl-*S*-(3-amino-3-oxopropyl)cysteine (Fig. 4). Glycidamide in turn can react with GSH via a first-order conjugation to ultimately yield *N*-acetyl-*S*-(3-amino-2-hydroxy-3-oxopropyl)cysteine and *N*-acetyl-*S*-(carbamoyl-2-hydroxyethyl) cysteine, or it can undergo further saturable metabolism via epoxide hydrolase (EH) to yield 2,3-dihydroxypropionamide (see Fig. 3). Acrylamide and glycidamide react with GSH, and deplete GSH following dermal exposure to acrylamide (Mukhtar et al., 1981). For this reason, the depletion and resynthesis of GSH were also incorporated in the model structure to assess this possibility (see D'Souza et al., 1988).

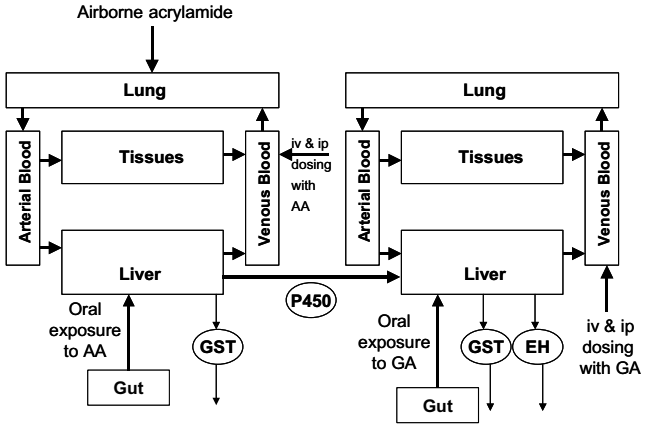


Fig. 4. The physiologically based pharmacokinetic model structure for acrylamide and glycidamide. The model developed by Kirman et al. (2003) was based on a simplified description of the body with a single lumped tissue compartment. Metabolism of AA occurred in the liver; cytochrome P450-mediated oxidation produced glycidamide that was introduced as input to a second model for GA. Phase II reactions were GSH conjugation via glutathione transferase (GST) with AA and both GSH conjugation with GST and hydrolysis by epoxide hydralase (EH) with GA. These models could account for inhalation, intravenous, intraperitoneal and oral dosing, depending on the study or exposure situation of interest. Due to high blood:air partitioning, exhalation was not a significant route of clearance of AA or GA. Venous and arterial blood compartments were included to permit modelling formation of hemoglobin adducts.

The binding of acrylamide and glycidamide with tissue macromolecules, including haemoglobin, was also accounted for in the model structure. Acrylamide and glycidamide binding with haemoglobin, liver macromolecules, and tissue macromolecules were all described as first-order processes. See Section 3.3 for further information on acrylamide and glycidamide adduct formation.

Physiological parameters (body weight, organ size, organ blood flow, etc.) for the rat were identified from information available in the published literature. Tissue:air and tissue:blood partition coefficients were estimated for acrylamide based on an algorithm derived by Poulin and Krishnan (1995, 1996a,b) using specific chemical properties. The Poulin and Krishnan algorithm could not be used for glycidamide because of inadequate chemical property information, so the glycidamide partition coefficients were extrapolated using a proportionality constant based on acrylonitrile and its epoxide metabolite (Kedderis et al., 1996). This approach to estimating partitioning should be strengthened by further direct research with glycidamide.

3.2.3. Model fit

In general, Kirman et al. (2003) found that a single set of kinetic constants for the metabolism of acrylamide and glycidamide fit the tissue concentration data following i.p. dosing of rats, the tissue concentration data following i.v. dosing of rats, and the amounts metabolised following gavage dosing of rats. Although the model simulations for liver concentration of acrylamide in rats following an i.v. dose correspond well with measured data, the model tended to under-predict the concentrations of acrylamide in blood and muscle by a factor of between two and four after about two hours post dosing. Model simulations of the amount of acrylamide and glycidamide metabolised by each of the four predominant pathways did agree well with measured data. However, no

single set of tissue macromolecular binding constants for acrylamide and glycidamide was able to describe all the radiolabel tissue concentration data following i.v. dosing of rats from the two studies (Miller et al., 1982; Ramsey et al., 1984). Hence, the use of a one-compartment model with a single rate constant for macromolecular binding based on kinetic data on rate of formation of acrylamide adducts to the N-terminal valine of haemoglobin are unlikely to accurately predict measured target tissue data (Ehrenberg et al., 1983; Calleman, 1996).

Despite gaps and limitations to the database, the developed PBTK model parameters have been estimated to provide a good description of the kinetics of acrylamide and glycidamide using a single set of values (with minor adjustments in some cases) (Kirman et al., 2003). Future studies will need to focus on the collection of key data for refining certain model parameters and for model validation as well as for developing a similar model for humans.

3.2.4. Conclusions

A PBTK model was developed for acrylamide and glycidamide kinetics in rats by integrating published values for physiology, estimated values for partition coefficients, and estimated values for metabolism and tissue binding based upon fitting model output to measured kinetics data. The fits of model output to the majority of the accessible data were quite good. Certain model output represented the data better than others since the fits were attempted for the entire available data using either a single set of metabolic constants or a single set of macromolecular binding constants.

The model can be used in its present form for the purposes of prioritising the collection of additional data. The model has already been used to identify dose regions where non-linear kinetics might be observed due to saturation of metabolism or depletion of cofactors. The present level of detail allows for an optimum design for model validation. Sensitivity analyses performed on the model output can also be used to prioritise data collection once hypotheses regarding the mechanisms of toxic action are identified and linked to appropriate tissue doses. The model parameters that most influence the tissue doses of interest can be identified by the sensitivity analysis and their measurement made a priority. Possible mechanisms of toxic action for acrylamide and glycidamide include genotoxicity, reactivity with sulphydryls (including GSH) and interaction with certain receptors. Although the current model was developed with data from studies utilising non-oral routes of exposure at relatively high doses of acrylamide, adjustments can be made for dietary exposures and dosages.

3.2.5. Uncertainties/gaps

Further improvement of the PBTK model will depend on the collection of key data for refining model parameters, including parameters that were estimated from studies of other chemicals. Extension of the model to humans will require measurements of selected parameters in human tissues, perhaps by in vitro studies, and incorporation of new data on adducts from human studies.

In addition, kinetic studies linked to mode of action research with acrylamide should be considered. These studies would define adducted target proteins and develop PBTK models to predict the concentrations of these adducts over time. For example:

(1) Acrylamide and glycidamide may act as carcinogens after acrylamide exposures by direct reaction with DNA bases leading to mutations during cell division and ultimately to cancer. In this case, the ability to simulate adduct concentrations with the PBTK model in animals with specific acrylamide-associated tumour burdens would form the basis for risk assessment calculations for these compounds. Interspecies extrapolation of DNA and protein reactivity from in vitro studies would support risk assessment calculations. However, other modes of action for these compounds have also been proposed, especially in view of the diverse group of target tissues and the tissue reactivity of acrylamide/glycidamide.

(2) A second mode of action related to GSH depletion, or GSH depletion associated with reactivity toward other proteins, could also be explored with the PBTK model already developed. This model could be improved by new studies of GSH status in various tissues following dosing with acrylamide. Some studies should be considered in relation to the oral cavity tumours, reported in female rats in one study (Johnson et al., 1986), where direct reactivity may be more important due to direct exposure of the epithelial tissues to acrylamide and glycidamide. PBTK modelling has also recently been applied to evaluate oral cavity tumours with vinyl acetate (Sarangapani et al., 2000), another compound that causes increases in oral cavity tumours. The extension of the present acrylamide/glycidamide model would need to include metabolic constants for acrylamide/glycidamide in the oral cavity epithelium and GSH levels in tissues during and after feeding.

(3) A more concerted effort is also likely to be necessary to determine the nature of specific protein targets that react with acrylamide/glycidamide in target tissues. Protein reactivity studies can now be more readily pursued due to continuing developments in protein mass-spectrometry. Such studies should permit more comprehensive evaluation of the interaction of acrylamide/glycidamide with multiple tissue constituents that may serve as primary cellular targets for these compounds.

(4) A possible mode of action for acrylamide/glycidamide is reaction with specific cellular targets, i.e., either with intracellular molecules or surface molecules, which

when adducted alter cell function. In any new toxicokinetic studies on acrylamide/glycidamide kinetics and disposition, it would be valuable to more fully catalog cellular protein adducts caused by acrylamide/glycidamide. As a first step in this direction, the nature of binding in peripheral nervous tissues could be studied in vitro and with high specific activity radiolabelled acrylamide in vivo. The chemistry of the binding and the formation of tissue adducts could be then included in the PBTK model within nervous tissues and direct research with other cells/tissues.

(5) The further elaboration of the interactions of these compounds in target tissues should also include examination of binding in other organs that develop tumours following acrylamide exposures. Cell lines from target tissues—thyroid, mammary tissues, uterus, scrotal mesothelial cells, and specific brain cell types—might also be used to assess specific binding, or alternatively, in vivo studies could be performed with radiolabelled acrylamide of high specific-activity. In addition, specific studies might be done to assess reactivity of acrylamide/glycidamide with specific molecules implicated in acrylamide/glycidamide toxicity, such as dopamine receptors or thyroid stimulating hormone (TSH) receptors in thyroid tissue.

3.3. Biomarkers of internal dose

3.3.1. Introduction

The internal dose of acrylamide may be estimated from determinations of concentrations of acrylamide or its metabolite glycidamide in bodily fluids, or alternatively by quantifying the extent of adduct formation of acrylamide or glycidamide with proteins or DNA. As indicated in Section 2.5, acrylamide forms adducts readily with sulphydryl groups in proteins and to a lesser extent with amino groups. The latter include the N-terminal amino groups of valine in globin, where adducts of acrylamide and glycidamide have been studied in detail as biomarkers of internal dose, in experimental animals and in humans. Acrylamide reacts very slowly with DNA, and thus DNA adduct measurement has not been used a biomonitor of internal dose of acrylamide. However, glycidamide forms adducts with guanine and adenine in DNA (see below), which are potential markers of the biologically active dose of acrylamide that reaches the DNA. Fig. 5 shows structures of several molecules used as biomarkers of dose of acrylamide.

3.3.2. Biomarkers of internal dose—haemoglobin adducts

3.3.2.1. Introduction. Several nucleophilic sites in proteins give rise to adducts with acrylamide (through Michael addition of the ethylenic group) and with glycidamide (through alkylation by either oxirane carbon) (reviewed by Friedman, 2003). Such reaction products with (adducts to) proteins are used for measurement of short-lived, reactive compounds in vivo. Haemoglobin

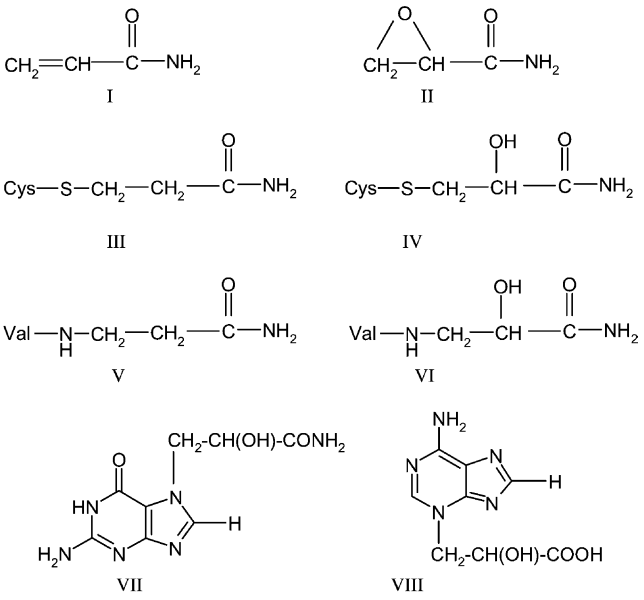


Fig. 5. Structures of molecules used as biomarkers of dose of acrylamide. (I) Acrylamide, (II) glycidamide, (III) *S*-(2-carbamoylethyl)cysteine, (IV) *S*-(2-carbamoyl-2-hydroxyethyl)cysteine, (V) *N*-(carbamoylethyl)valine, (VI) *N*-(2-carbamoyl-2-hydroxyethyl)valine, (VII) *N*7-(2-carbamoyl-2-hydroxyethyl)guanine, (VIII) *N*3-(2-carbamoyl-2-hydroxyethyl)adenine. Compounds III and IV have been analysed as their acidic hydrolysis products (carboxylic acids).

(Hb) has several advantages as monitor molecule such as a long and definite life span (about 120 days in humans). Furthermore, methods permitting identification and quantification of protein adducts as derivatised modified amino acids or detached adducts by mass spectrometry are well developed (reviewed by Törnqvist et al., 2002). Levels of Hb adducts could be used as a basis for toxicokinetic calculations and calculations of uptake and of dose in blood etc.

Indeed, measurement of a background level of a Hb adduct corresponding to acrylamide in persons without known exposure to acrylamide, gave the indication of a regularly occurring exposure to acrylamide in the population (Bergmark, 1997). A preliminary estimation of the uptake led to the conclusion that this exposure could lead to non-negligible cancer risk (Törnqvist et al., 1998). Studies of Hb adducts in rats fed fried feed thereafter showed that heated food was the probable origin of a background exposure to acrylamide (Tareke et al., 2000). This was then verified by the demonstration that acrylamide is formed during heating of a range of foods (Tareke et al., 2002).

3.3.2.2. Cysteine adducts. Cysteine-S is a relatively strong nucleophilic site in Hb. Studies of Hb adducts in rats could benefit from the high reactivity of the SH group of $Cys^{125\beta}$, which has a reactivity that is about 2 orders of magnitude higher than that of cysteine in human Hb (see references in Törnqvist et al., 2002, cf. Bergmark et al., 1993 for acrylamide).

Acrylamide. Hashimoto and Aldridge (1970) showed reaction of acrylamide with cysteine-S in Hb. Bailey et al. (1986) developed a method to determine this adduct in rat haemoglobin. Cysteine adducts were isolated after acid hydrolysis of the protein, also hydrolysing the amide group, as *S*-(2-carboxyethyl)cysteine from acrylamide. The modified cysteine was derivatised before the GC–MS analysis. This method has been applied for measurement of acrylamide as adducts in blood from acrylamide-exposed rats (Bergmark et al., 1991; Barber et al., 2001c).

Glycidamide. The formation of the epoxide-metabolite, glycidamide, from acrylamide was demonstrated in vivo and in vitro through analysis of adducts to cysteine. *S*-(2-carboxy-2-hydroxyethyl)cysteine was determined by GC–MS in Hb from acrylamide-exposed rats and after incubation of acrylamide and cysteine with liver microsomes (Calleman et al., 1990). Adducts from glycidamide to cysteine have also been measured in Hb from acrylamide- and glycidamide-exposed rats after total acid hydrolysis of the globin (Bergmark et al., 1991; Barber et al., 2001c).

3.3.2.3. Valine adducts. Valine is the N-terminus of Hb in all four globin chains in e.g. mice, rats and humans. The N-termini are major reactive nucleophilic sites for many electrophilic agents in Hb, particularly in human Hb, where, in contrast to rat Hb, there is no cysteine with high reactivity. In order to increase sensitivity a modified Edman degradation method (Törnqvist et al., 1986) for measurement of adducts to N-terminal valine in Hb by mass spectrometric methods has been applied for measurement of acrylamide exposure in humans. This procedure leads to detachment and derivatisation of the modified N-terminal valine in one step (Rydberg et al., 2002).

Adducts from acrylamide and glycidamide are measured as derivatives of *N*-(2-carbamoylethyl)valine and *N*-(2-carbamoyl-2-hydroxyethyl)valine, respectively (Bergmark et al., 1993). Adducts have been measured by GC/MS–MS, in negative ion chemical ionisation (NICI) mode or electron impact (EI) mode. For the analysis by GC/MS of the adduct from glycidamide the polar groups in the adduct have been further derivatised for improvement of analytical sensitivity (Licea Perez et al., 1999; Paulsson et al., 2003a). Analysis by liquid chromatography/tandem mass-spectrometry (LC/MS–MS) has recently been applied (Fennell et al., 2003). In rodents and humans exposed to acrylamide, adducts from acrylamide and glycidamide to N-termini of Hb have been measured with versions of the modified Edman degradation method. Adducts to N-termini have also been measured in humans without occupational exposure to acrylamide.

Acrylamide. The adduct from acrylamide to N-terminal valine in Hb has been measured in several studies of rodents exposed to acrylamide (as well as *N*-methylolacrylamide) (Paulsson et al., 2002; Paulsson et al., 2003a,b; Fennell et al., 2003; Sumner et al., 2003). The relation between exposure dose and internal dose, and the relation between internal dose and the frequency of induced micronuclei, have been studied in mice and rats.

In early studies of humans occupationally exposed to acrylamide (work in acrylamide production) adducts to N-terminal valine were explored for monitoring exposure using the above-mentioned method and analysis with GC–MS/MS (Bergmark et al., 1993; Calleman et al., 1994). This modified Edman degradation method has later been applied for monitoring of acrylamide exposures in different occupations (see Table 12).

Background levels of acrylamide-valine adducts in Hb from persons without occupational exposure to acrylamide have been measured in reference persons in studies of occupational exposures or in studies of background values (see Table 13). The background adduct from acrylamide in control persons (non-smokers) was found at about the same level (about 30 pmol/g) in different studies given in Table 13, as well as in other studies (Licea Perez et al., 1999, with $n = 2$; Paulsson and Törnqvist, unpublished, $n \sim 50$).

Bergmark (1997) found an average increment of the adduct level from acrylamide to N-terminal valine in

Table 12
Levels of adducts to N-terminal valine in haemoglobin from acrylamide measured in blood from workers in different exposure situations

Type of occupational exposure	Number of workers	Adduct level Hb range (nmol/g)	Reference
Acrylamide production (PR China)	41	0.3–34	Bergmark et al. (1993)
Acrylamide production (Korea)	11	0.071–1.8	Licea Perez et al. (1999)
Grouting in tunnel work (Sweden)	47	0.02–0.07	Hagmar et al. (2001)
	163	0.07–4.3 (18[a])	
Grouting in tunnel work (Norway)[b]	11 (non-smokers)	0.033–0.085 (0.28[a])	Kjuus et al. (2004)
	12 (smokers)	0.13–0.15 (0.89[a])	
Work with polyacrylamide gels (Sweden)	15 (non-smokers)	0.024–0.12	Bergmark (1997)
Work with polyacrylamide sealing	1	23	Paulsson et al. (2005)

[a] Extreme value.
[b] Blood sampling 63–143 days after discontinuation of grouting work.

Table 13
Background adduct levels from acrylamide to N-terminal valine in haemoglobin measured in non-smokers without occupational exposure to acrylamide

Adduct level (nmol/g)[a]	Number of persons	Reference
Mean: 0.031; Range: 0.024–0.049	8	Bergmark (1997)
Mean: ~0.04; Range: 0.02–0.07	18	Hagmar et al. (2001)
Mean: 0.033; Range: 0.020–0.047	6	Kjuus et al. (2004)
Median: 0.021; Range: 0.012–0.050	25	Schettgen et al. (2003)
Mean: 0.027 (SD: ±0.006)	5	Paulsson et al. (2003a)

[a] As given in the publication.

Hb of about 6 pmol/cigarette and day in smokers ($n = 10$). In the other studies cited in Table 13 enhanced adduct levels in smokers have been found at levels in agreement with Bergmark's (1997) results.

In the studies in Table 13 the modified Edman method for analysis of N-terminal valine adducts was used. It should be noted that the studies, except the study by Schettgen et al. (2003), in Table 13 are performed with the same MS-instrument (at Stockholm University) and the same standards for quantification. In the study of tunnel workers by Hagmar et al. (2001) a somewhat higher mean background adduct level was calculated in the non-smoking reference persons (Table 13). The somewhat higher value is probably due to a lower precision at low adduct levels in this study, aiming at disclosure of rather high occupational exposure. The background Hb adduct level from acrylamide to N-terminal valine could therefore, from the so far published studies, be estimated to be about 30 pmol/g, with a variation between individuals by a factor of about 2.

Glycidamide. Bergmark et al. (1993) measured glycidamide adducts to N-terminal valine in Hb with the modified Edman method in a few workers with high exposures to acrylamide. The analytical sensitivity and reproducibility of the method were later improved (cf. 3.3.2.3).

The adduct from glycidamide to N-terminal valine in Hb has been measured in rodents exposed to acrylamide or glycidamide for studies of metabolism or relation to genotoxic effects or dependence on exposure route (Paulsson et al., 2002, 2003a,b; Fennell et al., 2003; Sumner et al., 2003).

The relation between levels of adduct to N-terminal valine from acrylamide and glycidamide has been measured in humans in a few published studies. The data obtained in occupationally exposed humans by Bergmark et al. (1993) is in agreement with data from Paulsson et al. (in Paulsson et al., 2003b), showing an adduct level ratio of about 1:1. Considering that acrylamide reacts with valine-NH_2 about 3 times slower (Bergmark et al., 1993) than glycidamide, the ratio 1:1 in adduct level suggests that the dose of glycidamide is about 30% of the acrylamide dose. The same ratio has been published by Paulsson et al. (2003a) from studies of a few persons with only background exposure. However, lower relative levels of glycidamide adducts have been observed in studies of a few individuals (Licea Perez et al., 1999). Further studies are needed for careful clarification of the relation between internal doses of acrylamide and glycidamide in humans at background exposure levels.

3.3.2.4. Specificity of haemoglobin adducts. Acrylamide (and glycidamide) contains three carbons and is therefore a small molecule, the adducts and analyte derivatives of which might have different origins. A positive response in a biomarker reaction may therefore raise questions about the specificity. In studies of Hb adducts from acrylamide in rats fed fried feed, it was verified that the adduct measured really corresponded to acrylamide, by interpretation of fragment pattern in MS–MS analysis and by the estimation that the incremental adduct level was compatible with the intake of acrylamide in the rats (Tareke et al., 2000). Furthermore, using level of adducts to Hb N-termini as biomarker the formation of adducts by mis-incorporation of the adducted amino acid, valine, can be excluded, whereas incorporation in protein synthesis of amino acids, e.g. cysteine, histidine, with adducts in the side chains, may occur (Kautiainen et al., 1986). This is an advantage in studies of background levels of adducts corresponding to very low levels of exposure. Also, in humans, exposure to acrylamide at low to moderate levels leads to approximately equal levels of the valine adducts of acrylamide and glycidamide. The simultaneous measurement of adducts from the metabolite glycidamide would therefore support the identification of a signal as the acrylamide adduct. It should be noted, however, that in GC/MS–MS analysis, adducts from *N*-methylolacrylamide gives the same adduct as acrylamide (Paulsson et al., 2002).

3.3.2.5. Calculation of dose and uptake from haemoglobin adducts. A dose defined as the integral over time of the concentration (i.e. AUC, with the dimension $\text{mol} \times \text{L}^{-1} \times \text{h}$, that is M h) is applicable in estimations of risks of health effects (e.g. cancer) (Ehrenberg et al., 1983). The dose in vivo of an electrophilic compound could be inferred from the level of an adduct to a biomacromolecule if the rate of formation and the stability of the adduct is known. Using the in vivo dose concept, metabolic rates could be inferred from the Hb adduct level if the administered dose is known, as in animal experiments, or estimated, as in studies of humans. These data could then be used to calculate the uptake from a measured Hb adduct level.

The rates of formation of adducts from acrylamide or glycidamide to cysteine and N-terminal valine in Hb

were determined by Bergmark et al. (1993). These adducts are stable over the life-span of Hb. Using the in vivo dose concept, Calleman (1996) has estimated the rate of elimination of acrylamide in humans to 0.15 h^{-1} (half-life 4.6 h) and to approximately the same value for glycidamide. Strictly, the doses of acrylamide calculated from Hb adduct levels are the doses in blood. Due to rapid disposition of acrylamide in the body a one-compartment model is, however, approximately valid (Calleman, 1996). Using the rate constant for formation of adducts from acrylamide to N-terminal valine and the rate of elimination of acrylamide in humans the uptake could be inferred from the average steady-state level of adducts. According to the calculation below, an average adduct level of 30 pmol/g Hb would correspond to a daily uptake of about 80 μg by a 70 kg person (Törnqvist et al., 1998; cf. Bergmark, 1997). Using the same type of calculation it was estimated in the earlier feeding experiments with rat, that the content of acrylamide in the fried feed was compatible with the increment in the Hb adduct level from acrylamide (Tareke et al., 2000). This indicates a relatively good bioavailability of the acrylamide in food. The accuracy of these calculations could be improved by independent determinations of the equation parameters.

Calculated uptake at steady-state (background) level of 30 pmol/g globin of adduct from acrylamide to N-terminal valine in Hb:

$$\text{Uptake } (\mu g\ kg^{-1} day^{-1}) = \frac{[\text{adduct level}(30 \cdot 10^{-12}\ \text{mol/g globin})] \times [\text{elimination rate } (0.15\ h^{-1})] \times [71\ \text{g/mol}] \times [1\ \text{L/kg}]}{[\text{life span of erythrocytes} \times 1/2\ (63\ \text{days})] \times [\text{rate of adduct formation } (4.4 \cdot 10^{-6}\ \text{L(g globin)}^{-1}\ h^{-1})]} = 1.1\ (\mu g\ kg^{-1}\ day^{-1})$$

Species difference and individual differences in metabolic rates of acrylamide and glycidamide will influence the relation between exposure dose and internal dose in blood, measured as Hb adduct levels. This relation has been studied in mice and rats at relatively high exposure levels by several researchers. Studies of acrylamide exposure at lower dose rates are ongoing at several laboratories. The influence of polymorphism in detoxification enzymes is studied in humans (Paulsson et al., to be published).

3.3.2.6. Conclusion: haemoglobin adducts as biomarkers of internal dose. In most cases the dose as represented by AUC can be inferred from measured levels of adducts to the N-termini in Hb, from reactivity of the electrophilic compound and the rate constants for reaction with the valine amino group. Especially in studies of total and daily intake during protracted or chronic exposure, the well-known values of life-span of Hb (and stable adducts) are of distinct value. It is often desirable to measure the distribution of the internal dose to different organs or tumour sites, thus tissue analysis of DNA adducts should be useful.

The different versions of the method for measurement of adducts from acrylamide and glycidamide to N-terminal valine in Hb have sufficient sensitivity and reproducibility for studies of various issues, e.g. exposure, internal dose and metabolism, concerning dietary acrylamide. A ring-test between different laboratories of analysis of Hb adducts from acrylamide/glycidamide would be useful. Hb adduct measurement of a large number of samples in molecular epidemiological studies would benefit from a more rapid sample work-up of the modified Edman degradation method for analysis of N-terminal valine Hb-adducts.

3.3.3. Biomarkers of internal dose—DNA adducts

3.3.3.1. Acrylamide in vitro. Solomon et al. (1985) studied the formation of adducts when acrylamide reacts with 2′-deoxynucleosides and with calf thymus DNA. Adduct formation was slow (reaction time 40 days at pH 7, 37 °C) and was shown to occur at *N*1 and *N*6 of 2′-deoxyadenosine (the former being the major product in DNA), *N*3 of 2′-deoxycytidine, *N*1 and *N*7 of 2′-deoxyguanosine (the latter being detected as the depurinated alkylated base), and *N*3 of thymidine (not detected as a DNA reaction product). The reaction occurred via Michael addition, producing initially 2-carbamoylethyl adducts. In some cases (especially when the adduct is adjacent to an exocyclic nitrogen) hydrolysis of this occurs to yield the 2-carboxyethyl adduct. Fig. 6 shows the structures of the deoxynucleoside and base adducts identified.

3.3.3.2. Glycidamide in vitro. When glycidamide reacts in vitro with 2′-deoxyguanosine, the major adduct produced was identified by mass spectrometry, UV and ^{13}C NMR to be *N*7-(2-carbamoyl-2-hydroxyethyl)-

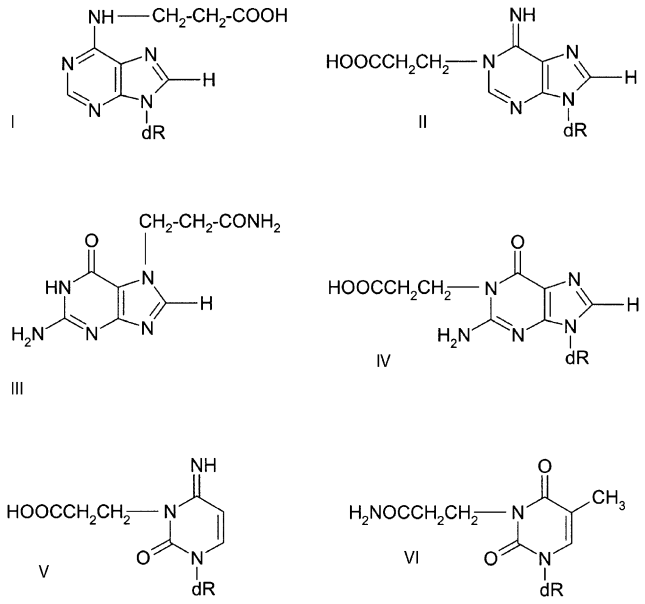


Fig. 6. Deoxynucleoside adducts of acrylamide: (I) *N*6-(2-carboxyethyl)-2′-deoxyadenosine; (II) 1-(2-carboxyethyl)-2′-deoxyadenosine; (III) 7-(2-formamidoethyl)-guanine; (IV) 1-(2-carboxyethyl)-2′-deoxyguanosine; (V) 3-(2-carboxyethyl)-2′-deoxycytidine; (VI) 3-(2-formamidoethyl)-thymidine.

guanine, (the glycidamide adduct with the *N*7 position of guanine) (Segerbäck et al., 1995) (VII in Fig. 5). Incubation of [^{14}C]acrylamide with DNA in the presence of S9 from rat liver, followed by depurinating hydrolysis yielded a product which eluted from HPLC similarly to synthetic *N*7-(2-carbamoyl-2-hydroxyethyl)guanine. More recently Gamboa da Costa et al. (2003) have carried out a detailed study of the interaction products of glycidamide with 2′-deoxyguanosine and 2′deoxyadenosine. For 2′-deoxyguanosine, the structure of the product *N*7-(2-carbamoyl-2-hydroxyethyl)guanine (VII, Fig. 5) was confirmed. The major adducts with 2′-deoxyadenosine were found to be *N*3-(2-carbamoyl-2-hydroxyethyl)adenine (VIII, Fig. 5) and *N*1-(2-carboxy-2-hydroxyethyl)adenine. All these three adducts were detected in DNA that had been incubated with glycidamide.

3.3.3.3. Acrylamide and glycidamide in vivo. The distribution and binding of [^{14}C]acrylamide was studied in SENCAR and BALB/c mice, after topical and oral administration, by Carlson and Weaver (1985). In the liver, which was the tissue of maximum DNA binding, the [^{14}C] content decreased by about one half between 6 and 24 h. SENCAR mice were reported to be more sensitive to skin papillomas following acrylamide treatment than BALB/c mice. However, the two strains did not differ in the amounts of binding of [^{14}C] to DNA in the tissues studied, including skin and liver.

Segerbäck et al. (1995) administered [^{14}C]acrylamide i.p. to rats and mice. Liver DNA was depurinated and the product was found to contain one radioactive component which coeluted on HPLC with *N*7-(2-carbamoyl-2-hydroxyethyl)guanine. The levels of this adduct were similar in different organs of the rat, indicating good distribution of glycidamide. Gamboa da Costa et al. (2003) developed a sensitive analytical system using LC–MS/MS and isotope dilution, and used this to study DNA adduct formation in mice (adult male C3H/HeNMTV, adult female C57Bl/CN and neonatal B6C3F$_1$) administered acrylamide or glycidamide i.p. Adducts were detected in liver, lung and kidney, with about 100-fold more *N*7-(2-carbamoyl-2-hydroxyethyl)guanine than *N*3-(2-carbamoyl-2-hydroxyethyl)adenine. *N*7-(2-carbamoyl-2-hydroxyethyl)guanine was detected in DNA of untreated mice, presumably reflecting the fact that acrylamide is present in rodent diets (Twaddle et al., 2004a). Administration of glycidamide produces higher levels of adducts than administration of acrylamide, and there was evidence that there was saturation of epoxidation at high doses.

In a comparison between adult F344 rats and B6C3F$_1$ mice administered the same dose of acrylamide (50 mg/kg), the levels of the *N*7-(2-carbamoyl-2-hydroxyethyl)guanine adducts in rat leukocyte DNA were 15–70/10^6 nucleotides, and in mouse tissues 10–20/10^6 nucleotides (McDaniel et al., 2004). *N*3-(2-carbamoyl-2-hydroxyethyl)adenine was about 90-fold less abundant.

3.3.3.4. Conclusion: DNA adducts as biomarkers of internal dose. *N*7-(2-carbamoyl-2-hydroxyethyl)guanine appears to date to be the most suitable biomarker of the dose of genotoxically active material that reaches DNA after exposure to acrylamide. The importance of the metabolite glycidamide, rather than the parent compound acrylamide, as the mediator of the genotoxic effects of acrylamide has been confirmed in many studies, including recently those showing its greater mutagenic potential in transgenic mouse embryonic fibroblasts (Besaratinia and Pfeifer, 2003, 2004) and in the HPRT locus in V79 cells (Baum et al., 2004), and greater DNA damaging potential measured by single cell gel electrophoresis in human lymphocytes (Baum et al., 2004). An administered dose of 50 mg/kg acrylamide yields the adduct *N*7-(2-carbamoyl-2-hydroxyethyl)guanine in DNA, with a concentration of approximately 20/10^6 nucleotides, in adult mouse tissues after 6 h (Gamboa da Costa et al., 2003). The limit of quantitation of this adduct using LC–MS/MS was 2/10^8 nucleotides, which is similar to that of many other biomarker procedures for measuring DNA damage in humans environmentally exposed to genotoxicants. The repair of this DNA lesion is unknown, although it would be expected to be lost by spontaneous or enzyme catalysed depurination. Thus, its use as a biomarker of internal dose is likely to be more appropriate for detecting recent

exposures to acrylamide. There is still a need for a longer term DNA adduct biomarker for determining internal doses over long periods. The relationship between the *N*7-(2-carbamoyl-2-hydroxyethyl)guanine adduct level and exposure dose in humans is so far unknown.

3.3.3.5. Biomarkers of internal dose-protamine adducts. Sega et al. (1989) conducted an evaluation of the time-dependence of the binding of radioactivity to protamine and DNA in sperm from mice administered 125 mg/kg [1-^{14}C]acrylamide. Dominant lethality reached a peak with conception at 5-8 days following administration of acrylamide (Shelby et al., 1986). The temporal pattern of radioactivity bound to sperm recovered from the *vas deferens* closely followed the temporal pattern of dominant lethal mutations, reaching a peak at day 9 (Sega et al., 1989). The radioactivity bound to DNA accounted for a small fraction of the total radioactivity bound to the sperm. In sperm from the caudal epididymis, total radioactivity bound reached a maximum on day 8 after dosing, as did the binding to protamine. The time difference between the sperm recovered in the *vas deferens* and the caudal epididymis was consistent with a two-day transit time. Two adducts were detected on acid hydrolysis of protamine, one of which was *S*-(2-carboxyethyl)cysteine (approximately 31% of the radioactivity bound), which would be produced by the reaction of acrylamide with cysteine residues in protamine. The second adduct peak (which accounted for approximately 69% of the radioactivity) was not characterised.

From this study, Sega et al. (1989) proposed that the dominant lethal effects of acrylamide could be caused by the binding of acrylamide to immature protamines, causing a blockage by the crosslinking of protamines, and preventing chromatin condensation within the nucleus. This in turn could result in DNA breakage, and dominant lethal events.

In evaluating this study, and its relevance to mode of action for acrylamide, the design may have limited the capability to detect DNA adducts. The specific activity of acrylamide used was 1 mCi/mmol. The amount of sperm DNA used in the measurements of DNA binding was not indicated for all samples, but was 44.2 μg DNA at 9 days. A total activity of 5.1 dpm was measured in this sample, and the alkylations were estimated at 16 alkylations per 10^6 nucleotides. The low levels of radioactivity in DNA are at the limit of detection, and would suggest the absence of significant adducts. However, DNA adducts from glycidamide have been detected recently using more specific methods, and have indicated that the amount of *N*7-(2-carbamoyl-2-hydroxyethyl)guanine formed by administration of acrylamide is in the range of 2000 adducts per 10^8 nucleotides in liver, lung and kidney in mice administered 50 mg/kg acrylamide by i.p. injection. Binding of acrylamide to protamines may be involved in the generation of DNA strand breaks. However, not all of the adducts in protamine have been characterised. Binding of glycidamide could be involved. More recent studies have indicated that administration of 1-aminobenzotriazole, an inhibitor of CYP 2E1 and oxidation of acrylamide to glycidamide, inhibits the acrylamide induced dominant lethal effects, suggesting a role for glycidamide (Adler et al., 2000). Glycidamide administered directly to mice caused a dominant lethal response (Generoso et al., 1996). Two modes of action were discussed: binding to protamine, and binding to DNA. Glycidamide could bind to cysteine residues in protamine, and account for the uncharacterised adduct described by Sega et al. (1989). Alternatively, glycidamide could bind to DNA, forming adducts as described by Segerbäck et al. (1995) and more recently by Gamboa da Costa et al. (2003).

3.3.4. Uncertainties/gaps

The major uncertainties in the use of adducts as biomarkers of internal dose are:

- improved analytical methods are needed for adducts (especially DNA adducts), that would be appropriate for human biomonitoring,
- lack of knowledge of the dose response for DNA adduct formation in humans,
- lack of extensive validation of some analytical methods,
- effect of polymorphisms in metabolising or detoxifying enzymes.

4. Conclusions

The aim of this effort was to develop a framework contributing to the overall risk assessment of acrylamide in food. It is based on the outcome of the ILSI Europe FOSIE (Food Safety in Europe—Risk Assessment of Chemicals in Food and Diet) project. This work is adding to this overall framework by focusing especially on the human exposure and internal dose assessments of acrylamide in food. Emphasis is being given to a review of the current knowledge on acrylamide formation and levels in food, the type of foods and consumption patterns of acrylamide containing food, intake in individuals and subgroups of the population, biomarkers of exposure and internal dose, bioavailability and a physiologically-based toxicokinetic model. The risk assessor should be provided with an overview of the current level of knowledge pertaining to acrylamide exposure, whilst highlighting sources of variability, gaps and uncertainties, and emphasising further research needs relevant to the overall acrylamide risk assessment.

4.1. Formation and food levels

The current hypothesis of the main route of acrylamide formation is the condensation of free asparagine with reducing sugars in the Maillard reaction. Intermediates of the reaction in foods are, however, not fully characterised. Minor pathways most probably contribute only little to overall food acrylamide levels, but should be kept in mind in the light of special food types, processing and/or ingestion by subgroups with high intakes.

Analytical methods for acrylamide are sufficiently developed, including procedures for difficult matrices such as coffee or cocoa beans. In general, early estimations of levels in the major food categories and exposure estimates of the average consumer were largely correct, increased reliability of analytical methods basically confirmed earlier findings. Proficiency tests are ongoing, and standardised reference materials should be available soon. Databases in Europe and North America collect reliable, quality-checked measurements covering relevant food categories that presumably contribute the most to overall exposure. The major food categories contributing to most of the human exposure are similar in North America and Europe, comprising French fries, potato fritter, potato chips, cereals, crispbread, bread, coffee, pies and pastry. However, data exist only for some European countries and North America, data for Asian and African countries are lacking. Moreover, effects of cultural differences within the EU and the US on human exposure have not yet been extensively addressed. Problems remaining are inherent to the large between-sample variability, requiring large sample numbers to reach statistical significance when analysing effects on reduction within or between laboratories, or within or between food categories. There is insufficient information of the acrylamide content of foods that have not been heated to high temperature (and the reason for the presence of this acrylamide). The impact and contribution to overall exposure of foods contain acrylamide at a level below the limit of quantification remains to be established.

Options to reduce levels of acrylamide in food, either industrially processed or prepared at home, are very limited to date. Some successes have been demonstrated using enzymatic digestion of asparagine in processed potato products, or applying more defined frying conditions. The use of these methods on the industrial scale will not be available in the short term. In addition, it has to be kept in mind that any change of the processing conditions will change the final product with respect to quality, taste and texture. Moreover, reduction of acrylamide levels in certain brands or products will not necessarily change the overall exposure of consumers.

Detailed assessment of the quantitative contribution to overall acrylamide exposure from food cooked at home is to date unavailable, as is the contribution of sources other than food, e.g. smoking. Thus an important task that remains is the deduction of overall exposure levels from the determination of biomarkers of exposure and internal dose, and their validation.

So far, adolescents have been identified to be exposed to significantly higher levels than the average adult consumer. However, the existence of possible susceptible subgroups is still unresolved. Pregnant women do not seem to represent a susceptible subgroup for developmental or neurodevelopmental effects at the current exposure levels.

4.2. Food consumption surveys

Rather than providing the risk assessor with an exhaustive inventory, this section was aimed to give examples of the type of study designs and dietary exposure data available and highlight the current methodological strengths and limitations for estimating and interpreting data.

While acrylamide levels were mostly gathered during the last two years by local, authorised laboratories, most of the dietary surveys used to estimate acrylamide exposure used national food survey data that partially dated back many years, and were thus not specifically designed for an acrylamide exposure assessment. Consequently, they do not necessarily reflect current dietary consumption habits and exhibit limitations concerning e.g. information on preparation and cooking method, or preparation at home versus industrial preparation.

The methods used to record dietary intakes varied considerably, with a large variability in the number of repeated measurements, the number and distribution of groups (age, gender), the number and detail of food items recorded, the aggregation of food categories etc. Different parameters of distribution were not systematically given, with different levels of coverage of the mean, median, standard deviations and percentiles. In addition, the amount of concentration data varied considerably. However, the overall results remain consistent with the first conclusions of the FAO/WHO consultation and the following meetings. In general, acrylamide intake values for adolescents are consistently higher than for adults, being inversely correlated with age when given on a body weight basis. Also the foods contributing the most to dietary exposure remain consistent between the studies, considering the foods that have been analysed to date.

However, the current methodological differences across studies do not permit full comparison and interpretation of results, and efforts should be made to harmonise them better. This is particularly important considering that dietary acrylamide relates to all dietary patterns and countries. The different levels of exposures across age categories and study populations need further investigations in order to identify in particular

the different determinants and characteristics of the populations potentially at risk, as well as the confounding factors and co-variables to be systematically considered in the statistical analyses such as for example age.

A better understanding of the within- and between-subject variability of acrylamide and its main sources is also needed in order to determine the number of repeated measurements actually needed to estimate acrylamide intakes, particularly when open-ended methods are used. Information on home cooking methods and industrial processes (e.g. through brand names and/or product names) and sufficient descriptive detail on the acrylamide food sources should be systematically requested in order to estimate dietary acrylamide intakes. Furthermore, the reliability of the external dietary exposures should be evaluated against validated biomarkers of internal exposures (e.g. haemoglobin or DNA adducts), which are still under development.

Biomarkers. The selection of biomarkers has to take into consideration several aspects, irrespective of the intended use as a biomarker of exposure or as a biomarker of internal dose. The rate of metabolism of acrylamide to glycidamide is important in the light of the toxic effects supposed to be induced by the two compounds, acrylamide being more relevant in binding to proteins and neurotoxicological effects, and glycidamide presumably being relevant for DNA binding and therefore for a genotoxic mode of action. Acrylamide itself is a potential biomarker that can be detected in human urine and blood. Glycidamide itself was not detected in human urine to date. The urinary metabolite *N*-acetyl-*S*-(2-carbamoylethyl)cysteine may be used, but the available analytical method is not specific for acrylamide. Acrylamide forms adducts readily with sulphydryl groups in proteins and to a lesser extent with amino groups. The latter include the N-terminal amino groups of valine in globin, where adducts of acrylamide and glycidamide have been studied in detail as biomarkers of internal dose, in experimental animals and in humans. Levels of Hb adducts could be used as a basis for toxicokinetic calculations and calculations of uptake and of dose in blood etc. The different versions of the method for measurement of adducts from acrylamide and glycidamide to N-terminal valine in Hb have sufficient sensitivity and reproducibility for studies of problems, e.g. exposure, internal dose and metabolism, concerning dietary acrylamide. Interlaboratory validation of the methodology for the analysis of Hb adducts from acrylamide/glycidamide is required.

*N*7-(2-Carbamoyl-2-hydroxyethyl)guanine appears to date to be the most suitable biomarker of the dose of genotoxically active material that reaches DNA. Its use as a biomarker of internal dose seems appropriate for detecting recent exposures to acrylamide. There is still a need for a longer term DNA adduct biomarker for determining internal doses over long periods. The relationship between the *N*7-(2-carbamoyl-2-hydroxyethyl)guanine adduct level and exposure dose in humans is so far unknown.

The use of biomarkers are related to studies of metabolic rates, interspecies extrapolation and effects of polymorphism in metabolic or detoxifying enzymes and other aspects of interindividual variability. Interlaboratory validation of the analytical methods appropriate for human biomonitoring would be of value.

Overall, there is an important need to have a comparison of external dietary exposure and validated biomarkers of exposure and internal dose in order to assess the use of these biomarkers as exposure biomarkers at these exposure levels. Similarly studies of the relationship of biomarkers of internal dose with later biological effects would indicate the extent to which these biomarkers can contribute to risk assessment.

4.3. Bioavailability and toxicokinetics

In general the bioavailability of acrylamide after oral administration is high, but the oral bioavailability of acrylamide in humans is not known with certainty. It is unclear whether various food matrices may influence the bioavailability of acrylamide.

A PBTK model has been developed for acrylamide and its oxidative metabolite, glycidamide, in the rat based on available information. Despite gaps and limitations to the database, model parameters have been estimated to provide a good description of the kinetics of acrylamide and glycidamide using a single set of values. Future studies will need to focus on the collection of key data for refining certain model parameters and for model validation, as well as for developing a similar model for humans.

Understanding of acrylamide toxicokinetics would be improved by additional kinetic studies related to the mode of action of acrylamide (e.g. interspecies comparison of adduct concentration), knowledge of metabolic constants for glutathione interactions such as in the oral cavity and information on specific proteins that react with acrylamide in target tissues (including nervous tissues and/or organs that develop tumours).

A validated human acrylamide/glycidamide PBTK model capable of predicting target doses at relevant dietary exposures, in combination with expanding data on mechanism of action, will allow for a substantive improvement in reducing the uncertainty in assessing the public health risk of exposure to acrylamide in food.

Acknowledgments

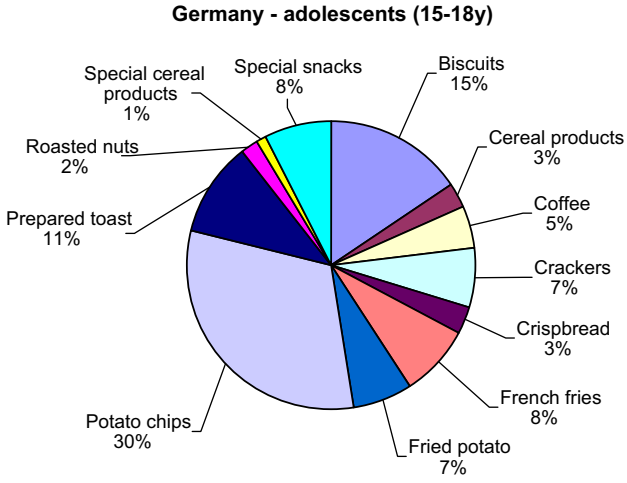
This project is a joint effort of the ILSI Europe Acrylamide Task Force and the ILSI North America Technical Committee on Food Toxicology and Safety

Assessment and its Subcommittee on Acrylamide. They would like to thank the expert group/authors for their commitment to this project and for preparation of the publication. In addition, they would like to thank the national and international institutes and individuals in countries in Europe and elsewhere who contributed to the inventory of existing databases. In particular they would like to thank Dr. Hambridge, Food Standards Australia New Zealand, Dr. De Henauw, University of Ghent (B), Dr. Ruprich, National Institute of Public Health (CZ), Dr. Volatier and Dr. Tard, AFSSA (F), Dr. Sommerfeld and Dr. Dehne, Federal Institute for Risk Assessment (D), Dr. Bergsten, Norwegian Food Control Authority (N), Dr. Becker, Swedish National Food Administration (S), Dr. Zoller, Swiss Federal Office of Public Health (CH), Dr. Konings, Dutch Food Authority (NL) and Dr. Dinovi, US FDA.

This work was supported by a grant from the Acrylamide Task Force of the European branch of the International Life Sciences Institute (ILSI Europe). Industry members of this task force are Barilla, Cereal Partners (Nestlé & General Mills), Danisco Sweeteners, Frito Lay, Group Danone, Kellogg, Kraft Foods, Masterfoods, Nestlé, Procter & Gamble, RHM Technology, Swiss Quality Testing Services (SQTS) and Unilever. The opinions expressed herein are those of the authors and do not necessarily represent the views of ILSI and ILSI Europe.

Appendix A

Selected examples on the contribution of acrylamide food sources according to different population studies and age categories



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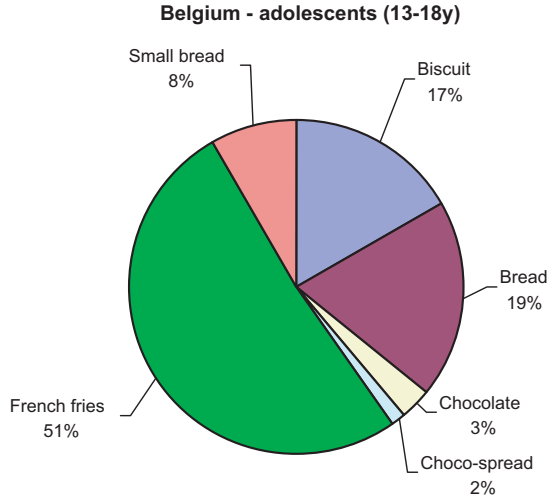

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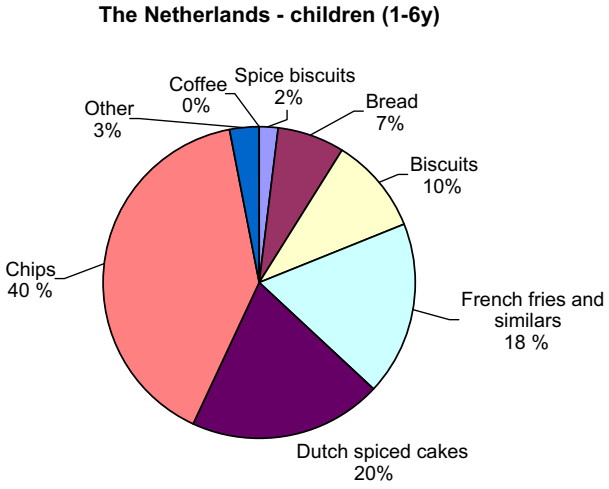

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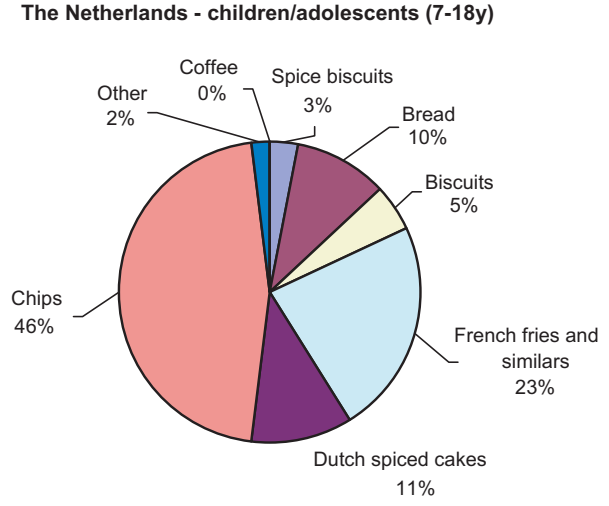

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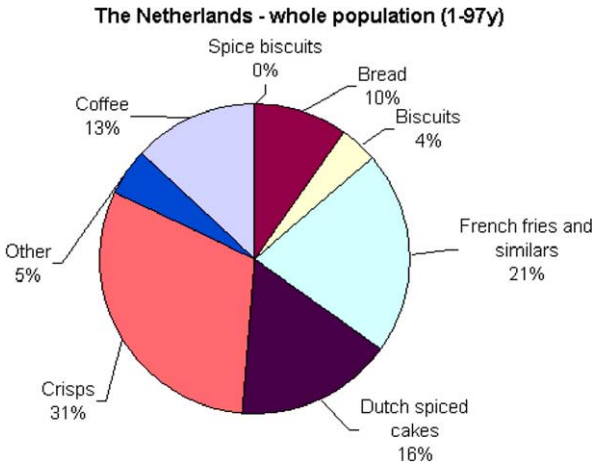

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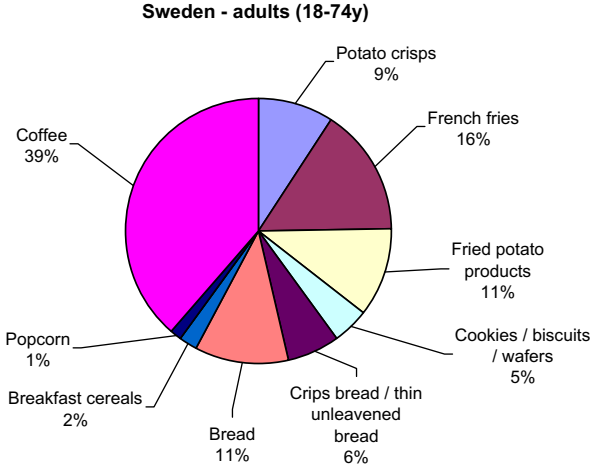

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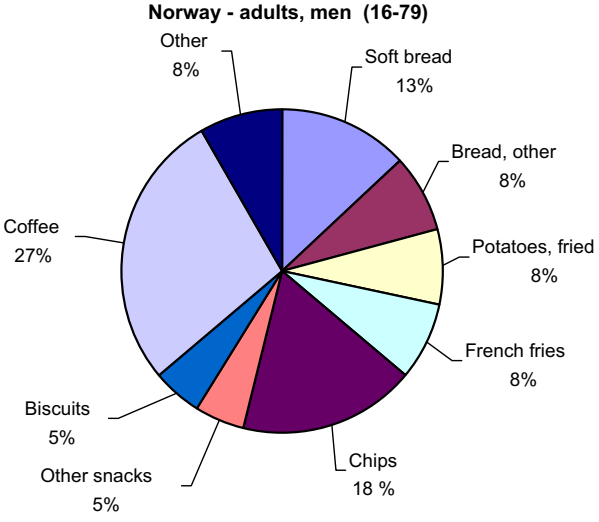

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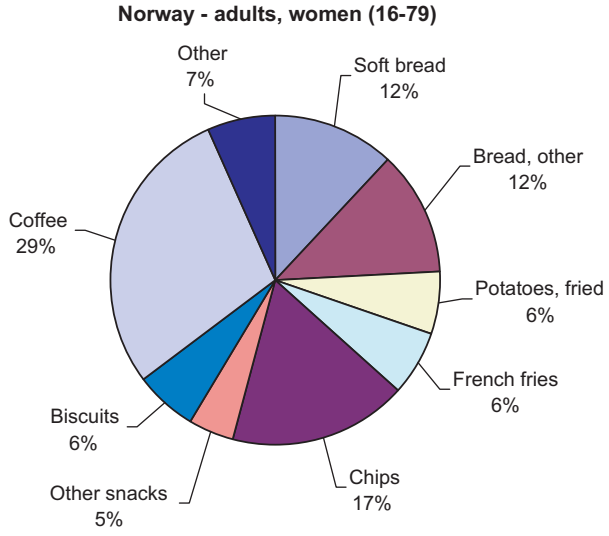

Data source: Dybing and Sanner (2003)

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