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Draft Scientific Opinion on Acrylamide in Food[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

EFSA was asked to deliver a scientific opinion on acrylamide (AA) in food. AA has widespread uses as an industrial chemical. It is also formed when certain foods are prepared at temperatures above 120 °C, especially in foods containing asparagine and reducing sugars. The CONTAM Panel evaluated 43 419 analytical results from food commodities collected and analysed since 2010. AA was found at the highest levels in 'Coffee and coffee substitutes', followed by 'Potato crisps and snacks' and 'Potato fried products'. Mean and 95th percentile dietary AA exposures across surveys and age groups were estimated at 0.3 to 1.9 µg/kg body weight (b.w.) per day and 0.6 to 3.4 µg/kg b.w. per day, respectively. Preferences in home-cooking can have a substantial impact on human dietary AA exposure. Upon oral intake, AA is absorbed from the gastrointestinal tract and distributed to all organs. AA is extensively metabolised, mostly by conjugation with glutathione but also by epoxidation to glycidamide (GA). Formation of GA is considered to represent the route underlying the genotoxicity and carcinogenicity of AA. Neurotoxicity, adverse effects on male reproduction, developmental toxicity and carcinogenicity were identified as possible critical endpoints for AA toxicity from experimental animal studies. The data from human studies were not adequate for dose-response assessment. The CONTAM Panel selected $BMDL_{10}$ values of 0.43 mg/kg b.w. per day for peripheral neuropathy in rats and of 0.17 mg/kg b.w. per day for neoplastic effects in mice. The Panel concluded that the current levels of dietary exposure to AA are not of concern with respect to non-neoplastic effects. However, although the human studies have not demonstrated AA to be a human carcinogen, the margins of exposure (MOEs) across dietary surveys and age groups indicate a concern with respect to neoplastic effects.

KEY WORDS

acrylamide, glycidamide, exposure, food, risk assessment, BMD, MOE

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SUMMARY

Following a request from the European Commission, the Panel on Contaminants in the Food Chain (CONTAM Panel) was asked to deliver a scientific opinion on acrylamide (AA) in food.

AA is a low molecular weight, highly water soluble, organic compound. It is used *inter alia* as an industrial chemical and in the production of polyacrylamides. Heightened concerns about exposure to AA arose in 2002 when it was discovered that it forms when certain foods are prepared at temperatures above 120 °C. It forms, at least in part, due to a Maillard reaction between certain amino acids, such as asparagine, and reducing sugars. However, several other pathways and precursors have also been proposed to contribute to AA formation. AA forms in numerous baked or fried carbohydrate-rich foods, including French fries/potato chips, crisps, breads, biscuits and coffee. AA is also known to be present in cigarette smoke.

The analytical determination of AA in food products is most frequently performed by high performance liquid chromatographic (HPLC) or gas chromatographic (GC) separation methods with mass spectrometric detection (MS), either in selected ion monitoring (SIM) mode or by tandem mass spectrometry (MS/MS) in multiple reaction mode (MRM) using isotope labelled standards.

In its exposure assessment, the CONTAM Panel evaluated a total of 43 419 analytical results from food commodities collected and analysed since 2010 and reported by 24 European countries and 6 food associations. Data provided by European countries and those provided by food associations gave overall consistent and complementary information. AA was found at the highest levels in 'Coffee and coffee substitutes' (average medium bound (MB) levels of 578 µg/kg), followed by 'Potato crisps and snacks' (average MB level of 389 µg/kg) and 'Potato fried products' (average MB level of 308 µg/kg). Lower AA levels were found in 'Processed cereal-based baby foods' (average MB level of 73 µg/kg), 'Soft bread' (average MB level of 42 µg/kg) and 'Baby foods, other than cereal-based' (average MB level of 24 µg/kg). The CONTAM Panel explored the possibility to perform a temporal trend analysis of the AA concentrations in certain foodstuffs across Europe on the basis of the data submitted to EFSA by the European countries. Because of gaps in the databases and the fact that results for different years are not always comparable, a reliable Europe-wide temporal trend analysis is not feasible. However, a dataset of manufacturers' measurements of AA levels in 40 455 samples of fresh sliced potato crisps from 20 European countries for the years 2002 to 2011 showed a substantial downward trend for mean levels of AA, from 763 ± 91.1 µg/kg in 2002 to 358 ± 2.5 µg/kg in 2011. For other food categories a similar downward trend was not observed.

Estimation of human exposure to AA revealed that infants, toddlers and other children were the most exposed groups. Depending on the survey and age group, chronic dietary exposure of children was estimated to be on average between 0.5 and 1.9 µg/kg b.w. per day and the 95th percentile was between 1.4 and 3.4 µg/kg b.w. per day. Chronic dietary exposure of adolescents, adults, elderly and very elderly was estimated to be on average between 0.3 and 0.9 µg/kg b.w. per day and the 95th percentile was between 0.6 and 2.0 µg/kg b.w. per day depending on the survey and age group.

The main contributor to the total AA exposure of infants was 'Baby foods, other than processed cereal-based' followed by 'Other products based on potatoes' and 'Processed cereal-based baby foods'. The main contributor to the total exposure of toddlers, other children and adolescents was 'Potato fried products' representing up to half the total exposure, followed by 'Soft bread', 'Biscuits, crackers, crisp bread', 'Other products based on cereals' and 'Other products based on potatoes'. These foods groups were also the main contributors to the total exposure of adults, elderly and very elderly together with 'Coffee and coffee substitutes'.

A sensitivity analysis was conducted in order to assess the influence of specific behaviours (brand loyalty, places of consumption, home-cooking habits) on the total dietary exposure to AA. Scenarios on the brand loyalty for potato crisps and coffee products resulted in a variation of less than 5 % and 15 %, respectively, of the total dietary exposure to AA. In scenarios on home-cooking behaviours,

degree of bread toasting resulted in variations of less than 5 %, while for conditions of potato frying the total dietary exposure to AA could be increased up to 80 %.

In both experimental animals and humans upon oral intake, AA that is not covalently bound to components of the food matrix is extensively absorbed from the gastrointestinal tract. After reaching the systemic circulation, AA is rapidly distributed into the tissues. AA is also able to cross the placenta and is transferred to a small extent into human milk. AA is extensively metabolised, mostly by conjugation with glutathione (GSH) (primarily mediated by glutathione-S-transferases) but also by epoxidation to glycidamide (GA), which is widely distributed into tissues. The formation of GA represents a metabolic activation pathway preferentially mediated by CYP2E1. Mice are more proficient in converting AA into GA than either rats or humans.

Formation of GA is considered to represent the route underlying the genotoxicity and carcinogenicity of AA. Covalent DNA adducts of GA have been amply demonstrated in *in vitro* models and experimental animals, and these have been used as biomarkers of AA exposure. The N7-guanine adduct derived from GA (N7-GA-Gua) is the most abundant DNA adduct following AA exposure. GA-DNA adducts in experimental animals are found at similar levels in various tissues of the body, although CYP2E1 is primarily located in the liver.

Covalent adducts of AA with DNA have been generated in chemical reactions, but have never been detected *in vivo* or *in vitro* in animal or human tissues.

Detoxification of both AA and GA can proceed through conjugation with GSH and the GSH adducts are subsequently converted to mercapturic acids (MA), which are excreted in urine. The MAs of AA and GA represent the major metabolites and their urinary excretion levels can be used as biomarkers of AA exposure. AA and GA can also react with proteins to form covalent adducts, e.g. with haemoglobin (Hb). Also these Hb adducts represent important biomarkers of AA exposure.

Several studies have reported various approaches to physiologically based pharmacokinetic (PBPK) modeling of AA absorption, metabolism, and disposition with the goal of predicting human internal exposures to AA and GA (i.e. area under the curve, AUC) for use in reducing the uncertainty in risk assessment inherent in animal to human extrapolations.

PBPK models allow derivation of human-equivalent doses (HEDs), which could be used to convert external doses of AA that produce critical effects in animal studies to the human external doses required to produce equivalent AUC values for either AA or GA, depending on the toxic endpoint used. The HEDs derived from equivalent AA-AUCs in rats and mice suggest that endpoints related to AA-mediated effects (e.g. neurotoxicity) require 4- to 6-fold higher doses in rats when compared to humans, based on inter-species differences in toxicokinetics. However, 0.5- to 0.7-fold lower doses of AA would be required in mice to produce equivalent GA-AUCs for genotoxicity-related endpoints when compared to humans.

Toxicological studies with AA have been conducted in rats, mice, monkeys, cats and dogs, using various dosing protocols and routes of exposure. Oral LD_{50} values for AA were reported to be > 150 mg/kg b.w. for rats, 107 mg/kg b.w. for mice, and 150-180 mg/kg b.w. for rabbits and guinea pigs.

Adverse effects reported in repeated dose toxicity studies of AA in rats, mice, monkeys, cats and dogs consisted of loss of body weight and effects on the nervous system reflected by hind-limb paralysis, reduction in rotorod performance and/or histopathological changes in peripheral nerves and nervous system structures. In mice, effects reported in addition to the neurotoxicity consisted of effects on the testes, including the degeneration of epithelia in spermatids and spermatocytes, the reduction of spermatozoa, and the presence of multinucleate giant cells, as well as forestomach hyperplasia, hematopoietic cell proliferation of the spleen and preputial gland inflammation, lung alveolar epithelium hyperplasia and cataract and for female mice ovarian cysts.

In rats, effects reported in addition to the neurotoxicity, included atrophy of skeletal muscle, testicular atrophy, distended urinary bladders, increased prevalence of duct ectasia in preputial glands, hematopoietic cell proliferation in the spleen, bone marrow hyperplasia, ovarian atrophy, degeneration of the retina, exfoliated germ cells epididymis, hepatocyte degeneration and liver necrosis, bone marrow hyperplasia, mesenteric lymph node cellular infiltration and pituitary gland hyperplasia.

Thirteen-week and 2-year studies in mice and rats dosed with GA revealed adverse effects that were generally similar to those reported for AA. Rats were more sensitive to the neurotoxic effects of AA and GA than mice, and neurotoxicity in rats (such as hind-leg paralysis and peripheral neuropathy) was consistently associated with lower AA doses and greater severity when compared to equimolar concentrations of GA.

Rodent studies have demonstrated adverse effects of AA on male reproductive parameters including reduced sperm counts and effects on sperm and testis morphology with a no-observed-adverse-effect level (NOAEL) of approximately 2 mg/kg b.w. per day.

Rat and mouse studies have shown some signs of developmental toxicity (increased incidence of skeletal variations, slightly impaired body weight gain, histological changes in the central nervous system, and neurobehavioural effects) at exposure levels that in some cases are also associated with maternal toxicity. The lowest NOAEL reported for developmental toxicity was 1.0 mg/kg b.w. per day from studies in rats exposed gestationally and neonatally.

The genotoxicity of AA, as well as of its reactive metabolite GA, has been extensively studied. *In vitro* genotoxicity studies indicate that AA is a weak mutagen in mammalian cells but an effective clastogen. GA is a strong mutagen and a clastogen. It induces mutations via a DNA adduct mechanism. *In vivo*, AA is clearly genotoxic in somatic and germ cells. AA exerts its mutagenicity via metabolism by CYP2E1 to GA. AA can also induce gene mutations by a pathway involving the generation of reactive oxygen species (ROS) and oxidative DNA damage.

AA is carcinogenic in multiple tissues in both male and female mice and rats. In rats, the major tumours produced by AA are adenomas, fibroadenomas and fibromas of the mammary gland, thyroid gland follicular cell adenomas or carcinomas, and testes or epididymis tunica vaginalis mesotheliomas. In mice, the major tumours produced by AA are: Harderian gland adenomas, mammary gland adenoacanthomas and adenocarcinomas, lung alveolar and bronchiolar adenomas, benign ovary granulosa cell tumours, skin sarcomas, and stomach and forestomach squamous cell papillomas in females, and Harderian gland adenomas and adenocarcinomas, lung alveolar and bronchiolar adenomas and carcinomas, and stomach squamous papillomas and carcinomas in males.

A similar spectrum of tumours is observed when equimolar concentrations of GA were administered in drinking water to rats and mice, which is consistent with GA being the proximate carcinogenic metabolite of AA.

AA is an electrophilic molecule which can undergo Michael addition-type reactions with nucleophilic target molecules. In particular, activated thiolate moieties in cysteine residues of enzymes and other functional proteins, e.g. in neuronal cells or spermatocytes, have been described as targets. The neurotoxic properties of AA are considered to originate mainly from this type of reactivity.

AA shows some reactivity towards nucleic acids, whereas reports on the formation of DNA adducts *in vivo* suggest that GA is mainly, if not exclusively, responsible for the formation of DNA adducts in AA-treated animals.

Evidence from the available studies in the literature on hormonal and endocrine effects of AA is equivocal. This is particularly true for changes in hormone levels in AA-treated animals which were reported in some studies. Mechanistic hypotheses on local endocrine effects of AA which may explain tumour formation in certain hormone or paracrine-regulated target tissues lack experimental proof.

There is a wide range of epidemiological studies investigating possible effects of AA in humans. Two cohort studies considered occupational exposure to AA and did not indicate an increased cancer risk. Associations between AA exposure through diet and cancer risk have been analysed in at least 34 publications, based on 16 epidemiological studies on several cancer sites. For most cancer sites there is no consistent indication for an association between AA exposure and increased risk. With respect to renal cell, endometrial and ovarian cancer a few studies have reported positive associations with AA intake, although the overall evidence is limited and inconsistent.

Two studies reported an inverse relation between AA exposure (measured by levels of AA and GA adducts) and birth weight and other markers of foetal growth. The CONTAM Panel noted that it has not been established whether the association between dietary AA exposure and these outcomes is causal.

Studies among workers occupationally exposed to AA showed an increased risk of neurological alterations, including mostly the peripheral, but also the central nervous system.

From all data available, the CONTAM Panel identified four possible critical endpoints for AA toxicity, i.e. neurotoxicity, effects on male reproduction, developmental toxicity, and carcinogenicity.

The data from human studies were not adequate for dose-response assessment. The CONTAM Panel performed benchmark dose (BMD) analyses on data for neurotoxicity and on the tumour incidences induced by AA in experimental animals. The CONTAM Panel selected the value of 0.43 mg/kg b.w. per day derived as the lowest $BMDL_{10}$ from the data on incidences of peripheral nerve (sciatic) axonal degeneration in male F344 rats exposed to AA in drinking water for two years as the reference point for non-neoplastic effects. Based on the fact that this $BMDL_{10}$ is lower than the NOAEL of approximately 2 mg/kg b.w. per day for adverse effects on male reproductive parameters and of 1.0 mg/kg b.w. per day for developmental toxicity, the CONTAM Panel concluded that using the $BMDL_{10}$ for neurotoxicity as the reference point is conservative when considering possible non-neoplastic effects of AA.

For neoplastic effects, the CONTAM Panel selected as a reference point the value of 0.17 mg/kg b.w. per day derived as the lowest $BMDL_{10}$ from data on incidences of Harderian gland adenomas and adenocarcinomas in male B6C3F_1 mice exposed to AA for 2 years. The CONTAM Panel noted that the Harderian gland is an organ absent in humans, but that in rodents this organ is a sensitive target tissue to detect compounds that are both genotoxic and carcinogenic. Taking into account that target tissues for tumour formation by a given genotoxic carcinogen may differ between rodents and humans, the CONTAM Panel considered the most sensitive target tissue in rodent bioassays, the Harderian gland, a conservative endpoint for assessment of the risk for neoplastic effects of AA in humans.

The fact that AA and its metabolite GA are positive in a variety of genotoxicity tests indicates that AA is of concern with respect to genotoxicity. Therefore, the CONTAM Panel considered it inappropriate to establish a tolerable daily intake (TDI).

Risk characterisation for non-neoplastic effects was performed using the margin of exposure (MOE) approach and the $BMDL_{10}$ value of 0.43 mg/kg b.w. per day for the most relevant and sensitive endpoint for neurotoxicity, i.e. the incidence of peripheral nerve (sciatic) axonal degeneration observed in F344 rats exposed to AA in drinking water for two years in the NTP study. MOE values for the neurotoxic effects ranged from 1 433 (minimum LB) to 226 (maximum UB) for the mean exposure, and from 717 (minimum LB) to 126 (maximum UB) for the 95th percentile exposure estimates across surveys and age groups). Taking into account differences between species and within the human population, the Panel concluded that the MOEs across surveys and age groups are not of concern. However, the Panel noted that the MOEs for the 95th percentile UB exposure estimates for toddlers and other children are close to the value that might be of concern for neurotoxicity.

For the risk characterisation for neoplastic effects, the MOE approach for compounds that are both genotoxic and carcinogenic is considered appropriate, using as the reference point the $BMDL_{10}$ of 0.17 mg/kg b.w. per day, i.e. the lowest $BMDL_{10}$ from data on incidences of Harderian gland adenomas and adenocarcinomas in male $B6C3F_1$ mice exposed to AA for two years in the NTP study. Comparison of the data on human exposure levels to AA across surveys and age groups reported above to this $BMDL_{10}$ of 0.17 mg/kg b.w. per day, reveals MOE values that range from 567 (minimum LB) to 89 (maximum UB) for the mean exposure estimates, and from 283 (minimum LB) to 50 (maximum UB) for the 95th percentile exposure estimates across all surveys and age groups. The EFSA Scientific Committee concluded that, for substances that are both genotoxic and carcinogenic, an MOE of 10 000 or higher, based on a $BMDL_{10}$ from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view. Since the MOEs calculated are all lower than the value of 10 000, the CONTAM Panel concluded that, although the available human studies have not demonstrated AA to be a human carcinogen, the MOEs across surveys and age groups indicate a concern with respect to neoplastic effects.

The CONTAM Panel noted that AA is a germ cell mutagen and that there are at present no established procedures for risk assessment using this endpoint.

Finally, the CONTAM Panel makes the following recommendations: The reporting of AA occurrence data should be improved regarding the mode of preparation of the products before analysis. Duplicate diet studies are recommended in order to improve exposure assessment, since they provide a more accurate indication of AA levels in food as prepared and consumed at home. Data on urinary metabolites levels from individuals participating in the duplicate diet studies should be generated for the purpose of validation of the biomarkers. Further epidemiological studies are required to confirm or refute the inverse relation between dietary AA intake and birth weight and other markers of foetal growth observed in two studies. Improved approaches for the detection and risk assessment of germ cell mutagens should be developed, and applied to AA and GA.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Acrylamide is a carcinogenic substance that is formed in foods that have undergone heat treatment, e.g. roasting, baking and frying, and that are high in certain amino acids and reducing sugars. Acrylamide is therefore an issue in French fries and potato crisps, but also in other foodstuff such as biscuits, coffee, etc.

Industry (Food and Drink Europe) have developed a so-called 'toolbox'[4] containing measures that can be applied by the different sectors of food industry to bring acrylamide levels down. Sector specific brochures have also been developed.

The Commission is monitoring acrylamide levels in food via specific monitoring recommendations[5]. The results of the monitoring are compiled by EFSA. Despite the fact that the industry toolbox is in place since 2006, levels in food are not systematically decreasing in all concerned food commodities, as demonstrated by the results compiled since 2007.

Therefore a second Commission recommendation[6] was adopted in January 2011 which asks Member States to carry out further investigations at food operator's premises in case high acrylamide levels are found. Indicative values have been established in that recommendation. If an indicative value is exceeded, an investigation should be carried out. The indicative values are not legal limits and do not require enforcement action if they are exceeded.

In the near future, the Commission will assess the approach taken and decide about the need for further appropriate measures.

However, since the Statement of the Scientific Panel on Contaminants in the Food Chain to a summary report on acrylamide in food of the 64th meeting of the Join FAO/WHO Expert Committee on Food Additives (Adopted on 19 April 2005) and the ESFA Scientific Colloquium on acrylamide carcinogenicity[7], new scientific information had become available.

In order to assess the need for further measures as regards acrylamide in food, EFSA is requested to assess the risk related to the presence of acrylamide in food.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Art 29 (1) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority for a scientific opinion on the risk to human health related to the presence of acrylamide in food.

[4] Food and Drink Europe Acrylamide toolbox available at: http://ec.europa.eu/food/food/chemicalsafety/contaminants/ciaa_acrylamide_toolbox09.pdf

[5] Commission Recommendation 2007/33/EC on the monitoring of acrylamide levels in food (OJ L123, 12.5.2007, p.33) and Commission Recommendation 2010/307/EU on the monitoring of acrylamide levels in food (OJ L137, 3.6.2010, p.4)

[6] Commission Recommendation of 10.1.2011 on investigations into the levels of acrylamide in food (document C(2010) 9681 final, available at: http://ec.europa.eu/food/food/chemicalsafety/contaminants/recommendation_10012011_acrylamide_food_en.pdf

[7] EFSA Scientific Colloquium no. 11 on acrylamide carcinogenicity – new evidence in relation to dietary exposure, held at Tabiano (PR), Italy from 22-23 May 2008.

ASSESSMENT

1. Introduction

Acrylamide (CH_2=$CHCONH_2$) (AA) is a low molecular weight, highly water soluble, organic compound. AA is used *inter alia* as an industrial chemical and in the production of polyacrylamides. Heightened concerns about exposure to AA arose in 2002 when it was discovered that it forms when certain foods are prepared at high temperatures (> 120 °C) (Tareke et al., 2002, CAC/RCP 67-2009[8]). It forms, at least in part, due to a Maillard reaction between certain amino acids, such as asparagine, and reducing sugars (Mottram et al., 2002; Stadler et al., 2002). However, several other pathways and precursors have been proposed to contribute to AA formation (reviewed by Keramat et al., 2011). AA forms in numerous baked or fried carbohydrate-rich foods, including French fries/potato chips, crisps, breads, biscuits and coffee. AA is also known to be present in cigarette smoke.

There is widespread human exposure to AA. The toxicological properties of AA have been well studied and include neurotoxicity, genotoxicity, carcinogenicity and reproductive toxicity. AA has been classified as a Group 2A carcinogen (probably carcinogenic to humans) by the International Agency for Research on Cancer (IARC, 1994). AA is an α,β-unsaturated carbonyl compound with electrophilic reactivity. It can therefore react with nucleophilic groups on biological molecules, which may contribute to the generation of its toxic effects. Reaction of AA with proteins is extensive (and such reaction products have been used as biomarkers of exposure to AA). *In vivo*, AA is metabolised to a reactive epoxide glycidamide (GA), which is thought to have a major role in the genotoxicity of AA through its binding to DNA.

In view of the known toxic effects of AA, the discovery of its presence in certain foods stimulated many new studies of its metabolism, bioavailability, toxicokinetics, DNA adduct formation, mutagenicity and experimental toxicity. Many new investigations of human exposure to AA have recently been made and a large number of epidemiological studies have also been initiated.

Many European countries monitor AA since 2002. In 2007, the European Commission (EC) launched a Recommendation that the Member States should perform the monitoring of AA in foodstuffs that are known to contain high AA levels and/or contribute significantly to human exposure. Based on the results of the monitoring in the Member States from 2007-2011, the EC has set 'indicative values' for AA in various foodstuffs. The 'indicative values' are not safety thresholds, but only intended to indicate the need for an investigation if the values are exceeded in order to explore whether appropriate measures have been taken to control the AA formation.

In order to assess the need for further measures as regards AA in food, EFSA has been requested to assess the risk to human health related to the presence of AA in food, taking into account the extensive new data on AA exposure and toxicity.

The CONTAM Panel based its evaluation on information submitted following public calls for data, original studies published in the open literature that have become available until May 2014, and previous evaluations performed by international bodies. The methodology for the literature search and selection criteria for the consideration of scientific data for the risk assessment of AA in food are described in Appendix A.

1.1. Previous risk assessments

In light of the findings of high concentrations of AA formed during the frying or baking of a variety of foods, several international bodies and scientific groups had carried out risk assessments related to the presence of AA in food.

[8] CODEX Alimentarius Commission, 2009. Code of practice for the reduction of Acrylamide in Foods. CAC/RCP 67-2009, 11 pp. Available at: www.codexalimentarius.org/input/download/standards/11258/CXP_067e.pdf

In 2002, the FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) held a consultation to collect the views of an international group of experts on the health implications of AA in food. The objective was to review and evaluate the new and existing data and research on AA, to identify needs for further information and studies, and to develop and suggest possible interim advice for governments, industry and consumers (FAO/WHO, 2002). Based on the available data at that time, food was estimated to make a significant contribution to total exposure of the general public to AA, with estimated average intakes for the general population ranging from 0.3 to 0.8 µg/kg body weight (b.w.) per day. It was anticipated that children would generally have intakes two to three times higher than those of adults when expressed on a b.w. basis (FAO/WHO, 2002). The FAO/WHO consultation recognized the presence of AA in food as a major concern for humans based on the ability of AA to induce cancer and heritable mutations in laboratory animals. Neurotoxicity was the key non-cancer, non-genotoxic effect of AA in humans and animals, although it was concluded that those effects were not to be expected from the levels of AA encountered in food. A range of recommendations was provided for further information and new studies to better understand the risk to human health posed by AA in food, as well as some advice to minimize whatever risk existed, such as avoiding excessive cooking of food and investigating possibilities for reducing levels of AA in food (FAO/WHO, 2002).

That same year, the Scientific Committee on Food (SCF) was asked to assess the implications for food safety of the new information on AA in foods (SCF, 2002). The SCF had previously evaluated AA as a monomer in food contact materials and maintained its previous conclusion that AA was a genotoxic carcinogen. The SCF recommended that levels of AA in food should be as low as reasonably achievable. Due to the lack of detailed knowledge about a number of aspects in relation to AA and food safety, the SCF provided only general advice on the scientific issues relevant to risk management and endorsed the interim advice given by the FAO/WHO consultation (FAO/WHO, 2002).

In 2005, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) at its 64th meeting performed an evaluation of the data available on AA. The full report was published in 2006 (FAO/WHO, 2006). Dietary intakes were estimated at 1 and 4 µg/kg b.w. per day for the general population and for consumers with a high intake, respectively. These estimates also included children. JECFA concluded that the epidemiological studies and data on biomarkers in humans and animals available at the time of the evaluation were inadequate to establish a dose-response relationship, and therefore performed the assessment on the basis of available studies in animals. JECFA considered as pivotal effects for the risk assessment the genotoxicity and carcinogenicity of AA, but also considered other non-cancer end-points of concern, such as effects on the nervous system. Increased incidence of tumours at a variety of sites was observed in two long-term studies in F344 rats administered AA in drinking water (Johnson et al., 1986; Friedman et al., 1995). Benchmark doses and the 95 % benchmark dose lower confidence limits for a 10 % extra risk of tumours (BMD_{10} and $BMDL_{10}$) were derived for both studies. It was noted that although the pathways of metabolism of AA are similar in rats and humans, quantitative differences such as the extent of bioactivation of AA to GA or detoxication of GA could result in species differences in sensitivity (FAO/WHO, 2006). The lowest range of $BMDL_{10}$s was found for total mammary tumours from the study by Johnson et al. (1986) ranging from 0.30 to 0.46 mg/kg b.w. per day. The no-observed-effect level (NOEL) for induction of morphological changes in nerves observed in a 90-day study in rats was 0.2 mg/kg b.w. per day (Burek et al., 1980), while the overall NOEL for reproductive and developmental effects and other non-neoplastic lesions was higher (2 mg/kg b.w. per day, Tyl et al., 2000a). Margins of exposure (MOEs) for the general population and for consumers with a high intake were calculated by comparing the estimated intake with the NOELs and $BMDL_{10}$s derived (Table 1). Considering the NOEL of 0.2 mg/kg b.w. per day for morphological changes in nerves, MOEs of 200 and 50 were obtained for the general population and consumers with a high intake, respectively. Comparison with the NOEL of 2.0 mg/kg b.w. per day for reproductive, developmental, and other non-neoplastic effects in rodents provided MOEs of 2 000 and 500, respectively. JECFA concluded that at the estimated average intakes, adverse effects based on these endpoints were unlikely, but that morphological changes in nerves could not be excluded for some individuals with very high intake (FAO/WHO, 2006). When considering the lowest $BMDL_{10}$ of 0.30 mg/kg b.w. per day for induction of mammary

tumours in rats, MOEs of 300 and 75 were obtained, respectively. JECFA considered these MOEs were low for a compound that is genotoxic and carcinogenic, and that they may indicate a human health concern (FAO/WHO, 2006). JECFA recommended that efforts to reduce AA concentrations in foodstuffs should continue, and that AA be re-evaluated when results of carcinogenicity and long-term neurotoxicity studies become available. JECFA also noted the potential of physiologically based pharmacokinetic (PBPK) modelling to better link human biomarker data with exposure assessments and toxicological effects in experimental animals.

In April 2005, the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) adopted a statement to the summary report released on 2 March 2005 of the above-mentioned risk assessment carried out by JECFA at its 64th meeting (EFSA, 2005a). The CONTAM Panel noted the use of the MOE approach that incorporated data from European countries, including information gathered under collaborative initiatives between the European Commission and EFSA. The Panel agreed with the principal conclusions and recommendations of JECFA and concluded that an additional evaluation by EFSA was not necessary at that time.

The French Food Safety Agency (Afssa, now Agency for Food, Environmental and Occupation Health and Safety, ANSES) was also asked in 2002 to give an advice on the presence of AA in food commodities with regard to the safety of food for consumers and published two reports synthesising the knowledge and available data at that time (Afssa, 2003, 2005). The last report published in 2005 included a refined estimation of the exposure to AA of the French population for different age groups. For adults (>15 years old) the exposure estimates were 0.5 and 0.98 µg/kg b.w. per day for average and high consumers, respectively. For children (3-14 years old) the exposure estimates were higher than those of adults, with values of 1.25 and 2.54 µg/kg b.w. per day for average and high consumers, respectively (Afssa, 2005). French fries were identified as the food commodity contributing most to the AA intake for young age groups. Afssa referred to the summary and conclusions of the sixty-fourth meeting of the JECFA published in 2005, and concluded that at the time of the report it was not possible to make special recommendations about the preparation of the food commodities or food consumption (Afssa, 2005).

In 2005, the National Toxicology program-Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) Expert Panel published a report on the reproductive and developmental toxicity of AA (Manson et al., 2005). The report concluded that there were no human data available on developmental or reproductive toxicity of AA and that available experimental data were sufficient to conclude that AA can produce developmental toxicity in rats and mice, and that AA is a reproductive toxicant in male rats and mice bred to untreated females. The NTP-CERHR Expert Panel also concluded that there are sufficient data to conclude that AA induces transmissible genetic damage in male germ cells of mice in the form of reciprocal translocations and gene mutations. Lowest-observed-adverse-effect levels (LOAELs) in the range of 4-45 mg/kg b.w. per day were established. The NTP-CERHR Expert Panel indicated that the data suggest that at these exposure levels (5-14 mg/kg b.w. per day) AA has no effect on female reproductive function in rats or mice. Considering the low level of estimated human exposure to AA derived from a variety of sources, the Expert Panel expressed 'negligible concern for adverse reproductive and developmental effects for exposures in the general population'.

In 2008, EFSA held a Scientific Colloquium on AA carcinogenicity and new evidence in relation to dietary exposure (EFSA, 2008). The objective of this EFSA colloquium was to debate the state and future challenges regarding the potential toxicity and cancer risk associated with dietary exposure to AA considering the new information that became available since the last risk assessment carried out by JECFA in 2005. In particular, four topics were considered: (i) epidemiological evidence relating AA exposure to cancer risk in humans, including discussions on uncertainties, (ii) the applications of biomarkers for AA and models in relation to the exposure, metabolism and elimination (toxicokinetics) and the mode of action of AA in experimental animals and humans (toxicodynamics), (iii) the state of the art on the genotoxic and non-genotoxic mechanisms of carcinogenicity of AA, and (iv) the current knowledge on dietary exposure to AA across Europe and the exploration of any new potential food source contributing to dietary exposure.

Concerning the epidemiological data, it was concluded that there was some evidence for an association between dietary exposure to AA and some types of cancer. However, the relative risks were low and the totality of all the epidemiological evidence was not consistent. It was cautioned not to expect tumour site concordance between animals and humans. For epidemiological studies the need to develop food frequency questionnaires (FFQ) that would focus on food processing, including home-cooking, was identified. The FFQs should be supplemented by biomarker measurements. The relevance of understanding and controlling confounders such as smoking (EFSA, 2008) was mentioned.

In the area of biomarkers, and to help in interspecies extrapolation, the importance of a better understanding of the overall fate of AA in humans was recognised, as well as the measurement of GA DNA adducts in humans. It was noted that biomarkers are indicators only of relatively short-term exposure and that they can be confounded by factors other than the diet (EFSA, 2008).

On the genotoxic/non-genotoxic mechanism of carcinogenicity, it was recognised that genotoxicity is an important mode of action, but there may be other, non-genotoxic mechanisms for certain tumour types observed in animals. Accurate dose-response analysis and the derivation of BMDs for each tumour site, together with information on mode of action, were found helpful in assessing which tumours are the most important for human risk assessment. Animal data also indicated some intermediate biomarkers that may reflect the biologically active dose of AA that could be useful (EFSA, 2008).

For dietary exposure, the analytical methods for establishing occurrence data and estimating human exposure available at the time of the colloquium were found to be adequate and, it was not anticipated that better data would change dramatically the margins of exposure estimated by JECFA in 2005. It was recommended to refine the FFQs to enable studies to focus on subpopulations considered to be more at risk. It was also noted that mitigation measures may prove to be more important than advice (EFSA, 2008).

Overall, the EFSA Colloquium concluded that it was not possible to improve the existing risk assessments but that the data anticipated to be available in the following years (e.g. a NCTR/NTP carcinogenicity study) would be valuable in adding weight to the current risk assessments and in reducing the uncertainties.

In March 2010, the United States Environmental Protection Agency (US-EPA) published a toxicological review on AA (US-EPA, 2010) to provide scientific support and rationale for the hazard and dose-response assessment to chronic exposure to AA. EPA used dose response data from animal toxicity testing to produce human health reference values for carcinogenicity in multiple tissues and degenerative peripheral nerve damage, the most sensitive non-cancer endpoint. Oral reference dose (RfD in mg/kg b.w. per day) values and inhalation reference concentrations (RfC in mg/m^3) were derived for the non-cancer endpoint and cancer potency estimates were derived as either an oral slope factor (the plausible upper bound on the estimate of risk per mg/kg b.w. per day of oral exposure) or an inhalation unit risk (an upper bound on the estimate of risk per $\mu g/m^3$ of air breathed) using methodology extensively documented in US-EPA guidance documents (2010).

The increased incidence of degenerative lesions of peripheral nerves was selected as the critical effect for derivation of the RfD for AA and the RfD value was based on the dose-response data from Johnson et al. (1986). BMD modelling of the incidence data for microscopically-detected degenerative nerve lesions in rats was performed. The 95 % lower confidence limits of the estimated dose associated with a 5 % extra risk ($BMDL_{05}$) for nerve lesions of 0.27 mg/kg per day for mild-to-moderate lesions was chosen as the most sensitive response, and was selected as the point of departure (i.e. reference point) for deriving the RfD. An internal dose metric of AA-area under the curve (AA-AUC) in the blood from an oral exposure in rat was estimated from *in vivo* rat data, and the administered dose in humans that would result in a comparable internal AA-AUC was calculated using conversion factors developed from human adduct data and second order adduct formation rate

constants. The estimated AA-AUC in rat blood following exposure at a $BMDL_{05}$ was used to derive a human equivalent dose (oral exposure, HED) of 0.53 mg AA/kg per day as the point of departure (see Section 7.1.5). The point of departure was divided by a total uncertainty factor (UF) of 30 (3 for animal-to-human extrapolation to account for toxicodynamic differences, and 10 for intra-individual variability in human toxicokinetics and toxicodynamics) to derive the RfD of 0.002 mg/kg per day.

For the oral cancer effects, an oral slope factor of 0.6 $(mg/kg\ per\ day)^{-1}$ derived from male rat data from Johnson et al. (1986) and the $BMDL_{10}$ of 1.5×10^{-1} for the combined risk of thyroid tumours or testicular tumours was selected for calculating a point of departure (i.e. reference point) and deriving a human oral slope factor. The rat $BMDL_{10}$ was converted to a HED_{BMDL10} based on comparable levels of GA-AUC in blood between the rat and human relative to their respective administered doses. The resulting HED_{BMDL10} (1.9×10^{-1} mg/kg per day) at the benchmark response (BMR) of 0.1 was used to derive a human oral slope factor of 0.5 $(mg/kg\ per\ day)^{-1}$.

In 2010, JECFA at its 72nd meeting reconsidered the studies described in its previous risk assessment carried out in 2005 (FAO/WHO, 2006), as well as new information on occurrence, dietary exposure and the completed toxicity studies on metabolism, genotoxicity, neurodevelopmental effects, long-term/carcinogenicity studies on AA and GA and new epidemiological studies. The full report was published in 2011 (FAO/WHO, 2011). JECFA noted that although mitigation measures applied after 2003 might have reduced the exposure for some individuals or population subgroups, neither the estimated average AA exposure for the general population, including children, of 1 µg/kg b.w. per day, nor the exposure for consumers with high dietary exposure of 4 µg/kg b.w. per day, had changed since its last evaluation in 2005.

As in its previous evaluation, the available epidemiological studies were not considered suitable for a dose-response analysis and therefore JECFA based the assessment on the available studies in experimental animals. JECFA reported that the increased incidence in the morphological changes in nerves in rats remained the most sensitive non-carcinogenic end-point (Burek et al., 1980), as no new studies in experimental animals observed non-carcinogenic effects at doses lower than 0.2 mg/kg b.w. per day. Therefore, the MOE relative to this end-point calculated in 2005 remained unchanged (200 and 50 for the general population and consumers with high dietary exposure, respectively). JECFA noted that although adverse neurological effects were unlikely at the estimated average exposure, morphological changes in nerves could not be excluded for individuals with a high dietary exposure to AA (FAO/WHO, 2011).

For the cancer effects, JECFA considered the just completed 2-year NCTR/NTP study in which $B6C3F_1$ mice and F344 rats were treated with AA in drinking water (Beland, 2010, as cited by FAO/WHO, 2011). It noted that the sites of tumours (thyroid and mammary gland, peritesticular mesothelium) induced in male and female rats were in agreement with those found in the two previous long-term studies in rats (Johnson et al., 1986; Friedman et al., 1995). Benchmark doses and the 95 % benchmark dose lower confidence limits for a 10 % extra risk were derived for the induction of the different tumours observed in both mice and rats. Although the range of values observed was similar to that obtained in its previous evaluation in 2005, the lowest $BMDL_{10}$ values were obtained for tumours in the Harderian gland in male mice (0.18-0.56 mg/kg b.w. per day) (FAO/WHO, 2011). Although humans have no equivalent organ to the Harderian gland, JECFA was not able to discount this effect and considered it appropriate to use a value of 0.18 mg/kg b.w. per day for male mice (the lowest values in the range of $BMDL_{10}$s). For rats, the lowest $BMDL_{10}$ was observed for mammary tumours in females with a value of 0.31 mg/kg b.w. per day. When considering the induction of mammary tumours in rats, MOEs of 310 and 78 for average and high dietary exposures were obtained, respectively. For Harderian gland tumours in mice, the MOE values were 180 and 45 for average and high exposures, respectively (FAO/WHO, 2011) (Table 1). JECFA noted that these MOE values were similar to those determined in its previous evaluation (FAO/WHO, 2006), and still considered that for a compound that is both genotoxic and carcinogenic, these MOEs indicate a human health concern (FAO/WHO, 2011).

JECFA also concluded that 'there was a poor correlation between the estimated dietary exposure and internal biological markers of AA exposure (AA-valine and GA-valine haemoglobin adducts) in humans and that worker cohort epidemiological studies did not provide any evidence that exposure to AA resulted in an increase in the incidence of cancer' (FAO/WHO, 2011). JECFA recommended that longitudinal studies on intra-individual levels of AA and GA haemoglobin (Hb) adducts should be measured over time in relation to concurrent dietary exposure, to provide a better estimate of the AA exposure for epidemiological studies to inform the risk associated with consumption of certain foods (FAO/WHO, 2011).

In June 2011, the Federal Institute for Risk Assessment (BfR) produced an opinion on AA (BfR, 2011). BfR summarised the available human and animal studies, and estimated the AA dietary intake. BfR concluded that the results of the evaluated epidemiological studies that reported on various cancer types in connection with AA intake, were inconsistent and hence, a correlation between AA intake and cancer development can neither be assumed nor excluded, and this risk, if existing, could hardly be proven given the intake level estimated (BfR, 2011). BfR compared various exposure estimates of the AA intake of consumers on the basis of German and European data representing the AA contents in food and the consumption frequency of specific food items. Estimates of 0.14 and 0.39 µg/kg b.w. per day for average and 95th percentile intake, respectively, were obtained. However, for the risk characterisation, the dietary intake estimates reported by EFSA (2011a) were used as they were considered more representative (0.34 and 0.83 µg/kg b.w. per day for average and 95th percentile intake, respectively). BfR considered cancer risk as the critical end-point, and used a $BMDL_{10}$ of 0.30 mg/kg b.w. per day for mammary tumours in female F344 rats, and the $BMDL_{10}$ of 0.16 mg/kg b.w. per day for tumours in the Harderian gland in male mice as points of departure, both based on the outcome of the 2-year NTP study. To characterize the cancer risk, BfR calculated the MOE between the estimated AA intake and the points of departure (i.e. reference points). For the induction of mammary tumours in female rats, MOE values were 882 and 361 for average and 95th percentile consumers, respectively. For the induction of Harderian gland tumours, the MOE values were 471 and 193, respectively. BfR concluded that the MOEs for consumers and children eating large amounts of foods with high AA contents may pose a health risk.

BfR noted that 'blood and/or urine biomarkers may be more suitable for determining the AA intake of consumers than the estimation via the AA contents in food and consumption data' (BfR, 2011). It recommended efforts to minimise the AA contents in industrially processed food, and other measures such as consumers and restaurants following the advise of 'baking golden brown instead of charring'.

In 2012, the Agency for Toxic Substances and Disease Registry (ATSDR) prepared a toxicological profile on AA (ATSDR, 2012) and derived acute-, intermediate- and chronic-duration oral Minimal Risk Levels[9] for AA. The male-mediated infertility was selected as the critical effect for acute-duration oral exposure (14 days or less), and a Minimal Risk Level was derived based on results of fertility testing of male rats administered AA by gavage for 5 days prior to 1-week mating sessions with untreated female rats (Sublet et al., 1989). PBPK modelling (Sweeney et al., 2010) and BMD analysis were performed to predict a HED of 0.31 mg/kg per day, that was divided by an uncertainty factor of 30 (3 for interspecies extrapolation using a PBPK model and 10 for human variability), resulting in an acute-duration oral Minimal Risk Level of 0.01 mg/kg per day. The intermediate-duration oral Minimal Risk Level was derived based on a no-observed-adverse-effect level (NOAEL) of 0.2 mg/kg per day and a LOAEL of 1 mg/kg per day for ultrastructural changes in peripheral nerve fibers in male rats (Burek et al., 1980). PBPK modelling (Sweeney et al., 2010) was used to estimate the rat internal dose metrics for blood AA and GA at the NOAEL and for estimating a HED of 0.038 mg/kg per day, that was divided by the uncertainty factor of 30, to result in a Minimal Risk Level of 0.001 mg/kg per day. The chronic-duration oral Minimal Risk Level was derived based on degenerative changes in sciatic nerves from male F344 rats receiving AA from the drinking water for up to 2 years, as detected by light microscopy (Friedman et al., 1995). PBPK modelling (Sweeney et

[9] According to ATSDR (2012), the 'Minimal Risk Level is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure.'

al., 2010) and BMD analysis were performed to estimate a HED of 0.042 mg/kg per day, that was divided by the uncertainty factor of 30, to result in a Minimal Risk Level value of 0.001 mg/kg per day.

Health Canada published in 2012 a revised exposure assessment of AA in food (Health Canada, 2012). Food samples were analysed within a monitoring program that started in 2009 to assess the effectiveness of AA reduction strategies. A probabilistic exposure assessment was conducted resulting in mean AA intakes ranging from 0.356 to 0.609 µg/kg b.w. per day for age groups from 1-18 years old, and from 0.157 to 0.288 µg/kg b.w. per day for adults (>19 years old). The 90th percentile exposure estimates ranged from 0.591 to 1.516 µg/kg b.w. per day, and from 0.307 to 0.740 µg/kg b.w. per day, respectively. The age group from 1-3 years old was the one with the highest intake estimates. Restaurant French Fries was the food commodity contributing most to the dietary intake. MOEs were calculated based on the NOAEL for morphological changes in nerves in rats and the $BMDL_{10}$s for mammary and Harderian gland tumours in female rats and male mice, respectively, proposed by JECFA (FAO/WHO, 2011). In the first case, the lowest MOE was obtained for the age group 1 - 3 years old with values of 328 and 132, when considering mean and 90th percentile exposure, respectively. For the cancer effects with the lowest $BMDL_{10}$ (0.18 mg/kg b.w. per day), the lowest MOEs were obtained for the same age group, with values of 296 and 119 for mean and 90th percentile exposure, respectively. Although Health Canada noted that those MOEs are higher than those reported by JECFA (FAO/WHO, 2011), it concluded that dietary exposure to AA is a potential health concern.

The Danish National Food Institute (DTU) published in 2013 a report with the results from the food monitoring of chemical contaminants for the period 2004-2011, and estimated the exposure to AA of the Danish population and calculated the MOEs (DTU, 2013). For adults, the mean (95th percentile) AA dietary intake was 0.21 (0.46) µg/kg b.w. per day, while for children (aged 4-14 years old) it was estimated at 0.39 (0.89) µg/kg b.w. per day. For adults, the food category contributing most to the intake was potato products, followed by coffee and cocoa. For children, potato products were also the highest contributor, followed by crisps, chocolate and bread. MOEs were calculated based on the NOAEL for morphological changes in nerves in rats and the $BMDL_{10}$s for mammary and Harderian gland tumours in rats proposed by JECFA (FAO/WHO, 2011) (Table 1). The lowest MOEs were obtained for children for the carcinogenic effect on the Harderian gland in mice, with values of 466 (mean exposure) and 202 (95th percentile exposure). The report concluded that the exposure to AA is of food safety concern (DTU, 2013).

The Food Standards Australia New Zealand (FSANZ) published in 2014 the results of its 24th Australian Total Diet Study (TDS), which included AA among other compounds. The study focused on 94 foods and beverages likely to contribute to the dietary exposure to AA. Mean (P90) AA intake estimates were 1-2 (1-3) µg/kg b.w. per day (lower bound exposure) and 2-4 (2-8) µg/kg b.w. per day (upper bound exposure). The highest dietary estimates were for the population aged 17 years old and above. The food categories contributing most to the exposure were cereal and grain-based foods (excluding cakes and biscuits), vegetables and pulses, and snacks and condiments. MOEs were calculated based on the NOAEL for neurotoxicity and the $BMDL_{10}$ for mammary tumours in rats and Harderian gland tumours in male mice as established by JECFA (FAO/WHO, 2011). The MOEs for the non-carcinogenic endpoint ranged from 30 to 310, and the FSANZ concluded that adverse neurological effects cannot be excluded for individuals with high AA dietary exposure. The MOEs for the cancer endpoint were in all cases lower than 500, and FSANZ concluded that these suggest a human health concern for a compound that is genotoxic and carcinogenic (FSANZ, 2014).

The International Agency for Research on Cancer (IARC) classified AA as a Group 2A carcinogen ('probably carcinogenic to humans') (IARC, 1994).

In 2011, the National Toxicology Program (NTP) determined that AA is reasonably anticipated to be a human carcinogen (NTP, 2011) and the US-EPA characterized AA as 'likely to be carcinogenic to humans' (US-EPA, 2010). The American Conference of Governmental Industrial Hygienists (ACGIH)

771 has classified AA as an A3 carcinogen (confirmed animal carcinogen with unknown relevance to
772 humans) (ACGIH, 2011, as cited in ATSDR, 2012).

773 Furthermore, AA is listed in Appendix 2, Entry 28 – Carcinogens: category 1B of the REACH
774 (Registration, Evaluation, Authorisation and Restriction of Chemicals) Regulation. Since 2010, AA
775 (EC No 201-173-7 and CAS No 79-06-1) is included in the Candidate List of Substances of Very High
776 Concern (SVHC) for authorisation.

777 AA is classified as Carc. Cat.1B H350: May cause cancer, Muta. Cat. 1B H340: May cause genetic
778 defects and Repr. Cat.2 H361f: Suspected of damaging fertility according to CLP Regulation (EC) No
779 1272/2008.

780

781 **Table 1:** Summary of previous risk assessments performed by international bodies

Reference	Critical end-point	Key study	Reference point [g] (mg/kg b.w. per day)	UF	Health-based guidance value (mg/kg b.w. per day)	Exposure estimates (µg/kg b.w. per day)	MOE
FAO/WHO (2006)	Morphological changes in nerves (rats)	Burek et al. (1980)	0.2 (NOEL)	n.a.	n.a.	General population: 1 Cons. high percentile: 4	200 50
	Reproductive and developmental effects and other non-neoplastic lesions	Tyl et al. (2000a)	2.0 (overall NOEL)	n.a.	n.a.		2 000 500
	Mammary tumours (rats)	Johnson et al. (1986)	0.30 ($BMDL_{10}$)	n.a.	n.a.		300 75
FAO/WHO (2011)	Morphological changes in nerves (rats)	Burek et al. (1980)	0.2 (NOAEL)	n.a.	n.a.	General population: 1 Cons. high percentile: 4	200 50
	Harderian gland tumours (male mice)	Beland (2010)	0.18 ($BMDL_{10}$) [(a)]	n.a.	n.a.		180 45
	Mammary tumours (female rats)	Beland (2010)	0.31 ($BMDL_{10}$) [(a)]	n.a.	n.a.		310 78
US-EPA (2010)	Increased incidence of degenerative lesions of peripheral nerves	Johnson et al. (1986)	0.27 ($BMDL_{05}$) 0.53 (HED)	30 (3 for inter-species differences, 10 for intra-species differences)	0.002 (RfD)	n.a.	n.a.
	Summed risks for thyroid or TVM tumours	Johnson et al. (1986)	0.15 ($BMDL_{10}$) 0.19 (HED_{BMDL10})[(e)]	n.a.	n.a.	n.a.	n.a.

782 Table continued overleaf.

783 **Table 1:** Summary of previous risk assessments performed by international bodies (continued)

Reference	Critical end-point	Key study	Reference point [g] (mg/kg b.w. per day)	UF	Health-based guidance value (mg/kg b.w. per day)	Exposure estimates (µg/kg b.w. per day)	MOE
BfR (2011)	Harderian gland tumours (male mice)	NTP Report [b]	0.16 ($BMDL_{10}$) [c]	n.a.	n.a.	General population: 0.34 Cons. high percentile: 0.83	471 193
	Mammary tumours (female rats)	NTP Report [b]	0.30 ($BMDL_{10}$) [d]	n.a.	n.a.		882 361
ATSDR (2012)	Male-mediated infertility	Sublet et al. (1989)	0.31 (HED)	30 (3 for interspecies extrapolation using a PBPK model, 10 for human variability)	0.001 (acute-duration oral Minimal Risk Level)	n.a.	n.a.
	Ultrastructural changes in peripheral nerve fibers in male rats	Burek et al. (1980)	0.038 (HED)	30 (3 for interspecies extrapolation using a PBPK model, 10 for human variability)	0.001 (intermediate-duration oral Minimal Risk Level)	n.a.	n.a.
	Degenerative changes in sciatic nerves in rats	Friedman et al. (1995)	0.042 (HED)	30 (3 for interspecies extrapolation using a PBPK model, 10 for human variability)	0.001 (chronic-duration oral Minimal Risk Level)	n.a.	n.a.

784 Table continued overleaf.
785

786 **Table 1:** Summary of previous risk assessments performed by international bodies (continued)

Reference	Critical end-point	Key study	Reference point [g] (mg/kg b.w. per day)	UF	Health-based guidance value (mg/kg b.w. per day)	Exposure estimates (µg/kg b.w. per day)	MOE
Health Canada (2012)	Morphological changes in nerves (rats)	FAO/WHO (2011)	0.2 (NOAEL)	n.a.	n.a.	Mean intake / 1-18 years old: 0.356-0.609 Mean intake / > 19 years old: 0.157-0.288 P90 intake / 1-18 years old: 0.591-1.516 P90 intake / > 19 years old: 0.307-0.740	562-328 1274-694 220-132 270-651
	Harderian gland tumours (male mice)	FAO/WHO (2011)	0.18 ($BMDL_{10}$)	n.a.	n.a.	Mean intake / 1-18 years old: 0.356-0.609 Mean intake / >19 years old: 0.157-0.288 P90 intake / 1-18 years old: 0.591-1.516 P90 intake / >19 years old: 0.307-0.740	506-296 1146-625 198-119 586-243
DTU (2013)	Morphological changes in nerves (rats)	FAO/WHO (2011)	0.2 (NOAEL)	n.a.	n.a.	Mean intake (adults): 0.21 P95 intake (adults): 0.46 Mean intake (children): 0.39 P95 intake (children): 0.89	930 438 518 225

787 Table continued overleaf.
788

789 **Table 1:** Summary of the previous risk assessments performed by international bodies (continued)

Reference	Critical end-point	Key study	Reference point [g] (mg/kg b.w. per day)	UF	Health-based guidance value (mg/kg b.w. per day)	Exposure estimates (µg/kg b.w. per day)	MOE
	Mammary tumours (female rats)	FAO/WHO (2011)	0.31 ($BMDL_{10}$)	n.a.	n.a.		1442 678 873 391
	Harderian gland tumours (male mice)	FAO/WHO (2011)	0.18 ($BMDL_{10}$)	n.a.	n.a.		873 391 466 202
FSANZ (2014)	Morphological changes in nerves (rats)	FAO/WHO (2011)	0.2 (NOAEL)	n.a.	n.a.		310 [f] 130 [f] 150 [f] 80 [f]
	Mammary tumours (female rats)	FAO/WHO (2011)	0.31 ($BMDL_{10}$)	n.a.	n.a.	Mean intake (LB): 1-2 Mean intake (UB): 1-3 P90 intake (LB): 2-4 P90 intake (UB): 2-8	480 [f] 210 [f] 240 [f] 130 [f]
	Harderian gland tumours (male mice)	FAO/WHO (2011)	0.18 ($BMDL_{10}$)	n.a.	n.a.		280 [f] 120 [f] 140 [f] 80 [f]

790 BMDL: the 95 % benchmark dose lower confidence limit; b.w.: body weight; HED: Human Equivalent Dose; NOAEL: no-observed-adverse-effect level; NOEL: no-observed-effect level;
791 PBPK: pharmacologically-based pharmacokinetic model; RfD: Reference Dose; TVM: Tunica Vaginalis Mesothelioma. UF: uncertainty factor.
792 (a): Results of the LogLogistic model.
793 (b): NTP Technical Report on the Toxicology and Carcinogenesis Studies of Acrylamide (CAS No. 79-06-1) in F344/N Rats and $B6C3F_1$ Mice (Drinking Water Study), as cited by BfR (2011).
794 (c): BMD modelling performed by the BfR. Results from the LogProbit model.
795 (d): BMD modelling performed by the BfR. Results from the LogLogistic model.
796 (e): The HED $_{BMDL10}$ at the benchmark response of 0.1 was used to derive a human oral slope factor of 0.5 $(mg/kg \text{ per day})^{-1}$ (US-EPA, 2010).
797 (f): MOEs reported for the age group 17 years and above.
798 (g): Defined as 'point of departure' by some international bodies.

1.2. Chemical characteristics

AA (CAS No 79-06-01) is an odourless white crystalline solid with the molecular formula C_3H_5NO and a molecular weight of 71.08 g/mol (Figure 1). Synonyms are *inter alia* 2-propenamide, acrylic amide and ethylene carboxamide.

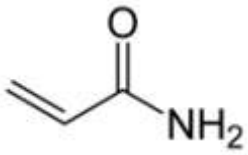


Figure 1: Chemical structure of acrylamide (AA)

The melting point is 84.5 °C and the vapour pressure is 0.9 Pa (7×10^{-3} mm Hg) at 25 °C (ATSDR, 2012). The physical properties, including solubilities, were summarized by IPCS in 1999 (IPCS, 1999). Solubility at 30 °C is high in water (2 155 g/L), methanol (1 550 g/L), ethanol (862 g/L), and acetone (631 g/L). AA is less soluble in chloroform (26.6 g/L) and benzene (3.46 g/L). The Log K_{OW} is -0.67, the log k_{oc} is 1, and the Henry's law constant at 25 °C is 1.7×10^{-9} atm-m^3/mol (US-EPA, 2010).

AA is stable at room temperature but readily polymerizes if heated to melting point or if exposed to ultraviolet radiation (WHO/IPCS, 1999).

The stability of AA and its reactivity with various food-relevant nucleophiles at elevated temperatures in model systems were studied by Adams et al. (2010). The results showed that AA was quite stable in aqueous solutions, but much less in dry reaction conditions. Buffer type and pH had a significant influence on the decrease of free AA. The presence of amino acids with a nucleophilic side chain considerably decreased the free AA. The highest reactivity was noted for cysteine, leading to the formation of the mono-addition product cysteine-S-β-propionamide, as well as to the double addition product. Other nucleophiles, such as lysine, arginine, serine and ascorbic acid, were less reactive, but yielded comparable condensation products (Adams et al., 2010).

1.3. Production, use and environmental fate

1.3.1. Industrial production and use

The industrial production of AA started in 1954 (WHO, 1999) and different methods have been used for its production. Initially, the reaction of acrylonitrile with hydrated sulfuric acid was used. Due to the relatively high levels of impurities this method was replaced by the catalytic hydration of acrylonitrile with a copper catalyst to form AA, a process with a lower yield of impurities (US-EPA, 2010).

AA is available in solid form with a purity reported to be greater than 98 % (w/w), or supplied as a 30-60 % (w/w) aqueous solution (EU, 2000). In the EU, AA is produced as a 30-50 % aqueous solution via the catalytic hydration of acrylonitrile, and the production capacity has been estimated at between 150 000 to 200 000 tonnes per annum[10]. In the US the annual production capacity has been reported to be 137 000 tonnes (Habermann, 2004, as cited in Beland et al., 2013).

AA is produced for a wide variety of industrial applications. In the EU the majority of AA is used in the production of polyacrylamides, with a residual content of AA in the polymers of < 0.1 % (w/w) (EU, 2000). Polyacrylamides are primarily used as flocculants for clarifying drinking-water and treatment of industrial effluents (FAO/WHO, 2011; ATSDR, 2012). AA and polyacrylamides are used

[10] Recommendation from the Scientific Committee on Occupational Exposure Limits for Acrylamide. SCOEL/UM/139. September 2011. Annex December 2012. 38 pp.

in the paper and pulp processing, in the dye synthesis, in cosmetics and food packaging (NICNAS, 2002; FAO/WHO, 2011). Other applications include its use as a grouting agent and soil stabilizer in the construction of dams and tunnels (ATSDR, 2012). It is also used in the cosmetic industry and for the preparation of polyacrylamide gels for electrophoresis (IARC, 1994).

1.3.2. Environmental fate

The release of AA into the environment may occur during its production and direct use, as well as from the production and use of polyacrylamides. Drinking water treated with polyacrylamides as flocculants can contain residual AA (US-EPA, 2010) and therefore a parametric value for AA in drinking water has been established (see Section 2). The use of AA as a grouting agent can cause the contamination of ground water and soil (WHO, 1985).

AA is not considered to be highly persistent in the environment (ATSDR, 2012). Due to its high solubility in water and log K_{oc} of 1, AA is expected to be highly mobile in water and soils (US-EPA, 2010). A higher mobility and lower rate of degradation in sandy soils than in clay soils has been reported. It is not expected that AA is removed from soils or water by volatilisation. The available data indicate that AA concentrations in the atmosphere are very low (ATSDR, 2012), and when present, its low vapour pressure makes it unlikely that AA will be transported in the atmosphere (US-EPA, 2010; ATSDR, 2012).

AA is not expected to bioconcentrate considerably in aquatic organisms (EU, 2000; ATSDR, 2012). Petersen et al. (1985) reported bioconcentration factors (BCFs) of 1.44 and 1.65 for the carcass and viscera of rainbow trout, while Fujiki et al. (1982, as reported in EU, 2000) estimated for carp and Japanese medaka BCFs of 0.77 and 2.53, respectively.

Biodegradation is likely to occur in soil to different degrees depending on the soil type, pH and temperature (NICNAS, 2002). Enzyme-catalyzed hydrolysis is one of the main mechanisms of removal of AA from soils, while in water, non-biological hydrolysis may play an important role. When present in the atmosphere, AA is highly reactive with hydroxyl radicals, and a half-life for this reaction has been reported to be 8.3 hours (EU, 2000; ATSDR, 2012).

1.3.3. Formation in food

The main formation mechanism of AA in food is the reaction of the free amino acid asparagine with reducing sugars at temperatures above 120 °C via the Maillard reaction, as was demonstrated by Mottram et al. (2002) and Stadler et al. (2002) soon after the first reports of the AA occurrence in certain foodstuffs. The Maillard reaction, also denoted as 'non-enzymatic browning' is long known to be responsible for the brown colour of processed food and the formation of a multitude of characteristic flavour compounds formed during food processing of bread, meat, coffee, nuts and others at higher temperatures (Maillard, 1912; Hodge, 1953). Besides the formation of these desirable substances, the Maillard reaction was also identified as the formation mechanism of a number of undesirable compounds, such as AA. The CONTAM Panel concluded however, that data on other undesirable heat-processed products are too limited to perform a group evaluation for all these Maillard reaction products formed in addition to AA.

Becalski et al. (2011) showed that substantial amounts of AA can be generated in model systems even at temperatures lower than 100 °C under conditions that resemble the drying of foods, such as prunes. The authors concluded that AA in prunes and prune juice very likely originates from sugars and asparagine which is present in considerable amounts in the starting material, i.e. plums.

Mestdagh et al. (2008) reported that the ratio of fructose to glucose impacted both color and AA levels of fried potato strips, with a relative higher fructose concentrations favoring AA formation.

AA can also be formed from 3-aminopropionamide, a transient intermediate during thermal degradation or enzymatic decarboxylation of asparagine (Zyzak et al., 2003). The occurrence of 3-

aminopropionamide, a minor but potent precursor in AA formation, in several potato cultivars in different amounts was described by Granvogl et al. (2004).

Other pathways of AA formation that do not require asparagine are also described in the literature. It was shown that AA can in principle be formed from acrolein and acrylic acid, especially in lipid rich foods. The different pathways via acrolein and acrylic acid in the formation of AA were summarized by Stadler and Scholz (2004).

The pyrolytic AA formation from purified wheat gluten and gluten-supplemented wheat bread rolls was demonstrated by Claus et al. (2006).

Casado et al. (2013) investigated AA precursors in sterilized table olives in model systems based on the aqueous fraction of olive pulp from untreated and lye-treated green olives, and demonstrated the formation of AA from commercial model peptides containing protein-bound aspartic acid, alanine and methionine, respectively, at 200 °C and different times in the absence of any carbonyl sources. The authors concluded that their results strongly support the role of peptides/proteins as precursors of AA formation in sterilized olives.

Granvogl et al. (2008) reported that in addition to AA, also GA is formed during the heat processing of foods, although only to a minor extent. In potato chips, the amount of GA was 0.5 % of the amount of AA, whereas this proportion was only 0.2 % in French fries without showing a clear dependence on heating time (see also Section 4.2.1).

In summary, although several pathways of AA formation in food were investigated and demonstrated, especially in model systems, AA forms predominantly from free asparagine and reducing sugars during high temperature cooking, such as frying, roasting and baking, and processing (Halford et al., 2012a).

2. Legislation[11]

In order to protect public health, Article 2 of the Council Regulation (EEC) No 315/93[12] stipulates that, where necessary, maximum tolerances for specific contaminants shall be established. Thus, a number of maximum tolerances for contaminants, natural plant toxicants as well as for process contaminants such as 3-monochloropropane-1,2-diol (3-MCPD) are currently laid down in Commission Regulation (EC) No 1881/2006[13]. AA in food is not regulated so far under this EU Regulation.

Commission Recommendation 2010/307/EU[14] which replaced Commission Recommendation 2007/331/EU[15] recommends that Member States should perform the monitoring of AA levels in certain specified foodstuffs and report the data annually in a prescribed format to EFSA. The monitoring exercise is targeted to those foodstuffs that are known to contain high AA levels and/or contribute significantly to the human dietary intake. Besides the type of product, also the sampling points and procedure, sample numbers and frequencies, analytical requirements as well as minimum additional information to be provided for each product, are each laid down in the two Commission recommendations.

[11] In this scientific opinion, where reference is made to European legislation (Regulations, Directives, Decisions), the reference should be understood as relating to the most current amendment, unless otherwise stated.
[12] Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, p. 1-5.
[13] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. OJ L 364, 20.12.2006, p. 5–24.
[14] Commission Recommendation 2010/307/EU of 2 June 2010 on the monitoring of acrylamide in food, OJ L 137, 3.6.2010, p. 4-10.
[15] Commission Recommendation 2007/331/EC of 3 May 2007 on the monitoring of acrylamide in food. OJ L 123, 12.5.2007, p. 33-40.

Based on the results of the monitoring in the Member States from 2007-2011, the EU Commission set 'indicative values' for AA in various foodstuffs. The most recent indicative values (Table 2) are laid down in Commission Recommendation 2013/647/EU[16]. According to the Recommendation, the 'indicative values' are not safety thresholds, but only intended to indicate the need for an investigation. Enforcement action and/or the issuing of a Rapid Alert should only be undertaken on the basis of a sound risk assessment carried out on a case by case basis, but not merely because an indicative value is exceeded. The Recommendation states: 'Investigations should continue to include the food business operator's Hazard Analysis and Critical Control Points (HACCP) or a similar system with a view to exploring with the food business operator whether relevant processing steps susceptible for the formation of acrylamide have been identified and whether appropriate measures have been taken to control them. In doing so, the competent authorities should assess the extent to which currently known options for the minimisation of acrylamide levels, e.g. those proposed in the Code of Practice for acrylamide adopted by the Codex Alimentarius Commission and in the acrylamide 'toolbox' developed by FoodDrinkEurope, have been implemented by the food business operator'.

These harmonized 'indicative values' provided a more uniform approach across the Member States than the application of values at national levels, such as the German 'Signalwerte' which were introduced in 2002 as part of a national minimizing concept for AA in foodstuffs (Section 4.5).

Table 2: Indicative values for AA in foodstuffs according to Commission Recommendation 2013/647/EU[16]

Foodstuff	Indicative value (µg/kg)
French fries ready-to-eat	600
Potato crisps from fresh potatoes and from potato dough Potato based crackers	1 000
Soft bread Wheat based bread Soft bread other than wheat based bread	 80 150
Breakfast cereals (excl. porridge) - Bran bran products and whole grain cereals, gun puffed grain (gun puffed only relevant if labelled) - wheat and rye based products (1) - maize, oat, spelt, barley and rice based products (1)	 400 300 200
Biscuits and wafers - Crackers with the exception of potato based crackers - Crispbread - Gingerbread - Products similar to the other products in this category	500 500 450 1 000 500
Roast coffee	450
Instant (soluble coffee)	900
Coffee substitutes (a) coffee substitutes mainly based on cereals (b) other coffee substitutes	 2 000 4 000
Baby food, other than processed cereal based foods (2) (a) not containing prunes (b) containing prunes	 50 80
Biscuits and rusks for infants and young children	200
Processed cereal based foods for infants and young children (3), excl. biscuits and rusks	50

(1): Non-whole grain and/or non-bran based cereals. The cereal present in the largest quantity determines the category.
(2): As defined in Article 1(2)(b) of Commission Directive 2006/125/EC[17].
(3): As defined in Article 1(2)(a) of Directive 2006/125/EC.

[16] Commission Recommendation of 8 November 2013 on investigation into the levels of acrylamide in food, OJ L 301. 12.11.2013, p. 15-17.
[17] Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 6.12.2006, p. 16-35.

Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption[18] has set a parametric value for AA of 0.10 µg/L. Parametric values are based on the scientific knowledge available and the precautionary principle and have been selected to ensure that water intended for human consumption can be consumed safely on a life-long basis, and thus represent a high level of health protection. The parametric value for AA refers to the residual monomer concentration in the water as calculated according to specifications of the maximum release from the corresponding polymer in contact with the water.

According to Article 6 of Council Directive 98/83/EC, the parametric values shall be complied with:

> (a) in the case of water supplied from a distribution network, at the point, within premises or an establishment, at which it emerges from the taps that are normally used for human consumption;
>
> (b) in the case of water supplied from a tanker, at the point at which it emerges from the tanker;
>
> (c) in the case of water put into bottles or containers intended for sale, at the point at which the water is put into the bottles or containers;
>
> (d) in the case of water used in a food-production undertaking, at the point where the water is used in the undertaking.

Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food[19] lists AA in Annex I as an authorized substance to be used as monomer. It is not authorized to be used as an additive or polymer production aid. A specific migration limit is not set for AA. Therefore, according to Article 11 of this Regulation, a generic specific migration limit of 60 mg/kg of food applies.

Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products[20] lists AA in Annex II as prohibited substance in cosmetic products. Annex III of this Regulation provides restrictions on the residual AA content in polyacrylamides used in cosmetic products. For polyacrylamide used in body-leave-on products, the maximum residual AA content is 0.1 mg/kg and for polyacrylamide put into other cosmetic products, the maximum residual AA content is 0.5 mg/kg.

Annex XVII of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)[21] provides that AA shall not be placed on the market or used as a substance or constituent of mixtures in a concentration, equal to or greater than 0.1 % by weight for grouting applications after 5 November 2012.

3. Sampling and methods of analysis

Detailed requisites for sampling points and procedures, sample numbers and frequencies, information to be provided for each product and analytical requirements are provided by Commission Recommendation 2010/307/EU on the monitoring of AA levels in food.

[18] Council Directive 98/83 of 3 November 1998 on the quality of water intended for human consumption. OJ L 330, 5.12.98, p. 32-54.
[19] Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. OJ L 12, 15.1.2011, 1-89.
[20] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. OJ L 342, 22.12.2009, p. 59-209.
[21] Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. OJ L 396, 30.12.2006, 1-849.

3.1. Sample collection and frequency

According to the Commission Recommendation, the sampling of the products should be carried out at market level (e.g. at supermarkets, smaller shops, bakeries, French fries outlets and restaurants), where there is a good traceability, or at production sites. Products with origin in one of the Member States should be sampled wherever possible. The distribution of samples per Member State is based on human population size with a minimum sample number of 4 per product category and Member State. A total of 10 different product categories have to be sampled. Depending on the population, between 40 and 230 samples have to be analysed by each of the 27 Member States. These numbers refer to the minimum number of samples to be taken annually and the Member States are invited to take more samples when possible. In order to see time trends, it is considered important that products with the same specifications (e.g. same type of bread) are sampled every year where possible. For each of the 10 product categories, the Recommendation gives detailed provisions concerning subcategories to be sampled and in case of French fries from potato dough also the time point (March/November) for sampling.

In order to ensure that the samples are representative for the sampled lot, Member States should follow the sampling procedures laid down in part B of the Annex to Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs[22].

3.2. Methods of analysis

3.2.1. Legal requirements

According to the EU Recommendations, Member States should carry out the analysis of AA in accordance with the criteria laid down in points 1 and 2 of Annex III to Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. This implies that the methods of analysis should be characterised at least by the following criteria: accuracy, applicability (matrix and concentration range), limit of detection (LOD), limit of quantification (LOQ), precision, repeatability, reproducibility, recovery, selectivity, sensitivity, linearity and measurement uncertainty. The precision values shall either be obtained from a collaborative trial which has been conducted in accordance with an internationally recognised protocol on collaborative trials (e.g. ISO 5725:1994 or the IUPAC International Harmonised Protocol) or, where performance criteria for analytical methods have been established, be based on criteria compliance tests. The repeatability and reproducibility values shall be expressed in an internationally recognised form (e.g. the 95 % confidence intervals as defined by ISO 5725:1994 or IUPAC). The results from the collaborative trial shall be published or freely available. To ensure comparability of analytical results, methods should be chosen that can achieve an LOQ of 30 µg/kg for bread and foods for infants and young children and 50 µg/kg for potato products, other cereal products, coffee and other products. Results should be reported corrected for recovery.

3.2.2. Analytical approaches

3.2.2.1. Food

A number of comprehensive reviews on analytical methods for the determination of AA in food products were published during the past decade (Wenzl et al., 2003; Stadler and Scholz, 2004; Zhang et al., 2005; Oracz et al., 2011; Keramat et al., 2011; Tekkeli et al., 2012; Arvanitoyannis and Dionisopoulou, 2014; Elbashir et al., 2014). The following paragraphs give a short summary on the general procedures and main conclusions, but do not claim for completeness. For more specific details, the reader is referred to the above reviews.

[22] Commission Regulation (EC) No 333/2007 of 28 March 2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs. OJ L 88, 29.3.2007, p. 29-38.

The low molecular weight, high reactivity and lack of chromophore are challenges in the analysis of AA at low concentrations in food products. The choice of optimal extraction conditions and subsequent clean-up steps is dependent on the food matrix of interest. Water and mixtures of water and organic solvents, such as n-propanol or 2-butanone are preferred extraction solvents, mostly at room temperature. A special aspect of the extraction procedure is swelling of the dry matrix in order to get better access of the extraction solvent to potentially absorbed or enclosed AA. Depending on the matrix, swelling yielded a recovery increase of up to 100-fold (Biedermann et al., 2002; Wenzl et al., 2003). To enhance extraction, it was also proposed to increase the temperature of the extraction solvent or to treat the sample in an ultrasonic bath for several minutes. For example, ionic liquid based ultrasonic assisted extraction (ILUAE) was developed to determine the AA content in food samples (Albishri et al., 2014). In any case, care must be taken during extraction to avoid artifactual formation of AA, particularly if conducted under reflux conditions in a low moisture environment (e.g. methanol) and for longer periods of time (Stadler and Scholz, 2004). For clean-up of the extracts, a number of combinations of solid phase extraction (SPE) materials were reported. These include *inter alia* reversed phase materials, anion exchange, mixed mode anion and mixed mode cation exchange, and graphitized carbon materials. Depending on the fat content, food samples may require a defatting step in the clean-up procedure. Although classical clean-up methods, such as SPE, are still widely used, other procedures, such as matrix solid phase dispersion methods (MSPD) and solid phase micro extraction (SPME) become increasingly popular as they require less chemical reagents and often allow a faster sample preparation (Oracz et al., 2011).

The analytical determination of AA in food products is most frequently performed by high performance liquid chromatographic (HPLC) or gas chromatographic (GC) separation methods with mass spectrometric detection (MS), either in selected ion monitoring (SIM) or by tandem mass spectrometry (MS/MS) in multiple reaction mode (MRM). A few authors also report on the application of high resolution mass spectrometry for the determination of AA in food products. As a low cost alternative for the determination of AA in various food stuffs, the application of HPLC coupled to a diode array detector (HPLC/DAD) was described (Michalak et al., 2013; Can and Arli, 2014).

HPLC based methods were reported for both ion trap systems and triple quadrupole systems after electrospray ionisation (ESI) as well as atmospheric pressure chemical ionisation (APCI). The HPLC separation is mostly performed on reversed phase columns or by ion exchange chromatography and the identification and quantification preferentially in MS/MS mode. In recent years the use of ultra-performance liquid chromatography (UPLC) became more and more popular. Because of the high sensitivity and selectivity without the need for derivatisation, HPLC-MS/MS and UPLC-MS/MS methods have nowadays become the methods of choice for the determination of AA in food products. Modern methods based on these analytical techniques fulfil the EU requirements regarding LOD and LOQ without any problem.

The determination of AA in food by GC-MS methods is either done with or without derivatisation. Derivatisation is most frequently done by bromination, but can also be performed with silylation followed by SPME. The brominated AA is less polar than the parent compound and thus better soluble in non-polar organic solvents. A subsequent liquid-liquid extraction between aqueous and organic phase can then be applied as an effective clean-up step. The advantage of derivatisation procedures is that the molecular weight of the derivative is increased which results besides the higher volatility in an improved selectivity. However, derivatisation is a time consuming process, since for example, the excess of bromine has to be removed after the reaction.

The major drawback of GC-MS methods without derivatisation is the lack of characteristic ions because of the low molecular weight of AA. In electron ionisation mode (EI), the major fragment ions for identification and quantification are *m*/*z* 71 and 55, respectively. Co-extracted substances, such as maltol or heptanoic acid produce almost the same ions and may therefore interfere. This makes higher efforts for clean-up mandatory. An increase in selectivity and decrease of interfering signals is possible by the use of GC coupled to tandem mass spectrometry (GC-MS/MS). LODs in the range of

1-5 µg/kg can thus be achieved. Alternatively, the selectivity can be improved by applying positive chemical ionisation (PCI) mass spectrometry with methane or ammonia as reagent gases.

Isotope labelled standards of AA are readily commercially available, either as AA-D_3, AA-D_5, $^{13}C_1$-AA or as $^{13}C_3$-AA. Addition of these internal standards at the beginning of the MS based analysis can improve the accuracy of the result as losses of the native analyte AA during sample treatment are corrected by use of the isotope labelled standard. The spiking approach is based on the establishment of equilibrium in the matrix interactions between the internal standard and the native analyte. As long as the equilibrium is not established, differences in the extraction procedure might have a great influence on recoveries (Wenzl et al., 2003).

In recent years several methods were published for the determination of AA based on electrophoresis. These include capillary electrophoresis (CE), capillary zone electrophoresis (CZE), non aqueous capillary electrophoresis (NACE) and micellar electrokinetic chromatography (MEKC). To increase sensitivity of the CE methods, the field amplified sample injection (FASI) was used and a tandem mass spectrometer was coupled (FASI-CE-MS/MS). Thus, LODs and LOQs of 8 and 20 µg/kg for AA in crisp bread could be obtained which were in a comparable range as from HPLC-MS/MS analyses. Further details of these analytical techniques are summarized by Oracz et al. (2011), Tekkeli et al. (2012) and Arvanitoyannis and Dionisopoulou (2014).

Other analytical techniques, such as pyrolysis gas chromatography/mass spectrometry (Py-GC-MS) and Fourier Transform Infrared Analysis (FT-IR) were frequently applied in the analysis of model systems in connection with studies to elucidate the formation of AA, but do not play a role in the determination of AA in food products.

Although a number of analytical approaches applying different analytical techniques have been published in the past decade, GC- and HPLC-based MS(/MS) methods are primarily used by private and official control laboratories for the routine determination of AA in food products. This is underpinned by the Scientific Report of EFSA 'Update on acrylamide levels in food from monitoring years 2007 to 2010' (EFSA, 2012a). While 56 % of the submitted results from the Member States were reported as performed with HPLC-MS based methods, about 37 % of the samples were analysed with GC-MS based methods.

3.2.2.2. Biological matrices

Analytical methods for the determination of free (unbound) AA in biological matrices, such as blood, urine, tissue or human milk, have been reported in the literature. The HPLC-MS/MS procedures for sensitive quantitative determinations follow the same general approach as used for the analysis of AA in food.

Sörgel et al. (2002) reported on the analysis of AA in urine, human milk and placenta perfusion medium. Following a liquid/liquid extraction, the analytical determination of the concentrated extracts was performed by HPLC-MS/MS. The authors reported concentrations down to 1 ng/mL for urine, 2 ng/mL for placenta perfusate and 5 ng/mL for human milk.

Doerge et al. (2005a, b) reported quantification of AA and GA in serum and tissues, and Doerge et al. (2007) reported quantification of AA and GA in urine after oral and intravenous dosing in mice and rats. Methodology included SPE prior to HPLC-MS/MS quantification. The LODs in serum for AA and GA, respectively, were 3 nM (0.2 ng/mL) and 30 nM (2.6 ng/mL), tissues LODs were 0.1 nmol/g for AA (7 ng/g) and GA (9 ng/g). In urine, LODs were 10 nM (0.7 ng/mL) for AA and 100 nM (8.7 ng/mL) for GA.

Fohgelberg et al. (2005) reported on the analysis of human milk for AA. After addition of a deuterated internal standard, AA was extracted with solvents and after clean-up on an SPE column measured by HPLC-MS/MS. The LOQ was 0.5 ng/g.

Annola et al. (2008a) developed a rapid and sensitive method using HPLC-MS/MS for the simultaneous determination of AA and GA in placental tissue and in perfusion medium from human placental perfusion studies. After addition of the internal standard ^{13}C-AA to the samples and a single step sample preparation, HPLC-MS/MS determination was performed. LOQs for AA and GA were reported as 0.5 µg/mL for the analysed matrices.

Motwani and Törnqvist (2011) used cob(I)alamin, (Cbl(I)) for trapping of GA. The trapping of GA by Cbl(I) results in the formation of an alkylcobalamin (GA-Cbl) that was used for quantitative analysis of the epoxide. The alkylcobalamin was analysed by LC–MS/MS using an electrospray ionisation source in the positive ion mode. The Cbl(I) method was validated for measurement of GA in liver S9 fractions from human and rat.

A number of papers have been published that describe the analysis of Hb adducts of AA and GA, and polar metabolites, such as mercapturic acids (MA) and their sulfoxides or 2,3-dihydroxypropionamide. Further information is given in Section 7.2.

3.2.3. Analytical quality assurance: reference materials, validation and proficiency testing

Certified reference materials (CRM) containing AA in crisp bread at a reported certified level of 0.98 ± 0.09 mg/kg (ERM®-BD272), AA in toasted bread at a reported certified level of 425 ± 29 ng/g (ERM®-BD273), and AA in rusk at a reported certified level of 74 ± 7 ng/g (ERM®-BD274) are commercially available. Complementary to these CRMs produced by the German Federal Institute for Materials Research and Testing (BAM) and the Institute for Reference Materials and Measurements (JRC-IRMM), Kim et al. (2010) reported on the development of a CRM containing AA in potato chips (KRISS CRM 108-10-003) at a certified concentration of 0.455 ± 0.012 mg/kg.

Several proficiency tests and interlaboratory studies comprising AA in various food products were performed, in particular shortly after the first reports on the occurrence of high AA levels in food. The results of these studies are published in a number of reports (Clarke et al., 2002; Klaffke et al., 2005; Owen et al., 2005; Wenzl and Anklam, 2005). In general, there was no evident trend in performance or bias in results obtained with GC-MS or HPLC-MS based methods. However, in some cases the results for samples with low concentrations indicated a bias of the results obtained by GC-MS without derivatisation. Moreover, each study revealed a number of laboratories for which results were outside the acceptable range for accuracy.

An inter-laboratory comparison study with 11 laboratories from 8 EU Member States was carried out by JRC-IRMM (Wenzl et al., 2008) to evaluate the effectiveness of a method that was standardised for the analysis of AA in bakery and potato products for the determination of AA in roasted coffee with the intention to extend the scope of the standardised method. AA levels in roasted coffee ranged between 160 and 585 µg/kg. The calculated method performance parameters were found to be satisfying with regard to internationally accepted criteria as Horrat values for reproducibility were between 0.5 and 0.6, which is much below the broadly accepted maximum value of 2.0. In September 2010, the European Committee for Standardisation (CEN) received a mandate to develop two standardised analytical methods for the determination of AA in food. These standardised analytical methods are ‘Determination of acrylamide in potato-based products, cereal based products and coffee with HPLC-MS (Deadline for requested deliverable is 31 December 2014)’ and ‘Determination of acrylamide in potato-based products, cereal based products and coffee with GC-MS (Deadline for requested deliverable is 31 December 2016)’.

4. Occurrence and patterns of AA in food

4.1. Current occurrence of AA in food – Occurrence results reported to EFSA

4.1.1. Overview of the datasets

Two sources of data were considered:

- data submitted by European countries in the framework of the EFSA continuous data call[23],
- data submitted by six food associations in the framework of the *ad-hoc* EFSA call for AA occurrence data in food and beverages intended for human consumption collected outside official controls launched during spring 2013[24].

4.1.2. Data management and validation

A detailed data quality control was performed in order to check for duplicate submissions, to correct errors in the food description and/or reporting the results, to assess the reliability of analytical results, and to ensure the overall comparability of the data. The quality control targeted especially the values greater than the 75th percentile plus 1.5 times the inter-quartile distance, or less than the 25th percentile minus 1.5 times the inter-quartile distance within each food group defined in Commission Recommendation 2010/307/EU. This data quality control was performed in close cooperation with data providers and laboratories. The data which could not be corrected due to lack of information and those which were considered as unreliable were not further taken into account.

4.1.2.1. Sampling requirements

Only samples taken from 2010 and afterwards were considered in order to avoid any bias related to recent improvement in the analytical methods, and not take into account products no longer available on the European market.

Samples taken in a country not belonging to the European Economic Area (EEA) were not taken into account, unless the data provider specifically indicated the same product (based on same supply chain, same recipe and same processing) to be also available in the European market.

When results were corresponding to different sub-samples of a same sample, an average was determined at the sample level, and this value was retained for further analysis.

4.1.2.2. Analytical requirements

Only results for which the analytical techniques were based on HPLC or GC were considered, provided that the laboratory was accredited and/or the method was validated.

Cut-off values for left-censored data were set at the indicative values defined in Commission Recommendation 2013/647/EU on investigations into the levels of AA in food (Table 2). Results associated with a LOQ above the corresponding cut-off values were not considered. In absence of an indicative value, the cut-off value was set at 50 µg/kg, which corresponds to the objective analytical performance set in Commission Recommendation 2010/307/EU on the monitoring of AA levels in food.

4.1.2.3. Food description

The data were classified according to the most detailed items available in the FoodEx1 system for food (EFSA, 2011b) and according to the food groups adapted from the food description used in Commission Recommendation 2010/307/EU on the monitoring of AA levels in food and in Commission Recommendation 2013/647/EU on investigations into the levels of AA in food.

[23] http://www.efsa.europa.eu/en/data/call/datex101217.htm
[24] http://www.efsa.europa.eu/en/dataclosed/call/130425.htm

In this opinion, and in line with the Commission Recommendations 2010/307/EU and 2013/647/EU, the terminology 'Potato crisps' refers to crunchy thin slices of deep-fried/baked potato usually eaten as snacks, whereas 'French fries' refer to batons of deep-fried potato usually served as an accompaniment during a meal.

'Potato fried', 'Potato croquettes' and 'Roasted potatoes' were classified together with the 'French fries' in a category called 'French fries and potato fried', the potato pancakes, patties, fritter and *rösti* were gathered in a category of 'Other potato fried products' and the other potato products ('Potato baked', 'Potato boiled', 'Potato powder', etc) as 'Other (non fried) potato products'. A distinction was made between the 'French fries and potato fried' sold as ready-to-eat and those sold as fresh or pre-cooked. It was also attempted to distinguish the products analysed as fresh or pre-cooked from those analysed after having been prepared for consumption.

4.1.2.4. Data submitted by European countries

All the data related to the presence of AA in food collected since 2010, submitted to EFSA and checked at the date of the 13th of November 2013 were taken into account. This represented a total of 8 240 samples, originating from 24 European countries (Table 3).

The data quality control process led to the exclusion of 10 % of the available samples:

- 35 samples submitted twice,
- 6 samples for which the food description was too ambiguous,
- 3 samples corresponding to sub-samples, for which an average value at the sample level was retained for further analysis,
- 197 samples for which the analytical technique was not indicated, 56 results for which the analytical method was not validated and/or the laboratory not accredited, one result not generated by HPLC or GC,
- 312 samples associated with a LOQ above the respective cut-off level,
- 182 samples considered as unreliable from an analytical point of view.

The final dataset contained 7 448 samples.

Table 3: Number of analytical samples for each sampling year by the respective country

Country	2010	2011	2012	2013	Total
Austria	99	73	82	-	**254**
Belgium	169	192	175	-	**536**
Bulgaria	-	44	-	-	**44**
Cyprus	41	43	42	-	**126**
The Czech Republic	45	78	63	-	**186**
Denmark	120	117	118	-	**355**
Estonia	30	42	12	-	**84**
Finland	-	120	10	6	**136**
France	56	165	176	-	**397**
Germany	812	986	1 065	-	**2 863**
Greece	80	52	50	-	**182**
Hungary	30	36	48	-	**114**
Ireland	66	54	49	-	**169**
Italy	165	155	217	-	**537**
Lithuania	10	42	40	-	**92**
The Netherlands	62	-	-	-	**62**
Norway	51	-	-	-	**51**

Table continued overleaf.

Table 3: Number of analytical samples for each sampling year by the respective country (continued)

Country	2010	2011	2012	2013	Total
Poland	-	141	-	-	**141**
Romania	-	80	86	40	**206**
Slovakia	125	99	115	54	**393**
Slovenia	41	128	77	-	**246**
Spain	107	41	76	44	**268**
Sweden	56	104	96	-	**256**
The United Kingdom	93	199	250	-	**542**
Total	**2 258**	**2 991**	**2 847**	**144**	**8 240**

The source of the data was indicated in 89 % of the samples. From these, 91 % were generated in the framework of official monitoring programs, the remaining results in the framework of other surveys and combinations of several programmes.

Information on the sampling strategy was provided for around 82 % of the samples. When it was reported, it appeared that overall 30 % of the samples were coming from random sampling and 70 % from selective, suspect or 'convenient'[25] sampling. Due to the overall targeting strategy, the data from the monitoring programs may overestimate the levels of AA in products available on the market.

Analytical techniques based on HPLC were used for 61 % of the samples, and techniques based on GC for 39 % of the samples.

4.1.2.5. Data submitted by food associations

A total of 37 552 data were received following the *ad-hoc* call for occurrence data on AA in foods and beverages intended for human consumption collected outside official controls. The data were submitted to EFSA by six organisations: four European food associations (European Coffee Federation, European Breakfast Cereals Association, European Snacks Association, FoodDrinkEurope) and two national associations (Finnish Food and Drink Industries' Federation and the German Plant Bakeries Association).

The data quality control process led to the exclusion of 4 % of the available analytical samples:

- 205 samples taken before 2010,
- 1 361 samples taken outside the EEA, without sufficient indication the corresponding product would be found in the European market,
- 15 samples for which the LOQ was missing.

The final dataset contained 35 971 samples.

The sampling point was indicated in more than 99 % of the samples. Most of them (97 %) were taken at the manufacturing or storage place, the rest being taken at the retail level. The four European food associations were asked to provide an indication of the overall representativeness of the products for which results were provided, regarding the entire European market. The information provided is summarised in Table 4. The data provided by the food associations covers a large percentage of the respective products in the EU market.

[25] Strategy based on the selection of a sample for which units are selected only on the basis of feasibility or ease of data collection (EFSA, 2010).

1262 **Table 4:** Overall representativeness of the food products within the European market

Food category	Estimation of the EU market/volume share
Baby food	80 % of the market in the EU by volume
Breakfast cereals	75 % of the market in the EU by volume
Coffee products	70-80 % of the market in the EU by volume
Potato crisps from potato dough	80 % of the market in the EU by volume (tonnes). A total of 20 countries are covered and in each case, the market leader is represented
Potato crisps from fresh potatoes	40-50 % of the market in the EU by volume (tonnes). A total of 20 countries are covered and in each case, the market leader is represented
Pre-cooked French fries	Around 50 % share of the marketed pre-cooked French fries in the EU. Data were submitted by the biggest French fries producers in the EU and some smaller companies
Crisp bread	Less than 50 % share of marketed crisp breads in the EU

1263

1264 Analytical techniques based on HPLC were used for 99.9 % of the samples, and techniques based on
1265 GC for 0.1 % of the samples.

1266 4.1.2.6. Comparison of both datasets

1267 The distribution of the LOQs for AA across the food groups, after the exclusion of the LOQs above
1268 the cut-off level, are illustrated in Figure 2 for both the data submitted by the European countries and
1269 the one submitted by the food associations.

1270 The median LOQs of the results provided by food associations are at 10 µg/kg for all food groups,
1271 except for 'Potato fried products', 'Soft bread' and 'Coffee and coffee substitutes' for which it stands
1272 around 30-40 µg/kg. The 95th percentile is below 50 µg/kg in all food groups, except for 'Potato fried
1273 products' for which it reaches 100 µg/kg. Overall, the LOQs of the results provided by European
1274 countries are higher than those provided by food associations. The median LOQs of results provided
1275 by the European countries are in the range of 20-30 µg/kg for all food groups. The 95th percentile is
1276 around 40-50 µg/kg for 'Soft bread', 'Baby foods' and the 'Other products', around 70 µg/kg for
1277 'Biscuits, crackers, crisp bread and similar' and around 100 µg/kg for the other food groups.

1278

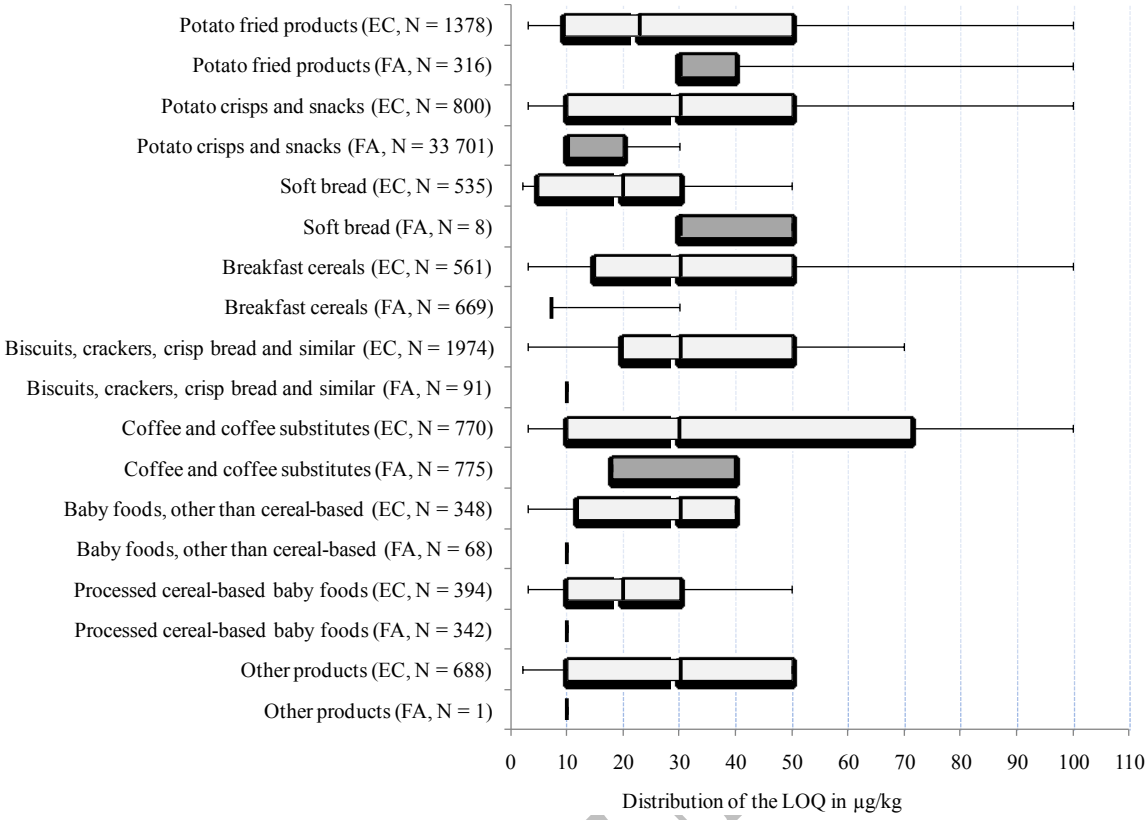


Figure 2: Distribution of the limits of quantification (LOQ) in µg/kg for AA across food groups and according to the origin of the data (European countries (EC) in light grey/food associations (FA) in dark grey) after applying the qualifying criteria (Box-plot: whiskers at P5 and P95, box at P25 and P75 with bold line at P50).

Table 5 shows the distribution of AA levels across the food groups, according to the origin of data. All the samples reported as below the LOD/LOQ were replaced by half their respective LOD/LOQ (middle bound (MB) estimates).

The two datasets are not directly comparable, as they have been generated for different objectives, with different practices regarding the sampling design, the preparation of the samples before analysis and different methods of analysis. Some high levels observed in the dataset provided by the European countries compared to the dataset provided by the food associations may be attributed to a selective sampling strategy towards food products known to contain high AA levels. The results provided by the food associations for 'Potato fried products' reflect levels of AA expected after the product has been prepared according to the instructions given on the package using both home and professional fryers. Regarding data provided by European countries, some of the 'Potato fried products' samples have not been prepared before analysis, and there are uncertainties relating to the exact conditions of preparation in a number of other samples of 'Potato fried products'. Such discrepancies in the protocol of preparation may explain differences in the AA levels observed between the two datasets. Samples provided by the European countries overall are associated with higher LOQs than those provided by food associations. This may explain the higher number of left-censored data observed in the dataset provided by the European countries compared to the one provided by food associations. Finally, differences in the number of samples, as observed for example for the 'Potato crisps and snacks' (33 701 samples provided by the food associations vs. 800 samples by the European countries) can also explain differences in the final estimates of AA levels.

1304 **Table 5:** Distribution of acrylamide (AA) (middle bound (MB) estimates) according to the origin of
1305 data, expressed in µg/kg

	Food category	Origin[a]	N[b]	LC[c]	Mean[d]	Median[d]	P95[d]
1	**Potato fried products**	**EC**	1 378	13.9	332	196	1 115
		FA	316	15.8	201	170	493
	French fries and potato fried, fresh or pre-cooked						
1.1	* sold as ready-to-eat	EC	877	12.7	308	218	904
1.2	* sold as fresh or pre-cooked, analysed as sold	EC	74	40.5	367	88	1 888
1.3	* sold as fresh or pre-cooked, prepared as consumed [e]	EC	241	14.5	288	103	1 059
		FA	316	15.8	201	170	493
1.4	* sold as fresh or pre-cooked, preparation unspecified	EC	90	15.6	368	174	1 468
1.5	Other potato fried products	EC	96	2.1	606	544	1 549
2	**Potato crisps and snacks**	**EC**	800	7.0	580	389	1 841
		FA	33 701	0.0	384	310	920
2.1	Potato crisps made from fresh potatoes	EC	498	6.6	654	431	2 050
		FA	30 969	0.0	388	310	934
2.2	Potato crisps made from potato dough	EC	63	7.9	316	191	870
		FA	2 732	0.3	338	298	747
2.3	Potato crisps unspecified	EC	216	7.9	519	348	1 465
2.4	Potato snack other than potato crisp	EC	23	4.3	283	149	-
4	**Soft bread**	**EC**	535	49.5	40	17	137
		FA	8	0.0	181	180	-
4.1	Wheat soft bread	EC	302	45.0	38	15	120
4.2	Other soft bread[f]	EC	99	43.4	46	25	203
		FA	8	0.0	181	180	-
4.3	Soft bread unspecified	EC	134	64.2	40	25	141
5	**Breakfast cereals**	**EC**	561	29.1	113	67	348
		FA	669	1.3	201	128	661
5.1	Maize, oat, spelt, barley and rice based products	EC	149	32.9	73	50	230
		FA	61	4.9	172	120	540
5.2	Wheat and rye based products	EC	33	21.2	142	140	-
		FA	118	0.8	178	141	460
5.3	Bran products and whole grains cereals	EC	151	9.3	164	135	413
		FA	369	0.3	230	135	770
5.4	Breakfast cereals, unspecified	EC	228	40.8	100	50	350
		FA	121	3.3	148	103	423
6	**Biscuits, crackers, crisp bread and similar**	**EC**	1 974	18.8	264	120	1 077
		FA	91	0.0	277	278	520
6.1	Crackers	EC	162	12.3	231	183	590
6.2	Crisp bread	EC	437	25.2	149	89	428
		FA	91	0.0	277	278	520
6.3	Biscuits and wafers	EC	682	21.1	201	103	810
6.4	Gingerbread	EC	693	14.1	407	155	1 600
7	**Coffee and coffee substitutes[g]**	**EC**	770	7.4	452	237	1 300
		FA	775	0.0	703	666	1 110
7.1	Roasted coffee (dry)	EC	566	7.2	244	203	563
		FA	29	0.0	363	360	-
7.2	Instant coffee (dry)	EC	116	8.6	674	620	1 133
		FA	746	0.0	716	670	1 115
7.3	Substitute (dry), based on cereals	EC	20	5.0	510	522	-
7.4	Substitute (dry), based on chicory	EC	37	0.0	2 942	3 100	-
7.5	Substitute (dry), unspecified	EC	31	16.1	415	377	-

1306 Table continued overleaf
1307

Table 5: Distribution of acrylamide (middle bound (MB) estimates) according to the origin of data, expressed in µg/kg (continued)

	Food category	Origin[(a)]	N[(b)]	LC[(c)]	Mean[(d)]	Median[(d)]	P95[(d)]
8	**Baby foods, other than processed cereal based**	**EC**	348	70.1	24	15	70
		FA	68	50.0	24	8	123
8.1	Not containing prunes	EC	294	70.7	21	15	60
		FA	63	54.0	15	5	46
8.2	Containing prunes	EC	8	0.0	81	31	-
		FA	5	0.0	133	127	-
8.3	Plum content unspecified	EC	46	78.3	33	9	-
9	**Processed cereal-based baby foods**	**EC**	394	48.2	103	15	200
		FA	342	4.7	38	17	154
9.1	Biscuits and rusks	EC	173	38.2	115	45	287
		FA	62	0.0	97	65	256
9.2	Other processed cereal-based foods	EC	208	53.8	99	15	62
		FA	24	66.7	8	5	-
9.3	Processed cereal-based foods unspecified	EC	13	92.3	13	15	-
		FA	256	0.0	26	14	86
10	**Other products based on potatoes, cereals and cocoa**	**EC**	568	32.6	97	36	370
		FA	1	100.0	5	5	-
10.1	Porridge	EC	8	12.5	33	16	-
		FA	1	100.0	5	5	-
10.2	Cake and pastry	EC	198	30.8	66	25	219
10.3	Savoury snacks other than potato	EC	135	13.3	171	88	690
10.4	Other products based on cereals	EC	143	53.1	68	25	293
10.5	Other (non-fried) products based on potatoes	EC	40	60.0	108	8	-
10.6	Other products based on cocoa	EC	44	11.4	104	65	-
11	**Other products not based on potatoes, cereals, coffee and cocoa**	**EC**	120	45.8	330	36	1 510
11.1	Roasted nuts and seeds	EC	40	55.0	93	25	-
11.2	Black olives in brine	EC	3	0.0	454	313	-
11.3	Prunes and dates	EC	18	27.8	89	47	-
11.4	Vegetable chips	EC	11	9.1	1846	1 511	-
11.5	Paprika powder	EC	30	56.7	379	25	-
11.6	Other	EC	18	55.6	68	25	-

(a): origin: EC: European countries; FA: Food associations.
(b): N: number of samples.
(c): LC: percentage of censored results.
(d): Mean, median, P95: mean, median and 95th percentile contamination level presented as the middle bound (MB) estimate. In case of too few observations (less than 60 for the 95th percentile), the estimation may be biased and is consequently not provided.
(e): Product prepared as consumed under laboratory standard conditions or at home. Results provided by food associations correspond to pre-cooked products dedicated for home-cooking but also for restaurants, which have been prepared according to the cooking instructions on the pack using both home and professional materials (fryers, oven). Pre-cooked products dedicated for restaurants can differ from those dedicated for home-cooking regarding the solids content (higher level of solids in products for restaurants than for home-cooking) and cut size of the potato. The final cooking is also different between home and restaurants as professional fryers have higher power than home-fryers and quicker cooking time.
(f): The samples of 'Other soft bread' provided by the food associations all correspond to *pumpernickel*.
(g): Results available for coffee beverage were expressed in powder equivalent, according to the dilution factor used to prepare the beverage before the analysis as indicated by the data provider. When such information was not indicated, the following dilution factors were considered (EFSA, 2012a): 0.017 for instant coffee, 0.053 for filtered coffee and 0.125 for espresso coffee.

Despite these differences, the two datasets overall provide consistent information. In both datasets, 'Coffee and coffee substitutes' and 'Potato crisps and snacks' appear with the highest MB levels, comprised between 384 and 703 µg/kg at the mean, and between 920 and 1 841 µg/kg at the 95th percentile. 'Potato fried products', 'Crisp bread' and 'Breakfast cereals' are in an intermediate

position, with MB levels between 113 and 332 µg/kg at the mean, and between 348 and 1115 µg/kg at the 95th percentile. The 'Processed cereal-based baby foods' and 'Baby foods, other than processed cereal based' have the lowest MB levels, being between 24 and 103 µg/kg at the mean and between 70 and 200 µg/kg at the 95th percentile.

Moreover, the two datasets provide complementary information. The dataset provided by the European countries gives information on the AA levels in a number of food products currently not covered by any indicative value, and these food products are not included in the dataset provided by the food associations. The dataset provided by the food associations contains less uncertainty than the dataset provided by the European countries regarding the mode of preparation of 'Potato fried products' before analysis. Alltogether, the two datasets cover all food groups potentially containing AA and consequently contributing to the dietary exposure of AA.

The CONTAM Panel considered that both sets of data are suitable for exposure assessment and provide complementary information. Therefore, the CONTAM Panel concluded that the datasets can be combined in order to perform the exposure assessment of the European population.

4.1.3. Description of the occurrence levels

Three estimates were produced depending on the assumption made on the results below the LOD/LOQ: (i) the lower bound estimate (LB), replacing all the result reported as below the LOD/LOQ by 0, (ii) the middle bound estimate (MB), replacing all the results reported as below the LOD/LOQ by half their respective LOD/LOQ and (iii) the upper bound estimate (UB), replacing all the results reported as below the LOD/LOQ to their respective LOD/LOQ. Mean and 95th percentiles of the three estimates (LB, MB and UB) were computed for all food groups (Table 6) and according to additional information available at the food groups levels (Appendix B, Table B1), such as the degree of roasting of the coffee, the main cereal composing the bread or the cooking of the French fries.

Table 6: Distribution of acrylamide (AA) levels in µg/kg

	Food category	N[(a)]	LC[(b)]	Mean MB [LB-UB][(c)]	P95 MB [LB-UB][(c)]
1	**Potato fried products**	**1 694**	**14.3**	**308 [303 - 313]**	**971**
1.1	French fries and potato fried, fresh or pre-cooked, sold as ready-to-eat	877	12.7	308 [302 - 314]	904
1.2	French fries and potato fried, fresh or pre-cooked, sold as fresh or pre-cooked, analysed as sold	74	40.5	367 [362 - 372]	1 888
1.3	French fries and potato fried, fresh or pre-cooked, sold as fresh or pre-cooked, prepared as consumed[(d)]	557	15.3	239 [236 - 242]	656
1.4	French fries and potato fried, fresh or pre-cooked, sold as fresh or pre-cooked, preparation unspecified	90	15.6	368 [361 - 375]	1 468
1.5	Other potato fried products[(e)]	96	2.1	606 [606 - 607]	1 549
2	**Potato crisps and snacks**	**34 501**	**0.2**	**389 [388 - 389]**	**932**
2.1	Potato crisps made from fresh potatoes	31 467	0.1	392	949
2.2	Potato crisps made from potato dough	2 795	0.5	338	750
2.3	Potato crisps unspecified	216	7.9	519 [516 - 521]	1 465
2.4	Potato snack other than potato crisp	23	4.3	283	-
4	**Soft bread**	**543**	**48.8**	**42 [36 - 49]**	**156**
4.1	Wheat soft bread	302	45.0	38 [33 - 44]	120
4.2	Other soft bread	107	40.2	57 [51 - 62]	240
4.3	Soft bread unspecified	134	64.2	40 [31 - 50]	141

Table continued overleaf.

1359 **Table 6:** Distribution of acrylamide (AA) levels in µg/kg (continued)

	Food category	N[(a)]	LC[(b)]	Mean MB [LB-UB][(c)]	P95 MB [LB-UB][(c)]
5	**Breakfast cereals**	**1 230**	**14.0**	**161 [157 - 164]**	**552**
5.1	Maize, oat, spelt, barley and rice based products	210	24.8	102 [96 - 109]	403
5.2	Wheat and rye based products	151	5.3	170 [169 - 172]	410
5.3	Bran products and whole grains cereals	520	2.9	211 [210 - 211]	716
5.4	Breakfast cereals, unspecified	349	27.8	117 [109 - 124]	367
6	**Biscuits, crackers, crisp bread and similar**	**2 065**	**18.0**	**265 [261 - 269]**	**1 048**
6.1	Crackers	162	12.3	231 [229 - 233]	590
6.2	Crisp bread	528	20.8	171 [166 - 176]	486
6.3	Biscuits and wafers	682	21.1	201 [197 - 206]	810
6.4	Gingerbread	693	14.1	407 [403 - 412]	1 600
7	**Coffee and coffee substitutes[(f)]**	**1 545**	**3.7**	**578 [577 - 578]**	**1 133**
7.1	Roasted coffee (dry)	595	6.9	249 [248 - 251]	543
7.2	Instant coffee (dry)	862	1.2	710	1 122
7.3	Substitute coffee (dry), based on cereals	20	5.0	510 [509 - 510]	-
7.4	Substitute coffee (dry), based on chicory	37	0.0	2 942	-
7.5	Substitute coffee (dry), unspecified	31	16.1	415 [414 - 415]	-
8	**Baby foods, other than cereal-based**	**416**	**66.8**	**24 [17 - 31]**	**72**
8.1	Baby foods, not containing prunes	357	67.8	20 [13 - 27]	48
	Infant formulae	*33*	*97.0*	*14 [3 - 26]*	-
	Fruit purée	*24*	*62.5*	*22 [15 - 29]*	-
	Juice	*3*	*100*	*12 [0 - 23]*	-
	Ready-to-eat meal and dessert	*291*	*64.3*	*20 [13 - 26]*	*51*
8.2	Baby foods, containing prunes	13	0.0	101	-
8.3	Baby foods, unspecified regarding prunes content	46	78.3	33 [25 - 40]	-
9	**Processed cereal-based baby foods**	**736**	**28.0**	**73 [70 - 76]**	**175**
9.1	Biscuits and rusks	235	28.1	111 [106 - 115]	287
9.2	Other processed cereal-based foods	232	55.2	89 [84 - 95]	60
	Cereals to be reconstituted	*159*	*54.7*	*125 [119 - 130]*	*86*
	Ready-to-eat meal cereal-based	*73*	*56.2*	*13 [8 - 17]*	*30*
9.3	Unspecified processed cereal-based foods	269	4.5	26 [25 - 26]	83
10	**Other products based on potatoes, cereals and cocoa**	**569**	**32.7**	**97 [92 - 101]**	**370**
10.1	Porridge	9	22.2	29 [28 - 31]	-
10.2	Cake and pastry	198	30.8	66 [61 - 71]	219
10.3	Savoury snacks other than potato-based (mostly maize-based)	135	13.3	171 [168 - 173]	690
10.4	Other products based on cereals	143	53.1	68 [61 - 76]	293
	Grains for human consumption	73	56.2	46 [39 - 54]	152
	Grains milling products	17	35.3	117 [112 - 121]	-
	Pasta	9	88.9	13 [0 - 25]	-
	Beer	11	100	14 [0 - 27]	-
	Composite dishes containing cereals	25	32.0	129 [122 - 135]	-
	Fine bakery wares for diabetics	1	0.0	139	-
	Other[(g)]	7	28.6	107 [104 - 109]	-
10.5	Other (non fried) products based on potatoes	40	60.0	108 [104 - 112]	-
	Potato bread	3	0.0	570	-
	Other[(h)]	37	64.9	70 [66 - 74]	-

1360 Table continued overleaf.
1361

1362 **Table 6:** Distribution of acrylamide (AA) levels in µg/kg (continued)

	Food category	N[(a)]	LC[(b)]	Mean MB [LB-UB][(c)]	P95 MB [LB-UB][(c)]
10.6	Other products based on cocoa	44	11.4	104 [103 - 105]	-
	Cocoa powder	13	7.7	178 [178 - 179]	-
	Other products based on cocoa [(i)]	31	12.9	73 [72 - 75]	-
11	**Other products**	**120**	**45.8**	**330 [321 - 339]**	**1 510**
11.1	Roasted nuts and seeds	40	55.0	93 [82 - 103]	-
11.2	Black olives in brine	3	0.0	454	-
11.3	Prunes and dates	18	27.8	89 [87 - 92]	-
11.4	Vegetable chips	11	9.1	1 846 [1843 - 1848]	-
11.5	Paprika powder	30	56.7	379 [365 - 393]	-
11.6	Other[(j)]	18	55.6	68 [59 - 77]	-

1363 (a): N: number of samples.
1364 (b): LC: percentage of censored results.
1365 (c): Mean MB[LB-UB], P95 MB[LB-UB], mean and 95th percentile contamination level presented as the middle bound
1366 estimate (lower bound estimate; upper bound estimate). When the middle, lower and upper bound estimates are equal,
1367 only one estimate is given. In case of too few observations (less than 60 for the 95th percentile), the estimation may be
1368 biased and is not consequently not provided.
1369 (d): Product prepared as consumed under laboratory standard conditions or at home.
1370 (e): Potato patties (*kartoffelpuffer*, n = 52), potato pancake (n = 28), *rösti* (n = 12), unspecified (n = 3).
1371 (f): Results available for coffee beverage were expressed in powder equivalent, according to the dilution factor used to
1372 prepare the beverage before the analysis as indicated by the data provider. When such information was not indicated, the
1373 following dilution factors were considered (EFSA, 2012b): 0.017 for instant coffee, 0.053 for filtered coffee and 0.125
1374 for espresso coffee.
1375 (g): Malt extract (n = 2), unspecified grain and grain-based products (n = 3), unspecified snack food (n = 2).
1376 (h): Potato flakes/powder (n = 20), potato boiled (n = 12), unspecified potato and potato products (n = 3), potato-based dish
1377 (n = 1), new potato (n = 1).
1378 (i): Chocolate and chocolate based confectionary (n = 30), unspecified cocoa beans and cocoa products (n = 1).
1379 (j): Composite dishes vegetable-based and unspecified (n = 7), confectionary (not chocolate based) (n = 4), seafood chips
1380 (n = 2), unspecified legumes, nuts and oilseeds (n = 2), dried bananas (n = 1), oil frying blend (n = 1), rhubarb and
1381 unspecified vegetable and vegetable products (n = 1).
1382

1383 4.1.3.1. Potato fried products

1384 The AA MB levels in 'Potato fried products' were on average at 308 µg/kg and at the 95th percentile at
1385 971 µg/kg. The lowest levels were observed in the group of 'French fries and potato fried, fresh or
1386 pre-cooked, sold as fresh or pre-cooked and prepared as consumed' (MB average at 239 µg/kg) and
1387 the highest levels in the group of 'Other potato fried products' (MB average at 606 µg/kg) which
1388 gathers *rösti*, *kartoffelpuffer* and pancake (Table 6). However, such observations may be biased by
1389 some misclassifications. In some cases the information on the condition of preparation of the French
1390 fries before analysis was collected separatedly from the transmission of the individual results.
1391 Moreover, the food classification used for the data collection only distinguishes the French fries sold
1392 as ready to eat taken in small outlets, fast food chains and restaurants, from the pre-cooked French
1393 fries/potato products for home cooking. This lead to some difficulties to handle the fresh/pre-cooked
1394 French fries/potato products sampled in restaurants before cooking and analysed as such or prepared
1395 under laboratory conditions and the fresh/pre-cooked French fries/potato products taken as ready-to-
1396 eat at home.

1397 Higher AA levels were observed in potato fried products made from fresh potatoes (MB average at
1398 275 µg/kg) than in potato fried products made from potato dough (MB average at 197 µg/kg), but no
1399 substantial difference was observed between potato fried products baked in the oven (MB average at
1400 257 µg/kg) and those deep fried (MB average at 243 µg/kg) (Appendix B, Table B1).

Insufficient information was available at the sample level to compare the levels of AA in French fries according to their size, the conditions of frying, whether the pre-cooked product was frozen or not, and the storage conditions of the potatoes

4.1.3.2. Potato crisps and snacks

The AA MB levels in 'Potato crisps and snacks' were on average at 389 µg/kg and at the 95th percentile at 932 µg/kg. AA was found in higher levels in 'Potato crisps from fresh potatoes' (MB average at 392 µg/kg) than in 'Potato crisps from potato dough' (MB average at 338 µg/kg). AA levels appeared to be lower in potato crisps from batch process (MB average at 327 µg/kg) than in potato crisps from continuous process (MB average at 387 µg/kg) (Appendix B, Table B1). However, such observations may be biased by the differences in the number of samples between the different categories of 'Potato crisps and snacks'. Lower levels were observed in 'Potato snacks other than potato crisps', including mostly puffed potato snacks (MB average at 283 µg/kg) than in the 'Potato crisps' (Table 6).

4.1.3.3. Soft and crisp bread

The AA MB levels were lower in 'Soft bread' than in 'Crisp bread', on average at 42 and 171 µg/kg and at the 95th percentile at 156 and 486 µg/kg respectively (Table 6). Samples of 'Toasted bread' taken as such from the market show similar levels as 'Soft bread' (Appendix B, Table B1).

Lower levels were observed in soft bread and crisp bread mainly made from wheat (38 and 126 µg/kg respectively), than in soft bread and crisp bread mainly made from rye (57 and 245 µg/kg respectively) (Appendix B, Table B1). No comparison could be made with the other varieties of cereals (barley, maize, etc) due to the lack of data.

4.1.3.4. Breakfast cereals

The AA MB level in 'Breakfast cereals' excluding porridge was on average at 161 µg/kg and at the 95th percentile at 552 µg/kg, whereas it was on average at 29 µg/kg in 'Porridge'. AA was found in higher levels in 'Bran and whole grains breakfast cereals' (MB average at 211 µg/kg) than in 'Wheat and rye based breakfast cereals' (MB average at 170 µg/kg) and in 'Maize, oat, spelt, barley and rice based breakfast cereals' (MB average at 102 µg/kg) (Table 6).

4.1.3.5. Biscuits, cracker and gingerbread

'Gingerbread' contained higher levels (MB average at 407 µg/kg and 95th percentile at 1600 µg/kg) than 'Crackers' (MB average at 231 µg/kg and 95th percentile at 590 µg/kg) and 'Biscuits and wafers' (MB average at 201 µg/kg and 95th percentile at 810 µg/kg) (Table 6).

4.1.3.6. Coffee and coffee substitutes

AA MB level in 'Coffee and coffee substitute' was on average at 578 µg/kg and at the 95th percentile at 1 133 µg/kg.

The 'Roasted coffee' (MB average at 249 µg/kg) was found to be less contaminated than 'Instant coffee' (MB average at 710 µg/kg) (Table 6). Despite the limited number of samples, the level of AA was found to be higher in light roasting (MB average at 374 µg/kg) than in medium (MB average at 266 µg/kg) and dark roasting (MB average at 187 µg/kg) (Appendix B, Table B1). Such observation is in line with the literature, which showed that AA appear in high concentrations during the first minutes of roasting, and then degrade with the continuation of the roasting (Lantz et al., 2006). Whereas lower AA levels are observed in regular roasted coffee (MB average at 245 µg/kg) compared to decaffeinated roasted coffee (MB average at 319 µg/kg), higher AA levels are observed in regular instant coffee (MB average at 718 µg/kg) compared to decaffeinated instant coffee (MB average at 630 µg/kg) (Appendix B, Table B1). These comparisons should be interpreted cautiously, due to the differences in number of samples between the regular and decaffeinated coffee categories.

Regarding substitute coffee, higher levels were observed in 'Substitute coffee, based on chicory' (MB average at 2 942 µg/kg) compared to the 'Substitute coffee, based on cereals' (MB average at 510 µg/kg) (Table 6).

4.1.3.7. Baby foods

AA MB levels were higher in 'Processed cereal-based baby foods' than in 'Baby foods, other than cereal based', on average at 73 and 24 µg/kg and at the 95th percentile at 175 and 72 µg/kg, respectively (Table 6). 'Biscuits and rusks' (MB average at 111 µg/kg) were found with AA in higher levels than 'Other processed cereal-based babyfood' (MB average at 89 µg/kg). Higher levels were observed 'Baby foods, containing prunes' (MB average at 101 µg/kg) than in 'Baby foods, not containing prunes' (MB average at 20 µg/kg).

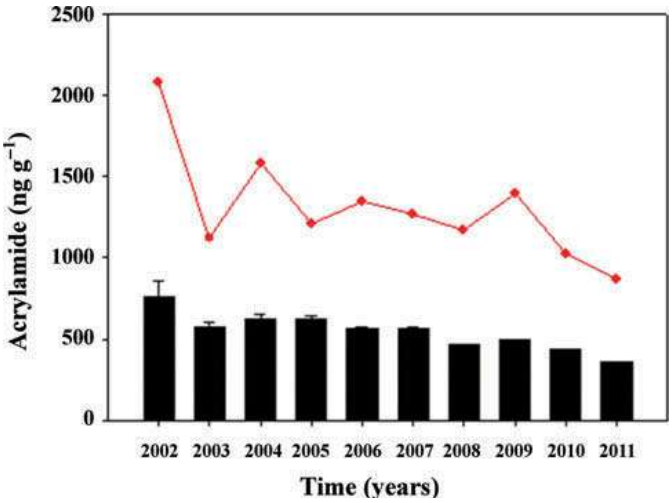
4.2. Temporal trend analysis of AA occurrence data in certain food categories

The CONTAM Panel explored the possibility to perform a temporal trend analysis of the AA concentrations in certain foodstuffs across Europe on the basis of the data submitted to EFSA by the Member States. Because of gaps in the database and the fact that results for the different years are not always comparable, a reliable Europe-wide temporal trend analysis is not feasible.

In 2012, EFSA compiled results from annual monitoring of AA levels in European foods carried out from 2007 to 2010 under Commission Recommendation 2007/331/EC of 3 May 2007 (EFSA, 2012a). Twenty-five European countries submitted a total of 13 162 results for the four-year period. During the monitoring period, time trends in AA levels for different food categories were estimated using a linear model. However, the trend analysis did not show any major changes in AA levels. While there was an indication of a decrease in AA levels in a few food categories (e.g. 'Processed cereal-based baby foods', 'Savoury snacks other than potato'), other food categories showed increased levels (e.g. 'Coffee and coffee substitutes', 'Crisp bread'). It was concluded that a more accurate trend evaluation of AA levels in European food categories would require an extended monitoring period and more detailed descriptions of sample sources. Specific recommendations to improve any future monitoring program for AA in foods included: consistently sensitive analytical methodology across the contributing laboratories; repeated sampling of the same type of products in different years; and sufficient number of samples per food group.

A dataset of manufacturers' measurements of AA levels in 40 455 samples of fresh sliced potato crisps from 20 European countries for the years 2002 to 2011 was compiled by Powers et al. (2013). Analysis of variance was applied to the data and showed a significant downward trend for mean levels of AA, from 763 ± 91.1 µg/kg in 2002 to 358 ± 2.5 µg/kg in 2011 (Figure 3). This was a decrease of 53 % ± 13.5 %. The yearly 95th quantile values were also subjected to a clear downward trend. The proportion of samples containing AA at a level above the indicative value of 1 000 µg/kg for potato crisps introduced by the European Commission in 2011 fell from 23.8 % in 2002 to 3.2 % in 2011. Nevertheless, even in 2011, a small proportion of samples still contained high levels of AA, with 0.2 % exceeding 2 000 µg/kg (Powers et al., 2013). The results of samples from 2010 onwards were submitted to EFSA through the industry call for data and are included in the occurrence and exposure assessment of this opinion.

1490



1491 **Figure 3:** Overall mean acrylamide (AA) levels (ng/g) in 40 455 samples of fresh sliced potato
1492 crisps from 20 European countries over years from 2002 to 2011, with standard errors and with trend
1493 in 95 % (Q95) quantiles (red) (figure from Powers et al., 2013[26]).

1494 **4.3. Previously reported literature data on AA in food**

1495 AA number of studies providing results for AA in different food commodities have been published in
1496 the literature since AA was first detected in food. Many of these have described the AA concentrations
1497 in different food groups while studing the influence of processing and/or approaches to reduce the AA
1498 content (see Section 4.4). Several international bodies have reported the occurrence of AA in different
1499 food commodities when estimating the dietary exposure to AA, both at a national and international
1500 level (see Sections 1.1 and 6.3). The paragraphs below, which do not claim for completeness, give an
1501 overview of some of the occurrence values reported.

1502 In Europe, Wenzl and Anklam (2007) reported the results collected by the Joint Research Centre
1503 during the years 2003-2006 to build up the European Union database of AA in food. Afterwards,
1504 EFSA has published a total of four scientific reports summarising the results from the annual
1505 monitoring of AA levels in European foods carried out from 2007 to 2010 under different Commission
1506 Recommendations[14, 15] (EFSA, 2009a, 2010, 2011a, 2012a).

1507 The last JECFA evaluation in 2010 reported the AA occurrence data from more than 12 500 samples
1508 reported from 31 countries, with 61 % coming from Europe, 28 % from Asia, 9 % from North
1509 America, 1 % from the Pacific, and 1 % from Latin America (FAO/WHO, 2011). National mean
1510 concentrations of AA in major foods ranged from 399 to 1 202 µg/kg for potato crisps, from 169 to
1511 963 µg/kg for potato chips (French fries), from 169 to 518 µg/kg for biscuits, from 87 to 459 µg/kg for
1512 crisp breads and crackers, and from 3 to 68 µg/L for coffee (prepared as ready-to-drink). JECFA noted
1513 that other food commodities generally had mean levels < 100 µg/kg. It also noted that since its
1514 previous evaluation in 2005 (FAO/WHO, 2006), AA levels in rye products had decreased
1515 significantly, but no differences were seen in products prepared using potato, barley, rice, wheat,
1516 maize or oats (FAO/WHO, 2011).

1517 The Danish food monitoring on chemical contaminants 2004-2011 (DTU, 2013) reported the highest
1518 mean AA values in instant coffee (580 µg/kg), popcorn (483 µg/kg), French fries sold as ready-to-eat

[26] This image has been reproduced from the publication: Powers SJ, Mottram DS, Curtis A, Halford NG, 2013. Acrylamide concentrations in potato crisps in Europe from 2002 to 2011. Food Addit Contam A, 30 (9) 1493-1500, doi: 10.1080/19440049.2013.805439. It is subject to the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/by/3.0/, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named authors have been asserted.

(472 µg/kg), potato crisps (448 µg/kg), potatoes prepared at home in the oven (312 µg/kg), biscuits (278 µg/kg), and roasted coffee beans (275 µg/kg). BfR (2011) reported the highest mean AA values from its 2010 monitoring of AA in coffee substitutes (739 µg/kg), followed by potato patties (*Kartoffelpuffer*) (692 µg/kg), soluble coffee (686 µ/kg) and ginger bread (*Lebkuchen*) and derived bakery products (522 µg/kg). Crisps and French fries were reported to have mean AA levels of 385 and 256 µg/kg, respectively. Afssa (2005) reported the highest AA levels in French fries (790 µg/kg), followed by salted biscuits (390 µg/kg) and potato crisps (298 µg/kg).

The survey carried out in Poland (Mojska et al., 2010), found the highest AA levels in potato crisps (904 µg/kg), followed by crackers (859 µg/kg) and French fries (from pre-cooked products, fried in the laboratory) (827 µg/kg). Crisp bread showed a mean value of 430 µg/kg and for the remaining food categories considered mean AA levels were lower. In Finland, Hirvonen et al. (2011) found the highest AA levels in crispbread (674 µg/kg), followed by potato crisps (539 µg/kg) and sweet biscuits (443 µg/kg).

In the US, Tran et al. (2010) reported the AA content in several food categories as part of a dietary exposure estimate. The highest mean values were reported for 'potato/other chips' (512 µg/kg), followed by 'French fries, fried potatoes' (442 µg/kg), 'other fruit juice (limied to prune juice) (224 µg/kg), crackers (221 µg/kg) and 'tortilla/corn chips' (190 µg/kg).

In Canada, mean AA levels in snack foods (including potato crisps) ranged from < 10 to 3 203 µg/kg, while in cookies and biscuits it ranged from 23 to 1 401 µg/kg. The mean AA levels in French fries ranged from 41 to 766 µg/kg (Health Canada, 2012). In another study in Canadian study, Normandin et al. (2013) reported mean AA concentrations of 1 053 µg/kg in French fries (deep fried, prepared as consumed), while lower concentrations were found in potato chips (French fries) (524 µg/kg) and oven-baked French fries (prepared as consumed, 358 µg/kg). The levels in coffee (brewed) were < 10 µg/kg.

In the national updated exposure assessment of AA completed in 2012 for New Zealand (MAF, 2012), the mean (range) AA levels in crisps was 581 µg/kg (112 - 1 460). The level were lower than those found in previous surveys (mean 1 570 (range: 370 - 2 320) µg/kg). Potato oven baked or roasted showed higher values specially those from supermarket wedges (1 278 (range: 435 - 2 252) µg/kg. Mean AA levels in biscuits and crackers was reported to range from 100 to 600 µg/kg. Conversely mean AA levels in cereal-based snacks products ranged from 150 to 600 µg/kg, representing an increase in levels from approximately 300 µg/kg compared to previous surveys.

In China, (Chen et al., 2012) reported AA levels in several food categories, with the highest mean values found in fried potato (604.3 µg/kg) and in other fried products such as fried prawn strips (341.4 µg/kg) and fried rice crust (201.5 µg/kg). Crisps were found at mean levels of 137.9 µg/kg).

Sirot et al. (2012) and Zhou et al. (2013) reported the AA levels in samples within total diet studies (TDS). Within the second French TDS, 2 280 individual food products were collected and further grouped in 192 analytical samples grouped in 16 food groups and prepared 'as consumed' (Sirot et al., 2012). The highest mean AA concentrations were found in potato crisps (954 µg/kg) followed by French fries (724 µg/kg) and salted biscuits (other than potato crisps) (697 µg/kg). Coffee showed mean values of 37 and 74 µg/kg for brewed and instant coffee, respectively. Bread and bread products showed values of 34 µg/kg while breakfast cereals showed lower levels (16 µg/kg). Zhou et al. (2013) performed total diet studies in four regions of China, analysed a total of 144 food composite samples grouped in 12 groups and prepared 'as consumed'. The highest mean concentration were reported in sugar (72.1 µg/kg), followed by potatoes (31.0 µg/kg) and vegetables (22.3 µg/kg). The levels in cereals were reported at 6 µg/kg. Cereals and potatoes were found to contain AA at lower levels compared to those reported in other countries, and the authors concluded that this was probably due to different cooking temperatures and raw materials.

The AA levels in infant foods have been reported in several studies. Mojska et al. (2012) analysed 111 commercially sold Polish baby food products, including follow-on formula, infant cereals, biscuits for infants and jarred baby food. The highest mean values were found in infant biscuits (219 µg/kg) and powdered cereals (148 and 129 µg/kg). When corrected to reconstitution with water to reflect ready to eat products, these levels fell into the 3-50 µg/kg range. The range of the mean AA levels in the remaining food types was 23-73 µg/kg. In another study in Poland, mean AA levels in instant cereal-based food and in cereal-based foods for infants were 34.7 and 13.4 µg/kg, respectively. Levels in candy-bars for young children were up to 53.5 µg/kg (Michalak et al., 2013). Mean AA levels in baby food products of 604 ± 694, 495 ± 403 and 290 ± 249 µg/kg were reported for crackers, biscuits and breakfast cereals purchased in Turkey (Cengiz and Gündüz, 2013).

Additionally, there have been studies to address local or individual food issues. Bent et al. (2012) analysed AA levels in commercially-available and home-made 'Caribbean' foods, including biscuits, breakfast cereals, banana chips, and home-prepared foods (breadfruit; *Artocarpus altilis*, banana fritters, and dumplings). Biscuits, breadfruits, and fried dumplings showed the highest levels, up to approximately 4 000 µg/kg. Sanganyado et al. (2011) determined the levels of AA in traditional foods in Zimbabwe with mean AA levels in roasted maize, roasted groundnuts and roasted soybeans of 460, 140 and 70 µg/kg, respectively. In boiled maize AA was found not detected. Özer et al. (2012) analysed the AA levels in Turkish traditional desserts reporting values of up to 150 µg/kg for baklava samples. Delgado-Andrade et al. (2010) determined the AA content in Spanish typical dishes such as lentil stew, paella, Spanish potato omelette and *churros*, with mean AA values per serving of 105 ± 32, 263 ± 38, 240 ± 15 and 160 ± 13 µg/kg, respectively.

In conclusion, a direct comparison between the occurrence data in the current assessment and the previously published occurrence data in literature should be done with caution, especially for processed products due to different analytical methodologies applied, and ingredients and processing methods which are not always reported. Nevertheless, the results of the various studies from different regions worldwide identified the same food commodities, such as processed potato and cereal based products that potentially contain high AA levels and thus are contributing substantially to human exposure. In addition, the results broadly suggest that AA levels have not changed considerably, despite efforts by food producers to improve and develop new processes that would mitigate AA formation during preparation.

4.3.1. Glycidamide in food

Using a stable isotope dilution assay and derivatisation with 2-mercaptobenzoic acid, Granvogl et al. (2008) reported for the first time on the occurrence of GA in processed food. Application of the method on several potato samples revealed amounts of GA between 0.3 and 1.5 µg/kg depending on the processing conditions. In potato chips, the amount of GA was 0.5 % of the amount of AA, whereas this proportion was only 0.2 % in French fries without showing a clear dependence on heating time. In a model experiment, the formation of GA by an epoxidation of the double bond in AA, that is, by a reaction with linoleic acid hydroperoxides, was established. This result was in good agreement with data showing that French fries processed in sunflower oil, which is high in linoleic acid, contained more GA as compared to fries prepared in coconut oil (Granvogl et al., 2008).

4.3.2. Data published by consumer organisations and consumer's magazines

Besides the determination of AA in the frame of official food control and by food business operators as part of their selfcontrol, analyses are also carried out by consumer organisations and consumer magazines. Following a consultation with the EFSA Stakeholder Consultative Platform, EFSA received information on the work undertaken by some consumers' organisations and published in consumer magazines related to AA (see Section on Documentation provided to EFSA). The main focus laid on the determination of AA in potato chips as these products initially showed relatively high concentrations and thus were a primary target for mitigation measures by food industry. To follow the contamination trend, between 2003 and 2012, a number of surveys, including a preferably representative range of products on the respective markets were conducted and the results published in

consumer magazines. In general, the data indicated a decreasing trend of AA concentrations in potato chips from the different countries where the analyses were performed. On the other hand, products such as gingerbread did not show a similar trend, possibly due to the use of traditional recipes and production processes often in small bakeries that are not applying the same mitigation measures as the bigger food industries.

A few consumer organisations also tested various deep fryers available on their market and examined the impact of the different cooking processes on the AA concentrations. For example, a national consumer organisation used a single brand of frozen pre-fried potatoes which were cooked in the various machines according to the respective instruction sheets. The main conclusion was that the hot air machines produced more AA (average: 710 µg/kg) than the conventional deep oil fryers (average: 518 µg/kg) tested (OCU-Compra Maestra (2013)). A similar result was observed by another consumer organisation when they tested an air fryer in 2011. The AA values were four times higher than the applied 'acceptable' value of 500 µg/kg (Test-achats (2008)). As a follow up of the published results, the producer adapted the instruction sheet of the machine. The effect was that the AA concentration was indeed much lower (below 500 µg/kg), but it affected the taste. In February 2013, another consumer organisation published a comparative study on fryers. The main conclusion was that the potatoes fried in conventional oil deep fryers were lower in AA formation (average: 477 µg/kg) than the potatoes fried in fryers that use hot air instead of oil (average: 710 µg/kg). For this study, a single brand of frozen pre-fried potatoes was used (Proteste (2013)).

4.4. Impact of raw material, storage and processing on AA levels in food

Shortly after the first report by the Swedish National Food Administration in April 2002 on high levels of AA in certain food commodities (SNFA, 2002), numerous research activities were initiated *inter alia* on the formation mechanism of AA, effects of processing and possible mitigation measures. It became soon clear that the main pathway of formation is the reaction between the amino acid asparagine and reducing sugars via the Maillard reaction (See Section 1.3.3). In many cooking processes, the Maillard reaction is the predominant chemical process that determines colour, flavour and texture of cooked foods, based on highly complex reactions between amino acids and sugars. In this process, the thermal input is pivotal for the AA formation, i.e. the combination of temperature and heating time to which the product is subjected (EU-Toolbox). The formation primarily takes place under high temperature, usually in excess of 120 °C, and low moisture (CAC/RCP 67-2009[8]). Therefore, AA is generally not detected at elevated amounts in boiled foods, but can be found at considerable concentrations in specific processed foods, especially where deep-frying or roasting is involved.

During the last decade, numerous papers were published that analysed various parameters that have a potential impact on the AA levels in food. The following paragraphs, which do not claim for completeness, give a short summary on the major findings.

4.4.1. Impact of raw material and storage

Early results on AA occurrence in food demonstrated considerable concentrations in carbohydrate-rich foods, in particular potato crisps and French fries. Stadler and Scholz (2004), Vinci et al. (2012) and Bethke and Bussan (2013) summarized the substantial findings of the various research groups concerning key factors that may impact AA formation and resulting potential measures to minimize AA in the final product. Following the finding that asparagine and reducing sugars are important determinants of AA formation, it was demonstrated that relative reductions in AA concentrations are possible by controlling the reducing sugar levels in potato cultivars (Biedermann-Brem et al., 2003). Besides the choice of the right cultivar, also the appropriate storage conditions are important. In this context, it was shown that storage below 8 °C will mobilize sugars in the tubers, e.g. concentrations can increase by up to a factor of 28 after 15-day storage at 4 °C (Noti et al., 2003). In their analysis of 40 455 samples of fresh sliced potato crisps from 20 European countries, Powers et al. (2013) showed that the effects of seasonality arising from the influence of potato storage on AA levels was evident, with AA in the first six months of the year being significantly higher than in the second six months.

Kumar et al. (2004) reported that in addition to temperature, also atmosphere can have an effect on sugar content in potatoes, because low oxygen levels supress sugar accumulation, while an increase of carbon dioxide concentration has the opposite effect.

Halford et al. (2012b) studied the effects of storage of nine potato varieties on precursors concentrations and their relationship with AA formation and showed the potential of variety selection for preventing unacceptable levels of AA formation in potato products and the variety-dependent effect of long-term storage on AA levels. The study also highlighted the complex relationship between precursor concentration and AA formation in potatoes.

Asparagine is the dominant free amino acid in potato tubers, typically accounting for approximately one-third of the total free amino acids. Its concentration is influenced by both genetic and environmental factors. Experiments by Elmore et al. (2007) with different potato varieties grown in a glasshouse in pots containing vermiculite showed that sulphur deficiency in the medium to water the plants caused an increase in free asparagine accumulation in one variety but a decline of asparagine concentration in two other varieties. However, in all three varieties, the glutamine concentration increased, indicating that potatoes preferentially accumulated free glutamine rather than free asparagine in response to sulphur deficiency. This resulted in asparagine concentration as a proportion of the total free amino acids falling and a concomitant reduction in AA formation during heating (Elmore et al., 2007; Halford et al., 2012a).

The effects of nitrogen and sulphur fertilisation on free amino acids, sugars and AA-forming potential in potatoes was investigated by Muttucumaru et al. (2013). In their study, 13 varieties of potato were grown in a field trial in 2010 and treated with different combinations of nitrogen and sulphur. Potatoes were analyzed immediately after harvest to show the effect of nitrogen and sulfur fertilisation on concentrations of free asparagine, other free amino acids, sugars, and AA-forming potential. The study showed that nitrogen application can affect AA-forming potential in potatoes but that the effect is type- (French fry, chipping and boiling) and variety-dependent, with most varieties showing an increase in AA formation in response to increased nitrogen but two showing a decrease. Sulfur application reduced glucose concentrations and mitigated the effect of high nitrogen application on the AA-forming potential of some of the French fry-type potatoes (Muttucumaru et al., 2013). Results from further agronomic practices, involving genetic modification and other genetic techniques to reduce relevant precursors in potatoes and other plants are summarized by Halford et al. (2012a).

4.4.2. Impact of processing

Potatoes and potato based products

The influence of frying temperature and processing duration on the AA content in French fries was analysed by Matthäus (2002). Figure 4 illustrates the substantial AA increase with rising temperature between 195-225 °C and processing duration between 16-24 minutes. The results show that the rise of temperature has a more pronounced effect than the increase of the processing time at the steady temperature. As a consequence, the frying temperature in oil-fried products should not exceed 170-175 °C to avoid extraordinary AA levels in the finished product. Matthäus (2002) also analysed the influence of the type of processing on the AA concentrations in fried potato patties (Figure 5). Although the highest temperature and the longest processing time is applied in the convection oven, the AA concentration in the finished product is considerably lower than in the fried potato patties prepared in the cooker at 215 °C for 6.5 minutes. This confirms that the efficacy of the heat transfer into the product is an important determinant. There are a number of further investigations on the impact of various parameters on AA formation in heat treated foods, such as type of frying oil, frequencies of oil use or addition of additives. The key findings of these studies which often showed minor effects on the AA levels were summarized by Stadler and Scholz (2004).

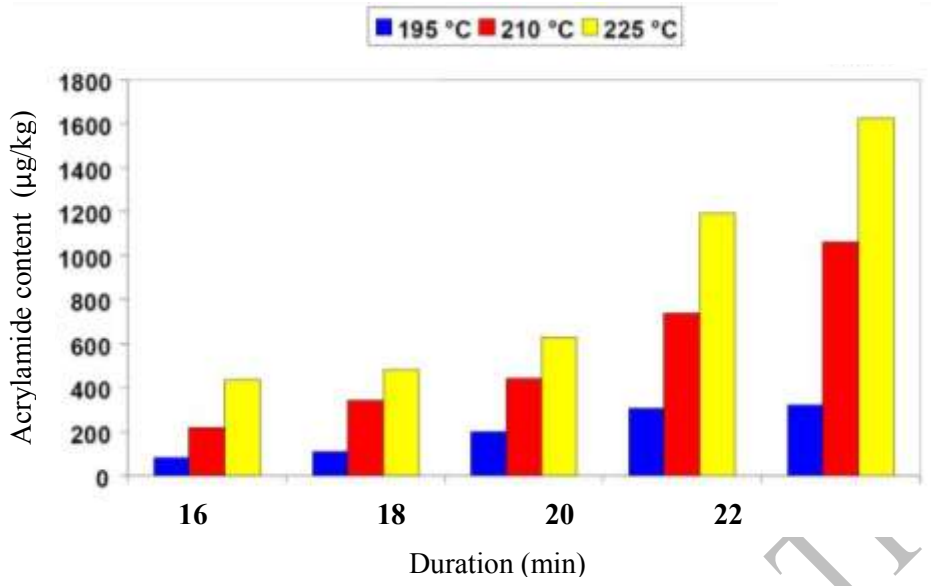


Figure 4: Influence of frying temperature and processing duration of the AA content in French fries. Source: Matthäus, 2002, modified.

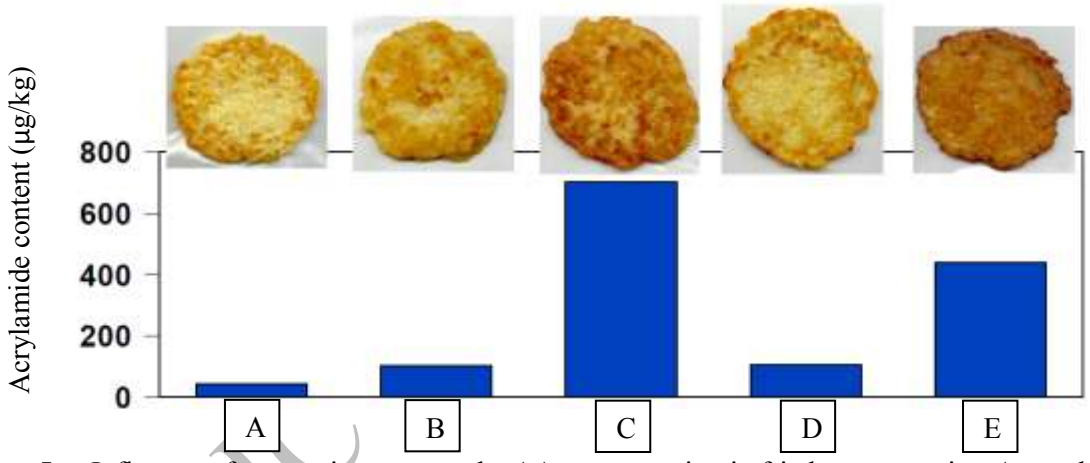


Figure 5: Influence of processing type on the AA concentration in fried potato patties. A: pre-baked; B: conventional baking oven 180 °C, 12 min; C: conventional baking oven 215 °C, 6.5 min; D: convection oven 220 °C, 17 min; E: deep fryer 180 °C, 3.5 min. Source: Matthäus, 2002, modified.

Truong et al. (2014) studied how the AA formation in sweetpotato French fries (SPFF) is affected by processing methods. AA levels in SPFF from untreated sweet potato strips fried at 165 °C for 2, 3, and 5 min were 124.9, 255.5, and 452.0 ng/g fresh weight, which were reduced by about 7 times to 16.3, 36.9, and 58.3 ng/g, respectively, when the strips were subjected to processing that included water blanching and soaking in 0.5 % sodium acid pyrophosphate before frying. An additional step of strip soaking in 0.4 % calcium chloride solution before par-frying increased the calcium content from 0.2 to 0.8 mg/g and decreased the AA levels to 6.3, 17.6, and 35.4 ng/g, respectively.

Lim et al. (2014) analysed the influence of deep frying using various vegetable oils on AA formation in sweet potatoes. The sweet potatoes contained 4.17 mg/g glucose, 5.05 mg/g fructose, and 1.63 mg/g free asparagine. Sweet potatoes fried in palm olein contained a lower AA concentration (1 443 µg/kg), compared to those fried in soya bean oil (2 019 µg/kg).

Yuan et al. (2014) examined the effect of immersion in different solutions on the AA content in potato slices after microwaving and frying. The results showed that immersing potato slices in water reduced

the amount of AA by 8-40 % after microwaving and 19-75 % after frying, respectively. For microwave processing, immersion in a NaCl solution at a concentration of 0.5 g/L caused a considerable reduction of the AA content by 96 %, followed by a treatment with a $CaCl_2$ solution of 2 g/L (80 %) and a citric acid solution at a concentration of 1 g/L (58 %) . For the frying process, the most effective method for acrylamide reduction was the immersion in a citric acid solution at a concentration of 1 g/L (77 %), followed by a $CaCl_2$ solution at a concentration of 2 g/L (72 %) and a NaCl solution at a concentration of 0.5 g/L (64 %). The optimal soaking treatments could effectively reduce the AA content while reasonably retaining the sensory attributes of the crisps.

Cereals and cereal based products

One important determinant factor for AA formation in cereals and cereal-based products is free asparagine, which shows a broad concentration range in different cereals depending on type of grain and year of harvest. An influence of the sugar levels in the flour may result from the extent of milling. In contrast to the processing of potato-based products, the processing of cereals and manufacturing of bakery products is more complex, because of the varying composition and moisture levels of the products and the baking technologies. Baking temperature combined with moisture content seems to be an important factor for AA formation. For example, the AA concentration in crispbread could be reduced by optimisation of the oven inlet and outlet temperatures, adhering to a maximum moisture content of the product at 7 % and also by decreasing the average longitudinal oven baking temperature and increasing the baking time (Stadler and Scholz, 2004). As mentioned earlier, the heat transfer during the baking process into the product is not as efficient as in products that are oil-fried.

Gingerbread may contain high AA concentrations. This can be attributed to the addition of ammonium bicarbonate which is a common baking agent in the production of gingerbread. Trials with gingerbread showed that the AA formation is proportional to the amount of ammonium bicarbonate added. The removal of ammonium bicarbonate resulted in a gingerbread with only traces of AA. The reaction pathway obviously does not proceed via amino-dehydroxylation of acrylic acid, as confirmed by spiking experiments using a stable isotope (^{15}N)-labeled baking agent. Rather, ammonium favors the reaction by accelerating the degradation of sugars and generating more reactive carbonyls that can condense with asparagine via the Maillard route. The research on the role of ammonium and addition of other additives in baking processes was summarized by Stadler and Scholz (2004).

Toasted bread

As part of the HEATOX project[27], AA levels in toasted breads (two white soft breads baked from wheat flour and two from wheat and rye flour) were investigated in order to estimate the AA intake from home-prepared foods. The bread loafs were toasted in a common household toaster for four different roasting times ('1'-'4'). The degree of toasting varied from no visible colour change to dark brown with black parts (see Figure 6). The two rye containing breads developed more browning than the white breads. The authors attributed this may be due to the fact that the ingredients for the darker breads included syrup, containing fructose and glucose in addition to sucrose, while ordinary sugar was used in the white breads. The AA levels before toasting were 3-8 µg/kg (fresh weight) in the white breads and 29-42 µg/kg in the rye containing breads. The levels for 'medium' toasting (based on colour: toasting time '3' for breads made of wheat flour and '2' for bread made from wheat and rye) were 16-61 µg/kg (Karl-Erik Hellenäs, 2014, personal communication) AA levels in bread toasted at toasting time '4' ranged between 31 and 118 µg/kg.

[27] HEATOX – Heat-generated food toxicants, identification, characterisation and risk minimisation. Project No. 506820. Deliverable reference number: 59. Guidelines to authorities and consumer organisations on hme cooking and consumption. Available at: http://www.slv.se/upload/heatox/documents/d59_guidelines_to_authorities_and_consumer_organisations_on_home_cooking_and_consumption.pdf

Figure 6: Left picture shows soft bread made of sifted wheat flour, right picture shows soft bread made from wheat and rye. The loaf on top is not toasted, those below are toasting times 1, 2, 3 and 4 on a common household toaster in order form left to right. AA levels reported to range from 3.0 to 31 µg/kg (left) and from 41.6 to 118 µg/kg (right) (Karl-Erik Hellenäs, 2014, personal communication).

Analyses by Jackson and Al-Taher (2005) indicated that for most types of bread, toasting to a 'medium' degree of doneness results in small to moderate AA levels (< 100 µg/kg). However, bread made from potato flour showed considerably higher AA levels after toasting than toasted bread made from wheat, rye or multi-grain flour. For example, 'dark' toast made from potato bread showed AA levels up to around 600 µg/kg, presumably due to the higher concentrations of asparagine compared to bread made with other flours. The same authors reported that scraping the surface of darkly toasted potato bread to remove the browned portions reduced AA levels from 483 µg/kg to 181 µg/kg. This finding supports earlier investigations by Surdyk et al. (2004) who reported that more than 99 % of AA in bread is found in the crust.

4.4.3. Concluding remarks

The different pathways and the various factors, including processing, that have an impact on the AA formation in the final products indicate that it is impossible to totally eliminate AA from food commodities. Therefore, the objective must be to control those parameters that have a potential impact on the AA formation (such as selection of raw material, storage condition, and food processing) in order to reduce the AA concentrations in food where feasible.

4.5. Initiatives for mitigation measures

An important initiative aiming to reduce AA in various food categories is the development of the FoodDrinkEurope 'Acrylamide toolbox'. The background and the aim of the toolbox are described in the summary of the latest toolbox publication 2013 as follows:

> 'The FoodDrinkEurope Acrylamide 'Toolbox' reflects the results of >10 years of cooperation between the food industry and national authorities of the European Union to investigate pathways of formation of AA and potential intervention steps to reduce exposure.The aim of the Toolbox is to provide national and local authorities, manufacturers (including small and medium size enterprises, SMEs) and other relevant bodies, with brief descriptions of intervention steps which may prevent and reduce formation of acrylamide in specific manufacturing processes and products. It is in particular intended to assist individual manufacturers, including SMEs with limited R&D resources, to assess and evaluate which of the intervention steps identified so far may be helpful to reduce acrylamide formation in their specific manufacturing processes and products. It is anticipated that some of the tools and

parameters will also be helpful within the context of domestic food preparation and in food service establishments, where stringent control of cooking conditions may be more difficult'[28].

Besides scientific publications on formation and reduction of AA in food and updated results from respective projects, the latest revision of the toolbox takes also into account the publication of the CODEX Code of Practice for the Reduction of Acrylamide in Foods (CAC/RCP 67-2009). The toolbox is not meant as a prescriptive manual nor formal guidance, but should be considered as a 'living document' with a catalogue of tested concepts at different stages that will be updated as new findings are communicated[29]. The latest 2013 toolbox focusses especially on the categories 'potatoes', 'cereals' and 'coffee' which were found to have a higher risk of AA formation. These are then sub-divided into compartments and the individual tools.

In addition to the AA toolbox, FoodDrinkEurope, in close co-operation with the European Commission and national authorities, has published pamphlets in 22 European languages. These pamphlets are short extracts of the toolbox in form of five sector specific brochures for 'Biscuits, crackers and crispbread', 'Bread products', 'Breakfast cereals', 'Fried potato products/Potato crisps' and 'Fried potato products/French Fries'. The brochures are designed to help food business operators to implement those parameters of the toolbox that are relevant for their specific sector[30].

In November 2013, the US-Food and Drug Administration (US-FDA) published for comments a draft guidance for industry on 'Acrylamide in Foods'[31]. As indicated in the draft document, the guidance 'provides information to help growers, manufacturers, and food service operators reduce acrylamide in certain foods' and 'is intended to suggest a range of possible approaches to acrylamide reduction and not to identify specific recommended approaches. This guidance also does not identify any specific maximum recommended level or action levels for acrylamide'. The document focuses on categories such as potato-based products (including raw materials, French fries, sliced potato chips, fabricated potato chips and other fabricated potato snacks), cereal-based foods (raw material, processing and ingredients) and other foods (i.e. coffee). It also tackles the preparation and cooking instructions on packaged frozen French fries and information for food service operations.

In Germany, a concept of minimising AA concentrations in foodstuffs was already introduced in 2002 (Göbel and Kliemant, 2007). Foodstuffs analysed within official food control were compiled and classified into certain food groups. Those foods which make up the 10 % most contaminated products in each group were identified. The lowest of the AA contents of these upper 10 % is the so-called 'signal value' for this group. If the signal value is higher than 1 000 µg/kg, the signal value will automatically be 1 000 µg/kg. Additionally, an observation of single products stemming from producers with an important market position was performed. If AA concentrations were found above the signal value, the competent authorities contacted the respective food producer and entered into the minimisation dialogue to check whether ingredients or processes could be changed to minimise AA contents, and which changes this could be. The signal values were updated annually by the German Federal Office for Consumer Protection and Food Safety (BVL). Once calculated, signal values were not raised as long as this minimisation concept was pursued, but were maintained or lowered. This means that AA contents in relevant foods will be continually reduced if the minimisation measures are successful. Food with AA contents of more than 1 000 µg/kg and from food groups for which no signal values have been set will automatically be included in the minimisation dialogue described above. The German national minimising concept with the calculation of signal values was widely replaced in 2011 with the introduction of EU wide indicative values.

[28] http://www.fooddrinkeurope.eu/uploads/publications_documents/FoodDrinkEurope_Acrylamide_Toolbox_2013.pdf
[29] http://www.fooddrinkeurope.eu/documents/brochures/ac_toolbox_20090216.pdf
[30] http://ec.europa.eu/food/food/chemicalsafety/contaminants/acrylamide_en.htm
[31] US-FDA. Draft guidance for industry: acrylamide in foods. Available at: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ChemicalContaminantsMetalsNaturalToxinsPesticides/ucm374524.htm

In 2009, BVL summarized the AA concentrations which formed the basis for the calculation of the German signal values in order to explore the effectiveness of the mitigation measures[32]. While the decrease of the mean AA concentrations in potato chips pointed to a successful application of the toolbox by food industry during the standardized industrial production of this potato product in the observed time span, the course of the mean AA concentration in French fries and potato patties showed either a stagnation or considerable variation. This may be partly due to the inclusion of samples from households and small snack-bars with no standardized food preparation.

A similar variation of the mean AA concentration over time was seen for gingerbread, a traditional spicy Christmas cookie and crispbread, which is probably due to the use of different traditional recipies and manufacturing processes, often in small bakeries, that are not applying the same effective mitigation measures as the bigger food industries.

AA is not only formed in commercially produced food but also in considerable amounts in food prepared in restaurants and in home produced food, especially in fried potato products (Michalak et al., 2011; Sanny et al., 2013; see also Section 4.3.2). In parallel with the increasing knowledge on the formation of AA in food, a number of recommendations for mitigation measures of AA concentration in food have been published by national authorities and the food industry. Comprehensive recommendations in newspapers, journals and the internet for frying potato-derived products and toasting at home were sometimes accompanied with punchy slogans, such as 'gilding rather than charring'. In addition, web pages were set up dealing with all aspects of AA, such as 'http://www.acrylamidefacts.org'. While these web pages are primarily addressed to the general population in the context of domestic cooking, tools, such as the FoodDrinkEurope 'Acrylamide Toolbox' are intended to assist industrial enterprises to keep the AA concentrations in foods as low as reasonably achievable.

5. Food consumption

5.1. EFSA's Comprehensive European Food Consumption Database

The EFSA Comprehensive European Food Consumption Database (Comprehensive database) provides a compilation of existing national information on food consumption at the individual level. It was first built in 2010 (EFSA, 2011c; Huybrechts et al., 2011; Merten et al., 2011) and then updated with new data available at the national level. In view of performing a chronic exposure assessment, in this opinion only individuals with at least two days of reporting were considered (Table 7). This represented 61 338 individuals from 28 surveys and 17 different European countries covering the following age groups: infants (< 1 year old), toddlers (≥ 1 year to < 3 years old), children (≥ 3 years to < 10 years old), adolescents (≥ 10 years to < 18 years old), adults (≥ 18 years to < 65 years old), elderly (≥ 65 years to < 75 years old) and very elderly (≥ 75 years old). There were four surveys available for infants, eight surveys available for toddlers, 17 surveys available for other children, 17 surveys available for adolescents, 16 surveys available for adults, 11 surveys available for elderly and nine surveys available for very elderly. According to the surveys, consumption data were collected either through repeated 24h or 48h dietary recalls, or through dietary records covering 3 to 7 days.

In some surveys, potatoes were reported as raw ingredient and/or without any indication regarding the preparation method. For all age groups, except infants, the following assumptions were made. If during a same meal both potato and oil or fat for frying were consumed, the oil/fat representing more than 5 % of the total consumption of potato and oil/fat, then the potato was assumed to be fried. If no oil/fat was consumed during the meal, or if it was representing less than 5 % of the total consumption of potato and oil/fat, then the potato was assumed not to be fried. The following oils/fats were considered as used for frying: 'Ghee', 'Corn oil', 'Cottonseed oil', 'Lard', 'Olive oil', 'Peanut oil', 'Sesame oil', 'Sunflower oil', 'Rapeseed oil' and 'Oil frying blend'. Regarding the infants, it was

[32] http://www.bvl.bund.de/DE/08_PresseInfothek/01_FuerJournalisten/01_Presse_und_Hintergrundinformationen/01_Lebensmittel/2009/2009_03_05_hi_erfolgreiche_bilanz_acrylamidminimierungskonzept.html;jsessionid=B191D082209442465A623E43DE06C474.1_cid322

considered that potato fried products were not yet introduced in their diet. The consumption events of potato raw/unspecified were assumed to correspond to non fried potato products. Overall, such assumptions were considered to be conservative, as the oil/fat consumed during a same meal as potato could also have been used to prepare another food, such as meat or fish, or used as a salad dressing.

Some consumption events of coffee beverages were not described very precisely, i.e. without any indication whether it was 'Espresso', 'Coffee Americano', 'Cappuccino' or 'Instant coffee'. These consumption events were considered to correspond to the kind of coffee beverage most frequently consumed at the survey level, if such information could be derived from the Comprehensive database.

The average consumption level was estimated at the individual level for the different food groups taken into account (see Section 6). Due to different methodologies, the data from the different surveys cannot be merged to produce one single estimate for the European population. The exposure is assessed at the survey level for each age group covered by the survey.

1910 **Table 7:** Dietary surveys considered for the chronic dietary exposure assessment and number of subjects in the different age groups

Country	Dietary survey acronym	Period[(a)]	Method	Days[(b)]	Number of subjects[(c)]	Age ranges (in year)
Belgium	Regional_Flanders	2002	Dietary record	3	661	2.5 – 7
Belgium	Diet National 2004	2004	24h dietary recall	2	3 083	14 – 105
Bulgaria	NUTRICHILD	2007	24h dietary recall	2	1 720	< 1 – 5
Cyprus	Childhealth	2003	Dietary record	3	303	11 – 15
Czech Republic	SISP04	2003 - 2004	24h dietary recall	2	2 353	4 – 64
Denmark	Danish Dietary Survey	2000 - 2002	Dietary record	7	4 120	4 – 75
Finland	DIPP_2001_2009	2001 - 2009	Dietary record	3	1 749 [(d)]	< 1 – 6
Finland	NWSSP07_08	2007 - 2008	48h dietary recall	4	306	13 – 15
Finland	FINDIET2012	2012	48h dietary recall	2	1 708	25 – 74
France	INCA2	2007	Dietary record	7	4 079	3 – 79
Germany	EsKiMo	2006	Dietary record	3	1 228	6 – 11
Germany	VELS	2001 - 2002	Dietary record	6	800	< 1 – 4
Germany	NVS II	2007	24h dietary recall	2	13 926	14 – 80
Greece	Regional Crete	2004 - 2005	Dietary record	3	838	4 – 6
Hungary	National Repr Surv	2003	Dietary record	3	1 360	18 – 96
Ireland	NANS_2012	2008 - 2010	Dietary record	4	1 500	18 – 90
Italy	INRAN SCAI 2005 06	2005 - 2006	Dietary record	3	3 323	< 1 – 98
Latvia	FC_PREGNANTWOMEN	2011	24h dietary recall	2	1 002	15 – 45
Latvia	EFSA TEST	2008	24h dietary recall	2	1 911	7 – 64
Netherlands	VCP kids	2006 - 2007	Dietary record	3	1 279	2 – 6
Netherlands	VCPBasis_AVL2007_2009	2007 - 2010	24h dietary recall	2	3 819	7 – 69
Spain	AESAN	1999 - 2001	Dietary record	3	410	18 – 60
Spain	AESAN FIAB	2009	24h dietary recall	2	1 067	17 – 60
Spain	enKid	1998 - 2000	24h dietary recall	2	382	1 – 14
Spain	NUT INK05	2004 - 2005	24h dietary recall	2	1 050	4 – 18
Sweden	NFA	2003	24h dietary recall	4	2 491	3 – 13
Sweden	Riksmaten 2010	2010 - 2011	Web record	4	1 797	18 – 80
United Kingdom	NDNS	2008 - 2011	Dietary record	4	3 073	1.5 – 94

1911 (a): Starting and ending years of the survey.
1912 (b): Maximum number of reporting days per subject.
1913 (c): Number of subjects with at least two reporting days.
1914 (d): One subject, exclusively breastfed, is not considered.

5.2. Specific consumption patterns in the total population and in 'consumers-only' in European countries

Consumption data for 'French fries and potato fried', 'Potato crisps' and 'Coffee' were analysed in all dietary surveys used for the exposure assessment as described in Table 7. For 'Coffee', both coffee consumed as a beverage and coffee consumed as an ingredient of a recipe was taken into account. The ranges of mean and 95th percentile of average consumption levels determined for the total population (all subjects of the survey) and for the 'consumers-only' as well as the percentage of consumers are detailed in Appendix C.

5.2.1. French fries and potato fried

According to the Comprehensive database, the percentage of 'French fries and potato fried' consumers varies from 14 up to 85 % across the surveys in the toddlers, other children, adolescents and adults age groups (Appendix C, Table C1). It is lower or even zero in some surveys covering the infants, elderly and very elderly age groups. When considering the consumers only, the highest consumption levels are observed in the groups of adolescents and adults, with a median average consumption levels at 68-69 g per day and a median 95th percentile at 148-155 g per day. Consumption levels are lower in the groups of toddlers and other children, with a median average consumption levels at 32-40 g per day and a median 95th percentile at 52-88 g per day. When considering all subjects, the highest consumption levels are observed in the adolescents age group, with a median average and 95th percentile consumption levels respectively at 26 g per day and 122 g per day. After the group of infants, for which the consumption levels are almost null, the lowest consumption levels are observed in the group of toddlers, with a median average and 95th percentile consumption levels respectively at 13 g per day and 47 g per day.

5.2.2. Potato crisps

According to the Comprehensive database, the percentage of potato crisps consumers varies from 0 up to 59 % across the surveys (Appendix C, Table C2). The highest percentages are observed in the groups of other children and adolescents. No consumption event of potato crisps was reported in the infants groups, so no statistics were derived for this age group. When considering the consumers only, the highest consumption levels are observed in the adolescents and adults age groups, with a median average and 95th percentile consumption levels at respectively 20-22 g per day and 48-50 g per day. The lowest consumption levels are observed in the groups of toddlers and very elderly, with a median average consumption levels at 8.3-8.6 g per day. Not enough data were available to derive a 95th percentile in these age groups. When considering all subjects, the highest consumption levels are observed in the adolescents age group, with a median average and 95th percentile consumption levels respectively at 4.7 g per day and 26 g per day. The lowest consumption levels are observed in the groups of elderly and very elderly, with a median average consumption level at 0.3 g per day. In this age group, the percentage of consumers being below 5 % in all groups with more than 60 subjects, the 95th percentile consumption levels estimated across the surveys are always at 0 g per day.

5.2.3. Coffee

According to the Comprehensive database, the consumers of coffee (both as beverage and as ingredient of a recipe) represents from 34 up to 96 % of the population across the surveys in the adults, elderly and very elderly age groups (Appendix C, Table C3). The percentage of coffee consumers is comprised between 2 and 58 % in the surveys covering the adolescents age group, between 0 and 14 % in the surveys covering the other children age groups, between 0 and 2.8 % in the surveys covering the toddlers and between 0 and 0.2 % in the surveys covering the infants. When considering the consumers only, the highest consumption levels are observed in the elderly age group, with a median average and 95th percentile consumption levels at respectively 14 and 32 g dry equivalent per day. Consumption levels are lower in the groups of toddlers, other children and adolescents, with a median average consumption levels standing respectively at 1.0, 2.4 and 4.3 g dry equivalent per day. When considering all subjects, the highest consumption levels are also observed in the elderly age

group, with a median average and 95th percentile consumption levels respectively at 12 g dry equivalent per day and 31 g dry equivalent per day. In the groups of toddlers and other children, the average consumption levels are below 1 g dry equivalent/day and the 95th percentile below 3.1 g dry equivalent per day. In the groups of adolescents, the average consumption levels are below 5.5 g dry equivalent per day and the 95th percentile below 13 g dry equivalent per day.

6. Human exposure assessment

6.1. Methodology

6.1.1. Food grouping

In a baseline scenario, exposure was assessed considering 112 food groups (see Appendix D, Table D1): 12 at Foodex level 2, 48 at Foodex level 3, 46 at Foodex level 4, and 6 specific food groups defined for a best matching between the consumption and the occurrence data:

- 'French fries and potato fried': French fries, potato fried, potato croquette and roasted potato;
- 'Other potato fried products': potato pancakes, potato fritter and *rösti*;
- 'Non fried potato products': potato boiled, baked, mashed, flakes and potato-based pasta;
- 'Other potato snacks': mostly puffed potato snacks;
- 'Fruit purée for infants and young children, without prunes': fruit purée made from fruits other than prunes;
- 'Fruit purée for infants and young children, unspecified': fruit purée without any indication regarding the kind of fruit.

'Potato fried products': the full occurrence dataset available for 'French fries and potato fried' was used, without any distinction between the products sold as ready-to-eat, those sold as fresh or pre-cooked and analysed as sold or as consumed. The 'Other potato fried products' were considered apart.

'Potato crisps and snacks': the full occurrence dataset was used, without any distinction between 'Potato crisps made from potato dough' and 'Potato crisps made from fresh potato', and between the kind of process (batch/continuous). The 'Other potato snacks' were considered apart.

'Soft bread' and 'Crisp bread': the full occurrence dataset was used, with a distinction between 'Soft (including toasted) bread' and the 'Crisps bread', and according to the main cereal used (wheat, rye). 'Potato bread' and 'Potato-rye bread' were considered apart.

'Breakfast cereals': the full occurrence dataset was used, with a distinction according to the main cereal composing the breakfast cereals. A distinction was also made between the 'Oat flakes' and 'Oat bran/wholemeal flakes'. 'Wheat flakes' were considered to be made from both wholegrain/bran grains and from refined grains, whereas the flakes from the remaining cereal varieties were considered to be made only from refined grains. 'Grits' and 'Porridge' were considered apart.

'Biscuits, crackers and similar': the full occurrence dataset was used, with a distinction between 'Gingerbread' (including *lebkuchen* and *speculoos*), 'Crackers', 'Biscuits and wafers' and the 'Other pastries and cakes'. The 'Fine bakery wares for diabetics' were considered apart.

'Coffee and coffee substitutes': the full occurrence dataset was used, without any distinction according to the degree of roasting and caffeine content. A distinction was made between 'Coffee roasted' and 'Instant coffee', between 'Malt and barley coffee' and 'Chicory coffee'. Concerning the coffee beverage, 'Coffee espresso', 'Coffee americano', 'Cappuccino', 'Coffee macchiato', 'Iced coffee' and 'Coffee with milk' were assumed to be exclusively made from roasted coffee with the respective dilution factors of 0.125, 0.053, 0.044, 0.063, 0.035 and 0.035 applied to the occurrence estimate

expressed in dry equivalent. Dilution factors of 0.017 and 0.125 were respectively applied to the 'Instant coffee powder' and to 'Coffee substitutes (solids)' in order to estimate the AA levels in 'Instant coffee beverage', 'Coffee substitutes beverage'.

'Baby food, other than processed cereal-based ones': the full occurrence dataset was used, with a distinction between 'Infant formulae', 'Ready-to-eat meal and dessert', and 'Fruit purée'. The 'Fruit juice and herbal tea' were not considered as all results available were reported as < LOQ. A distinction was made between the fruit purées which were explicitly indicated as being made from another fruit than prunes, and the fruit purées without any indication regarding the kind of fruit, which were considered to be made either from prunes or from another fruit. A conversion factor of 0.14 (Kersting et al., 1998) was applied to the consumption events of 'Infant/follow-on formulae, liquid' in order to express them in powder equivalents.

'Processed cereal-based baby foods': the full occurrence dataset was used, with a distinction between the 'Biscuits and rusks', 'Cereals to be reconstituted' and 'Ready-to-eat meal cereal-based'. Conversion factors of respectively 0.1 and 0.2 were applied to the consumption events of 'Simple cereals reconstituted with milk' and 'Cereals with an added high protein food reconstituted with water' (Kersting et al., 1998) in order to express them as 'Cereals to be reconstituted' equivalents.

'Other products based on cocoa': the full occurrence dataset was used, with a distinction between 'Cocoa powder' and 'Chocolate products'. A dilution factor of 0.028 was applied to the 'Cocoa powder' in order to estimate AA levels in 'Cocoa beverages'.

'Other products based on cereals' and 'Savoury snacks other than potato-based': the full occurrence dataset was used, except data available for 'Beer'. Data for eleven samples of beer available were all reported as <LOQ. From this the CONTAM Panel concluded that there was insufficient quantitative evidence to take this food group into account in the exposure assessment. A distinction was made between the 'Savoury snacks other than potato-based', 'Grains for human consumption', 'Grain milling products' and 'Cereal-based pasta'. Regarding the 'Composite dishes containing cereals', the occurrence level of 'Soft bread' was applied to the bread content (55 %) of 'Sandwich and sandwiches-like meals' and 'Pizza and pizza-like pies'. The breaded meat, fish and vegetable products were also taken into account.

'Other products based on potatoes": the full occurrence dataset available for 'Non-fried potato products' was used. The occurrence level of 'Non-fried potato products' was applied to the potato content (47.8 %) of other potato-based dishes (mainly potato and vegetable/cheese/meat meals and salads).

'Other products': the occurrence dataset available for 'Roasted nuts and seeds' was applied to the consumption of 'Peanut', 'Tree nuts' and 'Oilseeds', assuming these products would be entirely consumed in their roasted form. The occurrence dataset available for 'Black olives in brine' was applied to the consumption of 'Table olives'. The occurrence dataset available for 'Prune and dates' was applied to the consumption of 'Dried prunes', 'Dried dates', 'Jam, Plum', 'Canned fruit, Plum', 'Fruit compote, Plum', 'Juice, Prune'. The occurrence dataset available for 'Paprika powder' was also taken into account. Data available for 'Vegetable crisps' were not considered, as such food is not present in the Comprehensive Database.

6.1.2. Left-censorship management

According to the WHO guidelines on the censorship treatment (GEMS/Food-EURO, 1995), when more than 40 % of the results were quantified at the food and food group levels, the mean contamination level was estimated considering the non detected/quantified results at half of their respective LOD/LOQ (MB approach). For the food and food groups with less than 40 % of quantified results, the average contamination level was estimated at the LB and UB levels. The average estimates used in this exposure assessment are detailed in Appendix D, Table D1.

6.1.3. Exposure calculation

Chronic exposure to AA was assessed at the individual level by multiplying the mean daily consumption for each food with the corresponding mean contamination, summing up the respective intakes throughout the diet, and finally dividing the results by the individual's body weight. The mean as well as the 95th percentile of chronic exposures were then derived for each population group (i.e. [survey and age group] combinations). The contribution of each food group to total exposure to AA was determined for each population group, as the ratio between the average AA intake resulting from the consumption of the food group and the total average exposure to AA.

6.1.4. Sensitivity analysis

In addition to the baseline exposure scenario, a sensitivity analysis was conducted in order to assess the influence of specific home-cooking behaviours and to reflect brand loyalty and places of consumption on the total dietary exposure to AA (see Section 6.2.3).

6.2. Results

6.2.1. Acrylamide exposure levels across the different population groups

Table 8 presents the ranges (minimum, median and maximum) of the mean and 95th percentile exposure levels across the different surveys and age groups (detailed results by survey and age group are available in Appendix E). Whereas all surveys and age groups were used to derive the ranges of the mean exposure levels, only the ones with more than 60 subjects were used to derive the ranges of the 95th percentiles.

Infants, toddlers and other children were the most exposed groups. The mean exposure levels ranged from 0.6 (minimum LB) to 1.9 µg/kg b.w. per day (maximum UB), and the 95th percentile from 1.4 (minimum LB) to 3.4 µg/kg b.w. per day (maximum UB) depending on the survey and age group.

Adolescents, adults, elderly and very elderly had mean exposure estimates ranging from 0.3 (minimum LB) to 0.9 µg/kg b.w. per day (maximum UB), and the 95th percentile estimates from 0.5 (minimum LB) to 2.0 µg/kg b.w. per day (maximum UB) depending on the survey and age group.

Table 8: Exposure to acrylamide (AA) in µg/kg b.w. per day across the surveys and age groups

Age group	Mean		P95	
	Median [Minimum - Maximum]		Median [Minimum - Maximum]	
	LB	UB	LB	UB
Infants (N[(a)] = 4 / 3)	0.8 [0.5 - 1.4]	0.9 [0.7 - 1.7]	2.3 [1.4 - 2.3]	2.5 [1.7 - 2.8]
Toddlers (N = 8 / 5)	1.4 [1.1 - 1.9]	1.5 [1.2 - 1.9]	2.6 [2.3 - 3.4]	2.7 [2.4 - 3.4]
Other children (N = 17 / 17)	1.2 [0.9 - 1.6]	1.2 [0.9 - 1.6]	2.2 [1.4 - 3.2]	2.3 [1.5 - 3.2]
Adolescents (N = 17 / 16)	0.7 [0.4 - 0.9]	0.7 [0.4 - 0.9]	1.4 [0.9 - 2.0]	1.4 [0.9 - 2.0]
Adults (N = 16 / 16)	0.5 [0.4 - 0.6]	0.5 [0.4 - 0.6]	1.0 [0.7 - 1.3]	1.0 [0.8 - 1.4]
Elderly (N = 11 / 11)	0.5 [0.3 - 0.5]	0.5 [0.4 - 0.5]	0.8 [0.6 - 1.0]	0.9 [0.7 - 1.0]
Very elderly (N = 9 / 8)	0.5 [0.3 - 0.5]	0.5 [0.4 - 0.6]	0.9 [0.6 - 1.0]	0.9 [0.6 - 1.0]

LB: lower bound; N: number of samples; P95: 95th percentile; UB: upper bound.
Note: In order to avoid the impression of too high precision, the numbers for all exposure estimates are rounded to 2 figures.
(a): Number of surveys used to derive the minimum/median/maximum mean exposure levels / number of surveys used to derive the minimum/median/maximum 95th percentile exposure levels.

6.2.2. Food groups contributing to the total AA exposure

The contribution to AA dietary exposure for ten food groups and 6 food subgroups was assessed for each survey and age group. The results are reported as a number of surveys for the following

2087 contribution ranges: 0-5 %, 5-10 %, 10-25 %, 25-50 % and higher than 50 % (Tables 9 and 10). For
2088 each age group, the minimum and maximum relative contribution in percentage to the overall LB and
2089 UB mean AA exposure determined across the surveys are also provided in Appendix F (Tables F1 and
2090 F2).

2091 For infants, the main contributors to the total exposure were 'Baby foods, other than processed cereal-
2092 based', 'Other products based on potatoes' and 'Processed cereal-based baby foods', representing
2093 respectively up to 60, 50 and 31 % of the total LB average exposure level in some infants groups.
2094 These were followed by 'Other products based on cereals', 'Biscuits, crackers, crisp bread' and
2095 'Breakfast cereals', representing respectively up to 29, 20 and 17 % of the total LB average exposure
2096 level. The 'Soft bread' was not representing more than 7.2 % of the total LB average exposure,
2097 whereas the 'Potato fried products', 'Potato crisps and snacks', 'Porridge', 'Cake and pastry',
2098 'Savoury snacks other than potato-based' and 'Other products not based on cereals, potatoes and
2099 cocoa' were not contributing to more than 5 % of the total LB average exposure.

2100 For toddlers, other children and adolescents, the main contributor to the total LB average exposure
2101 was 'Potato fried products', always representing more than 5 %, and up to 51 % of the total exposure.
2102 The 'Soft bread', 'Biscuits, crackers, crisp bread', 'Other products based on cereals' and 'Other
2103 products based on potatoes' could also contribute to more than 25 % of the total LB average exposure
2104 in some population groups. 'Coffee', 'Baby foods, other than processed cereal-based', 'Porridge',
2105 'Savoury snacks other than potato-based', 'Other products based on cocoa' and 'Other products not
2106 based on cereals, potatoes and cocoa' did not contribute to more than 10 % of the total LB average
2107 exposure in any population group. 'Processed cereal-based baby foods' and 'Breakfast cereals'
2108 represented up to 14 and 21 % of the total LB average exposure in the groups of toddlers. 'Cake and
2109 pastry' represented up to 15 % of the total LB average exposure in the groups of other children and
2110 adolescents. 'Potato crisps and snacks' represented up to 11 % of the total LB average exposure in the
2111 groups of adolescents.

2112 The AA exposure patterns were more various in the groups of adults, elderly and very elderly. The
2113 two food groups always representing more than 5 % of the total LB average exposure were 'Potato
2114 fried products' and 'Soft bread', contributing respectively up to 49 and 22 % of the total LB average
2115 exposure in certain population groups. 'Coffee' and 'Other products based on potatoes' were the other
2116 main contributors, respectively, representing up to 33 and 31 % of the total LB average exposure.
2117 'Porridge', 'Breakfast cereals', 'Cake and pastry', 'Other products based on cereals' and 'Biscuits,
2118 crackers, crisp bread' were found to contribute up to respectively 11, 11, 13, 21 and 19 % of the total
2119 LB average exposure. 'Potato crisps and snacks' and 'Other products not based on cereals, potatoes
2120 and cocoa' were not representing more than 6.6 % of the total LB average exposure, whereas 'Savoury
2121 snacks other than potato-based', 'Baby foods other than processed cereal-based', 'Processed cereal-
2122 based baby foods' and 'Other products based on cocoa' were not contributing to more than 5 % of the
2123 total LB average exposure.

2124 **Table 9:** Number of surveys split according to their percentage contribution to chronic dietary exposure to AA using lower bound concentrations across the
2125 children age groups

Food group	Toddlers					Other children					Adolescents				
	0-5 %	5-10 %	10-25 %	25-50 %	≥ 50 %	0-5 %	5-10 %	10-25 %	25-50 %	≥ 50 %	0-5 %	5-10 %	10-25 %	25-50 %	≥ 50 %
Potato fried products	-	-	6	2	-	-	-	12	5	-	-	-	10	6	1
Potato crisps and snacks	7	1	-	-	-	10	7	-	-	-	6	10	1	-	-
Soft bread	1	3	4	-	-	1	5	10	1	-	1	2	14	-	-
Breakfast cereals	4	2	2	-	-	7	7	3	-	-	11	4	2	-	-
Biscuits, crackers, crisp bread	2	-	6	-	-	3	-	13	1	-	4	1	12	-	-
Coffee	8	-	-	-	-	16	1	-	-	-	17	-	-	-	-
Baby foods, other than processed cereal-based	7	1	-	-	-	17	-	-	-	-	17	-	-	-	-
Processed cereal-based baby foods	6	1	1	-	-	17	-	-	-	-	17	-	-	-	-
Other products based on cereals, potatoes and cocoa	-	-	-	7	1	-	-	2	14	1	-	-	4	12	1
Porridge	*8*	-	-	-	-	*17*	-	-	-	-	*17*	-	-	-	-
Cake and pastry	*4*	*4*	-	-	-	*5*	*10*	*2*	-	-	*4*	*11*	*2*	-	-
Savoury snacks other than potato-based	*8*	-	-	-	-	*17*	-	-	-	-	*17*	-	-	-	-
Other products based on cereals	*1*	*3*	*4*	-	-	-	*9*	*7*	*1*	-	*3*	*7*	*6*	*1*	-
Other products based on potatoes	-	*3*	*4*	*1*	-	*2*	*5*	*10*	-	-	*4*	*4*	*9*	-	-
Other products based on cocoa	*7*	*1*	-	-	-	*10*	*7*	-	-	-	*12*	*5*	-	-	-
Other products not based on cereals, potatoes and cocoa	8	-	-	-	-	17	-	-	-	-	17	-	-	-	-

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2128 **Table 10:** Number of surveys split according to their percentage contribution to chronic dietary exposure to AA using lower bound concentrations across the
2129 adults age groups

Food group	Adults					Elderly					Very elderly				
	0-5 %	5-10 %	10-25 %	25-50 %	≥ 50 %	0-5 %	5-10 %	10-25 %	25-50 %	≥ 50 %	0-5 %	5-10 %	10-25 %	25-50 %	≥ 50 %
Potato fried products	-	1	10	5	-	-	2	8	1	-	-	1	7	1	-
Potato crisps and snacks	14	2	-	-	-	11	-	-	-	-	9	-	-	-	-
Soft bread	-	2	14	-	-	-	1	10	-	-	-	1	8	-	-
Breakfast cereals	12	2	2	-	-	8	3	-	-	-	6	2	1	-	-
Biscuits, crackers, crisp bread	1	6	9	-	-	1	5	5	-	-	1	4	4	-	-
Coffee	4	3	7	2	-	2	1	5	3	-	2	1	3	3	-
Baby foods, other than processed cereal-based	16	-	-	-	-	11	-	-	-	-	9	-	-	-	-
Processed cereal-based baby foods	16	-	-	-	-	11	-	-	-	-	9	-	-	-	-
Other products based on cereals, potatoes and cocoa	-	-	4	12	-	-	-	1	10	-	-	-	-	9	-
Porridge	*15*	*1*	-	-	-	*9*	*1*	*1*	-	-	*8*	*1*	-	-	-
Cake and pastry	*5*	*9*	*2*	-	-	*3*	*6*	*2*	-	-	*3*	*3*	*3*	-	-
Savoury snacks other than potato-based	*16*	-	-	-	-	*11*	-	-	-	-	*9*	-	-	-	-
Other products based on cereals	*4*	*7*	*5*	-	-	*6*	*3*	*2*	-	-	*5*	*1*	*3*	-	-
Other products based on potatoes	*2*	*3*	*11*	-	-	-	-	*10*	*1*	-	-	-	*8*	*1*	-
Other products based on cocoa	*16*	-	-	-	-	*11*	-	-	-	-	*9*	-	-	-	-
Other products not based on cereals, potatoes and cocoa	15	1	-	-	-	11	-	-	-	-	9	-	-	-	-

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6.2.3. Exposure levels resulting from home cooking, brand loyalty and places of consumption

In addition to the baseline exposure scenario, a sensitivity analysis was conducted in order to assess the influence of specific home-cooking behaviours and to reflect brand loyalty and places of consumption on the total dietary exposure to AA. The occurrence levels used for each of these scenarios are described in Appendix D (Tables D2 to D8). In each scenario, the exposure is assessed in a similar manner as for the baseline exposure scenario (see Sections 3.1.1.2 and 3.1.1.3). Detailed results for each scenario are provided in Appendix G. Tables 11, 12, 13 and 14 present the ranges (minimum, median and maximum) of the mean and 95th percentile exposure levels across the different surveys and age groups, obtained from the different scenarios and expressed as percentage difference from the baseline exposure scenario. Whereas all surveys and age groups were used to derive the ranges of the mean exposure levels, only the ones with more than 60 subjects were used to derive the ranges of the 95th percentiles.

6.2.3.1. Home-cooking habits and places of consumption

French fries and fried potatoes

Three scenarios were considered in order to reflect the cooking habits (according to instructions in packaging or consumers' preferences) and the places of consumption of the product.

Scenario A1 simulated the situation where all the 'French fries and potato fried' consumed at home and in restaurants would be prepared according to the cooking instructions on the pack of pre-cooked products. For this scenario, only the dataset provided by food associations was used to estimate the average AA level in 'French fries and potato fried' (Appendix D, Table D2). Indeed, the corresponding products have been prepared as for consumption according to the instructions, whereas high uncertainty relies on the conditions of preparation of the 'French fries and potato fried' for the results provided by the European countries. As shown in Table 11, compared to the baseline scenario where all the data available on the 'French fries and potato fried' were considered, this scenario resulted in a decrease in the mean and 95th percentile exposure levels up to respectively 16 and 22 %, depending on the survey and age group.

Scenario A2 simulated the situation where all the 'French fries and potato fried' are consumed in restaurants and prepared according to the current cooking practices, as reflected in the samples taken by the European countries in the framework of the official monitoring programs. For this scenario, only the dataset on the 'French fries and potato fried, fresh or pre-cooked, sold as ready-to-eat' were considered (Appendix D, Table D3). As shown in Table 11, compared to the baseline scenario where all the data available on the 'French fries and potato fried' were considered, this scenario resulted in an increase in the mean and 95th percentile exposure levels up to respectively 3.1 and 4.8 %, depending on the survey and age group.

Finally, Scenario A3 simulated the consumers' preference for crispy and brown 'French fries and potato fried'. In this scenario, it was considered that the level of AA in all consumed 'French fries and potato fried' was at the 95th percentile of AA level observed in 'French fries and potato fried, fresh or pre-cooked, sold as fresh or pre-cooked and prepared as consumed', i.e. 656 µg/kg (Appendix D, Table D4). Figure 7 shows the AA levels according to colour and cooking time of some pre-cooked French fries products for home cooking to illustrate the appearance of 'French fries and potato fried' containing 656 µg/kg.

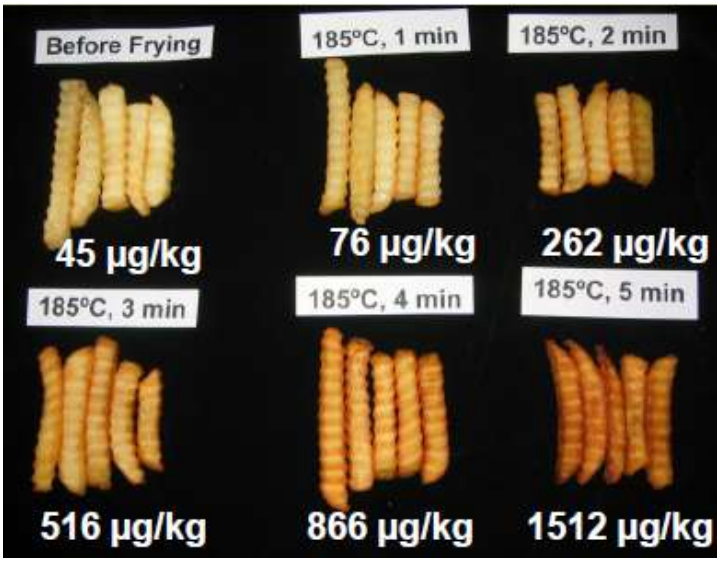

Figure 7: Level of AA according to colour and cooking time of some pre-cooked French fries products for home cooking (Lauren Jackson, 2014, personal communication)

As shown in Table 11, compared to the baseline scenario where all the data available on the 'French fries and potato fried' were considered, this scenario resulted in an increase in the mean and 95th percentile exposure levels up to respectively 64 and 80 %, depending on the survey and age group.

This sensitivity analysis illustrates the influence of the cooking habits of 'French fries and potato fried' on the total exposure to AA.

Table 11: Exposure to AA obtained from Scenarios A1, A2 and A3, expressed in percentage difference from the baseline exposure scenario

S	Age group	N	Mean Median [Min; Max] [(a)] LB	Mean Median [Min; Max] [(a)] UB	N	P95 Median [Min; Max] [(a)] LB	P95 Median [Min; Max] [(a)] UB
A1	Infants	4	>-0.5 [-0.5; 0]	0 [-0.4; 0]	3	0 [-1.3; 0]	0
A1	Toddlers	8	-6.6 [-9.9; -3.1]	-6.2 [-9.7; -2.9]	5	-6.2 [-16; -5.4]	-7.3 [-16; -5.6]
A1	Other children	17	-6.2 [-15; -3.2]	-6 [-15; -3.1]	17	-8.6 [-20; -4.6]	-8.5 [-18; -5.1]
A1	Adolescents	17	-6.8 [-16; -3.1]	-6.6 [-15; -3.0]	16	-11 [-21; -3.5]	-10 [-22; -3.6]
A1	Adults	16	-6.1 [-15; -2.9]	-5.9 [-15; -2.8]	16	-9.5 [-16; -2.6]	-9.5 [-16; -2.9]
A1	Elderly	11	-4.4 [-8.4; -1.8]	-4.2 [-8.2; -1.8]	11	-4.4 [-7.2; -2.1]	-5.5 [-9.0; -0.5]
A1	Very elderly	9	-3.4 [-7.9; -1.6]	-3.2 [-7.7; -1.5]	8	-6.9 [-14; -0.3]	-7.3 [-13; -0.3]
A2	Infants	4	<0.1 [0; 0.1]	<0.1 [0; 0.1]	3	0 [0; 0.3]	0
A2	Toddlers	8	1.3 [0.6; 2]	1.3 [0.6; 2.0]	5	1.2 [1.1; 1.8]	1 [0; 2.6]
A2	Other children	17	1.3 [0.7; 3.1]	1.2 [0.6; 3.0]	17	1.6 [0; 4.3]	2.2 [0.5; 3.9]
A2	Adolescents	17	1.4 [0.6; 3.1]	1.3 [0.6; 3.1]	16	2.9 [0.8; 4.8]	2.8 [1.4; 4.3]
A2	Adults	16	1.2 [0.6; 3]	1.2 [0.6; 3.0]	16	1.6 [0.7; 3.8]	1.9 [0.5; 3.2]
A2	Elderly	11	0.9 [0.4; 1.7]	0.9 [0.4; 1.7]	11	1.4 [0; 2.9]	1.6 [0; 2.3]
A2	Very elderly	9	0.7 [0.3; 1.6]	0.6 [0.3; 1.6]	8	1.3 [0.2; 3.3]	1.4 [0.6; 3.3]
A3	Infants	4	0.2 [0; 1.9]	0.2 [0; 1.5]	3	1.5 [0; 5.4]	1.8 [0; 3.3]
A3	Toddlers	8	27 [13; 41]	26 [12; 40]	5	43 [21; 65]	40 [19; 63]
A3	Other children	17	26 [13; 62]	25 [13; 61]	17	42 [29; 79]	41 [30; 80]
A3	Adolescents	17	28 [13; 64]	27 [12; 63]	16	46 [30; 80]	45 [27; 79]

Table continued overleaf.

Table 11: Exposure to AA obtained from Scenarios A1, A2 and A3, expressed in percentage difference from the baseline exposure scenario (continued)

S	Age group	Mean N	Mean Median [Min; Max][(a)] LB	Mean Median [Min; Max][(a)] UB	P95 N	P95 Median [Min; Max][(a)] LB	P95 Median [Min; Max][(a)] UB
A3	Adults	16	25 [12; 62]	24 [11; 61]	16	51 [20; 80]	50 [17; 76]
A3	Elderly	11	18 [7.6; 35]	17 [7.4; 34]	11	43 [17; 60]	43 [16; 57]
A3	Very elderly	9	14 [6.7; 33]	13 [6.4; 32]	8	42 [23; 68]	39 [22; 67]

LB: lower bound; N = number of population groups used to derive the corresponding statistics; P95: 95th percentile; S: scenario; UB: upper bound.
Note: when the median, min and max are equal, only one estimate is indicated. In order to avoid the impression of too high precision, the numbers for all percentages are rounded to 2 figures. A '0' means that the percentage difference is null.
(a): Expressed in percentage difference from the baseline exposure scenario.

Toasted bread

Scenario B1 simulated the consumers' preference for well toasted bread. In this scenario, it was considered that the level of AA in all toasted bread was 100 µg/kg (Appendix D, Table D5). According to Figure 8, it corresponds to almost highest value found in toasted bread (see Section 4.4.2).

As shown in Table 12, compared to the consumption of toasted bread taken from the market, consumption of such toasted bread would result in a percentage increase of the mean exposure levels up to 4.9 %, and of the 95th percentile up to 5.6 % depending on the survey and age group.

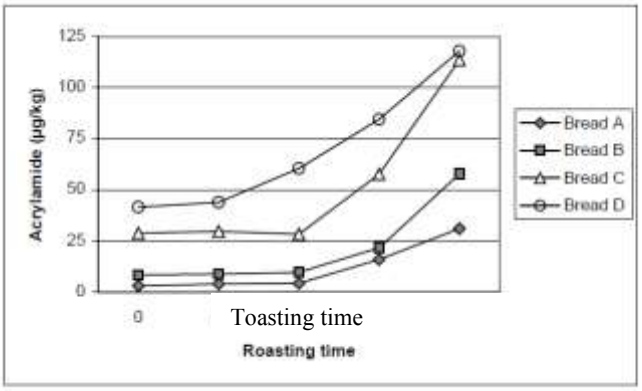

Figure 8: Level of acrylamide according to the toasting time. The picture corresponds to soft bread D (made of wheat and rye flour). The slice on the top is not toasted, those below are toasting time 1, 2, 3, 4 in order from left to right. Source: HEATOX project[27].

Table 12: Exposure to AA obtained from Scenario B1, expressed in percentage difference from the baseline exposure scenario

S	Age group	N	Mean Median [Min; Max][(a)] LB	Mean Median [Min; Max][(a)] UB	N	P95 Median [Min; Max][(a)] LB	P95 Median [Min; Max][(a)] UB
B1	Toddlers	8	0 [0; 0.2]	0 [0; 0.2]	5	0	0
B1	Other children	17	0 [0; 4.8]	0 [0; 4.6]	17	0 [0; 5.6]	0 [0; 4.7]
B1	Adolescents	17	0 [0; 4.9]	0 [0; 4.8]	16	0 [0; 4.2]	0 [0; 5]
B1	Adults	16	0.2 [0; 3.3]	0.2 [0; 3.2]	16	0 [0; 2.3]	0 [0; 2]
B1	Elderly	11	0 [0; 2.9]	0 [0; 2.8]	11	0 [0; 2.6]	0 [0; 4.1]
B1	Very elderly	9	< 0.1 [0; 2.5]	< 0.1 [0; 2.5]	8	0 [0; 2.5]	0 [0; 2.2]

LB: lower bound; N: number of population groups used to derive the corresponding statistics; P95: 95th percentile; S: scenario; UB: upper bound.
Notes: In absence of any consumption event of potato crisps reported for infants in the available consumption surveys, no sensitivity analysis was performed for this age group.
When the median, min and max are equal, only one estimate is indicated. In order to avoid the impression of too high precision, the numbers for all percentages are rounded to 2 figures. A '0' means that the percentage difference is null.
(a): Expressed in percentage difference from the baseline exposure scenario.

6.2.3.2. Brand loyalty

Potato crisps

Two scenarios were set in order to reflect the brand loyalty of consumers. Scenario C1 simulated the situation where all the 'Potato crisps' consumed are made from fresh potatoes through continuous process, whereas Scenario C2 simulated the situation where all the potato crisps consumed are made from potato dough. Only the occurrence data corresponding to the characteristics of the potato crisps considered in each scenario were used to estimate the average AA levels, as reported in Appendix D, Tables D6 and D7. As shown in Table 13, compared to the baseline scenario where all the data available on the potato crisps were considered (absence of brand loyalty), these scenarios on brand loyalty resulted in deviations representing less than 1.3 % of the mean exposure levels, and less than 3.9 % of the 95th percentile exposure levels. This low impact is explained by the fact potato crisps are not a major contributor to the total AA exposure.

Table 13: Exposure to AA obtained from Scenarios C1 and C2, expressed in percentage difference from the baseline exposure scenario

S	Age group	N	Mean Median [Min; Max] [(a)] LB	Mean Median [Min; Max] [(a)] UB	N	P95 Median [Min; Max] [(a)] LB	P95 Median [Min; Max] [(a)] UB
C1	Toddlers	8	< 0.1	<0.1	5	0	0
C1	Other children	17	< 0.1	<0.1	17	< 0.1 [0; 0.2]	0 [0; 0.3]
C1	Adolescents	17	< 0.1 [< 0.1; 0.1]	<0.1 [<0.1; 0.1]	16	0 [0; 0.2]	< 0.1 [0; 0.2]
C1	Adults	16	< 0.1	<0.1	16	0 [0; 0.2]	0 [0; 0.2]
C1	Elderly	11	< 0.1	<0.1	11	0 [0; 0.3]	0
C1	Very elderly	9	< 0.1	<0.1	8	0	0
C2	Toddlers	8	-0.3 [-1; 0]	-0.2 [-1; 0]	5	0	0 [-0.1; 0]
C2	Other children	17	-0.5 [-0.9; 0]	-0.5 [-0.9; 0]	17	-0.8 [-2.3; 0]	-0.3 [-2.6; 0]
C2	Adolescents	17	-0.7 [-1.3; -0.2]	-0.7 [-1.3; -0.2]	16	-0.8 [-3.9; 0]	-0.6 [-3.9; 0]
C2	Adults	16	-0.3 [-0.8; -0.1]	-0.3 [-0.8; -0.1]	16	-0.3 [-1.3; 0]	-0.3 [-1.9; 0]
C2	Elderly	11	0 [-0.3; 0]	0 [-0.3; 0]	11	0 [-0.4; 0]	0 [-0.8; 0]
C2	Very elderly	9	0 [-0.2; 0]	0 [-0.2; 0]	8	0 [-0.4; 0]	0 [-0.4; 0]

LB. lower bound; N: number of population groups used to derive the corresponding statistics; P95: 95th percentile; S: scenario; UB: upper bound.
Notes: In absence of any consumption event of toasted bread reported for infants in the available consumption surveys, no sensitivity analysis was performed for this age group.

When the median, min and max are equal, only one estimate is indicated. In order to avoid the impression of too high precision, the numbers for all percentages are rounded to 2 figures. A '0' means that the percentage difference is null.
(a): Expressed as percentage difference from the baseline exposure scenario.

Coffee

Scenario D1 simulated the consumers' preference for light roasted coffee. In this scenario, only the data corresponding to light roasted coffee were used to estimate the AA level in the different coffee beverages (Appendix D, Table D8). As observed in Table 14, compared to the baseline scenario where all kind of roasted coffee were considered (absence of consumer's preference), the consumption of light roasted coffee would result in an increase of the average and 95th percentile exposure levels of respectively up to 15 % and 16 % depending on the population group among the adults, elderly and very elderly age groups. Coffee not being a major contributor to the total AA exposure of toddlers, other children and adolescents, this scenario poorly impacted the total AA exposure estimates: less than 1.5 % of the average and 95th percentile exposure levels.

Table 14: Exposure to AA obtained from Scenario D1, expressed in percentage difference from the baseline exposure scenario.

S	Age group	N	Mean Median [Min; Max][(a)] LB	Mean Median [Min; Max][(a)] UB	N	P95 Median [Min; Max][(a)] LB	P95 Median [Min; Max][(a)] UB
D1	Toddlers	8	0 [0; 0.1]	0 [0; 0.1]	5	0	0
D1	Other children	17	< 0.1 [0; 0.5]	< 0.1 [0; 0.4]	17	0	0 [0; 1.3]
D1	Adolescents	17	0.1 [< 0.1; 1.5]	0.1 [< 0.1; 1.4]	16	0 [0; 0.4]	0 [0; 1.5]
D1	Adults	16	2.2 [0.2; 13]	2.1 [0.2; 12]	16	1.9 [0; 14]	1.4 [0; 13]
D1	Elderly	11	5.6 [0.2; 15]	5.3 [0.2; 15]	11	3.7 [0; 15]	3.6 [0; 16]
D1	Very elderly	9	4.7 [0.2; 13]	4.4 [0.2; 12]	8	1.6 [0; 14]	1.3 [0; 11]

S: scenario; N: number of population groups used to derive the corresponding statistics; LB. lower bound, UB: upper bound; P95: 95th percentile.
Notes: Due to the very low number of consumption events of coffee reported for infants in the available consumption surveys, no sensitivity analysis was performed for this age group.
When the median, min and max are equal, only one estimate is indicated. In order to avoid the impression of too high precision, the numbers for all percentages are rounded to 2 figures. A '0' means that the percentage difference is null.
(a): Expressed as percentage difference from the baseline exposure scenario.

6.2.4. Considerations on unspecified and/or raw potato products

A total of 13 267 out of 120 919 (11 %) consumption events of potato products (potato crisps and other snacks excluded) available in the Comprehensive database were indicated as raw or unspecified potato.

In the framework of this assessment, and for all age groups other than infants, these consumption events have been attributed to potato fried or not fried depending on whether oil/fat for frying was consumed in sufficient level during the same meal (see Section 5.1). Assuming that all these consumption events correspond to not fried potato and potato products, the average and 95th percentile exposure estimates would be up to 31 and 33 % lower, respectively, than in the baseline exposure scenario. On the other hand, assuming that all these consumption events correspond to fried potato and potato products, the average and 95th percentile exposure estimates would be up to 90 and 110 % higher, respectively, than in the baseline exposure scenario.

For infants, in the framework of this assessment, these consumption events have been attributed to non fried potatoes. If all these consumption events corresponded to fried potato and potato products, the average and 95th percentile exposure estimates would be up to 160 and 200 % higher, respectively, than in the baseline exposure scenario.

6.3. Previously reported human exposure assessments

Several international bodies have estimated the dietary exposure to AA while performing their risk assessments related to the presence of AA in food (see Section 1.1). The highest estimates were reported by JECFA, which concluded in 2005 that AA dietary exposure estimates were 1 µg/kg b.w. per day at the mean, and 4 µg/kg b.w. per day for a consumer at a high percentile of the distribution (FAO/WHO, 2006). These estimates also included children. In 2010, these estimates were updated using data submitted to JECFA and taken from the literature. Additionally, regional estimates were prepared using GEMS/Food data. Brazil, China, France, Ireland, New Zealand, Norway, Spain and the UK provided AA occurrence and dietary exposure information. The estimates of dietary exposure from these national data ranged from 0.2 to 1 µg/kg b.w. per day for the general population, including children, and from 0.6 to 1.8 µg/kg b.w. per day at high percentiles (95^{th} to 97.5^{th}). The major foods contributing to dietary exposure were potato products (crisps and chips), breads and rolls, and pastry and sweet biscuits (cookies). No trends could be evaluated between the 2005 and 2010 estimates. The regional estimates for the 13 GEMS/Food cluster diets ranged from 1.1 to 4.8 µg/kg b.w. per day. JECFA concluded that because of waste and collection of information at the household level, these estimates were comparable to the high percentile estimates made using individual data, as submitted for the national estimates. The JECFA further concluded that no changes had occurred in dietary exposure to AA between the 2005 and the 2010 evaluations and retained the 1 µg/kg b.w. per day and 4 µg/kg b.w. per day estimates for safety evaluation purposes (FAO/WHO, 2011).

Since then, studies of dietary exposure to AA for populations and sub-populations in several European countries and others have been published and are summarised below for the general population (adults) (Appendix H, Table H1) and for infants, toddlers, other children and adolescents (Appendix H, Table H2). In addition, other studies have focused on the intake of AA through typical dishes (Delgado-Andrade et al., 2010).

6.3.1. General population (adults)

Dietary exposure to AA in the general population of Poland was assessed in a study using a probabilistic approach using a household survey of food consumption and analytical values for AA levels in 225 food samples (Mojska et al., 2010). The highest AA levels were found in 'potato crisps'. The mean (95^{th} percentile) exposure to AA for adults (19-96 years old) was 0.33 (0.69) µg/kg b.w. per day. 'Fried potato products', 'breads' and 'coffee' were the major contributing food groups. In another study in a chosen population of South Poland, a 2-fold higher AA dietary exposure was found (0.85 µg/kg b.w. per day) (Zając et al., 2013). The authors used a semi-quantitative FFQ (1 470 participants) and the concentration of AA in each food group were from the European AA monitoring database from June 2006. The main contributors to the AA intake in all age groups were 'bakery products' followed by 'crisps'.

Claeys et al. (2010) estimated the AA intake of the Belgian population (15 years old or older) based on the AA monitoring data of the Belgian Agency for the Safety of the Food Chain. The consumption data were obtained from the Belgian Food Consumption Survey of 2004 (3 214 participants) based on a repetitive non-consecutive 24-h recall in combination with self-administered FFQ. The intake was estimated using a probabilistic approach resulting in an average intake (middle bound) of 0.35 µg/kg b.w. per day, and for high consumers (P97.5) of 1.58 µg/kg b.w. per day. The main contributors to the intake were 'chips', 'coffee', 'biscuits' and 'bread'.

In Finland, Hirvonen et al. (2011) estimated the dietary AA exposure of Finnish adults combining the Finnish occurrence data reported in the literature and the food consumption data from the FINDET 2007 survey (2 038 adult participants, 25-44 years old). The estimated median (P97.5) exposure for adults was 0.44 (1.16) µg/kg b.w. per day for women, and 0.41 (0.87) µg/kg b.w. per day for men. The main contributor to the AA intake was 'coffee' followed by 'starch-rich casserole', 'rye bread' and 'biscuits'.

Data from the Second French Total Diet Study (TDS) were used to estimate dietary exposure to AA for adults from different regions in France (Sirot et al., 2012; Anses, 2013). A total of 192 food samples were prepared 'as consumed' and analyzed for AA. The highest concentrations were found in fried potato products and salted biscuits. The mean dietary exposure to AA was 0.43 µg/kg b.w. per day and the corresponding 95th percentile exposure was 1.02 µg/kg b.w. per day. The main contributing foods were 'French fries', 'potato crisps', 'biscuits' and 'coffee'.

Chan-Hon-Tong et al. (2013) estimated the exposure to AA for French women before and during pregnancy. The consumption data were obtained from the EDEN mother-child cohort by means of a FFQ in the year before pregnancy and during the last 3 months of pregnancy (n = 1 861). The AA occurrence values were taken from the Second French TDS study as above. The mean (95th percentile) UB exposure before pregnancy was estimated at 0.404 (0.969) µg/kg b.w. per day, while at the third trimester of pregnancy the exposure estimates were lower, at 0.285 (0.712) µg/kg b.w. per day. The authors attributed the decrease in exposure partly to the increase of body weight that was not compensated by an increase of food intake. No significant difference in the intake of the major contributors to the AA intake was observed before and during pregnancy, although a decrease in the consumption of coffee was observed during pregnancy.

Within the European Prospetive Investigation into Cancer and Nutrition (EPIC) study, Freisling et al. (2013) estimated the AA intake in 27 centers of 10 European countries. A total of 39 994 participants, aged 35-74, completed a standardized 24-h dietary recall. The mean intake (minimally adjusted by gender) across centers ranged from 12 to 41 µg per day for women and from 15 to 48 for men. The main contributors to the intake were the food groups 'bread, crisp bread, rusks', followed by 'coffee' and 'potatoes'. It was observed that intakes were higher in northern European countries.

In New Zealand, two exposure assessment methods were employed by the Food Standards Australia New Zealand in their updated dietary exposure assessment for AA (MAF, 2012). The first was a deterministic method that employed model diets based on the New Zealand TDS. The second assessment used a dietary modelling approach with food consumption data from 24-hour dietary recalls taken from a national nutrition survey. The dietary modelling mean estimates for males 11-14, 19-24 and over 25 years old were 1.36, 1.01 and 0.84 µg/kg b.w. per day, respectively. The authors stated that it was not possible to say whether these estimates, which are higher than other national estimates, differed because of true differences in exposure or modelling methodology and assumptions. Using published 'usual intake' methods yielded 95th percentile estimates (1.15 - 3.31 µg/kg b.w. per day) that were within the range of equivalent estimates from other countries. Potato products, bread, breakfast cereals and beverages were the major foods contributing to the dietary AA exposure. It was noted that the contribution of potato crisps to AA exposure appears to have decreased between 2006 and 2011 and the contribution from potato, hot chips and oven baked/roasted potatoes appears to have increased (MAF, 2012).

In the US, Tran et al. (2010) estimated the mean exposure of adults at 0.39 and 0.33 µg/kg b.w. per day for male and females, respectively, based on the NHANES (2003-04) 24-h dietary recall consumption data and the occurrence in food products performed by the US-FDA.

In China, the average and 95th percentile AA dietary exposure (middle bound) in the general population has been estimated at 0.286 and 0.490 µg/kg b.w. per day, respectively (Zhou et al., 2013). A total of 144 food composite samples were analysed, based on TDS studies in four regions of China. The consumption data were collected by the Chinese Center for Disease Control and Prevention in 2000 based on a 3-day household dietary survey and 24 h recall. Levels of AA in most foods were similar to those reported in other countries, except for cereals and potatoes which were found to contain AA at lower levels. The authors concluded that this was probably due to different cooking temperatures and raw materials (Zhou et al., 2013). The main contributors to the dietary AA exposure were 'vegetables', followed by 'cereals' and 'potatoes', again showing a different pattern compared to other countries, pointing the authors to different food consumption habits. Dietary exposure to AA for adults 20-85 years old in Hong Kong was assessed using data from the 2005-2007 Hong Kong

Population-based Food Consumption Survey in combination with 95 food samples analyzed for AA (FEHD, 2012). The highest AA levels were found in 'potato chips' and 'snack biscuits'. Exposure was 0.13 µg/kg b.w. per day at the mean, and 0.69 µg/kg b.w. per day at the 97.5th percentile.

6.3.2. Infants, toddlers, other children and adolescents

Mojska et al. (2012) analysed 111 commercially made Polish baby food products including follow-on formula, infant cereals, biscuits for infants and jarred baby food. The estimated AA dietary intake for non breast-fed infants (6-12 months old) considering the minimum, average and maximum AA concentration found in those products ranged from 0.4 to 0.6, from 2.10 to 4.32, and from 7.47 to 12.35 µg/kg b.w. per day. For infants aged 6 months, jarred baby food and follow-on formula were the major contributors the AA intake despite their low AA content. For infants older than 6 months, cereals were the major contributor to the intake.

In Poland, Mojska et al. (2010) estimated the mean exposure to AA at 0.75 µg/kg b.w. per day for children 1-6 years old, and at 0.62 µg/kg b.w. per day for children 7-18 years old. The corresponding 95th percentile exposures were 2.88 and 2.45 µg/kg b.w. per day. The authors noted that exposures were higher for boys compared to girls. Zając et al. (2013) estimated the mean (95th percentile) exposure to children aged 6-12 years old at 1.51 (2.86) µg/kg b.w. per day) in a chosen population in South Poland.

The dietary intake of Finnish toddlers and children (1, 3 and 6 years old) was estimated by Hirvonen et al. (2011). The highest median (P97.5) AA intakes were estimated for 3 year-old children at 1.01 (1.95) µg/kg b.w. per day followed by the 6 year-old group at 0.87 (1.53) µg/kg b.w. per day. The 1-year old group showed median intakes of 0.4 µg/kg b.w. per day. The major contributor to the intake was casseroles, biscuits, chips and other types of fried potatoes.

In Turkey, Cengiz and Gündüz (2013) estimated the mean (95th percentile) AA intake for toddlers (1-3 years old) at 1.43 (3.76) µg/kg b.w. per day. Bread was reported to the the highest contributor to the exposure, followed by crakers, biscuits and baby biscuits.

Sirot et al. (2012) also estimated the AA intake for French children from 3 to 17 years old. The mean (95th percentile) dietary exposure to AA was 0.69 (1.80) µg/kg b.w. per day. The highest exposure was estimated for children 3-6 years old with a mean (95th percentile) exposure of 0.89 (1.864) µg/kg b.w. per day. Older children had lower estimates of mean exposure. The main contributing foods to the exposure were potatoes (French fries) and biscuits.

The mean exposure to AA of US children (3-12 years old) was estimated at 0.86 µg/kg b.w. per day, based on the NHANES (2003-04) 24-h dietary recall consumption data and the occurrence in food products performed by the US-FDA (Tran et al., 2010). Katz et al. (2012) published a study comparing estimates of dietary exposure to AA for teenagers (13-19 years old) from Western diets and 'guideline' diets (suggested food consumption from 'Dietary Guidelines for Americans, 2005'). The means from these analyses were 0.44 and 0.50 µg/kg b.w. per day, respectively. The 95th percentiles were 0.64 and 0.73 µg/kg b.w. per day, respectively.

Delgado-Andrade et al. (2012) estimated the AA intake of 20 Spanish male adolescents (11-14 years old) that participated in a 2-week trial consuming a designed diet based on their eating patterns aimed to compose a balance diet. The mean AA intake as estimated at 0.534 µg/kg b.w. per day. The highest AA intake was provided by the breakfast (32 %).

The mean dietary exposure for 10-17 year old non-smoking adolescents in Canada (using a 2-day food diary and a 1-month FFQ) was 0.58 µg/kg b.w. per day, with a 95th percentile exposure of 2.19 µg/kg b.w. per day (Normandin et al., 2013). The mean (95th percentile) estimates using the FFQ were 0.20 and 0.44 µg/kg b.w. per day, respectively. The authors noted that these results were similar to those published in other countries and attributed any differences to dietary survey design and differing

dietary patterns between the countries. The highest levels of AA occurred in deep-fried potatoes (French fries) and potato chips (Normandin et al., 2013).

6.3.3. Comparison between dietary exposure estimates made in this opinion and previously reported exposure assessments

The estimates of AA intake in the current assessment ranged for the mean exposure from 0.3 (minimum LB) to 1.9 µg/kg b.w. per day (maximum UB), and for the 95th percentile exposure from 0.6 (minimum LB) to 3.4 µg/kg b.w. per day (maximum UB) across survey and age groups. Direct comparison between these dietary exposure estimates and studies published in the literature should be made with caution due to the different methodologies used (sampling methods and food consumption surveys), food categories/products covered, as well as consumption habits in each country/region.

Considering the data above (see also Appendix H, Table H1 and H2), the exposure estimates in the current opinion are in the same range as those reported in the literature, both for adults and infants and children.

6.4. Potential non-dietary sources of exposure

Until AA was found in food, the main sources of human exposure to AA were considered to occur via specific occupations and smoking.

Occupational exposure might result from the manufacture and use of AA and polyacrylamides at the workplace, resulting in the dermal absorption of AA monomers from solution, or inhalation of dry monomers or aerosols of AA solution (IARC, 1994).

AA is a component of tobacco smoke (US-EPA, 2010; FAO/WHO, 2011), and hence smoking as well as passive smoking are an important source of human exposure to AA. Diekmann et al. (2008) speculated on the formation pathways of AA in cigarette smoke proposing three possibilities: (i) the (reversible) reaction of ammonia with acrylic acid and acetic acid (all present in mainstream smoke) that would result in AA (and acetamide) formation, (ii) through the Maillard reaction from the condensation of asparagine and reducing sugars (both reported to be present in tobacco), and (iii) through the oxidation of acrolein to acrylic acid, that would then react with ammonia to form AA.

The levels of AA in tobacco from cigarettes have been reported to range from 50.3 to 119.6 ng/g, while other tobacco products (snus, strips or sticks) showed levels ranging from 69.9 to 366.7 ng/g (Moldoveanu and Gerardi, 2011). Pérez and Osterman-Golkar (2003) reported concentrations up to 34 nmol/g (or around 2 420 ng/g) in snuff portion bags (water extract).

In mainstream cigarette smoke, the levels of AA have been reported to range from 1 100 to 2 340 ng per cigarette (Smith et al., 2000) or from 497.1 to 4 168.8 ng per cigarette (Moldoveanu and Gerardi, 2011).

Given the presence of AA in cigarette smoke, with concordance with Hb adducts consistently higher in smokers than non-smokers (von Stedingk et al., 2011; Phillips and Venitt, 2012), tobacco smoking represents a considerable source of total human exposure to AA.

Non-dietary exposure to AA for the non-smoking general population is thought to be low (ATSDR, 2012) and it can result from the ingestion of water treated with polyacrylamide containing residual AA monomers (ATSDR, 2012).

7. Hazard identifications and characterisation

7.1. Toxicokinetics

The characteristic features of the fate of AA in mammals were elucidated in one of the first toxicokinetic studies, which was performed in male Fischer-334 rats (Miller et al., 1982). After oral administration of doses of 1, 10 and 100 mg/kg b.w. of 2,3-^{14}C-AA dissolved in water, 53-67 % of the administered radioactivity was excreted within 24 h and 65 - 82 % within 7 days via urine and feces. More than 90 % of the excreted radioactivity appeared in the urine and consisted mostly of polar metabolites with less than 2 % as parent AA. After intravenous (*i.v.*) injection of a dose of 10 mg/kg b.w., urinary excretion of 62 % after 24 h and 71 % after 7 days was observed, which was virtually identical with that after oral administration. This suggests complete absorption of AA from the gastro-intestinal (GI) tract. When ^{14}C-AA was administered *i.v.* to bile duct-cannulated rats, 15 % of the dosed radioactivity was excreted in the bile within 6 h, again containing only trace amounts of parent AA.

In the same study, the kinetics of tissue distribution and elimination of radioactivity was studied after *i.v.* injection of 10 mg AA/kg b.w. After 1 h, the level of radioactivity plateaued in whole blood for 7 days. Blood plasma contained 3.4 % of the dose after 1 h, which declined thereafter with time in a biphasic manner. The administered radioactivity was rapidly distributed to liver, kidney, lung, muscles, brain, testes, sciatic nerve, spinal cord, skin, fat and small intestine. All tissues reached about the same concentrations of radioactivity, and no difference in distribution and elimination was noted between neural and non-neural tissues. Elimination from tissues was biphasic with half-lives of 5-8 hours for the initial phase and about 8 days for the second phase, which was believed to be due to the slow degradation of covalent adducts of AA with tissue proteins. A significant proportion of the radioactivity, i.e. about 12 % of the dose, was retained in red blood cells, which might be due to covalent binding of AA and its epoxide metabolite GA (Miller et al., 1982).

The propensity of AA for extensive absorption from the GI tract, rapid distribution into all organs, extensive metabolism, preferential urinary excretion of metabolites and covalent binding to tissue macromolecules have been confirmed in numerous subsequent studies.

7.1.1. Absorption and distribution

AA is rapidly ($t_{1/2}$ < 10 min) and extensively absorbed following gavage administration of aqueous solutions in mice (Doerge et al., 2005a) and rats (Doerge et al., 2005b). The determination of AA bioavailability is complicated because of the similarly rapid and extensive metabolism to GA in rodents. The GA/AA AUC ratios observed after *i.v.* and gavage administration of a common AA dose (100 µg/kg b.w.) were 32-52 % in mice and 60-98 % in rats (Doerge et al., 2005a,b). For comparison, the oral bioavailability of GA, for which no complicating metabolic conversion occurs, was quantitative in mice and rats (Doerge et al., 2005a,b). Oral administration of AA increases the metabolism to GA, relative to *i.v.* administration, by increasing the conversion efficiency. In mice, the GA/AA AUC ratio went from 0.42-1.1 following *i.v.*, to 2.4-2.9 following gavage, and 1.7-2.7 following dietary administration in mice (Doerge et al., 2005a), and from 0.14-0.13 following *i.v.*, to 0.57-0.96 following gavage, and 1.0 following dietary administration in rats (Doerge et al., 2005b). The route-dependence of GA formation was interpreted as reflecting pre-systemic metabolism by CYP2E1 in the liver following portal vein delivery from the GI tract.

Distribution of AA into tissues was measured in mice at 1 and 2 hours after gavage administration and GA levels were consistently higher at both time points (Doerge et al., 2005a). In rats, tissue levels of AA and GA were measured at 2 and 4 hours post-gavage dosing and AA levels were generally higher than GA (Doerge et al., 2005b). The levels of AA and GA in the tissues sampled (lung, muscle, brain in mice and muscle, brain, testes, and mammary in rats) were similar to the respective serum concentration, reflecting the distribution in total cellular water as suggested by the volumes of distribution of 0.6-0.8 L/kg b.w. and high aqueous solubilities (Doerge et al., 2005a,b). Tissue levels

were similar to each other except that AA and GA levels in liver were consistently lower. This difference was interpreted as reflecting the high metabolic detoxification capacity in liver (e.g. high glutathione (GSH) levels).

Studies in various mammalian species (Table 15) have confirmed that AA is rapidly and virtually completely absorbed from the GI tract. Due to its high aqueous solubility and distribution in the total body water, AA is freely distributed to tissues. AA also reaches the fetus and human milk (see further in this section).

Using the formation of Hb adducts in blood (see Sections 7.1.2.1 and 7.2.2) as endpoint, a study in Wistar rats of different age and sex suggested that the absorption of AA may be higher in female than in male and in younger than in older rats (Sánchez et al., 2008). The CONTAM Panel noted, however, that this study was conducted with very high doses of AA (25-100 mg/kg b.w.), which lead to systemic concentrations possibly causing enzyme and metabolic saturation, and may not reflect the absorption of AA at the dietary intake level.

In comparison with other animal species, hens appear to be less efficient in absorbing AA from the GI tract because the percentage of the dose found in tissues was almost an order of magnitude smaller than in rats (Blumenthal et al., 1995).

The high absorption of AA from the GI tract in most experimental animals and in humans after oral administration is indicated by: (i) the high recovery of the dosed AA as urinary metabolites, and (ii) the identical AUCs for the plasma concentrations of AA after oral and *i.v.* administration.

In vitro studies using differentiated Caco-2 cells which represents a well-established *in vitro* model for human intestinal absorption, also indicate that AA is readily absorbed and permeates through cell membranes via passive diffusion (Schabacker et al., 2004; Zödl et al., 2007).

When AA was preincubated with chicken egg albumin as a surrogate of dietary proteins, the AA uptake through the Caco-2 cell monolayer was significantly lower than without albumin (Schabacker et al., 2004). The authors assumed that AA reacts with nucleophilic groups of albumin and concluded that the food matrix may have a significant influence on the intestinal absorption of AA.

2534 **Table 15:** Studies on the absorption and tissue distribution of acrylamide (AA) in various species and strains after oral administration

Species (strain, gender)	Label of AA	Dose of AA (mg/kg b.w.)	Major findings	Reference
Rat (SD, F)	1-^{14}C	50	Peak plasma concentration of RA after 38 min, then decline with $t_{1/2}$ of 6 hours; concentration of RA comparable in various target- and non-target tissues but higher in blood	Kadry et al. (1999)
Rat (SD, M)	2,3-^{14}C	20	Peak plasma concentration of RA after 60-90 min, then decline with $t_{1/2}$ of 2 hours, equal concentrations in muscle, spinal cord and sciatic nerve but 2-fold higher in erythrocytes after 12 hours	Barber et al. (2001)
Rat (F344, M and F)	None	0.1	Peak plasma concentration of AA after 60 min (F) and 120 min (M); F have higher bioavailability and plasma concentration of AA; M have longer $t_{1/2}$ for plasma elimination of AA	Doerge et al. (2005a)
Mouse (SENCAR, M) (BALB/c, M)	2,3-^{14}C	100	Comparable concentrations of RA in lung, stomach, skin and liver, elevated in testis; no strain difference in concentrations and time courses of RA	Carlson and Weaver (1985)
Mouse (SW, M and F)	2,3-^{14}C	120	No notable accumulation of RA in peripheral nerves, but in the male reproductive tract (testis and epididymis), in epithelia of esophagus and stomach, and in fetal skin	Marlowe et al. (1986)
Mouse ($B6C3F_1$, M and F)	None	0.1	Peak serum concentration of AA after 15 min, then decline with $t_{1/2}$ of 1.5 hours; concentration of AA similar in serum, liver, lung, muscle, and brain after 1 or 2 hours	Doerge et al. (2005b)
Dog (Beagle, M)	1-^{14}C	1	Dosed RA mostly found in muscle, liver, and blood, little RA in brain, testis, lung, kidney, spleen, heart, bile, and fat; uniform distribution of RA in various areas of CNS	Ikeda et al. (1987)
Miniature pig (HH, M)	1-^{14}C	1	Dosed RA mostly found in muscle, gastro-intestinal tract, liver, blood, and fat, little RA in brain, testis, lung, kidney, spleen, heart, and bile; uniform distribution of RA in CNS	Ikeda et al. (1987)
Hen (White Leghorn)	2,3-^{14}C	50	Peak blood and plasma concentration between 4 and 12 hours; minimal binding of radioactivity to erythrocytes; 0.5 % of dosed radioactivity in eggs within 5 days in non-extractable form	Blumenthal et al. (1995)

2535 CNS: central nervous system; F: female; F344: Fischer-344; HH: Hormel-Hanford; M: male; RA: radioactivity; SD: Sprague-Dawley; SW: Swiss-Webster.

Effect of food matrix

The effect of the food matrix on the absorption of AA has been studied in experimental animals and in human volunteers *in vivo*. AA is virtually completely absorbed from the GI tract when ingested as an aqueous solution. When AA is formed during food processing, it can, in principle, undergo covalent binding to food amino acids, peptides and proteins, due to its electrophilic reactivity as an α,ß-unsaturated carbonyl compound, by forming Michael addition products. For example, Rydberg et al. (2003) reported that addition of various amino acids (35-140 mmol/kg of potato) or varying proportions of fish during the cooking process of potatoes could reduce the levels of AA found in the final food. Since only that proportion of AA that is unbound may be absorbed and is analytically determined, it is important in the design of studies on the effect of the food matrix on absorption of AA, to use food in which AA has been formed during food preparation and determined by chemical analysis. Spiking, i.e. addition of AA to the food, may lead to loss of part of the added AA due to covalent binding to food constituents, suggesting an apparent reduced absorption (Berger et al., 2011).

For example, in a study with male Sprague-Dawley rats, various food items containing AA due to their preparation at levels resulting in an oral exposure of 50 or 100 µg AA/kg b.w. per day were fed for 1, 3, 5, 7, and 9 days (Berger et al., 2011). The AA levels were analytically determined. The food items were French fries prepared by frying potato sticks from raw potatoes or from potato starch dough (dosage 100 µg AA/kg b.w. per day), bread crust from wholemeal rye loaf bread (dosage 50 µg AA/kg b.w. per day), and gingerbread made from a wheat and rye flour mixture (dosage 100 µg AA/kg b.w. per day). Positive control rats received the same doses of AA in water by oral gavage. 24 h after dosing AA for 1, 3, 5, 7 or 9 days, the amounts of AA, GA and the mercapturic metabolites of AA and GA (see Section 7.1.2.1) were determined in urine by HPLC-MS/MS, and the Hb adducts of AA and GA (see Section 7.2.2) were measured in blood by GC-MS of the N-terminal AAVal and GAVal. No difference in the amounts of urinary metabolites was noted between ingestion of AA from water and from French fries or gingerbread, whereas the excretion of urinary metabolites was about 20 % lower in the bread crust group. This suggests that among the food matrices studied, only bread crust causes some reduction in the intestinal absorption of AA in rats. In support of this notion, the levels of AA-Hb, which increased about linearly with the cumulative AA uptake via the food items and water, were nearly identical in the groups receiving AA in water and those exposed to AA in French fries and gingerbread, but about 17 % lower in the bread crust group (Berger et al., 2011). These results indicate that the absorption of food-borne AA from the intestinal tract of rats is not substantially influenced by the food matrix.

As discussed above in Section 7.1.1, when rats were fed a chow spiked with AA to achieve a bolus exposure of 100 µg AA/kg b.w., a reduced AUC for AA as compared to *i.v.* injection or oral gavage of an aqueous solution was observed (Doerge et al., 2005a). However, administration through the diet, relative to *i.v.* or gavage, also increased the conversion to GA, by altering pre-systemic metabolism. The AA content in the fortified chow was measured and did not change during the course of the experiment. The loss of AA from the fortified diet was determined to be slow at room temperature (half-time 41 days) and undetectable over 28 days at 5 °C (Twaddle et al., 2004).

In male C5BL/6J mice, the amounts of the major AA metabolites were determined by HPLC-MS/MS in the urine excreted during three days after subcutaneous (*s.c.*) injection of AA (at doses of 0.05, 0.5, 5 and 50 mg AA/kg b.w. per day) and after dietary AA exposure from crisp bread (at doses of 24, 143 and 289 µg AA/kg b.w. per day) (Bjellaas et al., 2007a). The AA in crisp bread was formed during baking. A linear relationship between AA intake and excretion of urinary AA metabolites was observed for both routes of administration. From crisp bread, 55 % of the ingested dose of AA was recovered as total urinary metabolites, whereas the respective recovery from urine was 54 % for the three lowest doses injected. The authors concluded that this indicates virtually complete oral absorption of AA from crisp bread (Bjellaas et al., 2007a).

The relative absorption of AA from water and from feed containing commercial potato chips with preparation-related AA was also determined in swine (Aureli et al., 2007). The levels of exposure

were 0.8 and 8 µg AA/kg b.w. per day, and the AAVal of the Hb adduct of AA was used to assess the amount of absorbed AA. Although the variation of these values was quite large, no statistically significant differences between the mean concentrations of the Hb adduct from swine receiving AA by drinking water or by potato chips in the feed were detected, again implying no restriction of absorption from this food item.

Home-prepared potato chips were fried to contain an AA concentration of 6.2 µg/g and fed to six young healthy Caucasian volunteers (three females and three males) at a dose of 12.4 µg AA/kg b.w. per day (Fuhr et al., 2006). AA and its major metabolites were quantified by HPLC-MS in the 72-h urine and found to account for about 60 % of the dose. The authors concluded that most of the AA present in the potato chips was absorbed by the human subjects, because the 60 % fraction of the dose excreted in urine is similar to that excreted in rodents and humans after oral exposure to an aqueous solution of AA (see Section 7.1.3).

In conclusion, part of the AA formed during preparation of food could react chemically with components of the food matrix to form stable adducts. However, the proportion of free (unbound) AA, which is the amount that can be determined by chemical analysis, is extensively absorbed from the GI tract even in the presence of common food matrices.

Placental transfer

In pregnant rats, rabbits, beagle dogs and miniature pigs *i.v.* injected with ^{14}C-AA, radioactivity has been demonstrated to reach the fetuses (Ikeda et al., 1983, 1985). Transfer of AA in human placenta has been reported in an *in vitro* study by Sörgel et al. (2002). The authors perfused the maternal side of three post-partum human placentas with AA at a concentration of about 1 µg/mL without recirculation of the perfusate and found concentrations of about 0.2 µg/mL in the fetal perfusate after 5 to 30 min of perfusion. In a later *in vitro* study, a dual recirculating human placental perfusion was used and the transfer rate of AA (at maternal concentrations of 5 and 10 µg/mL) and GA (5 µg/mL) through the placenta determined (Annola et al., 2008b). Antipyrine (100 µg/mL), which is known to pass through human placenta mainly by passive diffusion, was used as positive control, and all three compounds were determined by HPLC-MS/MS. AA and GA crossed the placenta from the maternal to the fetal side with similar kinetics as antipyrine, and the concentrations of AA and GA in the maternal and fetal circulation equilibrated within 2 hours. No metabolism of AA to GA was detected during an incubation time of 4 hours, nor was a DNA adduct of GA found by HPLC-^{32}P-postlabelling in the placental tissue perfused with AA or GA (Annola et al., 2008b).

In *ex vivo* perfusion studies with human placentas, AA and GA were also found to exhibit a high placental transfer (Mose et al., 2012).

In addition to the evidence from *in vitro* studies that AA and GA can easily cross the human placenta, trans-placental exposure has also been shown to occur *in vivo*. When the blood of 11 pregnant women taken a few hours before childbirth and the corresponding umbilical cord blood of their neonates was analyzed for the Hb adduct of AA by GC-MS analysis of AAVal, this Hb adduct could be found in all blood samples of the mothers and neonates (Schettgen et al., 2004a). The level of the Hb adduct was highest in a smoking mother, and its concentration in the blood of neonates was about half of that found in the blood of their mothers. Likewise, the mean ratio of the Hb adduct of AA in cord blood to maternal blood was 0.48 in another study involving 219 neonates and 87 mothers from Denmark (von Stedingk et al., 2011). Again, a highly significant correlation was observed between cord and maternal blood with regard to the Hb adducts of AA and also GA, for which the adduct ratio of cord to maternal blood was around 0.38. *In vitro* studies showed that the extent of Hb adduct formation of AA and GA with cord blood is about half of that with maternal blood, which was explained by structural differences between fetal and adult Hb.

The results of the studies of Schettgen et al. (2004a) and von Stedingk et al. (2011) indicate that the *in vivo* doses of AA and GA in fetal and maternal blood are about the same and that the placenta provides no protection of the fetus to exposure from these compounds if present in the maternal blood.

Transfer into milk and eggs

In cows' milk, a mean concentration of 175 µg AA/kg was found in a study administering a daily dose of 3.1 mg AA/kg b.w. in a gelatine capsule for 10 days (Pabst et al., 2005). After termination of the dosing, the AA concentration dropped below the LOQ of 5 µg AA/kg within two days. A mean carry-over of 0.24 % of the AA dose into the milk and a mean half-life of AA in the cow of 2.8 h were estimated from these data. In three commercial cow feed samples, AA concentrations in the range of 140-180 µg/kg were determined. Based on the carry-over rate of 0.24 %, a maximum AA concentration of 0.2 µg/kg would be expected in the milk of cows fed such feeds (Pabst et al., 2005).

The carry-over of AA from food into human milk was first reported by Sörgel et al. (2002) for two mothers who consumed potato chips containing about 1 mg of AA (approximate dosage 15 µg/kg b.w.). Concentrations of AA in the low µg/kg range were observed in the human milk between 3 and 8 h after the meal. When a series of 14 individual and 4 pooled human milk samples from non-smoking Swedish mothers exposed to a daily dietary AA intake of about 0.5 µg/kg b.w. were analyzed by HPLC-MS/MS, the concentration of AA was found to be below the LOQ of 0.5 µg/kg, except in one individual sample (0.51 µg/kg) (Fohgelberg et al., 2005).

A low carry-over into eggs (0.5 % of the dose) after oral administration of radiolabelled AA to white Leghorn hens was reported by Blumenthal et al. (1995). The radioactivity found in the eggs could not be extracted and was assumed to be protein-bound. Kienzle et al. (2005) reported on a carry-over of about 0.4 % of the dose into eggs of Japanese quails after *i.v.* injection of AA.

7.1.2. Metabolism

7.1.2.1. Metabolic pathways

Due to the broad industrial application of AA (see Section 1.3) and its neurotoxic and carcinogenic effects, this compound has been of interest to occupational medicine since the 1950s. Therefore, the metabolic pathways of AA in animals and humans are known for many years (Dearfield et al., 1988; Calleman, 1996) and are depicted in Figure 9.

In mammals, AA is converted to 2,3-epoxypropionamide (or GA), which is, in part, hydrolyzed to 2,3-dihydroxypropionamide (DHPA, also called glyceramide). Both AA and GA are conjugated with glutathione (GSH) and the GSH adducts subsequently converted to MA. AA gives rise to only one GSH adduct (AA-GSH), with the thiol group of GSH adding to the C3 position of AA in a typical Michael addition reaction. In contrast, GA forms two GSH adducts, because the thiol group of GSH can open the epoxide ring of GA either at C3, leading to GA-GSH, or at C2, leading to iso-GA-GSH. The conversion of AA-GSH yields N-acetyl-S-(2-carbamoylethyl)-L-cysteine (AAMA, also called N-acetyl-S-(3-amino-3-oxopropyl)-L-cysteine in the older literature). GA-GSH and iso-GA-GSH give rise to different MAs, i.e. N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine (GAMA, also called N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)-L-cysteine) and N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)-L-cysteine (iso-GAMA), respectively (Figure 9). Another MA, which has so far only been reported as an AA metabolite in humans, is the sulfoxide of AAMA, i.e. AAMA-SO (Figure 9). In rats, small amounts of the non-acetylated precursor of AAMA, i.e. S-(2-carbamoylethyl)-L-cysteine, have been found (Sumner et al., 1997). The MAs GAMA and iso-GAMA exist as diastereomers due to their chiral C-atom carrying the hydroxyl group (Figure 9). The relative amounts of the AA metabolites differ among species, as will be shown in Section 7.1.3.

Metabolic studies using AA labelled with either ^{14}C, ^{13}C or ^{2}H have unambiguously shown that the metabolites depicted in Figure 9 are derived from AA. This includes DHPA, which is thought to arise from GA through epoxide hydrolase (EH)-mediated hydrolysis (Miller et al., 1982; Sumner et al.,

1992). When $^{2}H_3$-AA was ingested at a dose of 13 µg/kg b.w. by a male volunteer, 5.4 % of the administered dose was excreted as $^{2}H_3$-DHPA in the 46-hour urine (Hartmann et al., 2011). However, the concentrations of DHPA in 30 urine samples from the general population were approximately ten times higher than expected from the metabolism of AA via GA (Latzin et al., 2012). Therefore, sources other than AA appear to contribute to the formation of DHPA, and DHPA cannot be considered a specific biomarker for the oxidative metabolism of AA.

As shown in Figure 9, both AA and GA can react with proteins to form covalent adducts, and *in vivo* adducts with Hb represent an important biomarker for AA exposure (see Section 7.2.2). AA itself has electrophilic properties and is intrinsically able to react with nucleophilic targets. Covalent adducts of AA with DNA have been generated in chemical reactions, but have never been detected *in vivo* or *in vitro* in animal or human tissues (Doerge et al., 2005c). In contrast, covalent DNA adducts of GA have been amply demonstrated *in vitro* and in experimental animals, and are used as biomarkers of exposure and thought to mediate the carcinogenic effects of AA (see Sections 7.2.3 and 7.3.6.4). Therefore, the formation of GA from AA represents a major metabolic activation reaction, although the reaction of AA with proteins may also significantly contribute to the toxicity.

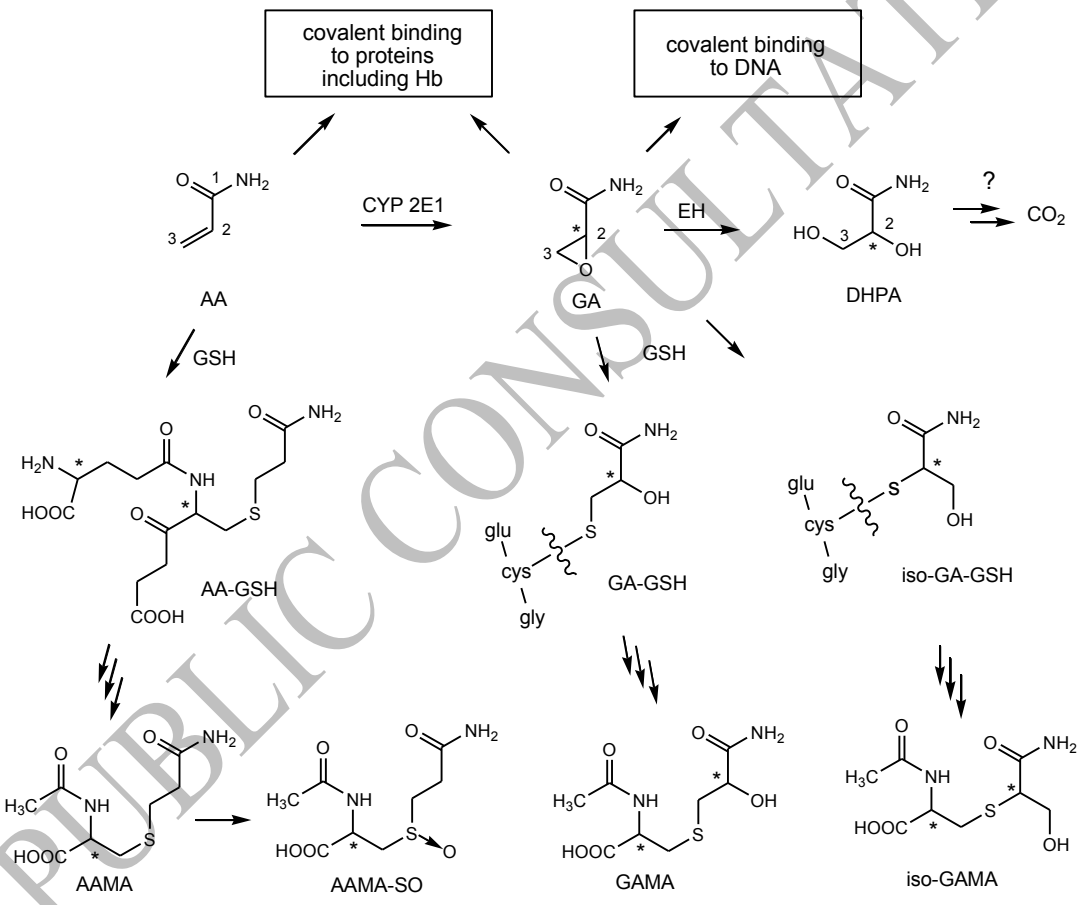

Figure 9: Overview of the metabolic pathways of AA in animals and humans. Asterisks denote chiral C atoms.

It is of interest to note that the first conclusive evidence for the epoxidation of AA came from the structure elucidation of the Hb adduct of GA in rats dosed with AA (Calleman et al., 1990; Bergmark et al., 1991).

In addition to the identified pathways depicted in Figure 9, there appears to exist a yet unknown albeit minor pathway leading to the release of the C1 of AA as carbon dioxide. This must be concluded from the observation that about 5 % of a dose of [^{14}C]-AA administered to rats is exhaled as [^{14}C]-carbon dioxide when AA is labelled in the carbonyl carbon (Hashimoto and Aldridge, 1970), in contrast to

when AA is labelled in the vinyl carbons (C2 and C3) and no [^{14}C]-carbon dioxide is exhaled (Miller et al., 1982). A possible intermediate of this decarboxylation pathway may be 2,3-dihydroxypropionic acid, which has been identified in small amounts in rat urine after oral gavage of AA (Sumner et al., 2003).

In all species tested the direct conjugation of AA with GSH eventually yields AAMA and AAMA-SO (in humans only), in contrast to the 'oxidative pathway', which involves epoxidation to GA and subsequent formation of GAMA, iso-GAMA and DHPA. Because the formation of GA represents a metabolic activation, whereas direct conjugation of AA prevents epoxidation, the ratio of the oxidative to the direct conjugation ('reductive') pathway is a measure for the extent of metabolic activation of AA. This ratio, which is an indicator of fluxes through activation and inactivation pathways, differs between species including humans (see Section 7.1.3).

As mentioned before, AA labelled with radioactive or stable isotopes was used in most studies on the metabolism or toxicokinetics of AA. The purpose of this approach was to circumvent the problem of 'background' levels of AA and its metabolites in urine and tissues. Several studies found that experimental animals and humans excrete AA metabolites in their urine even before the administration of AA (Kopp and Dekant, 2009; Watzek et al., 2012a). This may be due to the presence of AA and its reaction products in feed or food (see below).

In an animal diet prepared in-house no AA could be detected by HPLC-MS/MS with an LOD of 0.5 µg/kg (Watzek et al., 2012a). Based on this LOD, a maximum daily AA intake of 0.1 µg/kg b.w. would result from consumption of this diet by rats. However, female Sprague-Dawley rats on the AA-free diet excreted amounts of AAMA and GAMA corresponding to a daily ingestion of 0.6 µg AA/kg b.w. This caused Watzek et al. (2012a) to assume that small amounts of AA may be formed endogenously.

Tareke et al. (2009) reported the formation of AA when the amino acid asparagine was incubated with hydrogen peroxide and another study has shown that Hb adducts of AA are increased in mice treated with compounds known to induce free radicals (Tareke et al., 2008). The CONTAM Panel noted that methodological deficiencies in the Tareke et al. (2008, 2009) studies precluded conclusions regarding endogenous formation. As an alternative to the endogenous formation of AA, the possibility should be considered that adducts of AA with cysteine in dietary proteins are present in food and feed, which may be absorbed from the GI tract after proteolytic degradation and subsequently excreted in urine. Preliminary evidence for this possibility has been obtained from *in vitro* studies (Schwend et al., 2008).

7.1.2.2. Enzymology

Enzymes for metabolic activation

As GA is considered to be the ultimate genotoxic metabolite of AA (see Section 7.3.3), the enzymes involved in its metabolic formation and inactivation are of particular interest. Sumner et al. (1999) administered a single oral dose of AA to male and female mice and quantified the metabolites excreted in the 24-h urine. GA and its hydrolysis product DHPA, as well as AAMA and GAMA, derived from conjugation of AA and GA, respectively, with GSH (see Figure 9) were found as urinary metabolites of AA. When mice were pretreated with 1-aminobenzotriazole, a nonspecific inhibitor of cytochrome P450 (CYP), AAMA but neither GA nor metabolites derived from GA were detected in the urine. In mice devoid of CYP2E1 (CYP2E1-null mice), again AAMA was the only urinary metabolites of AA (Sumner et al., 1999). This study clearly indicated that the formation of GA is mediated by CYP enzymes (and can therefore be suppressed by 1-aminobenzotriazole), and that CYP2E1 is the predominant, if not sole, CYP isoform mediating the epoxidation of AA in mice. Consistent with this notion was the observation by Ghanayem et al. (2005a) that the plasma levels of GA in CYP2E1-null mice were only 5 % of that in wild-type mice after a dose of 50 mg AA/kg b.w. Moreover, only trace

levels of DNA and Hb adducts of GA were detectable in CYP2E1-null mice at this dose, which gave rise to high adduct levels in wild-type animals.

Two *in vitro* studies addressed the enzymology of GA formation in humans (Settels et al., 2008; Kraus et al., 2013). The study by Settels et al. (2008) demonstrated that GA was generated in incubations of AA with liver microsomes from two women and also with microsomes from insect cells expressing human CYP2E1. Moreover, genetically modified Chinese hamster V79 cells expressing human CYP2E1 were shown to convert AA to GA. The enzymatic activity for GA formation was highest in the insect microsomes containing human CYP2E1, followed by human liver microsomes and recombinant V79 cells. Microsomes from marmoset liver also generated GA from AA with an activity between that of human hepatic microsomes and recombinant V79 cells. A monoclonal antibody against human CYP2E1 and the CYP2E1-specific chemical inhibitor diethyldithiocarbamate (DDC) were shown to almost completely suppress the formation of GA from AA by human and marmoset liver microsomes and recombinant V79 cells, respectively.

Following this first report by Settels et al. (2008) on the involvement of CYP2E1 in the epoxidation of AA in humans, the formation of GA from AA was studied *in vitro* with four different specimens of human liver microsomes pooled from various male and female donors, and eight microsomal preparations expressing human CYPs, i.e. CYP1A1, CYP1A2, CYP2B6, CYP2C9*1, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 (Kraus et al., 2013). The maximum formation rate (V_{max}) and Michaelis-Menten constant (K_m) were determined using an AA concentration ranging from 0.2 to 20 mM. For human liver microsomes, the mean V_{max} was 199 pmol GA/mg protein/min and the K_m was 3.3 mM. No difference was observed between male and female donors. For the human CYP isoforms, pronounced GA formation was only observed for CYP2E1 with V_{max} of 5.4 nmol GA/nmol CYP2E1/min and K_m of 1.3 mM. The activities of CYP1A1, CYP1A2, CYP2C19 and CYP2D6*1 for GA formation were only measurable at 20 mM AA and ranged from 0.7 to 1.8 percent of the CYP2E1 activity, whereas CYP2B6, CYP2C9*1 and CYP3A4 did not lead to the formation of detectable amounts of GA.

The dominant role of CYP2E1 for the conversion of AA to GA was supported in the same study by the observation that GA formation by human liver microsomes and recombinant CYP2E1 could be suppressed by DDC (Kraus et al., 2013). At higher concentrations, DDC completely blocked GA formation in both enzyme systems, and the similar IC_{50} values obtained for microsomes and CYP2E1 (3.1 and 1.2 µM, respectively) further suggest that CYP2E1 may be the only human CYP mediating GA formation in this experimental setting.

Consistent with the importance of CYP2E1 for the bioactivation of AA is the observation by Taubert et al. (2006) that the formation of GA from AA in rat liver slices is greatly deminished in the presence of diallylsulfide, which is a specific constituent of garlic and a potent inhibitor of CYP2E1 (Brady et al., 1991).

In support of a major role of CYP2E1 for the metabolism of AA to GA, Kurebayashi and Ohno (2006) reported that the rate of GA formation was four-fold higher in primary hepatocytes from male Sprague-Dawley rats treated with acetone, a known inducer of CYP2E1, than in hepatocytes from untreated rats

In order to study whether AA itself could induce CYPs, cultured human HepG2 cells, which are derived from a hepatic adenocarcinoma, were treated with 1.25 and 2.5 mM AA (Sen et al., 2012). A 2.0-2.6-fold, 2.4-3.2-fold, and 1.4-1.9-fold increase in CYP2E1-associated enzyme activity, protein level and m-RNA level, respectively, was observed. A somewhat more pronounced inducing effect (up to 5.7-fold, depending on the endpoint used) was noted for the induction of CYP1A2, which is, however, not involved in the metabolism of AA. The expression of CYP3A4, another important hepatic CYP, was found to be slightly inhibited in HepG2 cells after treatment with AA.

Nixon et al. (2014) reported that the expression of the CYP2E1 gene was upregulated about 2.5-fold in spermatocytes of adult mice after treatment of the isolated cells for 18 hours with 1µM AA. The expression of CYP1B1 was elevated 3.3-fold under these conditions of exposure, which did not affect cell viability or morphology.

As ethanol is an important substrate of CYP2E1, the consumption of alcoholic beverages might have an influence on the metabolism of AA to GA. Ethanol has been shown to influence the carcinogenicity of several N-nitrosamines by competitively inhibiting hepatic metabolism, which increases distribution to other organs where bioactivation can occur (e.g. Griciute et al., 1981). Similarly, co-exposure of $B6C3F_1$ mice to 5 % ethanol and 90 ppm urethane in drinking water decreased levels of 1,N6-ethenodeoxyadenosine adducts in liver DNA. However, the co-administration of ethanol had minor impact on the carcinogenicity of urethane in mouse liver and lung observed after 2 years of exposure (Beland et al., 2003). When the ratio of the Hb adduct levels for AA and GA was compared to questionaire data for alcohol intake in 161 non-smoking Swedish men, a linear trend for a decrease of the ratio of the GA-Hb adduct to the AA-Hb adduct was observed with increasing alcohol intake, suggesting that ethanol competitively inhibits the CYP2E1-mediated epoxidation of AA (Vikström et al., 2010). Likewise, GA-Hb adducts were significantly lower in high alcohol consumers as compared to moderate consumers in a study involving 510 subjects from nine European countries, randomly selected and stratified by age, gender, and smoking status (Vesper et al., 2008). A weak correlation of alcohol consumption with increasing AA-Hb adduct concentration and decreasing GA-Hb adduct concentration was also reported in a representative sample of the U.S. population, although only the AA-Hb adducts were significantly correlated (Vesper et al., 2013).

The role of CYP2E1 for the metabolism of AA in humans has also been addressed by an *in vivo* study with 16 healthy Caucasian nonsmokers of both genders (Doroshyenko et al., 2009). A single meal of potato chips corresponding to a dose of 15 µg AA/kg b.w. was ingested by the volunteers without pretreatment and after inhibition of CYP2E1 with a single dose of 500 mg DDC or induction of CYP2E1 with ethanol (48 g per day for 1 week prior to ingestion of AA). Inhibition of CYP2E1 gave rise to an increased cumulative excretion of AA (1.34-fold compared to control) and AAMA (1.15-fold), whereas urinary excretion of GAMA was reduced 0.44-fold. Likewise, the ratio of urinary GAMA to AAMA dropped significantly from 0.026 to 0.010 after inhibition of CYP2E1. Induction of CYP2E1 with ethanol did not result in significant changes in the toxicokinetics of AA nor in the formation of Hb adducts. However, a low dose of ethanol was used in this study, expected to lead to only about 30 % increase in CYP2E1 activity, and the CYP2E1 induction was not measured in the human subjects. The authors concluded that the interindividual variability is too high to detect small difference in the AA toxicokinetics at CYP2E1 induction of this low level (Doroshyenko et al., 2009). In human hepatoma HepG2 cells *in vitro*, treatment with ethanol at concentrations of up to 240 mM gave rise to a weak induction of CYP2E1, as demonstrated by Western immunoblotting (Lamy et al., 2008). When the ethanol-treated cells were exposed to AA, the level of DNA strand breaks was about two-fold higher than that in AA-exposed non-induced HepG2 cells, demonstrating that the DNA strand breaking agent GA is formed to a larger extent when the level if CYP2E1 is increased (Lamy et al., 2008).

Based on all the evidence, the CONTAM Panel concluded that CYP2E1 is the predominant isoform for converting AA to GA.

In view of the importance of CYP2E1 for the formation of GA, it is of interest to consider the activity of this enzyme in different species and organs and its dependency on age (Overton et al., 2008). CYP2E1 is a highly conserved enzyme and constitutively expressed in the liver and in extrahepatic tissues of many species including human, mouse and rat. The highest activity of CYP2E1 is invariably found in liver, exceeding that in other organs by a factor of ten or more. Male rats and humans express CYP2E1 in liver in greater quantities than do females. In rat liver, CYP2E1 expression begins within one day after birth, whereas male and female human fetal liver at age 23-40 weeks exhibits about 1 % of the expression of adult livers (Nishimura et al., 2003). In the study by Nixon et al. (2014), the authors found that CYP2E1 is expressed in mouse testis at different developmental stages. Expression

was low in immature testis from day 2 or 6 after birth, but reached high levels between day 11 and 18, dropped to about half at day 22 and stayed there during adulthood. Spermatogonia had about 3-fold higher levels of CYP2E1 expression than spermatocytes or spermatids. This observation may be of interest for the reproductive toxicity of AA in male rodents.

Large inter-individual variation in CYP2E1 activities has been reported in humans and the basis is complex, relating to gene-environment interactions including enzyme induction and inactivation by dietary constituents, disease states, drug interactions, physical and hormonal status, and significant ethnic differences (Bolt et al., 2003).

Enzymes for metabolic inactivation

The reaction of AA and GA with the thiol group of GSH is generally considered a detoxification reaction, as is the hydrolysis of GA to DHPA. Conjugation with GSH appears to be more important as a detoxification pathway than epoxide hydrolysis for two reasons. Firstly, GSH conjugation of AA prevents epoxidation of AA to GA. Secondly, GSH conjugation of GA occurs at a faster rate than GA hydrolysis, because higher amounts of GAMA than DHPA are excreted in the urine in humans and rats (Table 16).

In view of the rapid and extensive urinary excretion of AAMA and GAMA, it is commonly assumed that GSH conjugation is mediated by glutathione S-transferases (GSTs). The participation of GSTs can also be concluded from species differences observed in the pattern of urinary metabolites (Gargas et al., 2009). For example, Fennell and Friedman (2005) found that 59 % of the urinary metabolites originated from direct conjugation of AA with GSH in rats orally dosed with 3 mg AA per kg b.w., whereas the same conjugation accounted for 86 % in humans under the same conditions (Fennell et al., 2005). Therefore, humans appear to conjugate almost 30 % more of the same dose of AA directly with GSH than do rats, thus producing lower amounts of GA.

To-date, it is unknown which GST isoforms are involved in the GSH conjugation of AA and GA. Studies on the *in vivo* toxicokinetics of AA and on the formation of Hb adducts of GA and AA in humans with different genotypes for GST isoforms have not provided a clear picture. For example, significant associations were disclosed between different genotypes for GSTM1 and GSTT1, and the ratio of Hb adducts of GA and AA (Duale et al., 2009). In general, individuals with null variants of these enzymes had a higher ratio of GA-Hb to AA-Hb in their blood than those with the wild-type genotypes. On the other hand, no obvious difference in the urinary excretion of metabolites was observed between individuals with different GST genotypes, including GSTP1 single nucleotide polymorphisms and even GSTM1- and GSTT1-null genotypes (Doroshyenko et al., 2009). When peripheral blood leukocytes from human donors with different GST genotypes were treated with GA *in vitro* and the extent of DNA damage measured by the alkaline Comet assay or the sister chromatid exchange (SCE) assay, no differences were observed between GSTM1- and GSTT1-null and wild-type genotypes (Pingarilho et al., 2012, 2013). However, the SCE results from the study of Pingarilho et al. (2013) suggest that GSTP1 (Ile105Val) and GSTA2 (Glu210Ala) polymorphisms may influence the detoxification of GA.

Data on the induction of GSTs by AA are also very rare. Sen et al. (2012) studied the effects of AA at 1.25 mM and 2.5 mM concentration on the activity and expression of GST-mu and GST-pi isozymes in cultured HepG2 cell. A slight induction (2.0-5.1-fold, depending on the endpoint) was observed for GST-mu, whereas the activity and expression of GST-pi were reduced. It would be important to know whether and to what extent GST isoforms are induced upon chronic exposure to low levels of AA.

Another gap of knowledge concerns the stability of the thiol adducts of AA. It is known that the conjugation of α,ß-unsaturated carbonyl compounds with a thiol group via a Michael addition can be reversible (Monks et al., 1990; Van Bladeren, 2000). As has been shown with several chemicals such as acrolein and benzoquinone, GSH adducts and MAs may not be final detoxification products but serve as transport form for the toxin to various organs, where it can be released to exert systemic

toxicity. No studies exist on the reversibility of AA conjugates. Although the high amount of AAMA commonly found in urine as an excreted metabolite of AA does not imply a significant instability, the situation may be different for AA adducts with thiol groups in tissue proteins.

As metabolic activation to GA is important for the genotoxic effects of AA, the ratio of GA formation to GSH adduct formation is an important aspect in the susceptibility of various species and organs to the carcinogenic effect of AA. In primary rat hepatocytes, the rate of AA-GSH formation has been reported to be 1.5-3 times higher than the rate of GA formation (Watzek et al., 2013).

7.1.3. Excretion

Early studies in rats using ^{14}C-labelled AA have shown that the major route of excretion is the urine (Hashimoto and Aldrich, 1970; Miller et al., 1982). In the study of Miller et al. (1982), more than 66 % of an orally administered dose of 1-100 mg AA/kg b.w. was excreted as AA-derived material with the urine and only 4 % with the feces after 7 days. Urinary excretion after 24 h accounted for about 54 % of the dose. In later studies with rats, mice and humans, the 24-h urine was invariably found to contain between 40 and 60 % of the orally administered dose (Table 16).

Only metabolism studies using AA labelled with stable isotopes (1,2,3-^{13}C-AA or 2,3,3-^{2}H-AA) are listed in Table 16, because metabolites cannot be accurately quantified in low-dose studies when unlabelled AA is used, due to background metabolites being observed in control animals and humans not dosed with AA (Boettcher et al., 2006a; Kopp and Dekant, 2009; Watzek et al., 2012a). These background metabolites most likely to arise from AA present in feed and food, from tobacco smoke, protein adducts of AA in food, or possibly from exogenous or endogenous sources other than AA, as may be the case for DHPA (see Section 7.1.1).

The pattern of urinary metabolites is depicted in Table 16. Parent AA and its oxidative metabolites GA and DHPA account for only a minor portion of the excreted material. The predominant urinary metabolites are the MAs derived from AA and GA, i.e. AAMA, GAMA and iso-GAMA (Figure 9). AAMA usually represents the major and iso-GAMA the minor MA. For example, 51.7 % of a single oral dose of 13 µg AA/kg b.w. was excreted as AAMA, 4.6 % as GAMA and 0.8 % as iso-GAMA with the 46-h urine by a human male volunteer (Hartmann et al., 2009).

The amounts of metabolites of AA shown in Table 16 refer to the 24-h urine. However, it should be noted that significant amounts of AAMA, AAMA-SO and GAMA are also excreted during the second day after oral administration to humans (Boettcher et al., 2006a; Kopp and Dekant, 2009). For example, the total urinary metabolites of AA excreted in the 46-h urine of humans dosed with 0.5 µg AA/kg b.w. accounted for 71.3 % of the dose (Kopp and Dekant, 2009) as compared to 52.4 % after 24 h (Table 16). Thus, 18.9 % of the dosed AA was excreted as urinary metabolites during the 24 to 46-h time period. In contrast to humans, urinary excretion of AA metabolites in rats appears to be virtually complete after 24 h (Kopp and Dekant, 2009).

Other studies on the profiling of AAMA and GAMA in the urine of human volunteers also indicate a relatively slow excretion of AA metabolites in humans. After ingestion of self-prepared or commercial potato crisps with AA at dose levels of 0.6-14.8 µg/kg b.w., elimination half-lives of 12-17 h and 20-38 h were observed for AAMA and GAMA, respectively, and considerable interindividual variations were noted (Fuhr et al., 2006; Doroshyenko et al., 2009; Watzek et al., 2012b).

Table 16: Excretion of urinary metabolites over 24 hours in male rodents and humans after a single oral dose of acrylamide (AA) dissolved in water. Data are molar percentage of dose.

Species- Dose	AA	AAMA	AAMA-sulfoxide	GA	GAMA[(a)]	DHPA	Total excretion	ΣAA[(b)]	ΣGA[(c)]	ΣGA/ ΣAA	Method	Reference
Mouse – 50 mg/kg	NQ	21 ± 1.1	ND	8.6 ± 1.1	17 ± 0.6	2.7 ± 0.6	50.4	21	28	1.3	^{13}C-NMR	Sumner et al. (1992)
Mouse – 0.1 mg/kg	0.6	7	ND	16	16	ND	40	7.6	32	4.2	HPLC-MS/MS	Doerge et al. (2007)
Rat – 50 mg/kg	NQ	34 ± 1.8	ND	2.8 ± 0.5	12 ± 0.6	1.2 ± 0.4	50.7	34	16	0.5	^{13}C-NMR	Sumner et al. (1992)
Rat – 50 mg/kg	NQ	38	ND	3.9	10.5	0.6	53	38	15	0.4	^{13}C-NMR	Sumner et al. (2003)
Rat – 3 mg/kg	NQ	29 ± 4.5	ND	ND	21 ± 2.4	ND	50.0	29	21	0.7	^{13}C-NMR	Fennell et al. (2005)
Rat – 0.1 mg/kg	2	31	ND	6	28	ND	67	31	34	1.1	HPLC-MS/MS	Doerge et al. (2007)
Rat – 0.1 mg/kg	NQ	35 ± 7.4	ND	ND	27 ± 4.6	ND	62	35	27	0.8	HPLC-MS/MS	Kopp and Dekant (2009)
Rat – 20 µg/kg	NQ	30 ± 5.1	ND	ND	25 ± 6.2	ND	55	35	27	0.8	HPLC-MS/MS	Kopp and Dekant (2009)
Human – 3.0 mg/kg	NQ	22.0 ± 5.3	4.2 ± 1.1	0.8 ± 0.2	ND	3.3 ± 1.1	34.0	26.2	4.1	0.16	^{13}C-NMR	Fennell et al. (2005)
Human – 3.0 mg/kg	3.2	27.8 ± 8.0	7.2 ± 2.4	0.7 ± 0.2	0.7 ± 0.2	ND	39.9	35.0	1.4	0.04	^{13}C-NMR	Fennell et al. (2006)
Human – 1.0 mg/kg	5.0	34.4 ± 5.2	8.7 ± 1.2	0.6 ± 0.3	0.8 ± 0.1	ND	39.9	43.1	1.4	0.03	^{13}C-NMR	Fennell et al. (2006)
Human – 0.5 mg/kg	4.7	31.2 ± 6.5	8.3 ± 2.4	0.4 ± 0.2	0.8 ± 0.2	ND	45.4	39.5	1.2	0.03	^{13}C-NMR	Fennell et al. (2006)
Human – 20 µg/kg	ND	37.4 ± 2.9	6.3 ± 1.8	ND	3.2 ± 0.7	ND	46.9	43.7	3.2	0.07	HPLC-MS/MS	Kopp and Dekant (2009)
Human – 13 µg/kg	ND	45.1	ND	ND	2.8	ND	47.7	45.1	2.8	0.06	HPLC-MS/MS	Boettcher et al. (2006a)
Human – 0.5 µg/kg	ND	41.4 ± 3.5	7.2 ± 1.4	ND	3.8 ± 0.8	ND	52.4	48.6	3.8	0.08	HPLC-MS/MS	Kopp and Dekant (2009)

AA: acrylamide; AAMA: N-acetyl-S-(2-carbamoylethyl)-L-cysteine; GA: glycidamide; GAMA: N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine; DHPA: 2,3-dihydroxypropionamide; NQ: not quantified; ND: not determined.
(a): includes iso-GAMA if determined;
(b): ΣAA, sum of AA+AAMA+AAMA-sulfoxide;
(c): ΣGA, sum of GA+GAMA.

From the rodent studies conducted with different doses of AA (Table 16), an inverse relation appears to exist between dose and epoxidation of AA to GA, i.e. the proportion of GA decreases with increasing dose, possibly due to enzyme saturation. However, the assumption that higher doses of AA are less efficiently metabolized to GA is not supported by a recent study in female Sprague-Dawley rats, where over a dose range from 0.1-10 000 µg/kg b.w., virtually the same value of about 0.4 was observed for the ratio of GAMA to AAMA excreted within 16 hours (Watzek et al., 2012a). In most of the studies involving humans, virtually the same ratio for the sum of all GA-related metabolites to the sum of all AA-related metabolites (ΣGA/ΣAA) of about 0.05 was determined for AA doses covering three orders of magnitude, i.e. ranging from 0.5 µg to 1 mg AA per kg b.w., which also speaks against an effect of the dose on the ratio of the metabolic pathways (Table 16).

Of particular interest is the sum of all excreted metabolites formed through the GA pathway, i.e. GA, GAMA and DHPA, and its relation to the sum of AA of excreted parent AA plus metabolites generated from AA via direct conjugation with GSH, i.e. AAMA and AAMA-SO. The ratio of ΣGA to ΣAA reflects the extent of oxidative metabolism, i.e. activation of AA to GA, in comparison to 'reductive' metabolism, i.e. direct GSH conjugation and inactivation of AA (see Section 7.1.2.1). Marked differences in the extent of oxidative to reductive metabolism appear to exist between mice, rats and humans (Table 16). Whereas oxidative biotransformation exceeds direct conjugation in mice, resulting in ΣGA/ΣAA ratios of > 1, reductive metabolism appears to be somewhat more efficient than oxidative metabolism in rats with ΣGA/ΣAA ratios of 0.4 - 1. Humans exhibit ΣGA/ΣAA ratios of 0.1 or less, apparently forming the smallest relative amounts of GA but the highest amounts of direct AA conjugates (Table 16).

The ratio of urinary GAMA to AAMA was studied in a group of 91 male and female Caucasian non-smokers of different age ranging from 6 to 80 years, who were exposed to AA through their normal diet (Hartmann et al., 2008). Each of the seven age groups comprised 11 to 15 individuals with an even distribution of males and females. The median value for the GAMA/AAMA ratio was 0.3 with a large range (0.004-1.4). There was no gender-related difference in GAMA/AAMA levels. In young children (age 6-10 years) the GAMA/AAMA ratio was higher (median 0.5, range 0.2-1.3). The higher ratio in young children and the large range probably reflects the differences in the enzymatic status of the individuals, as discussed in Section 7.1.2.2.

7.1.4. Conclusion on absorption, distribution, metabolism and excretion of AA

The proportion of AA that is not covalently bound to components of the food matrix is virtually completely absorbed from the GI tract of rodents and humans. After reaching the systemic circulation, AA is extensively distributed to all organs and transferred into the fetus and to a low extent to milk.

AA is extensively metabolised, mostly by conjugation with GSH but also by epoxidation to GA. The formation of GA represents a metabolic activation pathway preferentially mediated by CYP2E1, the activity of which can be affected by dietary components, lifestyle choices, disease and physical states, and genetic polymorphisms. Mice and rats appear to be more proficient in GA formation than humans, indicating that the extent of metabolic activation of AA is higher in rodents than in humans. Metabolic inactivation reactions comprise the hydrolysis of GA to DHPA as well as the GST-driven formation of GSH adducts of AA and GA, which are further processed to the respective MAs and excreted in urine. Conjugation of AA and GA with GSH appear to be the predominant detoxification pathway, while GA hydrolysis plays a minor role. It is presently unknown which GST isoforms are involved in the conjugation of AA and GA with GSH in animals and humans.

The AA metabolites are rapidly and almost completely excreted with the urine, mostly as MAs of the GSH conjugates of AA and GA, and there is no indication of tissue accumulation, except for residual protein adducts.

7.1.5. Physiologically Based PharmacoKinetic (PBPK) modelling

Several publications have reported various approaches to PBPK modelling of AA absorption, metabolism and disposition with the objective of predicting human internal exposures to AA and GA for use in reducing the uncertainty in risk assessment inherent in animal to human extrapolations.

Kirman et al. (2003) used male F344 rat data to model the distribution of AA and GA to five compartments (arterial blood, venous blood, liver, lung, and all other tissues lumped together) and linked the enzymatic metabolism by Michaelis-Menten kinetics of AA to GA in the liver by CYP2E1, epoxide hydrolase-catalysed hydrolysis of GA and glutathione-*S*-transferase-catalysed conjugation of AA and GA, followed by elimination in urine of their mercapturate conjugates. The reaction of AA and GA with Hb and other tissue macromolecules was also included. Physiological parameters for the rat (body weight, organ size, organ blood flow, etc.) were obtained from the published literature. Tissue/blood partition coefficients for AA and GA were estimated using chemical-specific properties. Input data were derived primarily from rodent measurements of total radioactivity from [^{14}C]AA administration in blood and tissues (Miller et al., 1982), AA concentrations in blood and nerve tissue (Raymer et al., 1993), and urinary excretion data (Sumner et al., 1992). Despite limited input data, the model parameters provided an adequate description for most of the kinetic data available for AA using a single set of input values. No kinetic data for GA were available. Although no human modelling was attempted, the reported rat model was considered by the authors as 'a first step in providing a tool to assist in developing (human) exposure limits'.

Young et al. (2007) used a general-purpose PBPK model to simulate a more extensive number of data sets. The PBPK model contained sub-models for AA, GA, and their GSH conjugates. Each PBPK sub-model was comprised of 28 organ/tissue/fluid components that were maintained independently or connected through metabolic pathways. Partition coefficients for AA and GA were derived from measured values obtained following gavage administration to F344 rats and B6C3F$_1$ mice (Doerge et al., 2005a,b). Tissues other than those specifically analysed for AA or GA were partitioned equally to the blood compartment. The specific organ/tissue weights and blood flows were based on literature values for the respective animal species, sex, and total body weight. Optimisation was based on minimizing the weighted sum of squares of the difference between each data point and its simulated value. The model was fit initially using a comprehensive plasma and tissue data set for AA and GA in blood and tissues from low-dose studies of AA (100 µg/kg b.w. single exposure by *i.v.*, gavage, and dietary routes (see Table 17); 1 mg/kg b.w. per day repeated drinking-water exposures) and equimolar GA administered by *i.v.* and gavage routes (Doerge et al., 2005a,b). Urinary excretion of parent AA, GA, and mercapturates for AA and GA was also measured (Doerge et al., 2005a,b). Subsequently, available rodent data from the literature were also modeled. In addition, a pharmacodynamic (PD) module was used to link circulating concentrations of AA and GA with the formation of Hb adducts (AA-Val and GA-Val) and the tissue concentrations of GA with formation of the major DNA adduct, N7-GA-guanine. First-order kinetics were used in all cases because of the very high K_m values for oxidation of AA to GA (4-14 mmol/L) reported from rodent and human hepatic microsomes (Tareke et al., 2006). The PBPK/PD model of Young et al. (2007) fit all of the data available for low and high doses of AA and GA in rodents and dietary doses of AA in humans. GA data were fit first because it was the simplest simulation, then the GA parameters were held constant to optimally fit the AA dosing data. Finally, adduct formation and decay data were simulated in the PD module holding the PK parameters constant. Inclusion of generalized tissue macromolecular binding parameters for AA and GA was evaluated but found to have little impact on the data fits. Human simulations focused on available exposure and elimination data from the literature that were specifically related to dietary administration of low AA doses (about 1 µg/kg b.w. per day). When possible, allometry was used to scale based upon body weights as an alternative means to validate parameters. No serum concentration data from human exposure studies were available at the time of publication of this study, so the individual urinary excretion kinetics for AA, AA-GS and GA-GS from a low-dose AA dietary administration (12.4 µg/kg b.w.) to three male and three female volunteers reported by Fuhr et al. (2006) were used as the foundation for the human model. An estimate of the excretion of GA and

DHPA was made based on the ratio of total GA to AA-GS excretion from a single oral AA dose of 3 mg/kg b.w. reported in Fennell et al. (2005).

Table 17: Experimental area under the curve (AUC) values for acrylamide (AA) and glycidamide (GA) determined following AA dosing with 0.1 mg/kg b.w. by gavage for F344 rats (Doerge et al., 2005a) and B6C3F$_1$ mice (Doerge et al., 2005b)

Species/Sex	AA-AUC	GA-AUC	GA-AUC/AA-AUC	Reference
Rat-M	2.4 ± 0.51	1.3 ± 0.20	0.5	Doerge et al. (2005a)
Rat-F	4.5 ± 0.31	4.4 ± 0.46	1.0	Doerge et al. (2005a)
Mouse-M	0.87	2.6	3.0	Doerge et al. (2005b)
Mouse-F	0.83	2.0	2.4	Doerge et al. (2005b)

AA: acrylamide; AUC: area under the curve; b.w.: body weight; GA: glycidamide; F: female; M: male.

The Young et al. (2007) model produced statistically significant differences in the metabolic parameters when comparing sex, dose and route of administration in rats. The values of metabolic parameters for the mouse were within the same range as the rat values. Human parameters derived from dietary administration studies, when at odds with the rodent parameters, appeared to scale appropriately based on allometry. Internal dosimetry in humans consuming dietary AA was simulated as steady-state concentrations in blood and specified tissues, using as input data the estimated mean dietary exposure and measurements of urinary metabolites and Hb adducts from AA and GA from non-smokers (Doerge et al., 2008). Steady state concentrations were converted to daily AUC values by integrating over a 24 hours time period.

Walker et al. (2007) used a recalibration of model parameters from Kirman et al. (2003), in order to improve upon the original model. Specifically, by reducing the uncertainty about the assumption for total urinary elimination of AA-derived species based on 24 hour urine collection, by incorporating Hb adduct measurements, and using a more valid partition coefficient for GA. Data for Hb adducts were incorporated by adapting methodology to calculate AUCs for AA and GA (Calleman, 1996). Partition coefficients for GA were assumed to be equal to those for AA and were used for both rodent and human simulations. These modifications led to recalibrated sets of model parameters that were used to fit rat and human data sets with the goal of simulating human AUCs from defined exposures to AA. The human model was calibrated against human Hb adduct and urinary metabolite data sets derived from human volunteers given a single oral dose of AA (0.5-3 mg/kg b.w., Fennell et al., 2005). Walker et al. (2007) also modeled the effect of perinatal development and inter-individual variability based on the ontogeny of CYP2E1 activity and hepatic GSH concentrations. These PBTK/Monte Carlo simulations suggested modest differences in internal dosimetry for AA and GA between children and adults, with early-life differences predicted to be greater for AA than for GA.

Sweeney et al. (2010) reported an updated physiologically based toxicokinetic (PBTK) model for AA in humans and rats that reportedly included all the relevant kinetic information available at that time. The resulting model parameters were expanded and refined from those in Kirman et al. (2003) and extended to humans. This modelling used all the male F344 rat data sets, including partition coefficients, blood and tissues, Hb adducts and urinary metabolites, previously fit by Young et al. (2007) and Walker et al. (2007). The human model was fit using the Hb adduct and urinary metabolite data from Fennell et al. (2005, 2006) derived from human volunteers given a single oral dose of AA (0.5-3 mg/kg b.w.); time courses of urinary mercapturic acid metabolites derived from human volunteers given a single oral dose of AA (20-100 µg/kg b.w.; Kopp and Dekant, 2009); urinary metabolites derived from human volunteers given a single oral dose of AA (12.4 µg/kg b.w.; Fuhr et al., 2006); and urinary metabolite and Hb adduct data derived from human volunteers given a single oral dose of AA (15 µg/kg b.w.; Doroshyenko et al., 2009). Output data for internal dosimetry (i.e. steady-state concentrations or AUCs for AA and GA) were not reported except for an interspecies comparison between male rats and humans. Using simulated AUCs for AA and GA as the comparators, administration of a single dose of 100 µg AA/kg b.w. to rats was reported to be

equivalent to a human dose of 23 µg AA/kg b.w. for AA and of 130 µg AA/kg b.w. for GA. This rat-to-human equivalent dose relationship was reported to be linear up to doses of 2 mg/kg b.w.

DeWoskin et al. (2013) compared internal dosimetry for AA and GA as AUCs derived from either PBPK modelling (Sweeney et al., 2010) or Hb adduct measurements in rats and humans (Doerge et al., 2005a,b,c; Fennell et al., 2005; Tareke et al., 2006). The human equivalent doses and reference values (i.e. points of departure) derived from rat and human data using both procedures were similar to each other for neurotoxicity and carcinogenicity endpoints and essentially identical to those reported in the IRIS review of AA (US-EPA, 2010, see Section 1.1).

7.1.5.1. Comparisons of PBPK model simulations of internal dosimetry

Although the format of model output data reported by Sweeney et al. (2010) was not directly equivalent to those reported by Young et al. (2007) and Walker et al. (2007), some comparisons of the three models' output for rat and human internal dosimetry for AA and GA are possible. When compared to the experimentally measured serum AUC value for AA and GA in rats treated by gavage with 0.1 mg AA/kg b.w. (Table 17), the predicted AUCs (Table 18) from Young et al. (2007) were similar, but the predictions from Walker et al. (2007) are consistently 2- to 3-fold higher than the measured values. Sweeney et al. (2010) did not report AUCs for rats.

Only Young et al. (2007) produced a mouse model (Table 17). The relative metabolism of AA to GA, as estimated by the GA-AUC/AA-AUC ratios, was higher in male and female mice than either male and female rats and particularly higher than in humans (Table 18). It should be noted that the GA-AUC/AA-AUC ratios are similar to the ΣGA/ΣAA ratios derived from analysis of total urinary metabolites in rodents and humans (see Section 7.1.3, Table 16).

Table 18: Physiologically based pharmacokinetic (PBPK) model-simulated areas under the curve (AUCs) for rodents and humans treated with a single oral dose of acrylamide (AA, 0.1 mg/kg b.w.)

Species/Sex	AA-AUC	GA-AUC	GA-AUC/AA-AUC	Reference
Rat-M	2.4	1.1	0.5	Young et al. (2007)
Rat-F	4.3	2.8	0.7	Young et al. (2007)
Mouse-M	1.5	3.3	2.2	Young et al. (2007)
Mouse-F	0.91	2.3	2.5	Young et al. (2007)
Human	15 ± 3.0[(a)]	1.6 ± 0.43[(a)]	0.1	Young et al. (2007)
Rat-M	6	5	0.8	Walker et al. (2007)
Human	25	6.2	0.2	Walker et al. (2007)
Human	Not reported (10 calc.)[(b)]	Not reported (1.0 calc.)[(b)]	0.1	Sweeney et al. (2010)

AUC: area under the curve; b.w.: body weight; PBPK: Physiologically Based PharmacoKinetic (PBPK) modelling; SD: standard deviation; M: male; F: female.
(a): Model-predicted AUCs (mean ± SD) were determined from urinary time course and Hb adduct data for 3 men and 3 women from Fuhr et al. (2006).
(b): Calculated from HEDs reported by Sweeney et al. (2010) using experimentally determined male rat AUC values from Doerge et al. (2005a).

It was also possible to compare model-predicted human AUCs across three models. Human AUCs were directly available from Young et al. (2007) and Walker et al. (2007) but were estimated by using the human equivalent doses reported by Sweeney et al. (2010) of 0.023 mg/kg b.w. for AA and 0.130 mg/kg b.w. for GA and the respective experimentally derived AUCs from a dose of 0.1 mg AA/kg b.w. in male rats (Table 17). Human AUC values for AA and GA were highest from the Walker et al. (2007) model, lowest for the Sweeney et al. (2010) model, and intermediate for the Young et al. (2007) model (Table 18). Simulated human AUCs for AA were consistently higher than the rodent values in all models but the human GA AUCs were similar to rodents from Young and Sweeney models but higher from the Walker model. This finding for AA is in accordance with inter-

species predictions from allometry (see below), where larger animals typically show higher AUCs for the parent compound from identical doses, when administered on a 'mg/kg b.w.' basis (US-EPA, 2011). However, the AUCs for GA in different species also vary due to inter-species differences in metabolism, as well as allometry (e.g. epoxidation of AA, conjugation by GSH, hydrolysis by water, excretion by the kidney, etc).

7.1.5.2. Inter-species extrapolation of dosimetrics for AA and GA using a Human-Equivalent Dose (HED) approach.

A critical aspect of this risk assessment is the extrapolation of findings from animal toxicology studies with AA to predict the potential for effects in humans. This extrapolation includes uncertainties surrounding inter-species and intra-species differences in toxicokinetics and toxicodynamics, which are often incorporated by using default uncertainty factors to convert reference points (e.g. BMDL, NOAEL) into health-based guidance values (e.g. tolerable daily intake or TDI). Derivation of a human-equivalent dose (HED) is an accepted method for incorporating toxicokinetic data from animal studies to reduce uncertainties in estimating human dosimetrics (US-EPA, 2011). The US-EPA default dosimetric adjustment factor uses the body weight ratio to the ¾ power as the scaling factor [(b.w.-human/b.w.-animal)$^{3/4}$] when toxicokinetic measurements from different species are not available. The HED concept is best understood as a prediction of the dose, that when administered to a human on a mg/kg b.w. basis, produces an identical AUC for the toxicologically important molecule, either the parent compound or an active metabolite, as observed in the test species used for the toxicological evaluation at some specified dose. For example, if the $BMDL_{10}$ in a rat toxicity test is determined to be 1 mg/kg b.w. and that dose produces an AUC for the test compound in the rat of 1 nM × hour but the same dose produces an AUC in human of 4 nM × hour, the HED is 0.25 (1/4). In order to incorporate inter-species differences in toxicokinetics into the derivation of a health-based guidance value, one would multiply the rat $BMDL_{10}$ value by the HED to give a reference dose of 1 mg/kg b.w. × 0.25 = 0.25 mg/kg b.w. for a 70 kg human (or lower if additional uncertainty factors were included, as is typical). In this example, the allometric relationship between rats and human, based on the difference in b.w. (0.25 vs. 70 kg), also predicts that a lower dose is required in humans to produce an internal exposure that causes a defined degree of toxicity in the rat model.

Since target tissue concentrations of AA and GA are thought to determine different toxicological effects observed from doses used in the critical animal studies (i.e. AA is associated with neurotoxicity and GA is associated with genotoxicity, see Sections 7.3.2.7 and 7.3.3.3) and measurements of serum AUC correlate with formation of the respective adducts (i.e. Hb adducts of AA and GA, and GA-DNA adducts, Tareke et al., 2006; see Sections 7.2.2. and 7.2.3.2), the AUCs for AA and GA derived from PBPK models for animals and human can be used as the basis to characterize inter-species differences in toxicokinetics.

HEDs for AA and GA were determined for rodent species and humans using simulations from the three PBPK models as shown in Table 19 for a common dose of 0.1 mg AA/kg b.w. The Young et al. (2007) model simulated data from male and female rats and mice and the Walker et al. (2007) and Sweeney et al. (2010) models simulated data only from male rats. HEDs were calculated using the data in Table 18 by dividing the rodent AUC by the corresponding human value. For example, the male mouse HED for AA was calculated from the mouse/human AA-AUC ratio of 1.5/15 = 0.10 and the corresponding HED for GA formation was determined from the GA-AUC ratio 3.3/1.6 = 2.1. The male rat-associated HED values for AA were similarly predicted by all three models (0.16-0.24) and for GA (0.69-1.3). The Young et al. (2007) mouse model produced HEDs of 0.06-0.10 for AA and 1.4-2.1 for GA. The HEDs for AA are similar to those predicted for rat (0.24) and mice (0.14) based on allometric relationships between pharmacokinetic parameters and body weights alone (i.e. using the US-EPA (2011) default factor of (BW_H/BW_A) raised to the ¾ power). However, the HEDs for AA metabolism to GA are larger than predicted by body weight scaling alone, particularly in the mouse, meaning that larger doses of AA, on a mg/kg b.w. basis, are required in a human to produce equivalent AUCs for GA (Table 17 and 18).

Table 19: Human-equivalent doses (HEDs) from PBPK modelling. HEDs were calculated from the Young et al. (2007), Walker et al. (2007) and Sweeney et al. (2010) PBPK model-predicted rodent AUC/human AUC ratios for a common dose of AA (0.1 mg/kg b.w.) and represent the multiple of the AA dose to a rodent that a human would require to obtain an equivalent AUC for either AA or GA (e.g. an HED of 0.1 means that humans require 1/10 of the dose given to an animal, on a mg/kg b.w. basis, to produce an equivalent AUC value).

Species/Sex	HED-AA	HED-GA	Reference
Rat-M	0.24	0.81	Walker et al., 2007
Rat-M	0.23	1.3	Sweeney et al., 2010
Rat-M	0.16	0.69	Young et al., 2007
Rat-F	0.29	1.8	Young et al., 2007
Mouse-M	0.10	2.1	Young et al., 2007
Mouse-F	0.06	1.4	Young et al., 2007

AA: acrylamide; AUC: area under the curve; GA: glycidamide; HED: Human Equivalent Dose; M: male. F: female.

PBPK modelling predicted that a 1.4 or 2.1-fold higher dose of AA was required in a human to achieve the same GA-AUC as that in the female or male mouse, respectively (see Table 19, Young et al., 2007). This reinforces the idea that mice are more proficient in converting AA to GA than humans (see Table 20). The HED for GA-related endpoints derived from the three PBPK models for male rats ranged between 0.69-1.3, whereas the HED from female rats was intermediate between the HEDs from mice.

7.1.5.3. Use of PBPK modelling for human cancer and neuropathy risk assessments

Three publications have used internal dosimetry simulations from PBPK models for risk assessment of neurotoxicity and cancer in an effort to reduce uncertainty in extrapolating across dose and species from animal toxicity testing to humans exposed to AA in the diet (Doerge et al., 2008; Tardiff et al., 2010; DeWoskin et al., 2013).

(i) Doerge et al. (2008) interpreted results from rodent studies as being consistent with a genotoxic mechanism for AA carcinogenesis by virtue of its metabolism to GA, DNA adduct formation (N7-GA-Gua), somatic cell mutagenesis, and ultimately, tumour formation. This study used the Young et al. (2007) PBPK model to simulate the levels of N7-GA-Gua DNA adducts in rat target tissues using $BMDL_{10}$ values (0.40 mg/kg b.w. per day for female mammary, 0.66 mg/kg b.w. per day for male peri-testicular mesotheliomas, 1.2 mg/kg b.w. per day for male thyroid, 1.3 mg/kg b.w. per day for female rat central nervous system (CNS), and 1.5 mg/kg b.w. per day for female thyroid) as the AA dose from BMD analysis of the chronic male and female F344 rat bioassay tumour incidence data from Johnson et al. (1986). These adduct levels in tumour target tissues were then compared with simulated N7-GA-Gua levels in the analogous human tissues predicted to result from daily consumption of AA in the diet at a level of 0.4 µg/kg b.w. The MOEs for thyroid, central nervous system, peri-testicular mesothelium and mammary gland were in the range of 260-960. These MOEs were consistent with those previously estimated by JECFA (FAO/WHO, 2006, 2011) for mean and high levels of AA consumption of 1 and 4 µg/kg b.w. per day, respectively.

Similarly, Doerge et al. (2008) used the Young et al. (2007) PBPK model to estimate the brain/nervous tissue concentrations of AA from several studies reporting neuropathy in rat bioassays (Burek et al., 1980; Johnson et al., 1986; Friedman et al., 1995). Dose-response analysis using the generalised multistage model provided $BMDL_{10}$ values for neuropathy of 0.65 mg/kg b.w. per day for males and 0.60 mg/kg b.w. per day for females from Johnson et al. (1986) for 2-year exposures; and of 0.37 mg/kg b.w. per day for males and 0.90 mg/kg b.w. per day for females from Friedman et al. (1995) for 2-year exposures. In addition, Doerge et al. (2008) reported a NOAEL of 0.20 mg/kg b.w. per day for males derived from the data obtained by Burek et al. (1980) upon 90-day exposure of male rats). The PBPK model then used those doses to predict rat brain/nervous tissue concentrations of AA.

Those concentrations in rats were then compared with the predicted value of brain/nervous tissue AA in humans from daily consumption of AA in the diet at a dose of 0.4 µg/kg b.w. to calculate MOEs. Using male and female rat neuropathy data from lifetime (2 years) exposures to AA, the MOEs were in the range of 130 - 320; for a 90-day exposure to AA, the MOE was 54 using the $BMDL_{10}$ values (Doerge et al., 2008). These MOEs were similar to those previously estimated by JECFA (FAO/WHO, 2006) for mean and high levels of AA consumption of 1 and 4 µg/kg b.w. per day, respectively.

(ii) Tardiff et al. (2010) used PBPK model simulations of internal dosimetry for AA and GA from Sweeney et al. (2010) to interpret results from chronic rodent studies as being primarily consistent with hormonal dysregulation in the carcinogenic mechanism. A nonlinear dose-response approach was applied for carcinogenicity (mixed: genotoxicity and epigenetic mode of action (MoA)). Using also the dose-response data for rats exposed to AA in drinking water (Johnson et al., 1986; Friedman et al., 1995), the authors calculated a geometric mean reference point from the $BMDL_{10}$ values for thyroid tumours, CNS tumours, mammary gland tumours and peri-testicular mesothelioma data which amounted to 0.022 mg/L*h for rat using the AUC for AA as the dose metric, equivalent to a human equivalent dose of 1.8 mg/kg b.w. per day. From these $BMDL_{10}$ values the authors derived TDI and MOE values. The TDIs for cancer were estimated to be 2.6 and 16 µg/kg per day based on AA and GA, respectively. MOEs were calculated for average AA consumers to be 300 and 500 based on AA and GA, respectively. For cancer, the MOE for average AA consumers was estimated to be 200 and 1 200 based on AA and GA, respectively. The CONTAM Panel noted that the establishment of a TDI is generally considered inappropriate for a chemical that is both genotoxic and carcinogenic.

Similarly, Tardiff et al. (2010) also applied a nonlinear dose-response approach for neurotoxicity (non-genotoxicity) and calculated MOEs for rat neuropathy results from 2-year exposures (Johnson et al., 1986; Friedman et al., 1995), of 300 for mean human consumption and 80 for high consumption, assuming that AA is the toxic species, and 500 or 130, respectively, assuming that GA is the toxic species (Table 19). The TDI for neurotoxicity from AA was estimated to be 40 µg/kg per day and for GA was 70 µg/kg per day.

(iii) DeWoskin et al. (2013) used HEDs for AA and metabolically produced GA derived from either the Sweeney et al. (2010) PBPK model or a Hb adduct-based approach to determine reference doses (RfD, see Section 1.1) for AA-induced neuropathy based on a $BMDL_{05}$ of 0.27 mg AA/kg b.w. per day from chronic exposure in male rats (RfD = 0.002 mg AA/kg b.w. per day), and for AA-induced carcinogenicity based on a $BMDL_{10}$ of 0.15 mg AA/kg b.w. per day for combined incidences of thyroid tumours and tunica vaginalis mesotheliomas in male rats from an oral chronic exposure study (oral slope factor of 0.5 per mg AA/kg b.w. per day).

In all cases, the reference point from rat dose response data was multiplied by the appropriate HED to determine the human equivalent reference point. The reference points reported were essentially identical to those reported in the US-EPA assessment of AA (US-EPA, 2010, see Section 1.1).

7.2. Biomarkers of exposure/effects

Biomarkers of exposure for AA include its urinary metabolites (notably AAMA, GAMA and iso-GAMA) and its adducts with Hb and with DNA. Data gained from urinary metabolite determinations reflect exposure to AA over recent days. As globin adducts are not repaired they are accumulated over the lifespan of the protein, which is circa 120 days in humans, and their measurement thus represents a more chronic exposure to AA. As the lifespan of Hb is lower in experimental animals than in humans, the lifetime of globin adducts is shorter in experimental animals than in humans. Loss of Hb adducts in mice and both sexes of rats follows apparent first-order kinetics, the half-times for loss of AA- and GA-Val both being 8.8 days in mice and 12-13 and 11-12 days, respectively in rats (Tareke et al., 2006). DNA adducts are less persistent than globin adducts, the half-life in mouse liver being 2.6 days and in rat liver 4.1 days (females) and 4.5 days (males). In leukocytes the half-lives for loss of the DNA adduct are 6.7 days (females) and 7.4 days (males) (Tareke et al., 2006).

7.2.1. Mercapturic acids

The urinary metabolites most commonly used as biomarkers are the MAs derived from AA and GA (AAMA, GAMA and iso-GAMA) which are stable compounds and which can be quantified with high specificity and sensitivity (see Section 7.1.3). In humans, AAMA can be partially sulphoxidized to AAMA-sulphoxide (Fennell et al., 2005; Kopp and Dekant, 2009). The metabolite DHPA has been reported not to be a specific biomarker for AA exposure (Latzin et al., 2012).

Data gained from urinary metabolite determinations reflect recent exposure to AA (i.e. up to circa 2 days prior to the biomarker measurement).

7.2.1.1. Analytical methods

AAMA and GAMA are generally analysed by HPLC methods with detection by tandem mass spectrometry (MS/MS) using multiple reaction mode (MRM). The procedures involve addition of stable isotopically labelled standards (d_3-AAMA, d_3-GAMA) to a urine sample, and HPLC-MS/MS with either positive or negative ESI (Bjellas et al., 2005; Boettcher and Angerer, 2005; Boettcher et al., 2005; Fennell et al., 2006; Kopp et al., 2008; Schettgen et al., 2008; Berger et al., 2011; Huang et al., 2011a,b). In most procedures SPE column purification of the metabolite prior to MS analysis has been used, although column switching techniques with an on-line trap column have also been developed (Kellert et al., 2006; Kopp et al., 2008). Metabolomic analysis of urine from rats, using nuclear magnetic resonance- (NMR)-based and HPLC-MS-based methods, has detected the MA metabolites, together with changes in endogenous metabolites (such as an increase in creatinine and a decrease in taurine) showing that metabolomics analysis may have potential to provide biomarkers of effect (Sun et al., 2010).

7.2.1.2. Use of mercapturic acids as biomarkers

Determination of AAMA and GAMA in urine has been widely used as a biomarker for AA exposure since the evaluation of AA by JECFA in 2005 (FAO/WHO, 2006). Studies which are particularly related to food consumption or oral administration of pure AA are summarised below.

Experimental animal studies

Dose-response studies by Kopp and Dekant (2009) and Watzek et al (2012a) for the excretion of MAs in experimental animals following the oral administration of AA are reported in Section 7.1.2.1. Studies on the excretion of MAs following administration of AA in food matrices by Bjellaas et al (2007a) and Berger et al (2011) are reported in Section 7.1.1.1.

In summary, these studies show that the urinary excretion of MAs from AA and GA increases in a dose-dependent manner following administration of AA.

Human studies

Detailed data have been obtained on the extent of excretion of MAs in humans following the oral administration of a low dose of stable isotopically labelled AA (see Table 18).

Boettcher et al. (2006b) studied the influence of an AA-free diet on the excretion of urinary mercapturic acid metabolites derived from AA in three healthy volunteers fasting for 48 h. Urinary AA mercapturic acid metabolites were considerably reduced after 48 h of fasting, confirming that the diet is the main source of non-occupational AA exposure in humans (apart from smoking).

In a study of 47 non-smoking individuals the estimated AA intake did not correlate with urinary biomarkers (Bjellaas et al., 2007b). The median (range) total excretion of AA as MA metabolites in urine during 24 hours was 16 (7-47) µg for non-smokers. The median intake estimate in the study based on 24 h dietary recall was 21 (13-178) µg AA. The total AA-derived urinary metabolites correlated with intake of aspartic acid, protein, starch and coffee (n = 53).

In a study of 119 pregnant non-smoking women by Brantsæter et al. (2008), the dietary median (95th percentile) intake of AA was 0.48 (0.92) µg/kg b.w. per day as estimated by FFQ, 0.41 (0.82) µg/kg b.w. per day as estimated by food diary, and 0.42 (0.70) µg/kg b.w. per day as estimated by probabilistic approach. The total 24 hours AA-derived urinary metabolites was 0.16 (0.50) µg/kg b.w. per day in non-smokers. A significant correlation was observed between biomarker and estimated dietary intake. The total AA-derived urinary metabolites correlated with intake of crisp bread, potato crisps, cooking oil and garlic.

In the study of Heudorf et al. (2009), the internal exposure to AA and GA was studied in 110 children with regard to their exposure through diet and/or environmental tobacco smoke. Median (95th percentile) urinary levels were 36.0 (152.7) µg AAMA/L and 13.4 (55.9) µg GAMA/L. Based on the metabolite levels, the median uptake of AA was calculated to be 0.54 µg/kg b.w. per day. A number of associations with the consumption of French fries, various potato products, as well as fried cereals were found. No correlations between the exposure to environmental smoke and cotinine levels in urine were found.

The effects of genetic polymorphisms in CYP2E1, GST and mEH on excretion of MAs of AA and GA were studied in 85 workers exposed to AA (Huang et al., 2011a). A high interindividual variability in the metabolism of AA to GA was observed in the population and the results suggested that mEH and/or GSTM1 may be associated with the formation of urinary AAMA and GAMAs.

The excretion of MA metabolites was measured in human urine collected up to 72 h after consumption of potato crisps (Watzek et al., 2012b). The intake was of home-prepared crisps corresponding to ca. 1 000 µg AA per 150 g portion (a protocol similar to that published by Doroshyenko et al., 2009) and also commercially available crisps corresponding to an uptake of 44 µg AA per portion of 175 g. Excretion of AA-related MAs was detected (see Section 7.1.3).

In the study of Ji et al. (2013) the levels of AAMA were measured in urine of Korean children. The concentrations of AAMA in urine ranged between 15.4 and 196.3 ng/mL, with a median level of 68.1 ng/mL. Children exposed to environmental smoke had significantly higher levels of urinary AAMA. Median (95th percentile) values of daily AA intake in Korean children were 1.04 (2.47) µg/kg b.w. per day (Ji et al., 2013).

Brisson et al. (2014) studied the relationship between dietary intake of AA and biomarkers of exposure to AA in a group of non-smoking teenagers (n = 195). AA and its metabolites, GA, S-(2-carbamoylethyl)-L-cysteine, AAMA, AAMA-SO, GAMA and iso-GAMA were quantified in urine samples by HPLC-MS/MS. The method was sensitive enough to detect AAMA, AAMA-SO, GAMA and S-(2-carbamoylethyl)-L-cysteine in almost all urine samples. The most abundant metabolites detected were AAMA and AAMA-SO with respective geometric mean concentrations of 81.7 and 39.7 µg/mL (31.2 and 14.2 µmol/mol creatinine). The daily intake of AA during the 2 days before urine sampling (based on a 2-day food diary) was significantly correlated with the sum of AAMA and AAMA-SO urinary concentrations ($p < 0.0001$).

7.2.2. Hb adducts

Adducts of AA and GA with Hb are formed at several nucleophilic sites, the major site of reaction for AA being the cysteine SH groups. Cysteine adducts were the first Hb adducts to be determined in AA biomarker studies, although the analytical approaches were complex and sometimes not specific for AA. Specifically, rat Hb contains a reactive cysteine residue that is not found in other species, including humans. For this reason, studies of AA-Cys are not useful for human risk assessment. AA and GA adducts are also formed at the N-terminal valine residue of Hb, which is conserved across many species including laboratory rodents and human. Therefore, determination of these adducts is now the optimal approach for biomarker purposes, in view of the development of sensitive and specific analytical approaches for these products (Section 7.2.2.1).

Globin adducts are not repaired and are accumulated over the lifespan of the erythrocyte (120 days in humans) and their measurement thus represents exposure to AA over this time period. A theoretical relationship between Hb adduct formation and circulating AUCs for AA and GA was published by Calleman et al. (1992) that was subsequently confirmed experimentally by linear correlations between Hb adduct levels in mice and rats dosed by oral and injection routes with 0.1 mg/kg b.w. AA or GA and the corresponding serum AUCs for either AA and GA (Tareke et al., 2006).

The EFSA colloquium (EFSA, 2008) noted from the study of Bjellaas et al. (2007c) that Hb adducts did not correlate well with estimated dietary intake of AA, and that there was only a weak association of Hb adducts with estimated dietary AA in two other studies of non-smokers (Wirfält et al., 2008; Kütting et al., 2008). JECFA also concluded that 'there was a poor correlation between the estimated dietary exposure and internal biological markers of AA exposure (AA-valine and GA-valine haemoglobin adducts) in humans' (FAO/WHO, 2011). However BfR noted that 'blood and/or urine biomarkers may be more suitable for determining the AA intake of consumers than the estimation via the AA contents in food and consumption data' (BfR, 2011). The reason for this conclusion is that the urinary biomarkers and the Hb adducts currently used as blood biomarkers reflect the internal dose of AA/GA that is present in the experimental animal or human subject, and it is likely that they may be a more reliable indicator of dose than that derived from dietary estimates, in view of the number of potential variables which might affect the accuracy of the intake determination for AA.

7.2.2.1. Analytical methods

Törnqvist et al. (1986) described a sensitive and simple method for the analysis of Hb adducts using a modified Edman degradation procedure. The method used a fluorinated Edman reagent (pentafluorophenyl isothiocyanate) to specifically detach the adducted N-terminal amino acid in Hb (valine in the α- and β-chains in adult human Hb). This reaction produces a thiohydantoin derivative suitable for chromatographic analysis (Figure 10). The procedure has had widespread applications for monitoring environmental and occupational exposure to alkylating agents. GC-MS/MS procedures (Paulsson et al., 2003; Schettgen et al., 2004a, 2010; Urban et al., 2006; Berger et al., 2011) and HPLC-MS/MS (Fennell et al., 2003) procedures have been developed for the analysis of Hb adducts with AA and GA. A typical HPLC-MS/MS procedure involves precipitation of globin from red blood cells, which is then reacted with pentafluorophenyl isothiocyanate, followed by addition of stable isotope-labelled analytes as internal standards, SPE purification and ESI HPLC-MS/MS (Fennell et al., 2005; Tareke et al., 2006; Bjellas et al., 2007c; Huang et al., 2012). In the procedure of Vesper et al. (2006) the analytes were isolated using liquid-liquid extraction on diatomaceous earth. Phenyl isothiocyanate was used as an alternative derivatising agent by Chevolleau et al. (2007).

Further developments of the assay used modified derivatisation methods. For example the 'FIRE procedure' uses the modified Edman reagent fluorescein isothiocyanate (FITC) (von Stedingk et al., 2011) and has an LOQ of 1 pmol/g Hb for the GA adduct analysis and 2 pmol/g Hb for the AA adduct analysis. In summary the procedure involves the incubation of lysed red blood cells with FITC, the addition of deuterium substituted (d7-) fluorescein thiohydantions as internal standards, SPE purification, and analysis using positive ESI HPLC-MS/MS.

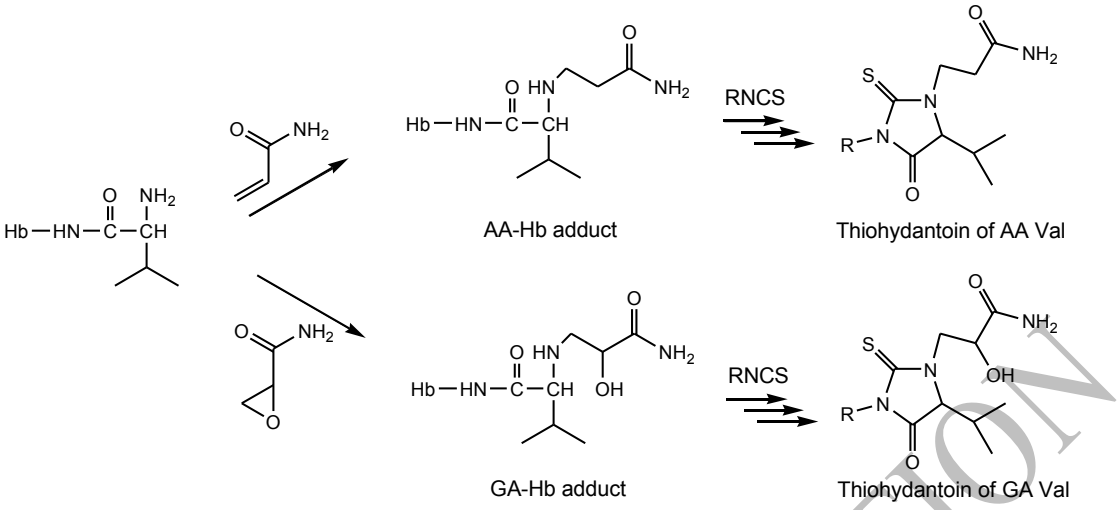

Figure 10: Adducts formed by AA and GA with N-terminal valine in globin, and the thiohydantoin products that are used for their analysis which are formed by a modified Edman degradation procedure (RNCS= pentafluorophenyl or fluorescein isothiocyanate)

Apart from the modified Edman degradation, other methods for the analysis of AA-Hb adducts have not been as well established. Preston et al. (2009) described the successful development of monoclonal antibodies specific for AA-adducted Hb, which was hoped by the authors to have potential for use in a high throughput analytical method; however this approach needs further development. Using MS/MS techniques to analyse whole globins or protein tryptic digests, Basile et al. (2008) characterised the sites in Hb that form adducts with AA, and suggested that this proteomic approach to analyse these adducts has potential for use as a biomarker.

7.2.2.2. Use of Hb adducts as biomarkers

The detection of background levels of Hb N-terminal valine adducts corresponding to AA in humans gave the first indication that there was a regularly occurring exposure to AA in the general population (Bergmark, 1997). Rats fed fried animal standard diet for 1 or 2 months showed an increase in the amount of the AA Hb adduct compared to control rats fed unfried diet, indicating that heated food was the probable origin of the background exposure to AA (Tareke et al., 2000). This was confirmed by demonstrating that AA is produced by heating a range of foods (Tareke et al., 2002).

Since then the modified Edman degradation technique has been widely used to determine the amount of the N-terminal AA or GA-adducts in Hb as a biomarker of AA exposure. Some of the key studies particularly related to dietary exposure to AA, or of oral administration of pure AA, are summarised below. In addition to these investigations, Hb adducts of AA have been used in epidemiological studies (described in Section 7.4), and PBPK modelling (described in Section 7.1.4).

Experimental animal studies

In the study of Tareke et al. (2006), F344 rats and B6C3F$_1$ mice were exposed to AA by repeat dosing with drinking water containing AA. The average daily doses of AA delivered were 2.59 ± 0.69 mg/kg b.w. per day (mice), 1.07 ± 0.28 mg/kg b.w. per day (female rats), 0.96 ± 0.28 mg/kg b.w. per day (male rats). AA-Hb and GA-Hb adducts accumulated to apparent steady state levels (Tareke et al., 2006). From an analysis of Hb adduct formation and serum toxicokinetics after single dose administration of either AA or GA by different routes of administration, significant linear relationships were observed between the AA- and GA-Hb adduct levels and the corresponding AUCs (Tareke et al., 2006).

Vikström et al. (2008) investigated mice (male, C57BL) fed diets containing 5 different levels of AA, with administered daily intakes of AA between 3 and 50 µg/kg b.w. per day. A linear relationship was observed between AA exposure and AA-Hb and GA-Hb adduct levels (Vikström et al., 2008).

In the study of Zeiger et al. (2009), AA was administered by gavage to male $B6C3F_1$ mice for 28 days at 12 doses, ranging from 0-24 mg/kg b.w. per day. The levels of both the AA-Hb and the GA-Hb adducts increased with dose. The GA-Hb to AA-Hb adduct ratio decreased from approximately 12-13 at the lower doses to 5.4 at the highest dose (Zeiger et al., 2009).

Berger et al. (2011) investigated if food matrices affect bioavailability and biological activity of AA in rats (see Section 7.1). Although AA-Hb adducts increased with increasing cumulative dose, there was no evidence for significant treatment-related effects on GA-Hb adduct formation.

In summary, these studies have shown that levels of AA- and GA-Hb adducts increase in a dose-dependent manner after oral treatment of experimental animals with pure AA or AA in food (Tareke et al., 2006; Vikström et al., 2008; Zeiger et al., 2009; Berger et al., 2011 (AA-Hb only)). Administration of AA by intraperitoneal (*i.p.*) injection to mice and rats has also shown dose-dependent increases in AA-Hb and GA-Hb adduct levels (Paulsson et al., 2002).

Human studies

A dose dependent increase in the level of AA- and GA-Hb adducts was seen in a study of humans orally administered [$^{13}C_3$]-AA (Fennell et al., 2005).

Schettgen et al. (2004a) determined the AA-Hb adduct levels in the blood of pregnant women (one smoker, ten non-smokers) and the umbilical cord blood of the corresponding neonates. There was a good correlation (r = 0.859) between adduct levels in non-smoking mothers and neonates. The Hb of the neonates contained approximately 50 % of the adduct level that was found in the Hb of the mother.

Hagmar et al. (2005) studied AA-Hb adducts in samples from a blood bank of the Malmö Diet and Cancer Cohort. The authors selected 142 individuals chosen to obtain the highest possible variation in the adduct levels from AA (none, random or high intake of coffee, fried potato, crisp bread and snacks, food items estimated to have high levels of AA). The levels of AA-adduct ranged between 0.02 and 0.1 nmol/g in non-smokers (n = 70), with considerable overlap between the different dietary groups. A significant difference was observed between men with high dietary exposure to AA compared to men with low dietary exposure (P = 0.04). This was not observed for women. A higher level of the AA-adduct was found in smokers (range: 0.03-0.43 nmol/g) (Hagmar et al., 2005).

AA-Hb and GA-Hb adducts were analysed in human Hb samples by Chevolleau et al. (2007) using ESI HPLC-MS/MS and phenyl isothiocyanate as derivatising agent. After assessment of the method on rats administered acrylamide by gavage (50 mg/kg b.w.), the procedure was applied to 68 Hb samples from the general French population. Mean levels of 33 and 23 pmol/g globin were observed for AA-Hb and GA-Hb adducts respectively, smokers giving higher mean values than non-smokers.

Bjellaas et al. (2007c) showed in a study of 50 subjects that, using multiple linear regression analysis, a significant positive correlation was found between the AA-Hb adduct concentration and the intake of chips/snacks and crisp bread. GA-Hb adduct levels did not correlate with consumption of any of the main food groups. Neither AA-Hb nor GA-Hb adduct concentration correlated with total dietary intake of AA as calculated from the reported food intake (Bjellaas et al., 2007c).

Wirfält et al. (2008) showed a significant association in non-smokers (n = 70) between Hb AA adducts and estimated AA from foods (P = 0.006). In smokers (n = 72) both AA from foods (P = 0.006) and the calculated amount of tobacco consumed (P = 0.003) were significantly associated with Hb AA adducts.

Kütting et al. (2008) showed a strong correlation of AA-Hb adducts with smoking and a weak but significant correlation with estimated dietary exposure in non-smokers (n = 828). Risk implications of these data were discussed in Kütting et al. (2009). The effect of smoking (n = 16) which increased the levels of AA-Hb and GA-Hb adducts compared to the levels observed in non-smokers (n = 13) was also reported by Schettgen et al. (2004a).

The study of Vikström et al. (2008) aimed to measure the relationship between dietary exposure to AA and internal doses of AA and its genotoxic metabolite GA at low levels of AA intake through the diet. A linear relationship was observed between the exposure to AA and Hb-adduct levels from both AA and GA at these low exposure levels.

The same authors also investigated whether alcohol consumption could have an influence on the metabolism of AA to GA in humans exposed to AA through food (Vikström et al., 2010). Alcohol intake estimates were obtained from questionnaire data (161 non-smoking men) and compared with the ratio of Hb-adduct levels for AA and GA. A negative, linear trend of GA-adduct to AA-adduct level ratios with increasing alcohol intake was observed. The strongest association between alcohol intake and GA-adduct to AA-adduct level ratios was obtained in the group of men with the lowest adduct levels (< 47 pmol/g globin) (*p*-value for trend = 0.02).

In order to assess human exposure to AA and GA in the general U.S. population, AA- and GA-Hb adducts were measured in 7 166 subjects from the National Health and Nutrition Examination Survey (NHANES) (Vesper et al., 2010). Exposure to AA was detectable in > 99 % of all participants. There was a high variability of adduct levels among individuals but modest differences between population subgroups. AA-Hb and GA-Hb levels ranged from 3 to 910 and from 4 to 756 pmol/g Hb, respectively, smokers having higher geometric mean levels than non-smokers. Tobacco smoke exposure in non-smokers had a small but significant effect on AA-Hb and GA-Hb levels. In non-smokers the highest adjusted geometric mean levels were seen in children 3-11 years of age and lowest in adults aged > 60 years.

Von Stedingk et al. (2011) used the adduct FIRE procedure to determine the adducts of AA and GA with Hb in maternal blood samples (n = 87) and umbilical cord blood samples (n = 219). Adduct levels from AA and GA were increased in tobacco smokers, and there was a significant correlation between cord and maternal blood adduct levels (ratio cord: maternal 0.48 (range 0.27-0.86) for AA and 0.38 (range 0.20-0.73) for GA).

In the study of Outzen et al. (2011), 537 non-smoking women aged 50-65 years were investigated to assess the dietary determinants of AA-Hb and GA-Hb adduct levels. Information on dietary and lifestyle variables was obtained from questionnaires. The median level for AA-Hb was 35 pmol/g globin and for GA-Hb was 21 pmol/g globin. Only a few dietary determinants of Hb-AA and Hb-GA were identified. Intakes of coffee and chips were statistically significantly associated with higher AA-Hb adduct levels and intakes of coffee and biscuits/crackers were statistically significantly associated with higher GA-Hb adduct levels. The authors concluded that the study implies that dietary intake measured by an FFQ explains only to a limited extent the variation in AA-Hb and GA-Hb concentrations.

In a study of Vikström et al. (2011), AA-rich foods were given to non-smokers and Hb adduct levels from AA and GA were measured in blood samples donated before and after exposure. These were used for calculation of AA-AUC and GA-AUC using reaction rate constants for the adduct formation measured *in vitro*. Two treatment schedules were used: a high intake of 11 µg AA/kg b.w. a day for 4 days or an extra (medium) intake of 2.5 µg AA/kg b.w. a day for a month. The AA-Hb and GA-Hb adducts both increased about two-fold after the enhanced intakes of AA. The AUCs for the high and medium groups, respectively, were for AA 212 and 120 nanomolar hours per µg AA/kg b.w., and for GA 49 and 21 nanomolar hours per µg AA/kg b.w. (Vikström et al., 2011).

The intra-individual variations of AA- and GA-adduct levels measured in blood samples from 13 non-smokers, collected over 20 months, were up to 2-fold and 4-fold, respectively (Vikström et al., 2012). The corresponding interindividual variations between 68 non-smokers, with large differences in AA intake, were 6-fold and 8-fold, respectively. The intra-individual variation of the GA- to AA-adduct level ratio was up to 3-fold, compared to 11-fold between individuals (n = 68). From AA-adduct levels the mean AA daily intake (n = 68) was in relatively good agreement with that estimated from dietary history methodology, 0.52 and 0.67 µg/kg b.w. per day, respectively. A low association of these measures was observed at an individual level (Rs = 0.39). The authors concluded that dietary AA is the dominating source for measured AA-adduct levels and corresponding inter- and intra-individual variations in non-smokers, and that measurements from single individual samples are useful for calculation of average AA intake (Vikström et al., 2012)

Huang et al. (2012) studied 51 AA-exposed workers and 34 controls to explore the effect of genetic polymorphisms of CYP2E1, mEH3, mEH4, GSTT1 and GSTM1 on AA and GA-Hb adduct levels. The results suggest that mEH4 and the combined genotypes of CYP2E1, GSTM1 and mEH4 may be associated with the formation of AAVal and GAVal (Huang et al., 2012)

The effects of prenatal exposure to AA measured by determinations of AA- and GA-Hb adducts on birth weight and head circumference (Pedersen et al., 2012) is described in Section 7.4.2.

Brisson et al. (2014) studied the relationship between dietary intake of AA and biomarkers of exposure in a group of non-smoking teenagers (n = 195). The geometric mean concentration for the AA adduct with N-terminal valine in Hb was 45.4 pmol/g globin and for the GA adduct 45.6 pmol/g globin. The daily intake of AA during the 2 days before urine sampling was not correlated with the Hb adducts. However AA intakes (based on FFQ) during the month before blood collection and passive smoking were associated with the sum of AA and GA adduct levels ($p < 0.0001$ and $p < 0.05$, respectively).

The adducts of AA and GA in Hb have been measured as biomarkers of exposure in the NHANES 2003-2004 sample of the US population (Vesper et al., 2013). Adduct data for AA were available for 4 093 participants and for GA for 4 152 participants. The population was estimated to consist of 48 % men and 71 % non-smokers. The association of sociodemographic and lifestyle factors with these biomarkers was assessed. Smoking was strongly and significantly correlated with both the AA and GA Hb adduct concentrations, and age was negatively correlated with both biomarkers. BMI was weakly negatively correlated with AA-Hb adducts levels. Alcohol consumption was weakly positively correlated with AA-Hb adduct levels but negatively associated with GA-Hb adduct levels after adjusting for sociodemographic and lifestyle variables.

In the study of Ferrari et al. (2013), dietary estimates of AA from questionnaires and from 24-h dietary recalls were compared with levels of AA adduct in Hb in 510 participants in the EPIC study. Estimates of total AA intake based on self-reported diet correlated only weakly with the AA-Hb adduct levels used as biomarker.

7.2.3. DNA adducts

DNA adducts derived from AA and GA have been investigated in *in vitro* studies and in experimental animals, particularly for mode of action studies (Section 7.3.6) and for their use in PBPK modelling (Section 7.1.5). AA reacts extremely slowly with DNA *in vitro*, producing a range of Michael addition products (Solomon et al., 1985), whereas GA is much more reactive by virtue of its electrophilic epoxide group. The N7-guanine adduct derived from GA, N-7-(2-carbamoyl-2-hydroxyethyl)guanine (N7-GA-Gua), is the major product following the incubation of AA with DNA in the presence of rat liver S9 (Segerbäck et al., 1995) and *in vivo* following the administration of AA to experimental animals (Figure 11). Minor amounts of adenine adducts of GA (N3-(2-carbamoyl-2-hydroxyethyl) adenine (N3-GA-Ade) and N1-(2-carboxy-2-hydroxyethyl)-2'deoxyadenosine) (N1-GA-dA) are also formed *in vitro* when DNA is incubated with GA (Gamboa da Costa et al., 2003). N3-GA-Ade is a minor product in DNA of tissues of mice and rats administered AA (Doerge et al., 2005c).

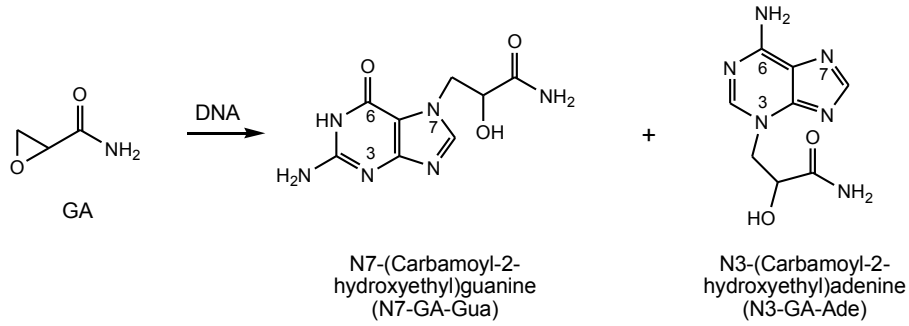

Figure 11: Adducts formed by GA with DNA.

Kotova et al. (2011) reported that N1-GA-dA, which was analysed by postlabelling after conversion to N6-(2-carboxy-2-hydroxyethyl)-2'-deoxyadenosine, was detectable in DNA reacted with GA and in DNA from cells exposed to GA, but not in DNA from mice treated with AA (7 week-old male CBA mice, single oral dose of AA (2 animals received 60 mg and 2 received 40 mg/kg b.w.)).

Thus N7-GA-Gua appears to be the most suitable biomarker of exposure for the genotoxic metabolite of AA, GA. *In vivo* N7-GA-Gua is removed from DNA, the reported half-lives for this in rats and mice being in the range 2.6 days (mouse liver) to 7.4 days (male rat leukocytes) (Tareke et al., 2006). The use of N7-GA-Gua as a biomarker is thus likely to be more appropriate for detecting exposures to AA within the week prior to the biomarker measurement. A causal link between internal exposures to GA and DNA adduct formation was inferred from the linear correlation observed for N7-GA-Gua adduct levels in liver DNA from groups of mice and rats dosed by oral and injection routes with 0.1 mg/kg b.w. AA or GA and the corresponding serum AUCs for GA, but not AA (Tareke et al., 2006).

7.2.3.1. Analytical methods

Determination of N7-GA-Gua is carried out by HPLC-MS/MS with positive ESI. DNA is subjected to neutral thermal hydrolysis to release the N7-GA-Gua adduct which is quantified using MRM with [$^{15}N_5$]-N7-GA-Gua as the internal standard.

7.2.3.2. Use of DNA adducts as biomarkers

Experimental animal studies

AA was administered to male Sprague Dawley rats by oral gavage at doses of 18 and 54 mg/kg b.w. (Manière et al., 2005). Tissue samples from brain, liver and testes were analysed for N7-GA-Gua 5, at 24, 48, and 72 hours after dosing. N7-GA-Gua adducts increased with dose and a relatively consistent organ distribution of the adduct in brain, testes and liver was observed, but there was no accumulation of adducts in the liver where the activating enzyme CYP2E1 is primarily located.

In the study of Tareke et al. (2006) (see Section 7.2.2.2), F344 rats and B6C3F$_1$ mice were exposed to AA by repeat dosing with drinking water containing AA. The level of N7-GA-Gua in DNA accumulated to apparent steady state levels (Tareke et al., 2006).

In the study of Zeiger et al. (2009), AA was administered by gavage to male B6C3F$_1$ mice for 28 days at 12 doses, ranging from 0-24 mg/kg b.w. per day. The levels of N7-GA-Gua in liver DNA increased with dose (Zeiger et al., 2009).

The effect of age was studied in rats by Koyama et al. (2011a). Three and 11 week old male gpt delta transgenic F344 rats were treated with 0, 20, 40 or 80 ppm AA via drinking water for 4 weeks. N7-GA-Gua levels were measured in DNA from liver, testes, mammary gland and thyroid, where they all increased in a dose-dependent manner. Adduct levels in young and adult animals did not differ significantly in thyroid and mammary glands, but were higher in liver and testis of young compared to

adult rats. The effect was particularly notable in the testis where young rats had a 6-fold higher level of N7-GA-Gua.

Watzek et al. (2012a) carried out a dose-response study with AA in female Sprague-Dawley rats. After a single oral dose of 0.1-10 000 µg/kg b.w., N7-GA-Gua was determined in DNA from liver, kidney and lung 16 hours after dosage. Adducts could not be detected below the dose of 1 µg/kg b.w. but at this dose were detectable in kidney and lung (significantly different from control $p < 0.001$). Adducts at doses of 100 µg/kg b.w. and above showed a significant increase above those at the next lower dose level ($p < 0.001$) (Watzek et al., 2012a).

In summary, levels of N7-GA-Gua in DNA have been shown to increase in a dose-dependent manner after oral treatment of experimental animals with AA and GA (Manière et al., 2005; Tareke et al., 2006; Zeiger et al., 2009; Koyama et al., 2011a; Watzek et al., 2012a). Administration of AA by *i.p.* injection to mice and rats has also been shown to cause dose-dependent increases in N7-GA-Gua in DNA (Gamboa da Costa et al., 2003; Doerge et al., 2005c).

Human studies

No data are available on the effects of dietary exposure to AA on adduct levels in human DNA.

7.2.4. Correlation between biomarkers

In F344 rats and B6C3F$_1$ mice exposed to a single dose gavage administration of AA (0.1 mg/kg b.w.) or an equivalent gavage dose of GA, a significant correlation was observed between GA-Hb adducts and N7-GA-Gua adducts in liver DNA (Tareke et al., 2006). Significant correlations were also observed in F344 rats and B6C3F$_1$ mice between urinary AAMA and N7-GA-Gua adducts and between urinary GAMA and N7-GA-Gua adducts (Doerge et al., 2007).

In a dose-response study AA in female Sprague Dawley rats, which encompassed human diet related exposure, levels of urinary AAMA and GAMA and of N7-GA-Gua in tissue DNA all showed significantly higher levels than controls at oral doses of 1 µg/kg b.w. (and above), and generally increased with dose. However no calculated correlation between these biomarkers was reported. (Watzek et al., 2012a).

In the study of human volunteers carried out by Hartmann et al. (2008) (Section 7.1.3) no significant correlation was found between AA-Hb adducts and AAMA (Pearson, $p = 0.02$, $r = 0.25$) or between GA-Hb adducts and GAMA (Pearson, $p = 0.03$, $r = 0.24$). The authors thought that this might be because of the different exposure periods being monitored by the two biomarkers.

An association of the urinary levels of AAMA with a biomarker of oxidative stress, 8-hydroxy-deoxyguanosine (8-OHdG), was observed in adolescents and young adults by Lin et al. (2013). Eight hundred subjects (mean age 21.3 years, range 12-30 years) were recruited, and urinary AAMA and 8-OHdG were measured by HPLC-MS/MS, the mean (SD) concentrations being 76.54 (76.42) µg/L and 3.48 (2.37) µg/L, respectively. Urinary AAMA was positively asociated with urinary 8-OHdG, and sub-population analyses showed the association to be significant in males, subjects aged 12-19 years, BMI ≥ 24, homeostasis model assessment of insulin resistance (HOMA-IR) ≥ 0.9, non-current smokers, and subjects who did not consume alcohol.

In the study of Zeiger et al. (2009) in male B6C3F$_1$ mice, a linear correlation was seen between GA-Hb adducts and N7-GA-Gua in liver DNA, following the administration of AA by gavage for 28 days at doses from 0-24 mg/kg b.w. per day.

Very few correlation studies have been carried between these AA exposure biomarkers and biomarkers of biological effect. In the Zeiger et al. (2009) study in male mice described immediately above, micronuclei (MN) in normochromatic erythrocytes (NCEs) increased linearly with dose. However when Hb or DNA adducts were used as the dose matrix a non-linear dose-response

relationship with a threshold at low doses was claimed by the authors to be the most appropriate model. Paulsson et al. (2002) showed that male CBA mice treated *i.p.* with 25, 50 or 100 mg/kg b.w. AA showed dose-dependent increases in both Hb adduct level and MN frequency in peripheral blood erythrocytes. In male Sprague Dawley rats treated with 100 mg/kg b.w. AA-Hb adducts were increased but no effect was seen on MN frequency in bone marrow erythrocytes (Paulsson et al., 2002).

7.2.5. Conclusion

Urinary MAs, Hb adducts and DNA adducts (N7-GA-Gua) all have different advantages as biomarkers for exposure to AA. Urine is non-invasive to collect for the MA analysis, compared to blood/tissue collection for Hb and DNA adducts. The biomarkers reflect different timescales for the detection of exposure. For MAs this is only recent days prior to the biomarker measurement. For N7-GA-Gua the half-lives of the adduct in mice and rats are in the range of 3-7 days. The Hb adduct will accumulate over the lifespan of the erythrocyte, which is circa 120 days in humans. N7-GA-Gua is the most appropriate biomarker to use for the interaction of GA, the genotoxic metabolite of AA, with DNA. The weight of evidence is that there are correlations both between these types of biomarkers, and between them and exposure to AA (see PBPK modelling, Section 7.1.5). Some evidence also exists in experimental animals for a correlation of these biomarkers with biomarkers of effects (e.g. MN in mice) but confirmation of this is needed.

7.3. Toxicity in experimental animals

7.3.1. Acute toxicity

Oral LD_{50} values for AA were reported to be >150 mg/kg b.w. for rats (McCollister et al., 1964; Fullerton and Barnes, 1966; Tilson and Cabe, 1979), 107 mg/kg b.w. for mice (Hashimoto et al., 1981), and 150-180 mg/kg b.w. for rabbits and guinea pigs (McCollister et al., 1964).

7.3.2. Repeated dose toxicity

In this section, the toxicological studies with repeated doses of AA conducted in rats, mice, monkeys, cats and dogs are reported. These studies were carried out using various dosing protocols and routes of exposure, mostly via oral exposure or injection. Some studies evaluated only neurotoxicity, while other studies also examined other effects, e.g. reproductive and developmental toxicity (see Section 7.3.5). A few studies examined the time course of AA toxicity along with overt, morphological, and biochemical changes. Only those repeated-dose toxicity studies using oral exposure to AA are presented and discussed in this section. The biochemical changes observed are presented and discussed in Section 7.3.6.

7.3.2.1. Mice

ddY male mice (6 per group) were given 0 (control) or 36 mg/kg b.w. AA by oral gavage two times per week for 8 weeks (Hashimoto et al., 1981). Exposed mice showed weakness and ataxia of the hindlimbs. Rotarod performance showed a progressive decrease from 3 weeks onwards. Relative testicular weight was reduced by 17 %. Microscopical analysis of the testes revealed some degeneration of epithelia in spermatids and spermatocytes, reduction of spermatozoa and the presence of multinucleate giant cells.

Female BALB/c mice (5 per group) were given 0 (control) or 26 mg/kg b.w. per day AA in drinking water for 12 days (Gilbert and Maurissen, 1982). After a 44 days recovery period the treated animals were given 20 mg/kg b.w. per day for 19 days. Some body weight loss and reduction in water consumption were noted. For the exposed mice hindlimb foot splay was increased from 6 days onwards, and rotarod retention time decreased from 8 days onwards. After the 44 day recovery period all these parameters were again at control values. A similar pattern of effects was noted for the second exposure period.

Chapin et al. (1995) investigated the neurotoxicity of AA in Swiss mice via application in the drinking water at concentrations of 0, 3, 10, or 30 ppm (estimated doses: 0.81, 3.19, or 7.22 mg/kg per day) for 14 weeks. At the highest dose level, male mice exhibited a deficit in forelimb and hind-limb grip strength in comparison to the untreated controls.

Tyl et al. (2000a,b) treated male mice with AA in drinking water at concentrations resulting in estimated doses of 0, 5, 15, 30, 45, or 60 mg/kg per day for 5 days. Hind-limb grip strength was significantly reduced in the 60 mg/kg per day dose group, while forelimb grip strength was unaffected in any dose group. No histopathological changes were found in the sciatic nerves of any dose group.

The NTP conducted a 13-week study (drinking water and diet) and a 2-year study (drinking water) in $B6C3F_1$ mice administered AA (NTP, 2012). In the 13-week range finding study, groups of eight male and eight female mice were treated with AA at a concentration of 0, 0.14, 0.35, 0.70, 1.41 or 3.52 mM in drinking-water (0, 10, 25, 50, 100 or 250 mg/L equivalent to 0, 3.2, 6.9, 13.3, 32.8 and 70.0 mg/kg b.w. per day for males, and to 0, 3.5, 7.8, 16.4, 31.4 and 83.1 mg/kg b.w. per day for females) or 0, 18.5, 37, 74, 185 or 370 mg/kg diet (equivalent to 0, 3.3, 6.6, 12.0, 32.1 and 59.4 mg/kg b.w. per day for males and 0, 3.7, 7.5, 13.9, 35.1 and 64.0 mg/kg b.w. per day for females). Small body weight reductions were noted at the two highest dose levels in the drinking water study and at the highest dose in the feeding study. Hindlimb paralysis was observed in all mice treated with AA at 3.52 mM/L or 370 mg/kg diet. Radiculoneuropathy involving the sciatic nerve, lumbar spinal cord or both was observed in all mice treated with 3.52 mM/L, in one out of eight mice fed 185 mg/kg diet and in mice fed 370 mg/kg diet. The neuronal degenerative changes were accompanied, at times, by atrophy in skeletal muscle of the hind-limb and urinay bladder dilation. The NOAELs were 0.70 mM for exposure via drinking water (equivalent to 13.3 mg/kg b.w. per day) and 74 mg/kg diet (equivalent to 12.0 mg/kg b.w. per day).

In the 2-year study (NTP, 2012), group of 48 male and 48 female $B6C3F_1$ mice were administered AA *ad libitum* in drinking water at concentrations of 0.0875, 0.175, 0.35 and 0.70 mM (equivalent to 1.04, 2.20, 4.11 and 8.93 mg/kg b.w. per day for males, and to 1.10, 2.23, 4.65 and 9.96 mg/kg b.w. per day for females). Control animals received the same tap water with no chemical added. A trend towards a decrease in survival was observed at the highest dose level for males and females, and for females also at the second highest dose level. Changes in body weight were only sporadic, and food and water consumption was generally not affected. Cataracts of the eyes in both male and female mice were observed at the highest dose administered, and for female also at the second highest dose level. A dose-related increasing trend in forestomach epithelium hyperplasia in both sexes was observed (significant at the higehst dose level). Increasing dose-related trends in hematopoietic cell proliferation of the spleen were reported for both sexes being significant at the highest dose, and for females also at the second highest dose. Other non-neoplastic lesions in male mice included: preputial gland inflammation (significant at the two highest doses) and lung alveolar epithelium hyperplasia (significant at the highest dose level), and for female mice: ovarian cysts (increased at the lowest dose and the two highest dose levels) (NTP, 2012). A NOAEL could not be established for the non-neoplastic effects in this study based on the increase of ovarian cyst observed at the lowest dose. The neoplastic findings of this study are described under Section 7.3.3.2.

NTP also conducted a 13-week and a 2-years study in $B6C3F_1$/Nctr mice administered GA in drinking water (NTP, 2013, draft report[33]). In the 13-week study, groups of eight male and eight female mice were administered GA in drinking water at doses of 0.0, 0.14, 0.35, 0.70, 1.41 or 3.52 mM (equivalent to 0, 12.2, 30.6, 61.2, 122 or 306 mg/L GA, and corresponding to approximately 0, 1.83, 4.59, 9.18, 18.3 and 45.9 mg/kg b.w. per day). Some body weight reduction in males was observed in the group given the highest dose. Two of eight male mice at the highest dose of 3.52 mM displayed hindlimb paralysis. Radiculoneuropathy involving primarily the sciatic nerve in both sexes was observed in the

[33] Draft Technical Report available at http://ntp.niehs.nih.gov/ntp/about_ntp/trpanel/2013/october/draft_tr-588.pdf. Notice by NTP: *This draft Technical Report is distributed solely for the purpose of predissemination peer review under the applicable information quality guidelines. This report has not been formally disseminated by the NTP. It does not represent and should not be construed to represent NTP determination or policy*.

highest dose group, at times accompanied by atrophy in skeletal muscle of the hindlimb and urinary bladder dilation. Degeneration of the germ cells in the testes was observed in seven of eight male mice given 3.52 mM GA. The NOAEL was 1.41 mM, equivalent to 18.3 mg GA/kg b.w. per day based on hindlimb paralysis, axon degeneration and germinal epithelium degeneration observed at the highest dose.

In the 2-year study, groups of 48 male and 48 female mice were administered GA *ad libitum* in drinking water at 0, 0.0875, 0.175, 0.35 or 0.70 mM (equivalent to 0, 1.20, 2.65, 5.13 or 9.55 mg/kg b.w. per day for males, and to 0, 1.37, 2.89, 5.64 or 12.99 mg/kg b.w. per day for females) (NTP, 2013, draft report). No changes in body weights, water and food consumption were in general observed. A significant dose-related decreasing trend in survival was reported for male mice at all doses except at the lowest one, and for female mice at the two highest dose levels. Cataracts in both sexes were observed (significant at all administered doses except the lowest one) as well as a dose-related increasing trend in epithelial hyperplasia of the forestomach (significant for males at the highest dose level, and for females at the second highest dose level). An increasing dose-related trend in hematopoietic cell proliferation of the spleen was reported for both sexes being significant at the two highest dose levels. Other non-neoplastic lesions for males were degeneration, ductal dilation and inflammation of the preputial gland, and for females ovarian cysts, hepatic angiectasis and necrosis, and axonal degeneration of the cervical spinal cord (NTP, 2013, draft report). The NOAEL for non-neoplastic effects was 0.0875 mM, equivalent to 1.20 mg GA/kg b.w. per day. The neoplastic findings of this study are described under Section 7.3.3.2.

7.3.2.2. Rats

In several studies, male rats were used only, while both genders were treated in others. In those studies directly comparing the neurotoxic effects in both genders, no substantial differences in sensitivity were found in most studies, e.g. by Gold et al. (2004) while the dose-response relationship suggested possible differences in another study (NTP, 2012) indicating that male rats may be more sensitive than female rats.

In a study by McCollister et al. (1964), Dow-Wistar male and female rats received AA in the feed. The calculated doses were 0, 0.3, 0.9, 3, 7, 9, 11, 30, or 40 mg/kg b.w. per day for up to 90 days. Up to 7 mg/kg per day there were no, at 9 mg/kg sporadic, and at 11 mg/kg slight but consistent signs of neurotoxicity characterized by hind limb weakness. Higher doses resulted in complete loss of hind limb function which, however, recovered after cessation of treatment.

Fullerton and Barnes (1966) investigated the neurotoxicity of AA in male and female Porton rats. A single oral dose of 100 mg/kg resulted in fine tremor, a second dose of 100 mg/kg 24 h later led to general weakness and most rats died within the following three days. In repeated-dose experiments, male and female rats received 12 daily gavage doses of 50 mg/kg over 15 days. Treatment resulted in severe weakness and rats died within days. When rats received 25 mg/kg applied 5 days/week, leg weakness became apparent after 20 doses and severe weakness developed after 28 doses. When the AA administration was stopped, the rats almost completely recovered within four weeks. After daily treatment of female rats with 10 mg/kg for up to 116 doses, no neurotoxicity was observed.

In the same study, male rats of 6 to 8 weeks of age were exposed to 0, 100, 200, 300 or 400 ppm AA in the feed (estimated doses: 0, 5, 10, 15, or 20 mg/kg b.w. per day) for up to 48 weeks (Fullerton and Barnes, 1966). In the 20 mg/kg per day dose weakness of the legs was observed after 3 weeks, in the 15 mg/kg b.w. per day dose group after 4 weeks, and in the 10 mg/kg b.w. per day dose group after 12 weeks of treatment. The 5 mg/kg b.w. per day dose group exhibited symptoms of slight leg weakness after 40 weeks. In the dose groups having received 10 mg/kg b.w. per day and higher, a significant decrease in nerve conductivity, and histopathological signs of peripheral nerve degeneration in the sciatic nerve, posterior tibial nerve, and sural nerve fibers were reported.

Hashimoto and Ando (1973) investigated Sprague-Dawley rats fed a diet containing AA at a concentration of 500 ppm (estimated dose: 25 mg/kg b.w. per day) for 4 weeks followed by 4 weeks of recovery. Animals were sacrificed on weeks 1, 2, 3, 4, 6 or 8. Weakness in the hind limbs and walking pertubations were observed after 2-3 weeks, complete hind limb paralysis after 4 weeks of treatment.

Tilson et al. (1979) administered AA by gavage to male F344 rats at doses of 0, 5, 10 or 20 mg/kg b.w. per day, 3 days per week, for 13 weeks. Neurotoxicity was assessed by determination of hind-limb extensor ability, locomotor activity, and forelimb grip power prior to the AA administration and after 1, 4, 7, 10, and 13 weeks of treatment. AA treatment at 20 mg/kg b.w. per day resulted in decreased hind-limb extensor strength and locomotor activity during weeks 7 to 13 and weeks 10 to 13 of treatment, while forelimb grip power was not affected. Microscopic examination after 13 weeks of treatment revealed mild distal nerve fiber (sciatic and tibial) damage and moderate (Schwann-cell column formation) damage at 10 mg/kg b.w. per day. These changes were moderate to severe in the 20 mg/kg b.w. per day group. No histopathological examination was carried out in the 5 mg/kg b.w. per day group.

In a study by Tilson and Cabe (1979) in male F344 rats, AA at 200 mg/kg given as a single dose by gavage produced a significant perturbation of hind-limb strength, while 50 and 100 mg/kg had no significant effect. The authors also investigated the effects of repeated administration of AA at 10 or 20 mg/kg per day, 5 days per week, for up to 4 weeks. Statistically significant decreases in the hind-limb strength were noted with 10 and 20 mg AA/kg per day, while the effect was sustained in the 20 but not in the 10 mg/kg group when dosing was ceased. Forelimb function was not affected by the treatment, indicating a more severe effect on distal axons than on proximal ones.

Burek et al. (1980) investigated AA in a 90-day toxicity study and a 92-day toxicity study with recovery periods in groups of 6-week-old male and female F344 rats, administering AA in the drinking water at concentrations designed to deliver doses of 0.05-20 mg/kg b.w. per day. Group sizes of 10 males and 10 females were used. Effects were observed mainly in the highest dose group (20 mg/kg b.w. per day in both male and female rats) and included reduction in body weight, dragging of the rear limbs and decrease in packed cell volume, total erythrocyte count and Hb concentrations. The primary target tissue was the peripheral nerve with lesions consisting of severe degeneration, characterized by demyelinisation and axonal loss. Slight spinal cord degeneration was also noted as well as atrophy of skeletal muscle, testicular atrophy and distended bladders. The electron microscopic substudies of the 90-day toxicity study and a 92-day toxicity study with recovery periods processed and examined the left sciatic nerves in a dose-response design with 3 animals per dose group where 3 blocks of nerve fibres (two longitudinal and 1 transverse) were selected and in each 50 fields were examined. The authors reported no treatment-related effects at 0.05 and 0.2 mg/kg b.w. per day in any of the parameters monitored (axolemna invaginations and Schwann cells of with different types of alterations). The percentages of fields examined showing any alterations were 15, 9, 12, 25, 34 and 55 % at levels of 0 (control), 0.05, 0.2, 1, 5 or 20 mg/kg b.w. per day. The main alterations observed were sciatic nerves showing axolemma invaginations or axolemma invaginations with cell organelles and/or dense bodies. Total incidences were 36, 24, 27, 30, 33, 8, or 32, 15, 17, 78, 109, 48, respectively, at the levels of 0 (control), 0.05, 0.2, 1, 5 or 20 mg/kg b.w. per day when pooling the 435-450 fields from the 3 blocks and the 3 rats in 90-day toxicity study. No individual data per animal were reported. From these findings JECFA (FAO/WHO, 2006, 2011) identified a NOAEL of 0.2 mg/kg b.w. per day and a LOAEL of 1 mg/kg b.w. per day for morphological changes in the sciatic nerve of male rats. The CONTAM Panel noted that the NOAEL of 0.2 mg/kg b.w. per day was derived from electron microscopic observations on several types of sciatic nerve lesions from a low number of animals per group (n = 3), and obtaining 150 dependent data points from each rat. It was also noted that the data showed no clear dose-response, and that the determination of the NOAEL was not based on a statistical analysis of the data, e.g. by testing for differences between dose groups and the controls.

Tannii and Hashimoto (1983) administered AA to male Wistar rats (4 animals per group) at concentrations of 0, 0.73, 1.12, 1.76 or 2.81 mM (estimated 0, 5.19, 7.96, 12.51 or 19.97 mg/kg b.w.

per day) in the drinking water for up to 90 days. All treated animals showed a slight reduction in body weight, and rotarod performance at 90 days exposure was affected for the two highest dose groups. Other signs of toxicity included weakness, tendency towards spreading and dragging hindlimbs and occasionally urinary incontinence. Light microscopy examination revealed moderate to severe changes in tibial and sural nerves including shrinkage and loss of myelinated fibres, myelin retraction and corrugation of myelin sheaths at the highest dose level.

Johnson et al. (1986) applied AA to male and female F344 rats in the drinking water at levels resulting in estimated doses of 0.01, 0.1, 0.5, or 2.0 mg/kg b.w. per day for 2 years. Although there were no overt signs of neurotoxicity in any of the dose groups, tibial nerve degeneration was detected histopathologically in the dose groups receiving 2 mg/kg b.w. per day after 18 and 24 months. The CONTAM Panel noted that the data on tibial nerve degeneration did not show a clear dose-response since only the value in the highest dose group was increased, but without statistical significance (Table 20). The NOAEL was 0.5 mg/kg b.w. per day.

Schulze and Boysen (1991) administered AA by gavage to male and female Sprague-Dawley rats (10 animals per group) at doses of 10 or 30 mg/kg b.w. per day, 7 days per week over 35 days. After 24 days of treatment and a recovery period of ten days, survivors in the high-dose group received 20 mg/kg b.w. per day for the remaining 11 days. After 2 weeks of treatment, the high-dose group but not the 10 mg/kg b.w. per day group, showed a significant decrease in rearing, significant changes in posture, increased mean gait scores, a significant decrease in forelimb and hind-limb grip strength, and a significant decrease in locomotor activity. Histopathological changes such as axonal fragmentation, degeneration, and swelling were found in both dose groups. Most severly affected sites were the tibial nerve, and the cervical and lumbar portions of the spinal cord

Yoshimura et al. (1992) analyzed the effects of dose level and duration of treatment on histopathological changes related to neurotoxic effects. When female Sprague-Dawley rats received AA via gavage at doses of 50 mg/kg b.w. per day signs of neurotoxicity emerged after 2 to 3 weeks of treatment. The animals exhibited vacuolisation of cells in the cerebellar cortex and degeneration of Purkinje cell organelles. Also, a distal axonopathy was restricted to the long tracts of their spinal cord and peripheral nerves. Also in rats that received 20 mg/kg per day (7 to 8 weeks), a distal axonopathy with accumulation of neurofilamentous material in the axoplasm of the central and peripheral nerve fibers was observed.

Male and female Sprague-Dawley rats (10 rats per group) received 0 (control), 12.5, 25 or 50 mg/kg b.w. per day AA by oral gavage for 7 days (Newton et al., 1992; Hughes et al., 1994, as cited in FAO/WHO, 2006). Reduced activity was recorded for all exposed animals with the highest prevalence in the highest dose group. Body weight gain was reduced for all exposed animals but this was only statistically significant for the highest dose group. At this highest dose level hindlimbs were splayed with a corresponding impairment of mobility and a reduced number of rearing counts. Mean forelimb and hindlimb grip strength were reduced for both males and females in the highest dose group. Landing foot splay was increased in a dose dependent manner. Histopathological examination revealed axonal degeneration in all animals at the highest dose level and to a lesser extent in a small number of animals in the 25 mg/kg b.w. per day group.

Friedman et al. (1995) exposed male and female F344 rats to AA for 2 years. AA was administered via the drinking water at concentrations resulting in estimated dose levels of 0, 0.1, 0.5 or 2.0 mg/kg b.w. per day in males, or 0, 1.0 or 3.0 mg/kg b.w. per day in females. Although there were no symptoms of overt neurotoxicity, histopathological examination revealed sciatic nerve degeneration in the male rats receiving 2.0 mg/kg b.w. per day and in the female rats receiving 3.0 mg/kg b.w. per day. The CONTAM Panel noted the data on sciatic nerve degeneration in male and female F344 rats did not reveal a clear dose-response since only the value in the highest dose group was increased, but without statistical significance (Table 20). The NOAEL was 0.5 mg/kg b.w. per day.

Male Fischer 344 rats (n = 7 or 8) were treated with AA at 44 mg/kg b.w. per day (Bowyer et al., 2009). Changes in mRNA levels in the striatum, substantia nigra and parietal cortex were measured by complementary DNA (cDNA) array and/or reverse transcriptase polymerase chain reaction (RT-PCR) analysis. Treatment resulted in significantly decreased body weight and reduced locomotor activity. These physiological effects were not accompanied by prominent changes in gene expression in the forebrain.

The NTP conducted a 13-week study (drinking water and diet) and a 2-year study (drinking water) in F344/N rats administered AA (NTP, 2012). In the 13-week range finding study, groups of eight male and eight female rats were treated with AA at concentrations of 0, 0.14, 0.35, 0.70, 1.41 or 3.52 mM/L in drinking-water (0, 10, 25, 50, 100 or 250 mg AA/L, equivalent to 0, 0.8, 2.1, 4.5, 8.6 and 22.3 mg/kg b.w. per day for males, and to 0, 1.1, 2.7, 6.0, 12.3 and 26.3 mg/kg b.w. per day for females) or 0, 7.4, 18.5, 37, 74 or 185 mg/kg diet (equivalent to 0, 0.5, 1.4, 2.8, 5.5 and 14.2 mg/kg b.w. per day for males and to 0, 0.6, 1.6, 3.2, 6.6 and 17.9 mg/kg b.w. per day in females). Body weights were affected at the highest dose level of AA (both administrations), as well as hind-leg paralysis. Four out of eight females administered 1.41 mM/L also displayed hind-leg paralysis. Radiculoneuropathy involving the sciatic nerve and lumbar spinal cord was observed in all rats treated via drinking water or dietary with the high dose of AA. A low incidence of radiculoneuropathy was also noted in females fed 74 mg AA/kg diet. The neuronal degenerative changes were accompanied, at times, by atrophy in skeletal muscle of the hind-limb and luminal dilation of the urinary bladder. All rats treated with 3.52 mM/L AA displayed increased hemosiderin pigment in their spleens and hyperplasia of red blood cell precursors in their bone marrow. Two of eight male rats fed 185 mg AA/kg diet also had increased hemosiderin pigment in their spleens. Degeneration of the germ cells in the testes was observed in all male rats given 3.52 mM and 1.41 mM AA and in five of eight male rats treated with 0.70 mM AA, and in all dose groups of male rats fed diet containing AA, with the incidence increasing with increasing dose. The NOAEL was 0.35 mM for exposure via drinking water, equivalent to 2.1 mg/kg b.w. per day, and the LOAEL was 7.4 mg/kg diet, equivalent to 0.5 mg/kg b.w. per day.

In the 2-year study (NTP, 2012), groups of 48 male and 48 female F344/N rats were administered AA *ad libitum* in drinking water at concentrations of 0.0875, 0.175, 0.35 and 0.70 mM (equivalent to 0.33, 0.66, 1.32 and 2.71 mg/kg b.w. per day for males, and 0.44, 0.88, 1.84 and 4.02 mg/kg b.w. per day for females). Control animals received the same tap water with no chemical added. No effect on the male survival was observed. For females, a decreased survival compared to control was observed in all groups except at the lowest dose. A significant dose-related decreasing trend in body weight was observed for both male and female rats. Feed consumption was in general not affected while water consumption was increased at later time points in female rats. Degeneration of the retina in the eyes of both sexes was observed at the highest dose levels, and for females also at the second highest dose level. A dose-related increasing trend in axonal degeneration of the sciatic nerve in male and female rats was reported, being significant at the highest dose administered (Table 20). Mean severity of the sciatic nerve axon degeneration was not dose-related and was minimal-mild in all dose groups. The doses of AA used in the chronic study did not produce hind-leg paralysis in either male of female rats. In male rats, a significant increased prevalence of duct ectasia in preputial glands was observed at all but the lowest dose. In female rats, the following nonneoplastic effects were observed: lesions involving the adrenal cortex (significant at the highest dose administered), an increased prevalence of excessive hematopoietic cell proliferation in the spleen, bone marrow hyperplasia and ovarian atrophy, the last two significant at the two highest dose levels. The NOAEL for the non-neoplastic effects was 0.0875 mM, equivalent to 0.33 mg/kg b.w. per day, based on the increase of duct ectasia in preputial glands observed at 0.175 mM. The neoplastic findings of this study are described under Section 7.3.3.1.

Table 20: Incidences of peripheral nerve (sciatic) and tibial nerve degeneration in F344 rats exposed to AA in drinking water for 2 years (Johnson et al., 1986; Friedman et al., 1995; NTP, 2012)

Endpoint	Gender	Dosage (mg/kg b.w. per day)	Incidence	Reference
Tibial nerve degeneration in F344 rats (slight and moderate)	Males	0 0.01 0.1 0.5 2.0	27/60 (45 %) 27/60 (45 %) 33/60 (55 %) 32/60 (53 %) 33/60 (55 %)	Johnson et al. (1986)
Tibial nerve degeneration in F344 rats (slight and moderate)	Females	0 0.01 0.1 0.5 2.0	3/60 (5 %) 7/60 (12 %) 5/60 (8 %) 7/60 (12 %) 16/60 (26 %)	Johnson et al. (1986)
Sciatic nerve degeneration in F344 rats	Males	0[(a)] 0 0.1 0.5 2.0	30/83 (36 %) 29/88 (33 %) 21/65 (32 %) 13/38 (34 %) 26/49 (53 %)	Friedman et al. (1995)
Sciatic nerve degeneration in F344 rats	Females	0[(a)] 0 1.0 3.0	7/37 (19 %) 12/43 (28 %) 2/20 (10 %) 38/86 (44 %)	Friedman et al. (1995)
Peripheral nerve (sciatic) axonal degeneration in F344/N rats	Males	0 0.33 0.66 1.32 2.71	5/48 (10 %)* 7/48 (15 %) 7/48 (15 %) 11/48 (23 %) 23/48 (48 %)**	NTP (2012)[(b)]
Peripheral nerve (sciatic) axonal degeneration in F344/N rats	Females	0 0.44 0.88 1.84 4.02	4/48 (8 %)* 3/48 (6 %) 1/48 (2 %) 4/48 (8 %) 19/48 (40 %)**	NTP (2012)[(b)]

(a): The control groups were split into two groups to better establish the variability of low-incidence of background tumours (Friedman et al., 1995).
(b): The concentrations in drinking water correspond to daily consumed doses over the entire 2-year study of 0, 0.0875, 0.175, 0.35 and 0.70 mM.
* Significant dose-related trend ($p < 0.001$).
** Significantly different from control ($p < 0.001$).

NTP also conducted a 13-week and a 2-years study in F344/N Nctr rats administered GA in drinking water (NTP, 2013, draft report). For the 13-week study, rats were administered GA in the drinking water at doses of 0.0, 0.14, 0.35, 0.70, 1.41 or 3.52 mM (or 0, 12.2, 30.6, 61.2, 122 or 306 mg/L, corresponding to approximately 0, 1.10, 2.75, 5.51, 11.0, 27.5 mg/kg b.w. per day). Male rats at the two highest doses showed a reduction in the body weight. At the highest dose of 3.52 mM, all rats showed hind-leg paralysis and low incidence of radiculoneurophathy involving the sciatic nerve and lumbar spinal cord, accompanied at times, by atrophy in the skeletal muscle of the hindlimb and urinary bladder dilatation. Degeneration of the germ cells in the testes was observed in all male rats given 0.70, 1.41 or 3.52 mM GA. A lower incidence of this lesion was also detected in all other doses of GA. The LOAEL was 0.14 mM, equivalent to 1.1 mg GA/kg b.w. per day, based on the testicular germ cell degeneration observed at the lowest dose.

In the 2-year study, groups of 48 male and 48 females rats were administered GA *ad libitum* in drinking water at 0, 0.0875, 0.175, 0.35 or 0.70 mM (equivalent to 0.39, 0.79, 1.56 or 3.34 mg/kg b.w. per day for males, and to 0.54, 1.08, 2.23 or 4.65 mg/kg b.w. per day for females) (NTP, 2013, draft report). A significant dose-related decreasing trend in body weight in both sexes was observed. No effects in food and water consumption were reported. A decreased survival compared to controls was

reported at the two highest dose levels for both sexes. There was no hind-leg paralysis observed for any dose in this study and axonal degeneration of minimal severity was observed in the lumbar spinal cord only in female rats treated with the highest dose of GA. A dose-related increase in fibrosis in the spleen was observed (for male rats significant at all doses except the lowest one, and for females at the highest dose level only). In addition, for male rats the following lesions were observed: exfoliated germ cells epididymes, hepatocytes degeneration and liver necrosis, and for females: bone marrow hyperplasia, mesenteric lymph node cellular infliltration, pituitary gland hyperplasia, axonal degeneration of the lumbar spinal cord, and uterine endometrial hyperplasia. The NOAEL for non-neoplastic effects was 0.0875 mM, equivalent to 0.39 mg GA/kg b.w. per day, based on the increases in fibrosis in the spleen and bone marrow hyperplasia observed at 0.175 mM. The neoplastic findings of this study are described under Section 7.3.3.1.

Rawi et al. (2012) investigated the hematological, biochemical, neurological and histopathological effects of AA on immature male and female rats given 0 (control) or 15 mg AA/kg b.w. per day for 28 days. The results obtained indicate that AA administration induced some behavioral disorders in the movement of immature male and female rats as well as loss of body weight. AA treatment also induced a significant decrease in hemoglobin, erythrocytes, hematocrit and lymphocyte levels of young female rats. AA significantly increased serum glucose, total cholesterol and triglycerides concentrations of both immature male and female rats. In the immature rats AA also caused a significant increase in the total urea concentration. Moreover, AA induced a marked increase in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the immature male and female rats. The activities of serum alkaline phosphatase (ALP) and acetylcholinesterase (AChE) were significantly decreased in both treated groups. In addition, AA caused a significant increase in norepinephrin, glutamate, aspartate and taurine, while it reduced dopamine and serotonin levels.

Toker et al. (2013) investigated serum homocysteine, arginine, citrulline and asymmetric dimethyl arginine levels and conducted a histopathological examination of the abdominal aorta in rats given AA via drinking water at dose levels of 2 or 5 mg/kg b.w. per day for 90 days. At the highest dose level, serum homocysteine, citrulline and asymmetric dimethyl arginine levels were significantly higher than in controls. Serum levels of citrulline were also significantly increased at the lowest dose level. Histopathological examination of the abdominal aorta revealed degeneration of the external elastic lamina in rats at the highest dose level. The authors concluded that long term ingestion of high dose AA with food might contribute to the development of atherosclerosis.

Nurullahoglu-Atalik et al. (2013) evaluated the influence of AA on urinary bladder responses to carbachol and potassium chloride. Animals of each gender were segregated into three groups each containing 6 animals, one control group and two groups treated with AA at 2 or 5 mg/kg b.w. per day for 90 days. In rats treated with AA, the EC_{50} values of carbachol and KCl, but not the maximal response to both agents, were significantly higher than in control group. Histopathological parameters such as edema, congestion, inflammatory cells, microvascular proliferation, fibrosis, eosinophils, mast cells and epithelial damage were all higher in the AA-treated groups than in the controls treated with carbachol or KCl. The authors concluded that the results demonstrate that AA-treatment can induce urinary bladder injury.

7.3.2.3. Cats

McCollister et al. (1964) carried out a feeding study in groups of cats of unknown origin (2 cats per AA dose group) receiving 0 (control), 0.03, 0.1, 1, 3 or 10 mg/kg b.w. per day AA by dietary administration 5 days per week up to 1 year. In the 10 mg/kg b.w. per day group progressive hindlimb weakness was noted over a period of 52 days leading to complete hind limb paralysis. In the 3 mg/kg per day dose group, hind limb weakness was observed after 26 days progressing over the whole treatment period. In the 1 mg/kg b.w. per day group, the same symptoms were seen at 26 days but disappeared later on. Lower dose levels did not result in any observed effects. There were no pathological abnormalities attributable to AA at any exposure level. However the animals in this study

were in poor condition and several animals died due to intercurrent infections. The CONTAM Panel also noted the limited number of animals included in the study and concluded the study was of too limited quality to derive a NOAEL.

A group of 17 cats of unknown breed were administered AA at a dose level of 15 mg/kg b.w. per day by dietary administration for 7 days per week up to 16 weeks (Post and McLeod, 1977). A control group of 23 animals received control diet. In the exposed group abnormal gait (hindlimbs) was observed within 4 to 6 weeks. From 12 to 16 weeks animals were unable to walk and showed weight loss and diarrhea. Motor conduction velocity in several nerves was significantly reduced and the amplitude of externally recorded muscle action potential for foot muscles as well as the action potential in the greater splanchnic nerve were reduced. Fibre density of large diameter nerve fibres in the left gastrocnemius muscle and small fibres in the vagus nerve and greater splanchnic nerves were reduced. Histophatological examination of several nerve fibres showed a reduced number of myelinated fibres. Electron microscopy revealed an increased density of neurofilaments and abnormal membraneous configurations between the axolemma and Schwann cell membrane. Degenerating fibres of the gastrocnemius muscle, loss of myelin for the splanchnic nerve and signs of degeneration for unmyelinated fibres were also reported.

7.3.2.4. Dogs

Fourteen dogs received AA at a dose level of 7 mg/kg b.w. per day by dietary administration for about 10 weeks (Satchell and McLeod, 1981). No control animals were included in the study. The dogs showed severe impairment of hindlimb function, 'toe-folding', ataxia, muscle weakness, and regurgitation.

Four dogs received 6 mg/kg b.w. per day AA via gelatin capsules for 6-7 weeks, followed by up to 8 weeks recovery (Hersch et al., 1989). Parameters of the animals pre-exposure served as control. Animals showed loss of use of hindlimbs and 'toe-folding'. Several respiratory parameters were also affected but were restored during the recovery period.

7.3.2.5. Hamsters

Imai and Kitahashi (2014) reported a 13-week toxicity study of AA administered in drinking water to Syrian hamsters, of which the authors indicated that they are sensitive to the induction of pancreatic ductal carcinogenesis. Male and female hamsters were exposed to AA at concentrations in drinking water required to provide 0 (control), 20, 30 and 50 mg/kg b.w. per day. At 50 mg/kg b.w. day, AA treatment caused abnormal gait advancing to hind limb paralysis in all males and females. Body weights at the two highest dose levels in male and the highest dose level in females were reduced. Microscopically, a dose dependent axonal/myelin degeneration of sciatic nerves was observed in all AA-treated groups. No obvious changes were found in pancreatic ducts/ductules in any group of animals.

7.3.2.6. Monkeys

Non-human primate female monkeys (species not specified, 1 animal per group) received 0 (control), 0.03, 0.1, 0.3 (2 animals), 1, 3 or 10 mg /kg b.w. per day AA by oral gavage or dietary administration 5 days per week for up to 1 year (McCollister et al., 1964). The animal in the highest dose group showed clear and severe clinical signs of neuropathy. At 3 mg/kg b.w. per day occasional abnormalities were reported. Due to limited reporting and the use of only one animal per dose groups clear conclusions cannot be derived from this study.

Four macaque monkeys were given 10 mg/kg b.w. per day AA in fruit juice for 5 days per week and 44-61 days until the onset of clinical signs of toxicity and two control animals received tap water for about 13 weeks (Maurissen et al., 1983). Animals were allowed to recover and examined up to 146 days. Body weight loss occurred in 3 of 4 exposed animals and 1 of 2 control animals. Clinical signs of toxicity detected included loss of balance, deceased activity, hindlimb weakness and forelimb tremor. All clinical signs (except the forelimb tremor) resolved within 2 weeks post treatment. A

decreased sensitivity towards a vibration stimulus was noted during the treatment phase. An increased time taken to pick up a food reward was observed in exposed animals towards the end of the treatment period. Sural nerve biopsies revealed no visible axons in some areas and myelin that had formed balls. Light microscopical evaluation of nerve fiber showed some abnormalities.

In a second study, three adult macaque monkeys were given 10 mg/kg b.w. AA in fruit juice for 5 days per week during 6-9 weeks and two additional monkeys served as controls (Maurissen et al., 1990). After a 30 week recovery period the animals were exposed a second time. Changes in body weight, time taken to pick up a food reward, response to an electrical stimulus and response to a vibration stimulus were essentially similar as reported in the earlier study described above (Maurissen et al., 1983).

Seven macaque monkeys were administered 10 mg/kg b.w. per day AA in fruit juice for 5 days per week and 13 weeks followed by a 20-30 weeks recovery period and two additional monkeys were used as control (Eskin et al., 1985). Histopathological examination of brain sections, optic nerve and eyes revealed various adverse effects including distal axonal swelling, disproportionally thin myelin sheaths, degeneration of myelin and shrunken axons. Swellings were not seen in the retinal nerve fiber layer and there were very few in the optic nerve.

Three adult macaque monkeys were administered 10 mg/kg b.w. AA in fruit juice 5 days per week for 6 to 10 weeks and one control animal was included in the study (Merigan et al., 1982). Induction of ataxia was pronounced. After 4 weeks of AA exposure a marked increase in cortical evoked potential was observed which preceded a decrease in visual acuity and flicker-fusion frequency which were apparent 2 weeks later. Towards the end of the treatment period a marked increase in time taken for a pick-up test was observed. AA exposure also resulted in weight loss, hindlimb weakness, gait disturbances and tremors.

Three macaque monkeys were administered AA at 10 mg/kg b.w. in fruit juice for 5 days per week and about 6-10 weeks with one animal used as control (Merigan et al., 1985). Parameters for visual capacity were evaluated. Reduced contrast sensitivity, impaired visual acuity, reduced flicker-fusion frequency, and impaired visual evoked potentials were noted. The authors suggested that the effects observed related to a conduction block in large-diameter optic nerve fibers.

7.3.2.7. Summary of repeated dose toxicity

The repeated dose toxicity of AA has been investigated in various animal models, including rats, mice, cats, dogs, hamsters and monkeys, and by numerous dosing regimens and durations of dosing. Adverse effects reported in all these species consisted of loss of body weight and neurotoxicity reflected by hind-limb paralysis, reduction in rotorod performance and/or histopathological changes in peripheral nerves and CNS structures.

In mice, effects reported in addition to the neurotoxicity consisted of effects on the testes, including degeneration of epithelia in spermatids and spermatocytes, reduction of spermatozoa and the presence of multinucleate giant cells, as well as forestomach hyperplasia, hematopoietic cell proliferation of the spleen and preputial gland inflammation, lung alveolar epithelium hyperplasia and cataract and for female mice ovarian cysts.

In rats, effects reported in addition to the neurotoxicity, included atrophy of skeletal muscle, testicular atrophy, distended urinary bladders, increased prevalence of duct ectasia in preputial glands, hematopoietic cell proliferation in the spleen, bone marrow hyperplasia, ovarian atrophy, degeneration of the retina in the eyes, exfoliated germ cells epididymis, hepatocyte degeneration and liver necrosis, bone marrow hyperplasia, mesenteric lymph node cellular infiltration and pituitary gland hyperplasia.

The 13-week and 2-year NTP studies in mice and rats dosed with GA revealed adverse effects that were generally similar to those reported for AA, including body weight reduction, cataracts, increasing trend in epithelial hyperplasia of the forestomach, hematopoietic cell proliferation of the spleen, ductal

dilation and inflammation of the preputial gland, and for females ovarian cysts, hepatic angiectasis and necrosis, and axonal degeneration of the cervical spinal cord atrophy in the skeletal muscle of the hindlimb and/or urinary bladder dilatation. In addition, for male rats exfoliated germ cells epididymes, hepatocytes degeneration and liver necrosis, and for females: bone marrow hyperplasia, mesenteric lymph node cellular infliltration, pituitary gland hyperplasia, axonal degeneration of the lumbar spinal cord, and uterine endometrial hyperplasia were reported.

Rats were more sensitive to the neurotoxic effects of AA and GA than mice, and neurotoxicity in rats, such as hind-leg paralysis and peripheral neuropathy, was consistently associated with lower doses and greater severity from treatment with AA when compared to equimolar concentrations of GA.

7.3.3. Genotoxicity

The genotoxicity of AA, as well as of its reactive metabolite epoxide GA, has been studied extensively. It has been reviewed by IARC (1994), JECFA (FAO/WHO, 2002, 2006, 2011), EFSA (2008), UK Committee on Mutagenicity of Chemicals in Food, Consumers Products and the Environment (COM, 2009), US-EPA (2010), ATSDR (2012) and Dearfield et al. (1995) and Carere (2006). A summary of the genotoxicity of AA and GA is reported hereafter and the more recent studies are described in more detail.

7.3.3.1. *In vitro* genotoxicity of AA and GA

Table 21 summarises the experimental design and results of recent *in vitro* gene mutation tests, Comet assays, micronucleus tests, chromosomal aberration tests and SCE assays with AA and GA.

In general, AA did not induce reverse mutations in bacterial gene mutation assays with and without activation. The available literature includes the use of strains of *Salmonella typhimurium* (Ames test), *Escherichia coli* and *Klebsiella pneumonia* (see ATSDR, 2012). One exception was a weakly positive result in a study where TA98 and TA100 strains were used, but only with S9 activation (Zeiger et al., 1987). The absence of mutagenic effects in presence of a metabolic activation system (rat liver S9) is very likely related to the scarce presence or lack in the S9 mix of the specific isozyme (the P450 CYP2E1) capable of metabolizing small hydrophilic molecules like AA. Therefore, the conversion of AA to GA will not be in sufficient quantities for an effect to be detectable in the Ames test. Negative results have also been obtained in *S. typhimurium* YG7108pin3ERb5 (a strain engineered to express CYP2E1). However, a number of compounds which should have been mutagenic following activation by CYP2E1 also gave negative results suggesting that the test system may not be adequate (Emmert et al., 2006). AA did not cause gene mutations in umu *S. typhimurium* strain OY1002/2E1 (a strain that does express human CYP2E1, reductase and bacterial O-acetyl-transferase) in the absence of exogenous metabolic activation, or in its parental strain *S. typhimurium* strain TA1535/pSK1002 (a strain that does not express these enzymes) either with or without exogenous metabolic activation. GA clearly produced a dose-related increase in mutations in *S. typhimurium* strain TA1535/pSK1002 in the absence of exogenous metabolic activation (Koyama et al., 2011b). GA induced gene mutations in *Salmonella typhimurium* strains TA1535 and TA100 with and without metabolic activation (S9 mix) (Hashimoto and Tanii, 1985), but not in *Klebsiella pneumonia* (Voogd et al., 1981).

AA showed equivocal, negative or weakly positive results in mammalian gene mutation assays (TK locus mouse lymphoma cells or HPRT locus V79 cells), whereas GA was clearly positive (Baum et al., 2005, 2008; Thielen et al., 2006; ATSDR, 2012) (see Table 21).

In human lymphoblastoid TK6 cells AA was negative in the absence of metabolic activation in a first TK assay but statistically positive in the second (at cytotoxic concentrations), whereas GA was significantly genotoxic even at concentrations that were not severely cytotoxic (Koyama et al., 2006). Two distinct phenotypic classes of TK mutants were detected: normally growing (NG) and slowly growing (SG) mutants which are associated with large-scale mutagenic effects. AA did not affect the proportion of SG mutants, while GA treatment lowered it. This implies that GA induced primarily point mutations. Genomic DNA extracted from the mutants was subjected to the PCR-based LOH

(loss of heterozygosity) analysis thus allowing the classification of the mutants into 3 types: non-LOH, hemizygous LOH and homozygous LOH. In general, hemi-LOH is the result of deletion and homo-LOH of inter-allelic homologous recombination. The fraction of hemi-LOH in AA-induced mutants was higher than in spontaneous mutants, indicating that AA induced primarily deletions. GA induced primarily NG mutants and most of them were the non-LOH type, which is presumably generated by point mutations and other small intragenic mutations. These results indicate that the genotoxic characteristics of AA and GA are distinctly different: AA is clastogenic and GA is mutagenic. The cytotoxicity and genotoxicity of AA were not enhanced by metabolic activation (rat liver S9), implying that the rat liver S9 did not activate AA.

Koyama et al. (2011b) reported a weakly positive result for gene mutation in human lymphoblastoid cell lines (TK6, h2E1v2 (a cell line that overexpresses human CYP2E1) and AHH-1 (its parental cell line) at high AA concentrations (> 10 mM) and in the absence of exogenous activation. In assays with the TK6 cell line with exogenous activation, it was noted that human liver microsomes induced a stronger positive response than rat liver S9 mix, however the induction of mutations was still weak. GA induced a dose-dependent increase of TK mutations starting from the lowest concentration of 0.5 mM. AA induced trace amounts of N7-GA-Gua adducts in TK6 cells (with and without S9) (about 22 N7-GA-Gua/10^8 nucleotides at 14 mM AA without S9 and up to 40 N7-GA-Gua/10^8 nucleotides at 10 mM AA with S9) and in AHH-1 (about 22 N7-GA-Gua/10^8 nucleotides at 1.4 mM AA without S9) and h2E1v2 cells (about 15 N7-GA-Gua/10^8 nucleotides at 1.4 mM AA without S9), whereas GA induced a substantial number of N7-GA-Gua adducts in TK6 cells (about 1100 N7-GA-Gua/10^8 nucleotides at 4.8 mM GA). These results suggest that genotoxicity is associated with DNA adduct formation and that AA was not metabolically activated to GA *in vitro*.

A concentration related increase in mutation frequency in the cII transgene assay was observed in cultured Big Blue mouse embryonic fibroblasts following exposure to AA (3.2, 32 and 320 µM) (Besaratinia and Pfeifer, 2003). The mutations may be ascribed to AA DNA-adduct-inducing property. Specific mutations were induced (an excess of A→G transitions and G→C transversions).

Besaratinia and Pfeifer (2004) compared the mutagenicity and DNA adduct formation by AA and GA in normal human bronchial epithelial cells and Big Blue mouse embryonic fibroblasts that carry a λ phage cII transgene. Human and mouse cells were treated *in vitro* with water, AA (3.2, 32 and 320 µM) or GA (50 and 500 nM, 5, 50, 500 µM and 5 mM) and then subjected to terminal transferase-dependent PCR to map the formation of DNA adducts within the p53 gene and the cII transgene. DNA adduct formation was higher after GA than AA treatment at all doses tested and occurred at similar specific locations within p53 and cII. It is of note that AA induced adduct formation was saturable suggesting that the conversion of AA to DNA reactive intermediates is limiting whereas the GA adduct formation was dose-dependent. Treatment with either AA or GA led to significant increases in mutation frequency and GA was more mutagenic than AA at all doses tested. The spectrum of GA induced cII mutations was statistically significantly different from the spectrum of spontaneously occurring mutations in the control cells. Compared with spontaneous mutations in control cells, cells treated with AA or GA had more A→G transitions and G→C transversions and GA-treated cells had more G→T transversions as distinct mutational signature. The mutational specificity of GA may reflect differences in the pathways of DNA adduct formation between AA and GA. However, the overall mutational spectra of GA and AA are consistent with N7-GA-Gua as predominant DNA adduct induced by both chemicals and N3-GA-Ade and N1-GA-dA as minor adducts induced by GA and AA, respectively. These adducts may be mutagenic either by depurination or by inducing directly mispairing. The overlapping in the location of mutations induced by AA and GA supports the idea that GA-induced adducts are involved in AA-induced mutagenesis.

Ao et al. (2008) examined HPRT mutation frequencies in HL-60 and NB4 leukemia cell lines exposed to AA. A significant increase in mutation frequency was only observed at the highest concentration (10 mM = 700 mg/L). The mutation spectrum was different from the spontaneous mutation spectrum in control cells. The most frequent spontaneous mutations were point mutations, whereas AA-induced mutations were mainly single exon deletions besides point mutations, and an increase in the proportion

of partial deletions was associated with the increase of AA treatment. There was no difference in mutational spectra between the two cell lines. There was no evidence of metabolic competency of these cell lines.

Mei et al. (2008a) evaluated the mutagenic potential of 4 h treatment with AA (2-18 mM) and GA (0.125 to 4 mM) on the TK locus in L5178Y mouse lymphoma cells. DNA adduct formation, mutant frequencies and the types of mutations were examined. Dose-dependent increases for both cytotoxicity and mutagenicity were induced by AA or GA treatment. Both small and large colony TK mutants were induced by both chemicals. GA was much more mutagenic than AA. To determine the types of mutations, loss of heterozygosity analysis of mutants was conducted using 4 microsatellite loci spanning the entire chromosome 11. Compared to GA-induced mutations, AA induced more mutants whose LOH extended to more than half of the chromosome. Statistical analysis of the mutational spectra revealed a significant difference between the types of mutations induced by AA and GA treatments. GA induced DNA adducts of adenine and guanine (N3-GA-Ade and N7-GA-Gua) in a linear dose-dependent manner. The levels of guanine adducts were consistently about 60-fold higher across the dose range than those of adenine. In contrast, no GA-derived DNA adducts were found in the cells treated with any concentrations of AA, consistent with a lack of metabolic conversion of AA to GA. Based on these results, the authors suggested that although both AA and GA generate mutations through a clastogenic mode of action in mouse lymphoma cells, GA induces mutations via a DNA adduct-mediated mechanism whereas AA induces mutations by a mechanism not involving the formation of GA adducts.

Johansson et al. (2005) investigated the mutagenicity and DNA repair of GA-induced adducts in Chinese hamster cell lines deficient in base excision repair (BER) (EM9), nucleotide excision repair (NER) (UV4) or homologous recombination (irs1SF) in comparison to parent wild-type cells (AA8). The DRAG (detection of repairable adducts by growth inhibition) assay, monitoring growth inhibition and reduced survival in these cell lines, was used to provide information of the type of DNA repair pathways needed for sustained growth after GA treatments. A DNA repair deficient cell line is expected to be affected in growth and/or survival more than a repair proficient cell line. The alkaline DNA unwinding (ADU) assay was used to investigate induction of SSBs. The *hprt* assay was applied to assess mutagenic potential in an endogenous locus. The authors reported a significant induction of mutations by GA in the *hprt* locus of wild-type cells but not in BER deficient cells. The authors emphasized the low mutagenic effect of GA in the assay in wild-type cells. The detection of mutations in BER-deficient cells could be hampered by the high cytotoxicity observed when compared to wild-type cells. Cells deficient in homologous recombination or BER were three or five times, respectively, more sensitive to GA in terms of growth inhibition than were wild-type cells. A similar dose-dependent increase in the level of SSBs was observed in wild-type (AA8) versus XRCC1 defective (EM9) cells (although the authors claim that there is an enhanced levels of SSB in EM9 cells but do not provide adequate statistical analysis) and the SSB level was not modulated by the PARP inhibitor 1,5-isoquinolinediol (ISQ). An apparently increased level of SSB was observed in the NER defective (UV4) cells as compared to AA8 cells but no effect on SSB level was detected when using inhibitors of replicative DNA polymerases. Although on the basis of these data the authors concluded that short-patch BER is involved in GA-induced repair, the CONTAM Panel noted that these data do not allow identifying the pathway involved in the repair of GA-induced lesions. In conclusion, this study suggests that BER and homologous recombination are involved in the cytotoxic processing of GA-induced lesions but what type of lesions are their substrate and/or lead to mutagenesis cannot be concluded from these results.

In mammalian cells, AA induced chromosomal aberrations, micronuclei (containing whole chromosomes or acentric fragments), sister chromatid exchanges (SCE), polyploidy, aneuploidy and other spindle disturbances (e.g. c-mitosis) in the absence of metabolic activation (Baum et al., 2005; Koyama et al., 2006, Jiang et al., 2007; Martins et al., 2007; Katic et al., 2010; ATSDR, 2012; Pingarilho et al., 2013) (Table 21). GA induced also chromosomal aberrations, micronuclei or SCE (Table 21), however at lower concentrations that are not severely cytotoxic. These data confirm that AA is a direct acting clastogen in mammalian cells *in vitro*, and is also an aneugen.

Pingarillo et al. (2013) studied the induction of SCE by AA and GA in human lymphocytes (see Table 21). In addition, the authors studied the role of individual genetic polymorphisms in key genes involved in detoxification and DNA-repair pathways (BER, NER, HRR and NHEJ) on the induction of SCE by GA. They compared the induction of SCE at a dose of 250 µM GA (a dose inducing an increase in SCEs of approximately 4-5 fold when compared with non-treated control lymphocytes) in cultured lymphocytes from 13 donors. The results show that lymphocytes from certain donors clearly responded to a GA insult to a lesser extent that did other donors. GSTM1 and GSTT1 deletion polymorphisms did not influence the level of SCE induced by GA. Conversely, for GSTP1 Ile105Val, lymphocytes from wild-type individuals have a higher level of GA-induced SCE than those with at least one variant allele. For GSTA2 Glu210Ala the level of SCE was lower for lymphocytes of heterozygous individuals when compared with wild-type homozygous individuals. For the DNA-repair pathways studied, no associations with the level of GA-induced SCE were found. By combining DNA damage in GA-treated lymphocytes and data on polymorphisms, the authors suggested associations between the induction of SCEs with GSTP1 (Ile105Val) and GSTA2 (Glu210Ala) genotypes in this exploratory study.

Koyama et al. (2011b) exposed human lymphoblastoid cell lines (TK6, AHH-1, and h2E1v2 lines) to AA at concentrations in the range of 5-15 mM (TK6 cells) or up to 3 mM (AHH-1 and h2E1v2 cells). The micronucleus assay in TK6 cells was performed with and without exogenous S9 mix. AA induced micronuclei in the absence, but not in the presence, of S9. A weak induction of micronuclei was observed in the AHH-1 and h2E1v2 cell lines that were assayed only in the absence of exogenous metabolic activation.

Several studies were conducted to investigate DNA damage induced by AA or GA by using the Comet assay (Table 21). Negative results have been reported in TK6 cells or human lymphocytes treated with AA, while GA indicated clearly positive results (Baum et al., 2005, 2008; Koyama et al., 2006; Thielen et al., 2006). The only exception is a positive result that was observed in human HepG2 cells treated for 1 h with 2.5, 5, 10 and 20 mM AA (Jiang et al., 2007). Several studies have shown that after post-treatment with the DNA repair enzyme formamidopyrimidine-DNA-glycosylase (fpg), DNA damage induced by GA became detectable at much lower doses than in the absence of the fpg treatment (Thielen et al., 2006; Baum et al., 2008). Disappearance of DNA strand breaks was measured after 4h treatment with 100 and 800 µM GA (concentrations that have been shown to induce significant DNA damage in the Comet assay). Within 8h, strand breaks induced by GA (100 µM) decreased by 80 %. The authors indicated that this is probably the result of efficient BER (Baum et al., 2008).

Galdo et al. (2006) investigated the induction by AA of DNA damage in rat thyroid cell lines PC Cl3 (more stable and normal karyotype) and FRTL5 (cells displaying a high level of aneuploidy which could make them more prone to DNA alterations) and in thyrocytes primary cultures from dog, human and sheep. The detection of DNA damage was done by the Comet assay under alkaline conditions. AA treatment (14 µM, 3h) increased significantly the number of PC Cl3 cells with a comet. In addition, dose-related increases were observed after 3 h exposure from 10 µM to 3 mM AA. In FRTL5 cells, an overnight treatment with 140 µM AA increased approximately twice the percentage of cells showing a comet; in dog, sheep and human thyrocyte primary cultures, similar increases were observed after overnight exposure to 1 mM AA. Dose-related increases in the % of comet were also observed in human thyrocytes after 48h exposure from 10 µM to 3 mM. The positive control H_2O_2 also induced DNA damage in these different cells. The authors also investigated the persistence of DNA damage. The damage induced by H_2O_2 was transient; H_2O_2 being quickly degraded by the cells and the damage induced being quickly repaired. In contrast, no degradation of AA by the cells was observed and no repair of the DNA lesions induced by AA occurred. The effect of AA, GA and etoposide were studied on H2AX phosphorylation by using an anti-phosphohistone H2AX (ser 139) antibody. Etoposide, an inhibitor of topoisomerase II, increased the phosphorylation of histone H2AX measured after 1h incubation, whereas AA (140 µM, 17 hours) and H_2O_2 were without effect. The effect of AA (14 or 140 µM) and GA (115 µM) was also studied at shorter times (15, 30 or 60 min) and longer time of action (3, 17, 40 hours). No effect was observed although in each experiment a strong effect of

4308 etoposide was always present. The authors concluded that the absence of effect of AA on H2AX
4309 histone phosphorylation suggests that the positive Comet assay observed with AA does not reflect the
4310 induction of DNA double strand breaks. The aim of the study was to develop an *in vitro* model for the
4311 tumorigenic action of AA on thyroid cells. The authors concluded also that the effects of AA
4312 demonstrated in this study could explain the *in vivo* tumorigenesis but not its relative thyroid
4313 specificity.

4314 Hansen et al. (2010) investigated the induction and repair of DNA damage induced by AA and GA in
4315 mouse male germ cells (prior to spermatid elongation) and in human and mouse peripheral blood
4316 lymphocytes, to assess inter-species and cell-type differences in DNA damage susceptibility. The
4317 Comet assay was used in combination with the DNA repair enzymes fpg and hOGG1 to measure
4318 specific DNA lesions. Millimolar concentrations of AA (up to 6 mM) did not induce a detectable
4319 increase in DNA lesions (strand breaks and alkali-labile sites) in mouse testicular cells and human
4320 peripheral blood lymphocytes. In contrast to AA, GA induced significant levels of DNA lesions at
4321 doses ≥ 1 mM. Using fpg, the GA-induced DNA damage was measured at 20-50 fold lower
4322 concentrations, in all cell types investigated. GA-induced DNA damage could not be recognised by
4323 hOGG1, suggesting that, based on the known affinities of these repair enzymes, alkylation rather than
4324 oxidation of guanine is involved. Early spermatogenic and somatic cells from mice showed similar
4325 susceptibility to GA. Human lymphocytes showed two-fold higher levels of GA-induced DNA lesions
4326 than mouse cells. The repair of GA-induced DNA lesions was explored with cells from mice either
4327 proficient (Ogg1+/+) or deficient (Ogg1-/-) in Ogg1 (mouse 8-oxoguanine DNA glycosylase). Low
4328 repair of GA-induced fpg-sensitive lesions was observed in primary male germ cells and lymphocytes
4329 from both $Ogg1^{+/+}$ and $Ogg1^{-/-}$ mice, showing that these lesions are highly persistent. The authors
4330 concluded that there may be differences between mice and humans in the susceptibility to AA and
4331 GA-induced DNA damage, and in this study, mouse male germ cells do not appear to be more
4332 sensitive to GA than somatic cells. The authors suggested that the inter-species differences in AA
4333 susceptibility may be related to differences in DNA repair or other cellular defense factors such as
4334 GSH status and activity of glutathione-S-transferases (Hansen et al., 2010).

4335 Oliveira et al. (2009) analyzed the cytotoxicity and clastogenic potential of AA in V79 cells (cells
4336 essentially devoid of CYP2E1 activity). The experiments include the evaluation of the effect of BSO,
4337 an effective inhibitor of GSH synthesis, GSH-monoethyl ester (GSH-EE), a compound that is taken up
4338 by cells and intracellularly hydrolysed to GSH (thus producing an intracellular GSH enrichment), and
4339 also GSH exogenously added to culture medium. Pre-treatment with BSO increased the cytotoxicity
4340 and the frequency of aberrant cells excluding gaps (ACEG) induced by AA. While pre-treatment with
4341 GSH-EE did not modify the cytotoxicity or the frequency of ACEG induced by AA (fail to show a
4342 protective effect of GSH-EE), co-treatment with AA and GSH decreased both parameters. *In vitro*
4343 studies in a cell-free system, using monochlorobimane (MCB), a fluorescent probe for GSH, were also
4344 performed in order to evaluate the role of AA in GSH depletion. Based on these results, the authors
4345 concluded that spontaneous conjugation of AA with GSH in the extracellular medium is involved in
4346 the protection given by GSH, reinforcing the role of GSH in the modulation of the cytotoxic and
4347 clastogenic effects induced by AA.

4348 AA did not induce unscheduled DNA synthesis (UDS) in rat hepatocytes and a weak response in
4349 human mammary epithelial cells, while GA induced a strong UDS response in both cell types
4350 (Butterworth et al., 1992).

4351 AA induced cell transformation in three different mouse cell lines (BALB/c, 3T3, NIH/3T3 and
4352 C3H/10T1⁄2) in the presence as well as in the absence of exogenous metabolic activation (ATSDR,
4353 2012).

4354 Bandarra et al. (2013, 2014) addressed the cytotoxicity, generation of ROS, formation of MN and
4355 induction of specific GA-DNA adducts in human mammary cells exposed to GA. For the cytokinesis-
4356 blocked micronucleus assay, human MCF10A epithelial cells were exposed to GA at concentrations
4357 up to 2 mM. These cells express multiple CYPs, including low levels of CYP2E1. The impairment of

cell proliferation by GA was first evaluated using the % of binucleated cells and the nuclear division index (NDI). A linear concentration-dependent decrease in cell viability was observed from 0.25 to 2 mM GA. The highest concentration tested profoundly affected the cell proliferation indices. The % of binucleated (BN) cells was so low at this concentration (< 10 %) that a correct assessment of MN was precluded. In the presence of GA, a dose-response increase of the frequency of micronucleated BN (MNBN) cells was observed, although a significant increase of (more than 2-fold) was only detected at the highest concentration tested (1 mM). The depurinating adducts, N7-GA-Gua (major) and N3-GA-Ade (minor), were assessed by HPLC-ESI-MS/MS. A linear dose-response relationship was observed for N7-GA-Gua up to 1 mM GA. The adduct was quantified at GA concentrations as low as 1 µM. N3-GA-Ade could only be quantified at GA concentrations ≥ 250 µM due to the fact that it is found at ~ 1 % of the level of N7-GA-Gua. At these very high concentrations, a linear dose-response relationship was also found. By integrating GA adducts, MN and cell viability data, it becomes clear that while the primary DNA damage (i.e. DNA adducts) is evident at low levels of exposure, its consequences, either in terms of MN formation or in decreased cell proliferation kinetics, are only observed at much higher GA concentrations. DNA repair mechanisms may be contributing to this differential outcome. The formation of double stand breaks (DSBs) may be responsible for the clastogenic effects of GA observed using the CBMN assay. The accumulation of DSBs eventually leads to cell-death. In view of this, and with the purpose of understanding DNA repair mechanisms triggered in GA-exposed cells, the authors used a specific inhibitor at the ATM kinase, KU55933. ATM is recruited and activated by DSBs thus playing a controlling role in homologous recombination. When ATM was inhibited, the cytotoxicity of GA in MCF10A cells was significantly increased, suggesting an important role of this kinase and homologous recombination in the repair of GA-induced lesions. The homologous recombination pathway has been reported as a possible repair pathway for GA-induced lesions (Johansson et al., 2005; Martins et al., 2007).

Nixon et al. (2014) examined the mechanisms of AA toxicity in male germ cells *in vitro*. The authors showed that CYP2E1 is expressed particularly in spermatocytes and the expression is up-regulated following exposure to AA. The induction of DNA adducts was investigated using the alkaline Comet assay after exposure of spermatocytes for 18 hours to 1 µM AA or 0.5 µM GA. A significant increase in tail DNA % was observed following GA exposure, however, only a modest effect was found in cells exposed to AA. The inclusion of fpg in the Comet assay led to enhanced detection of DNA damage in both AA and GA treated cells, with slightly higher levels of damage after GA exposure. Further characterisation of oxidative DNA damage using hOGG1 in the Comet assay failed to identify significant increases in the AA treated spermatocytes and only a modest increase was found in GA treated cells indicating that oxidative stress plays a minor role in the induction of this damage. No significant differences in GSH levels were also observed following treatment with either AA or GA. The analysis of damage by the fpg modified Comet assay following treatment of spermatocytes with AA (1 µM) or GA (0.5 µM) in the presence of resveratrol, an inhibitor of CYP2E1, showed that in the case of AA the co-treatment reduced the level of DNA adducts to control levels whereas no effect was observed in the case of GA. These data show that spermatocytes are able to metabolise AA to GA via CYP2E1 and that direct adduction by GA is a major source of DNA damage induced by AA in male germ cells.

4399 **Table 21:** Experimental design and results for selected *in vitro* genotoxicity test with acrylamide (AA) and glycidamide (GA)

Test	Cell system	Dose	Metabolic activation	Exposure time	Result	Reference
Gene mutation test	V79 cells/*hprt*	AA 100-10000 µM C+: MNNG C-: DMSO 0.1 % GA 400-2000 µM C+: MNNG C-: DMSO 0.1%	w/o S9	Incubation time: 24 h	No significant induction of mutations Dose-related significant induction of mutations from 800 µM upwards	Baum et al. (2005)
Gene mutation test	V79 cells/*hprt*	GA: 400-2000 µM C+: NOZ-2 C-: DMSO 0.1 %	w/o S9	Incubation time: 24 h	Dose-related increase mutations from 800 µM	Baum et al. (2008)
Gene mutation test	V79 cells/*hprt*	GA: 400-2000 µM C+: (±)-BPDE and α-A-NDELA	w/o S9		GA: dose-related increase mutations from 800 µM (±)-BPDE and α-A-NDELA: significant increase mutations at 3 µM and 10 µM, respectively	Thielen et al. (2006)
Gene mutation test	Human lymphoblastoid TK6 cells/TK	AA: 2.5-14 mM C-: PBS C+: DBN GA: 0.5-2.2 mM C-: PBS C+: DBN	w and w/o S9	Exposure: 4 h	Negative in 1st experiment. Positive at 14 mM in 2nd experiment. Dose-dependent cytotoxicity (about 20 % RS at high dose) Dose-dependent increase Positive even at non cytotoxic concentrations	Koyama et al. (2006)
Gene mutation test	Human promyelocytic leukemia HL-60 and NB4 cell lines	AA: 50-700 mg/L C-: distilled water	w/o S9	Exposure: 6 h	Dose-related increase of cytotoxicity (decrease plating efficiency): statistically significant at ≥ 50 mg/L in HL-60 and ≥ 300 mg/L in NB4 cells. Dose-related linear increase in mutation frequency: statistically significant at the highest dose in two cell lines (about 5 times higher than in the control)	Ao et al. (2008)

4400 Table continued overleaf.
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4402 **Table 21:** Experimental design and results for selected *in vitro* genotoxicity test with acrylamide (AA) and glycidamide (GA) (continued)

Test	Cell system	Dose	Metabolic activation	Exposure time	Result	Reference
Comet assay (ASCGE)	Human lymphocytes	AA: 1 000 - 6 000 µM C+: bleomycin C-: DMSO 1 % GA: 100-3 000 µM C+: bleomycin C-: DMSO 1 %	w/o S9	Incubation: 1-4 h	Negative Dose-dependent significant increase DNA damages from 300 µM upwards	Baum et al. (2005)
Comet assay (ASCGE) w and w/o fpg treatment	Human lymphocytes	GA: 3-300 µM C+: (±)-BPDE and α-A-NDELA	w/o S9		GA: w/o fpg: DNA damage at 300 µM (4 h) w fpg: significant increase DNA damage at 10 µM (4 h) (±)-BPDE and α-A-NDELA: genotoxic at 30 µM and 10 µM, respectively (1h) Genotoxicity not enhanced by fpg treatment	Thielen et al. (2006)
Comet assay	Human lymphoblastoid TK6 cells	AA: 2.5-14 mM C-: PBS C+: DBN GA: 0.5-2.2 mM C-: PBS C+: DBN	w/o S9	Exposure: 4 h	Negative Positive (from 0.6 mM) even at non cytotoxic concentrations	Koyama et al. (2006)
Comet assay	Human hepatoma G2 cells	AA: 0-20 mM C-: PBS C+: H_2O_2	w/o S9	Exposure: 1h	Dose-related increase in DNA damage from 2.5 mM	Jiang et al. (2007)
Comet assay	Chinese hamster lung fibroblasts V79 cells	GA: 3-300 µM	w/o S9		w/o fpg: statistically significant increase DNA strand breaks at 300 µM after 4 h and at 100 µM after 24 h (detectable at 300 µM after 1h) w fpg: statistially significant increase DNA strand breaks at 10 µM after 4 h	Baum et al. (2008)

4403 Table continued overleaf.
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4405 **Table 21:** Experimental design and results for selected *in vitro* genotoxicity test with acrylamide (AA) and glycidamide (GA) (continued)

Test	Cell system	Dose	Metabolic activation	Exposure time	Result	Reference
Comet assay with and w/o fpg	Mouse spermatocytes	AA: 1 µM GA: 0.5 µM C-: untreated C+: H_2O_2	w/o S9	Exposure: 18 h (5 min for C+)	w/o fpg: modest increase in tail DNA % with AA (about 2 times), significant increase in tail DNA % with GA (about 3-4 times) w fpg: significant increase in tail DNA % with AA and GA (about 9-10 times)	Nixon et al. (2014)
Comet assay with fpg	Mouse spermatocytes	AA: 10 nM- 10 µM GA: 5 nM – 5 µM C-: untreated C+: H_2O_2	w/o S9	Exposure: 18 h (5 min for C+)	Significant dose-related increases in tail DNA % following exposure to ≥100 nM AA or ≥ 5 nM GA	Nixon et al. (2014)
Comet assay with and w/o hOGG1	Mouse spermatocytes	AA: 1 µM GA: 0.5 µM C-: untreated C+: H_2O_2	w/o S9	Exposure: 18 h (5 min for C+)	AA +/- hOGG1: no significant levels of DNA damage GA - hOGG1: modest but significant increase in tail DNA % (about 1.5 x) GA + hOGG1: greater statistically significant increase in tail DNA % (about 2 x)	Nixon et al. (2014)
Comet assay with fpg	Mouse spermatocytes	AA: 1 µM GA: 0.5 µM C-: untreated C+: H_2O_2 Resveratrol: 0.1 µM	w/o S9	Exposure: 18 h (5 min for C+)	AA w fpg: significant increase in tail DNA % (about 2 x control w fpg) GA w fpg: significant increase in tail DNA % (about 1.5 x control w fpg) Resveratrol w fpg: no effect AA + resveratrol w fpg: no effect GA + resveratrol w fpg: significant increase in tail DNA % (about 2 x control w fpg)	Nixon et al. (2014)

4406 Table continued overleaf.
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4408 **Table 21:** Experimental design and results for selected *in vitro* genotoxicity test with acrylamide (AA) and glycidamide (GA) (continued)

Test	Cell system	Dose	Metabolic activation	Exposure time	Result	Reference
Comet assay With hOGG1	Mouse spermatocytes	AA: 1 µM GA: 0.5 µM C-: untreated C+: H_2O_2 Resveratrol: 0.1 µM	w/o S9	Exposure: 18 h (5 min for C+)	AA w hOGG1: significant increase in tail DNA % (about 1.2 x control) GA w hOGG1: no effect Resveratrol: no effect AA + resveratrol w hOGG1: no effect GA + resveratrol w hOGG1: no effect	Nixon et al. (2014)
MN test	Human lymphocytes (15 donors)	AA: 500-5 000 µM C+: bleomycin C-: untreated	w/o S9	Exposure: 23 h	5 000 µM: about 2-fold increase in mean MN frequency (7/15 donors) Overall: no significant increase MN frequency	Baum et al. (2005)
		GA: 50-1000 µM C+: bleomycin C-: untreated			No significant increase MN frequency	
MN test	Human lymphoblastoid TK6 cells	AA: 2.5-14 mM C-: PBS C+: DBN	w/o S9	Exposure: 4 h	Statistically significant positive increase in two experiments	Koyama et al. (2006)
		GA: 0.5-2.2 mM C-: PBS C+: DBN			Dose-dependent increase. Positive even at non cytotoxic concentrations	
MN test	Human hepatoma G2 cells	AA: 0, 0.625, 1.25, 2.5 mM C-: PBS C+: CP	w/o S9	Exposure: 24 h	Dose-dependent increase MN in BNC from 0.625 mM (3-fold increase at high dose)	Jiang et al. (2007)
MN test	Human lymphocytes	AA: 500 µM, 5 and 50 mM	w/o S9	Exposure: 20 h Harvesting at 72 h	Cytotoxicity at 5 and 50 mM Statistically significant increase MNBN at 500 µM (1/2 laboratory)	Katic et al. (2010)

4409 Table continued overleaf.
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4411 **Table 21:** Experimental design and results for selected *in vitro* genotoxicity test with acrylamide (AA) and glycidamide (GA) (continued)

Test	Cell system	Dose	Metabolic activation	Exposure time	Result	Reference
CA	Chinese hamster V79 cells	AA: 250-2 000 µM GA: 1-2 000 µM C+: MMC	w/o S9	Exposure: 16 h Harvesting 2.5 h after end of treatment	AA and GA induced dose-dependent cell death (MTT cytotoxicity assay) GA more cytotoxic than AA (survival values < 5 % at ≥ 4 mM GA and 10 mM AA) Increased CA by AA (especially at 2 000 µm) and GA (especially at ≥ 500 µM)	Martins et al. (2007)
SCE assay	Chinese hamster V79 cells	AA: 250-2 000 µM GA: 1-2 000 µM C+: MMC	w/o S9	Exposure: 27 h Harvesting 2.5 h after end of treatment	GA consistently induces SCE (≥ 10 µM) AA: significant increase SCE/cell at 2 000 µM	Martins et al. (2007)
SCE assay	Human lymphocytes	AA: 0-2 000 µM C-: PBS C+: MMC	w/o S9	Incubation 46 h	Slight increase SCE/metaphase at 2 000 µM	Pingarilho et al. (2013)
		GA: 0-2 000 µM C-: PBS C+: MMC			Marked dose-dependent increase SCE/metaphase up to 750 µM.	

4412 (±)-BPDE: (±)-anti-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide; α-A-NDELA: α-acetoxy-N-nitroso-diethanolamine; ASCGE: alkylating single cell electrophoresis; C+: positive control; C-:
4413 negative control; CA: chromosomal aberrations; fpg: formamido-pyrimidine-DNA-glycosylase; h: hour(s); MN: micronucleous; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium
4414 bromide; SCE: sister chromatid exchange; w: with; w/o: without.

7.3.3.2. *In vivo* genotoxicity of AA and GA

Table 22 summarized the experimental design and results of recent *in vivo* Comet assays, and micronucleus tests with AA.

Induction by AA of structural chromosome aberrations, micronuclei or polyploidy was observed in various studies in mice treated *in vivo* (doses 25-100 mg/kg b.w.) (Witt et al., 2008; ATSDR, 2012). The effects were observed in various tissues: bone marrow, spleen lymphocytes, splenocytes, peripheral blood reticulocytes, erythrocytes, spermatocytes, etc. AA produces chromosome damage in mouse, but not in rat somatic cells (Paulsson et al., 2002; Rothfuss et al., 2010; ATSDR, 2012) (Table 22). However, in some tests an increase in MN frequency has also been reported in rat somatic cells (Witt et al., 2008; Yener and Dikmenli, 2009). Low doses of AA (*i.p.* doses of 0-100 mg/kg b.w. and oral doses of 4-6 mg/kg b.w. per day) have been shown to induce MN in the flow cytometer-based MN assay in mice (Abramsson-Zetterberg, 2003; Zeiger et al., 2009). A clear increase of the frequency of micronucleated erythrocytes was seen. The dose-response was found to be linear with a tendency to have a steeper rise at the lowest doses. This tendency is opposite as compared to what detected with typical aneugenic agents (i.e. colchicine) which have no effect below a threshold dose. A significant dose response increase in the frequency of MNs in PCE was observed within an interval as low as 0-6.5 mg/kg b.w. The low DNA content of the MN from AA-treated mice supports the view that AA has no typical aneugenic effects (Abramsson-Zetterberg, 2003).

Paulsson et al. (2003) showed that GA induces MN in bone marrow cells of mice and rats treated with GA by *i.p.* injection.

Induction of DNA damage by AA as measured by the Comet assay has been reported in several tissues of mice or rats (Manière et al., 2005; Dobrzynska, 2007; Recio et al., 2010; Rothfuss et al., 2010) (Table 22).

Both the presence of DNA damage, measured by the Comet assay, and the formation of N7-GA-Gua and N3-GA-Ade were assessed in selected tissues from rats treated by gavage with 0, 18, 36 or 54 mg AA/kg b.w. (Manière et al., 2005). DNA damage was recorded in blood, brain, bone marrow, liver, testes and adrenal glands. AA induced DNA lesions in blood leucocytes and brain at the two highest doses and in testes at the highest dose 24 hours after exposure. No statistically significant increase in Comet parameters was observed in the other organs. No histopathological findings were observed in liver, brain and testes at the highest dose tested. In order to study a possible earlier increase in DNA damage in organs showing no response in the Comet assay 24 hours after exposure to AA, the extent of DNA migration was recorded 2 and 5 hours after administration of 54 mg AA/kg b.w. A statistically significant increase in DNA damage was observed in leucocytes, bone marrow, liver and adrenals 5 hours after administration of AA and 2 hours after administration in testes and adrenals. For the DNA adduct assay, tissue samples from brain, liver and testes were collected 5, 24, 48 and 72 hours after dosing and samples of blood were taken 5, 24 and 48 hours after dosing of rats treated with mg AA/kg b.w. In rat organs, the N3-GA-Ade DNA adduct was found in much less quantity than the N7-GA-Gua one (about 50-100 fold). After administration of the high dose, N7-GA-Gua was the major DNA adduct detected, at similar levels in brain and liver and at lower levels in testes. The profiles of N7-GA-Gua DNA adducts were similar in all the tested organs, with adduct levels peaking between 5 and 24 hours after dosing and then slightly decreasing until 72 hours. The N7-GA-Gua DNA adduct disappeared slowly from the rat organs, remaining at relatively high levels 3 days after treatment. The formation and elimination rate of N7-GA-Gua DNA adducts in the liver, brain and testes after administration of the low dose were very similar except that the levels reached were about 2-fold lower than with the highest dose of AA. The half-lives for N7-GA-Gua were 50 - 70 hours and 50 - 80 hours at high- and low dose, respectively. For N3-GA-Ade, they were about 20 hours and 20-30 hours, at high and low dose, respectively. The results of this study relate the occurrence of AA-induced DNA damage detected by the Comet assay to the formation of DNA adducts in selected organs and tissues.

A weak, positive result was reported in the mouse spot test after single or 3 daily *i.p.* injections of 0, 50 or 75 mg AA/kg b.w. in pregnant females indicating that AA is able to induce somatic mutations in foetal cells after transplacental absoption of the test compound (IARC, 1994; FAO/WHO, 2002; Carere, 2006).

AA did not cause UDS in the liver of rats receiving single or repeated (5 x) *i.p.* injections of 0, 30 or 100 mg/kg b.w. (see IARC, 1994; FAO/WHO, 2002; ATSDR, 2012).

UDS was measured in early spermatids of mice receiving single *i.p.* injections of 0-125 mg AA/kg b.w. (Sega et al., 1990). The testes were injected with tritiated thymidine either at the time of AA treatment or at later times, up to 48 hours following AA exposure. Sperm were recovered from the caudal epididymes 16 days after treatment. In addition, groups of male mice received a single *i.p.* injection of 0 or 125 mg AA/kg b.w. with tritiated thymidine injected into the testes 6 hours later. Sperm were then recovered from the caudal epididymes at 2 or 3 days intervals between 1 and 30 days after treatment (spermatozoal to early spermatocyte stages). Also, groups of 4 male mice received *i.p.* injections of 46 mg ^{14}C-AA/kg b.w.; DNA was extracted from liver and testes samples 1-24 hours after treatment and analysed for radioactivity. In the first experiment, a clear increase of UDS was noted in the testes with the maximum response occurring 6 hours after tritiated thymidine injection. The UDS response appeared to be linear over exposures up to 125 mg AA/kg b.w. For the second experiment non-significant increases of UDS were observed during the first 10 days after exposure to AA (late spermatid through spermatozoal stages at time of treatment) but an increase of UDS was noted from days 12-27 (early spermatocyte through mid spermatid stages at time of AA treatment). In the third experiment, DNA alkylation was observed (by measuring DNA adduct formation), with the maximum levels 4-6 hours post-administration in the testes and 1-2 hours post-administration in the liver. The DNA alkylation levels in the testes were about 10-fold lower than in the liver. Based on these results, the authors concluded that AA or, more likely a metabolite, is able to interact with DNA and elicit a UDS response in early spermatocytes through mid-spermatid stages (Sega et al., 1990).

UDS induction in hepatocytes and spermatocytes was studied in an *in vivo* test in F344 rats that received single oral doses (by gavage) of 100 mg AA/kg b.w. or repeated (5x) oral doses of 30 mg AA/kg b.w. (Butterworth et al., 1992). A statistically significant increase of UDS was noted in spermatocytes only after repeated administration of 30 mg AA/kg b.w. No UDS response was observed in the liver.

Dominant lethal mutations were induced following administration of AA via oral route, dermal application or *i.p.* injection in rodents (ATSDR, 2012). Exposure via *i.p.* injection was also associated with specific locus mutations in offspring of male mice exposed to 50-125 mg AA/kg before mating to untreated females and in offspring of pregnant female mice exposed to 50 or 75 mg/kg. Heritable or reciprocal translocations were noted in offspring of male mice exposed to 50-100 mg AA/ kg or 100 mg GA/kg via *i.p.* injection or dermal application before mating to untreated females (Favor and Shelby, 2005). The authors concluded that these results indicate an increase in the frequency of translocation carriers in offspring following paternal exposure to AA or GA in spermatid or spermatozoa. The *i.p.* exposure to AA on PND 1-8 also increased mutations at the TK and HPRT loci in spleen lymphocytes of mice (von Tungeln et al., 2009) and at lac Z loci in transgenic mice (Muta®Mouse) (Hoorn et al., 1993).

Three studies showed positive results in the specific locus mutation assay in mice, which allowed detection of both small and large gene deletions (ATSDR, 2012). In one study (Russell et al., 1991), male mice received 5 repeated *i.p.* injection doses of 50 mg/kg b.w. per day. Increased frequencies of specific locus mutations were observed for males mated with females on days 8-14 and 15-21 after treatment suggestive of specific locus mutations in the late stages of spermatogenesis (spermatids and spermatozoa).

Favor and Shelby (2005) reviewed seven published studies that assessed the effectiveness of AA and GA in inducing transmitted reciprocal translocations or gene mutations in the mouse. Results indicated

an increase in the frequency of translocation carriers in offspring following paternal exposure to AA or GA in spermatid and spermatozoa (Shelby et al., 1987; Adler, 1990; Adler et al., 1994, 2004; Generoso et al., 1996). Even the dose of 50 mg AA/kg b.w. *i.p.*, significantly increased the frequency of translocation carriers when compared to the historical control (0.6 % compared to 0.04 %) (Adler et al., 1994). A detailed cytogenetic analysis of the semi-sterile and sterile offspring recovered in the heritable translocation test indicated that the animals were often carriers of more complicated chromosomal rearrangements (reciprocal translocations between two chromosomes, translocation among three chromosomes, two independent reciprocal translocations each between 2 chromosomes). Two studies screened for specific-locus mutations (Russell et al., 1991; Ehling and Neuhäuser-Klaus, 1992). In addition to the specific-locus results, the studies included data on fertility (in the first study: litter size and number of offspring/male and in the second study: % fertile matings, number of corpora lutea/female, number of implants/female, number of live embryos/female and % of dead implants) and data on dominant lethality. The results were consistent between both laboratories and indicated that AA was mutagenic in the first 2 weeks post-treatment, corresponding to germ cells exposed in the spermatozoa or spermatid stages. Results for AA treatment of earlier spermatogenic stages differed between the two studies. Russell et al. (1991) reported no increase in the frequency of specific-locus mutations in offspring derived from germ cells exposed as stem-cell spermatogonia. By contrast, Ehling and Neuhäuser-Klaus (1992) observed a significant increase in the frequency of specific-locus mutations following exposure of spermatogonia to AA. The heritable translocation test detects per se clastogenic events. The extensive cytogenetic and fertility analyses of the heritable translocations and specific-locus mutations recovered in offspring of AA treated parental male mice clearly indicates that the transmitted mutations are associated with clastogenic events. The authors also concluded that in these spermatogenic stages AA is mainly or exclusively a clastogen; per unit dose, *i.p.* exposure is more effective than dermal exposure; and per unit dose, GA is more effective than AA.

The *in vivo* genotoxicity of AA and GA was investigated in Big Blue mice. Manjanatha et al. (2006) exposed mice to 0, 100 or 500 mg/L AA or an equimolar amount of GA (120 and 600 mg/L) dissolved in drinking water for 7 days per week and for up to 4 weeks. Micronucleated reticulocytes were assessed in peripheral blood within 24h of the last treatment and lymphocyte HPRT and liver cII mutagenesis assays were conducted 21 days following the last treatment. In addition, the types of cII mutations were determined by sequence analysis. The frequency of micronucleated reticulocytes was increased in males treated with the high doses of AA (3.3 fold) and GA (1.7 fold). Both doses of AA and GA produced increased HPRT mutant frequencies in lymphocytes, with the high doses inducing mutation frequencies 16-25 fold higher than the controls. The high doses of AA and GA produced significant 2-2.5 fold increases in cII mutant frequency in the liver. Molecular analysis of the mutants indicated that AA and GA produced similar mutation spectra and that these spectra were significantly different from that of spontaneous mutants. The predominant types of mutations in the liver cII gene from AA and GA treated mice were G:C → T:A transversions and -1/+1 frameshifts in a homopolymeric run of Gs. The authors concluded that the mutation frequencies and types of mutations induced by AA and GA in the liver were consistent with AA exerting its genotoxicity in Big Blue mice via metabolism to GA (Manjanatha et al., 2006). In the study of Mei et al. (2010), Big Blue rats were exposed to 0, 0.7 and 1.4 mM AA or GA dissolved in drinking water (50 or 60 mg/L and 100 or 120 mg/L, respectively; equivalent to approximately 4 and 12 mg/kg b.w. per day of AA and GA) for 7 days per week and for up to 60 days. The concentrations of AA and GA in drinking water were equal to and double the maximum dose used in 2-year carcinogenesis studies of AA and GA in F344 rats (see Section 7.3.3.2). After 2 months of dosing, the rats were euthanized and blood was taken for the micronucleus assay; spleens for the lymphocyte HPRT mutant assay; and liver, thyroid, bone marrow, testis (from males), and mammary gland (females) for the cII mutant assay. Neither AA nor GA increased the frequency of micronucleated reticulocytes, and the authors speculated that the doses used in the study were too low to produce a measurable positive response and that rats may be relatively insensitive to MN induction by AA and GA. Both compounds produced small (approximately 2-fold to 3-fold above background) but significant increases in lymphocyte HPRT mutant frequency. The spectrum of mutations from AA-treated rats was significantly different from that of control rats and of GA-treated rats, while the spectrum of mutations from GA-treated rats was marginally different from that of control rats. Neither compound increased the cII mutation frequency

in testis, mammary gland (tumour target tissues), or liver (non-target tissue), while both induced weak positive increases in bone marrow (non-target tissue) and thyroid (target tissue). The overall patterns of mutations in the thyroid cII mutants in the AA and GA-treated groups did not differ significantly from the controls. In addition, there were no significant differences between the spectra for AA and GA treated rats in either the male or the female groups. The authors concluded that although the genotoxicity in tumour target tissue was weak, in combination with the responses in surrogate tissues, the results were consistent with AA being a gene mutagen in the rat via metabolism to GA.

Wang et al. (2010a) investigated whether AA and GA induced mutagenic effects in the germ cells of male mice. Male Big Blue transgenic mice were administered 1.4 or 7.0 mM of AA or GA (100 or 120 mg/L and 500 and 600 m/L, respectively, equivalent to approximately 19 or 35 and 88 or 111 mg/kg b.w. per day) in the drinking water for up to 4 weeks. Testicular cII mutant frequency (MF) was determined 3 weeks after the last treatment, and the types of the mutations in the cII gene were analyzed by DNA sequencing. The testes cII MFs were significantly increased in all treatment groups. There was no significant difference in the cII MFs between AA and GA at the low exposure concentration. At the high concentration, the MF induced by GA was higher than that induced by AA. The mutation spectra in mice treated with AA (1.4 mM) or GA (both 1.4 and 7.0 mM) differed significantly from those of controls, but there were no significant differences in mutation patterns between AA and GA treatments. The mutagenic effect was found at exposure concentrations of AA and GA where no testicular atrophy was observed, indicating that the mutagenic effect is independent of the reproductive toxicity of AA and GA. The Big Blue mouse may be more sensitive for detecting the mutations in testes caused by AA and GA than the Big Blue rat since there was no increase in the MFs in testes of rats treated with AA or GA at 1.4 mM for 60 days and killed after 60-day treatment (Mei et al., 2010). However, this may be related to the difference in received effective doses in rats and mice. Comparison of the mutation spectra between testes and livers (data from previous study Manjanatha et al., 2006) showed that the spectra differed significantly between the two tissues following treatment with AA and GA, whereas the mutation spectra in the two tissues from control mice were similar. In testes, the treatments with AA and GA induced substantial increases in A:T → G:C transition and G:C → C:G transversion, whereas in liver, the predominant types of mutations were G:C → T:A transversions and -1:+1 frameshifts. The authors concluded that these results suggest that AA possesses mutagenic effects on testes by virtue of its metabolism to GA possibly targeting spermatogonial stem cells, but possibly via different pathways when compared to mutations in liver.

El-Bohi et al. (2011) measured CYP2E1 protein and transcript levels as well as DNA damage as measured by the *in vivo* alkaline single cell gel electrophoresis (Comet assay) in hepatic tissues of rats treated with AA. Male albino rats received 0 (distilled water), 50 or 100 mg/kg b.w. per day AA by oral gavage for 21 days. AA caused marked alterations in animal behaviour, revealing nervous manifestations and induced mortality in both treated groups which reached 30 % (in the first group) and 40 % (in the second group). AA elicited a highly significant increase in serum AST and ALT activities, and a significant decrease of total protein, albumin and globulin levels were recorded. AA caused down regulation of both CYP 2E1 protein and mRNA levels concomitant with a dose dependent significant increase in the number of DNA single strand breaks. Histopathological investigation revealed necrotic and degenerative changes in the liver of AA treated rats.

To explore the role of CYP2E1 metabolism in the germ cell mutagenicity of AA, CYP2E1-null and wild-type male mice were treated by *i.p.* injection with 0, 12.5, 25 or 50 mg AA/kg b.w. per day for 5 consecutive days. At defined times after exposure (2 days or 1 week), males were mated to untreated $B6C3F_1$ females. Females were killed in late gestation (about 13 days after the end of cohabitation period) and uterine contents were examined. No changes in any fertility parameters (% pregnant females, mean number of implants per pregnant female, % live fetuses per pregnant female and % resorptions per pregnant female) were seen in females mated to AA-treated CYP2E1-null mice. In contrast, AA exposure induced marked reproductive effects in wild-type male mice. The clearest indicator of dominant lethality is the % resorptions in females mated to treated males. Dose-related increases in resorptions (chromosomally aberrant embryos) and decreases in the numbers of pregnant females and in the proportion of living fetuses were seen in females mated to AA-treated wild-type

mice. AA exposure produced dose-related decreases in the mean number of implantation sites per pregnant female in females mated with wild type males in the second mating period (mating 1 week after exposure). These results demonstrated that AA-induced germ cell mutations in male mice require CYP2E1-mediated epoxidation of AA. Thus, induction and polymorphisms of CYP2E1 in human populations, resulting in variable enzyme metabolic activities, may produce differential susceptibilities to AA toxicities (Ghanayem et al., 2005b).

Female wild-type and CYP2E1-null mice were treated by *i.p.* injection with 0, 25, or 50 mg AA/kg b.w. per day for 5 consecutive days (Ghanayem et al., 2005c). Twenty-four hours after the final treatment, blood and tissue samples were collected. Erythrocyte micronucleus frequencies were determined using flow cytometry and DNA damage was assessed in leukocytes, liver, and lung using the alkaline Comet assay (pH > 13). Significant dose-related increases in micronucleated erythrocytes and DNA damage in liver cells and leukocytes were induced in AA-treated wild-type but not in the CYP2E1-null mice. No increases in the percentage of cells with low molecular weight (LMW) DNA (indication of necrosis or apoptosis) were seen in leukocytes of either genotype of mice, indicating an absence of cytotoxicity. No significant increases in LMW DNA in liver cells was seen in wild-type mice, however, in CYP2E1-null mice treated with 50 mg/kg b.w. per day AA, a small but statistically significant increase in the percentage of cells with LMW DNA was detected. No treatment-related increases in DNA damage were seen in lung cells of wild-type or CYP2E1-null mice and no treatment-related increases in the percentage of cells with LMW DNA were observed in either genotype of mice. This result is unexpected since the lung has been reported to be a target for AA-induced carcinogenesis in female mice and significant amounts of GA in lung tissue of female B6C3F$_1$ mice treated with AA have been detected (Doerge et al., 2005b). Lack of exposure of lung tissue is unlikely to be a factor in the negative results for DNA damage in this tissue. According to the authors, it is possible that the relatively small sample of cells analysed in the Comet assay may not have contained sufficient numbers of the particular cell type in which DNA damage is induced and from which tumours derive. These results support the hypothesis that genetic damage in somatic and germ cells of mice treated with AA is dependent upon metabolism of the parent compound by CYP2E1 (Ghanayem et al., 2005a).

Von Tungeln et al. (2009) compared the extent of DNA adduct formation and induction of micronuclei and mutations in mice treated neonatally with AA and GA. Male and female B6C3F$_1$/*tk* mice were treated *i.p.* on postnatal day (PND) 1, 8 or 15 or PND1-8 with 0, 0.14 or 0.70 mM AA or GA/ kg b.w. per day (corresponding to about 0, 10 and 50 mg AA/kg b.w. per day and 0, 12 and 61 mg GA/kg b.w. per day). On PND9, small samples of tail tissue were obtained to establish the genotype of the mice (to distinguish between B6C3F$_1$/*tk*+/+ and B6C3F$_1$ *tk* +/- mice). On PND16, the B6C3F$_1$/*tk* +/+ mice were killed to measure DNA adduct (N7-GA-Gua and N3-GA-Ade) levels in liver, spleen, lungs and bone marrow and blood was obtained by cardiac puncture to assess the induction of micronuclei. Three weeks after the last treatment, the B6C3F$_1$/*tk*+/- mice were killed to determine the mutant frequency in the *hprt* and *tk* genes. In the second experiment, male and female B6C3F$_1$ mice were treated *i.p.* on PND1-8 with 0, 0.14 or 0.70 mM AA of GA/kg b.w. per day. Tail samples were taken on PND8 to establish the genotype of the mice. The B6C3F$_1$/*tk* +/+ mice were killed on PND9 to determine the induction of MN in peripheral blood and DNA adducts levels in lung, liver and spleen. The B6C3F$_1$/*tk*+/- mice were killed 3 weeks after the last treatment to determine the mutant frequency in the HPRT and TK genes in splenic T-lymphocytes. An ENU-treated positive control group was included in the experiment. Both adducts were readily detected in mice treated with AA or GA. The highest levels of N7-GA-Gua were found in lung DNA, followed by the liver, spleen and bone marrow DNA. The levels of N7-GA-Gua decreased in the order 0.70 mM/kg b.w. per day GA > 0.70 mM AA/kg b.w. per day > 0.14 mM GA/kg b.w. per day ~ 0.14 mM AA/kg b.w. per day. The levels of N3-GA-Ade were approximately 100-fold lower than the levels of N7-GA-Gua. The relative ranking of N3-GA-Ade levels within tissues was similar to that observed with N7-GA-Gua and the levels of adducts decreased in the same order. Only the high dose of GA increased the frequency of micronucleated reticulocytes (MN-RET) and normochromatic erythrocytes (MN-NCE). In mice treated on PND1, 8 and 15 the HPRT mutant frequency was increased by 0.70 mM GA/kg b.w. per day. None of the treatments affected the TK mutant frequency. In mice dosed on PND1-8, 0.70 mM

GA/kg b.w. per day caused extensive mortality; each of the other treatments increased the TK mutant frequency, whereas AA (at both doses) increased the HPRT mutant frequency. Treatment with 0.14 mM GA/kg b.w. per day caused an increase in MN-NCE in peripheral blood at PND9. None of the other treatments affected the percentage of MN-RET or MN-NCE. The authors concluded that the mutagenic response in neonatal mice treated on PND1, 8 and 15 was due to GA, whereas mutations resulting from dosing on PND1-8 were due to another mechanism.

In a follow-up study, Von Tungeln et al. (2012) examined the tumourigenicity of AA and GA in mice treated neonatally. Male B6C3F$_1$ mice were injected *i.p.* on PND 1, 8 and 15 with 0 (deionized water), 0.14 or 0.70 mM AA or GA/kg b.w. per day and the tumourigenicity was assessed after 1 year (see Section 7.3.3.2). There was an increased incidence of combined hepatocellular adenoma or carcinoma in the group treated with the high dose GA. Analysis of the hepatocellular tumours indicated that the increased incidence observed in mice administered 0.70 mM GA/kg b.w. per day was associated with A→G and A→T mutations at codon 61 of H-ras. The authors concluded that these results, combined with the previous data on DNA adduct formation and mutation induction, suggested that the carcinogenicity of AA was dependent on its metabolism to GA, a pathway that is deficient in neonatal mice.

To compare the susceptibility to AA-induced genotoxicity of young versus adult animals, Koyama et al. (2011a) treated 3- and 11-week-old male gpt delta transgenic F344 rats with 0, 20, 40 or 80 mg/L AA via drinking water (equivalent to 0, 3.01, 5.95 and 12.19 mg/kg b.w. per day in young rats and 0, 1.83, 3.54 and 7.05 mg/kg b.w. per day in adult rats) for 4 weeks and then examined genotoxicity in the bone marrow, liver and testis. They also analysed the level of N7-(2-carbamoyl-2-hydroxyethyl)-guanine (N7-GA-Gua), the major DNA adduct induced by AA, in the liver, testis, mammary gland and thyroid. At 40 and 80 mg/L, both age groups yielded similar results in the Comet assay in liver, but at 80 mg/L, the bone marrow micronucleus frequency and the gpt-mutant frequency in testis increased significantly only in the young rats. AA did not increase the gpt-mutant frequency in the liver of either age group at any dose. The N7-GA-Gua adduct levels were increased in a dose-dependent manner in all tissues. In the mammary glands and thyroid, adduct levels did not differ significantly between young and adult rats. In the liver and testis, the level was higher in the young rats than in the adult rats (about 6 times that of adult rats). The authors concluded that these results implied that young rats were more susceptible than adult rats to AA-induced testicular genotoxicity.

To determine whether chronic AA exposure produces genetic damage in male germ cells *in vivo*, male Swiss mice (3 animals per group) were exposed to AA via drinking water (Nixon et al., 2012, 2013). AA was administered at doses of 0-10 µg/mL (equivalent to 0.0001-2 mg/kg b.w. per day) for up to 1 year. At 1, 3, 6, 9 and 12 months, DNA damage in isolated spermatocytes was measured using an alkaline Comet assay with the addition of formamidopyrimidine-DNA glycosylase (fpg), a restriction enzyme used to detect and cleave sites of GA-DNA adducts and γH2A.X expression, a marker of double-strand breaks. AA treatment did not significantly affect body weight or testis weight. A significant dose-dependent increase in DNA damage was observed in mouse spermatocytes following 6 months of exposure in the two highest dosage groups (0.15 and 1.4 mg/kg b.w. per day). After 12 months of exposure, increases in damage were detected at doses as low as 0.001 mg/kg b.w. per day. γH2A.X staining was more prominent in testes from mice exposed to AA, particularly at 0.17 and 1.53 mg/kg b.w. per day and was predominantly localized in spermatocytes. Thus AA exposure in male mice leads to an increased frequency of double-strand breaks in spermatocytes. The authors concluded that the results of this study demonstrated that chronic exposure to AA, at doses equivalent to human exposures, generated DNA damage in male germ cells of mice in the absence of major effects on mouse health or defects in spermatogenesis (Nixon et al., 2012). The CONTAM Panel noted that the Comet assay is indicative for DNA damage via genotoxic events but also via apoptosis. However, at the chronic exposures used in this study, no signs of germ cell apoptosis were observed in testis histology of mice. The CONTAM Panel considered that the small number of mice used per dose

group constitutes a potential limitation of the study[34] . The Panel also noted that the higher sensitivity of the Comet assay when used in conjunction with fpg for the detection of GA-induced DNA damage (Hansen et al., 2010) might explain why an effect was observed at so low doses in this study in comparison with the Manière et al. (2005) study, in which effects were only detected at higher doses. The CONTAM Panel, however, considers that the findings of the Nixon et al. (2012) study are of potential relevance for the evaluation of male germ cells genotoxicity following chronic exposure to AA and that further studies should be carried out to better characterize the genotoxicity at doses equivalent to human exposure using additional endpoints for DNA damage, including especially DNA adducts, and with an appropriate number of animals as indicated by EFSA (2012b).

Altaeva et al. (2011) studied the mutagenic effect of AA on thyroid gland cells by an extended micronucleus test (Cytoma test). Male Wistar rats received AA solution in distilled water orally (by gavage) 48, 96 and 144 h after hemithyroidectomy (HTE, to stimulate proliferative activity of the thyroid gland). Cells were collected on day 9 after HTE. The following doses of AA were studied: 0, 0.496, 2.48 and 12.4 mg/kg b.w. (corresponding to 0.004, 0.02 and 0.1 LD_{50}). Cytogenetic and cytotoxic parameters were recorded. No thyroid gland (TG) cells with MN were detected in intact rats after HTE (control). The relative content of cells with protrusions was 0.83 ‰, with internuclear bridges 0.33 ‰, the sum of cytogenetic disorders 1.16 ‰, the proliferation index 13.2 ‰ and the apoptic index 3.5 ‰. The incidence of TG cells with cytogenetic disorders increased in AA exposed cells but decreased with increasing dose (respectively 8-fold, 3.6 fold and 4.9 fold higher vs. the control). A few micronuclei (small size, presumably the result of clastogenicity) were detected in the thyroid gland cells of treated rats (not dose-related). Mainly the number of cells with protrusions (an indication of aneugenicity) were increased, again the effect was maximal in the low dose rats. The low dose of AA led to a 2-fold increase of the apoptosis, whereas the medium and high dose led to a 4.8 and 4.7 fold increase of the apoptotic index, respectively. The proliferative activity also increased 2-fold in the low dose AA cells, was inhibed in the medium dose (reached the control level) and again increased by 2.5 times in the high dose cells. Cell elimination and restoration in the population were balanced in the low dose, but this was paralleled by the maximum level of cytogenetic effects. In the medium dose there was a significant elimination of cells with cytogenetic aberrations, which did not recover in the absence of proliferative activity. The apoptotic index remained high in response to further increase in AA dose; the total count of cells in the populations presumably decreased and this fact triggered the compensatory reaction and hence proliferative activity. Increase of proliferation indicates a stronger toxic effect of the test substance, leading to compensatory reactions and necessitating cell population restoration. This was again paralleled by an increase in the level of cytogenetic effects. The authors concluded that a mutagenic effect of AA on thyroid gland cells was detected which along with an increase in proliferative activity leads to the development of tumours in this organ.

Yener (2013) investigated whether long term low dose exposure to AA increased micronucleus frequency in rat bone marrow polychromatic erythrocytes. Doses of 0, 2 or 5 mg AA/kg b.w. per day were administered to Wistar rats in their drinking water for 90 days. No obvious symptoms of sickness or decreased activity and no mortality were observed during the study. Cytotoxicity, indicated by a decrease in polychromatic erythrocytes/normochromatic erythrocytes ratio, was observed at both AA doses. Both doses of AA significantly increased the frequency of MN in polychromatic erythrocytes in both males and females. The difference between the two AA doses was not statistically significant.

Marchetti et al. (2009) used multicolour fluorescence *in situ* hybridization painting to investigate whether paternally transmitted chromosomal aberrations result in mosaicism in mouse two-cell embryos. Male $B6C3F_1$ mice were exposed by *i.p.* to 5 consecutive daily doses of 0 (distilled water) or 50 mg/kg AA. Groups of treated males were mated with untreated superovulated females at 2.5, 6.5,

[34] According to EFSA (2012b), 'Five scorable animals for each dose group, including vehicle and control groups, should be used (JaCVAM, 2009). Providing clear positive response is observed in the positive control group, a lower number of animals would be acceptable in this group. EFSA is aware that the use of a lower number of animals might be possible but the slight decrease in the statistical power might require additional testing, therefore for the time being, the use of five animals is recommended. If a lower number of animals is used, this should be scientifically justified.)'.

9.5, 12.5, 20.5, 27.5, 41.5 and 48.5 days after the last AA injection. Control males were mated with untreated females at 2.5, 6.5, 9.5 and 12.5 days after the last injection. AA treatment of male mice prior to mating induced prefertilization toxicity only after matings that sampled late spermatids (9.5 days post treatment, dpt) (reduction of frequency of fertilized embryos from 83 % in controls to 71 %). It induced also a significant reduction in the numbers of two-cell embryos that reached the metaphase stage in matings within the repair deficient window of spermatogenesis (2.5-12.5 dpt) with respect to control value. The percentages of asynchronous two-cell embryos were not affected by paternal exposure to AA. Paternal treatment with AA induced high frequencies of chromosomally abnormal two-cell embryos (> 50 %) after treatment of epididymal sperm (2.5 dpt), testicular sperm (6.5 dpt), and late spermatids (9.5 dpt). Testicular sperm were the most sensitive cell type for the induction of chromosome structural aberrations (statistically different from 2.5 and 9.5 dpt) with 82 % of the two-cell embryos presenting with chromosome structural aberrations. The frequencies of embryos with structural aberrations decreased to 19 % after treatment of elongating spermatids (12.5 dpt) and 5.0 % after treatment of round spermatids (20.5 dpt). Chromosomal aberrations were not significantly increased with respect to the control value after treatment of pachytene spermatocytes (27.5 dpt), differentiating spermatogonia (41.5 dpt), or stem cells (48.5 dpt). Testicular spermatozoa were also the germ-cell type with the highest amount of chromosomal damage. Each type of aberration was most prevalent at 6.5 dpt mating time when the total number of chromosomal aberrations per embryo was above 3.3 by both PAINT and DAPI analyses, whereas it was less than 2.4 per embryo at 2.5 dpt and decreased to around 1.5 at 9.5 dpt. PAINT/DAPI analysis of mouse two-cell embryos showed that paternal treatment with AA induced mosaicism for structural chromosomal aberrations. The majority of abnormal embryos, irrespective of mating time point, were mosaics, that is, the two metaphases had a different karyotype. In the treated groups, the majority of two-cell embryos had chromosomal structural aberrations in both metaphases, but the aberrations were of different types. In a second group, mosaic embryos had chromosomal aberrations in only 1 metaphase. A third group of embryos were structurally abnormal with the apparently same chromosomal aberration in both metaphases. Conversely, all abnormal embryos found in the control group had one metaphase with a chromosomal aberration, whereas the other appeared to be normal.

Paternal exposure to AA also induced numerically abnormal two-cell embryos. The majority of the embryos were hypodiploid in one or both metaphases. At 6.5 dpt, the most sensitive time for the induction of chromosomal structural aberrations, over 72 % of the embryos were hypodiploid. Hyperdiploid nonmosaic embryos and triploid nonmosaic embryos were also found, but their frequencies were not different with respect to controls. An increase of numerically abnormal mosaic two-cell embryos was also observed. The authors also analysed the persistence of the various types of chromosomal structural aberrations in two-cell embryos. Their results show that there is a tendency for loss of acentric fragments during the first mitotic division, whereas both dicentrics and translocations apparently undergo proper segregation without loss.

In previous studies (Marchetti et al., 1997, 2001, 2004, 2007), the authors reported a correlation between the percentages of zygotes with unstable chromosomal aberrations and embryonic lethality and between stable chromosomal aberrations and the percentages of offspring with reciprocal translocations. They therefore determined whether PAINT/DAPI analysis of two-cell embryos also provided good estimates of embryonic lethality and of translocation carriers at birth. Using PAINT/DAPI analysis, the frequencies of two-cell embryos with unstable aberrations, which are expected to die in utero because of loss of genetic material, were: 66 %, 80 %, 48 % and 19 % at 2.5, 6.5, 9.5 and 12.5 dpt mating times, respectively. The proportion of two-cell embryos with stable aberrations (24 %, 8 out of 33, two-cell embryos) is in agreement with the frequencies of offspring with heritable translocations (HT) reported using the standard HT method and paralleled the findings obtained in zygotes (Marchetti et al., 1997). The authors concluded that the frequencies of chromosomal aberrations in zygotes and two-cell embryos are consistent with each other and are a good predictor of embryonic fate for death after implantation and birth with reciprocal translocations.

The authors also concluded that embryonic development can proceed up to the end of the second cell cycle of development in the presence of abnormal paternal chromosomes. The high incidence of

chromosomally mosaic two-cell embryos suggests that the first mitotic division of embryogenesis is prone to missegregation errors and that paternally transmitted chromosomal abnormalities increase the risk of missegregation leading to embryonic mosaicism.

Ao and Cao (2012) reviewed the relevant mutations induced by AA and GA on both HPRT and TK gene loci in various test systems involving *in vivo* and *in vitro* tests. The individual studies have already been reported in this section. The authors conluded that mutation changes at the HPRT gene and TK gene confirm that AA is mainly a directly-acting clastogen, causing chromosomal aberrations. AA also produces weakly mutagenic effects at the HPRT gene by metabolic conversion of AA to GA. The genotoxic characteristics of GA are distinctly different from AA. GA is a strong mutagen with high reactivity to DNA, inducing predominantly point mutations.

In a review of the toxicity of AA, Exon (2006) reported that it was postulated by several investigators that the clastogenic effects of AA on germ cells may not be by direct interaction with DNA. These effects may be mediated through interference with the kinesin motor proteins that are involved in spindle fiber formation and chromosomal segregation during cell division or alkylation of protamines in sperm (Shiraishi, 1978; Costa et al., 1992; Adler et al., 1993, 2000). Alternatively, AA may alkylate DNA proteins via its affinity for sulfhydryl groups, resulting in clastogenesic effects (Sega et al., 1989; Sega, 1991).

4837 **Table 22:** Experimental design and results for selected *in vivo* genotoxicity tests with acrylamide (AA)

Test	Species	Route of administration	Dose	Exposure time	Result	Reference
Micronucleus test	Male B6C3F$_1$ mice. Peripheral blood and bone marrow Male F344/N rats. Peripheral blood and bone marrow	Oral (gavage)	0 (PBS), 12.5, 25, 37.5 or 50 mg AA/kg b.w. 0 (PBS), 12.5, 25, 37.5 or 50 mg AA/kg b.w.	3 days at 24 h intervals, sacrifice 24 h after last administration	Positive from 25 mg/kg b.w. in bone marrow and peripheral blood (microscopy and flow cytometry) No increase MN in bone marrow or peripheral blood (flow cytometry). Positive in PB (microscopy) at 25 and 50 mg/kg b.w.	Witt et al. (2008)
Micronucleus test	Male Sprague Dawley rats. Bone marrow	Oral (gavage)	0, 125, 150 or 175 mg AA/kg b.w.	48 h treatment	Dose-related increased frequency of MN in PCE (3.75-fold at high dose) AA decreased PCE/NCE (bone marrow cytotoxicity)	Yener and Dikmenli (2009)
Comet assay	Male Sprague Dawley rats. Blood, brain, liver, bone marrow, adrenals, testes	Oral (gavage)	0 (dist. water), 18, 36 or 54 mg AA/kg b.w. C+: MMS	Single dose, sacrifice 24 h after dosing and additional sacrifice for 0 and 54 mg AA/kg b.w. 2 h and 5 h after dosing	DNA lesions in blood leucocytes and brain at 36 and 54 mg/kg b.w. and in testes at 54 mg/kg bw 24 h after exposure. No statistically significant increase in comet parameters in liver, bone marrow and adrenals. 5 h after dosing: statistically significant increase in DNA damage in leucocytes, bone marrow, liver and adrenals. 2 h after dosing: increased DNA damage in testes and adrenals.	Manière et al. (2005)
Comet assay	Male Pzh:SFIS mice. Bone marrow, testes, liver, kidneys, spleen, lungs	*i.p.* injection	0, 50, 75, 100 and 125 mg AA/kg b.w.	Single exposure Sacrifice 24 h after dosing	Dose-related increase DNA damage (statistically significant from 75 mg/kg b.w. in spleen, liver, kidneys and testes, and from 50 mg/kg b.w. in lungs and bone marrow)	Dobrzynska (2007)

4838 Table continued overleaf.
4839

4840 **Table 22:** Experimental design and results for selected *in vivo* genotoxicity tests with acrylamide (AA) (continued)

Test	Species	Route of administration	Dose	Exposure time	Result	Reference
Comet assay	Male B6C3F$_1$ mice. Cell types: blood leukocytes, liver, duodenum and testes (somatic and germ cells)	Oral (gavage)	0 (PBS), 12.5, 25, 37.5 or 50 mg AA/kg b.w.	4 days at 24 h intervals, sacrifice 4 h after last administration	Positive (based on olive tail moment) in all tissues at all dose levels except at 12.5 mg/kg b.w. in testicular somatic cells	Recio et al. (2010)
	Male F344/N rats. Cell types: blood leukocytes, liver, thyroid, duodenum and testes	Oral (gavage)	0 (PBS), 12.5, 25, 37.5 or 50 mg AA/kg b.w.	4 days at 24 h intervals, sacrifice 4 h after last administration	Positive (based on olive tail moment) in blood leukocytes, testicular somatic cells, thyroid and duodenum. (magnitude lower than in mice) No increase DNA damage in liver cells or in presumptive sperm cells	
Comet assay	Male Wistar rats. Liver cells Bone marrow cells	Oral (gavage)	0, 10, 20 or 100 mg/kg b.w. per day	3 days, sacrifice 2 h after final treatment	Positive at the two highest doses Positive at high dose	Rothfuss et al. (2010)
	Male Wistar rats. Liver cells Bone marrow cells		0, 5, 10 or 20/20* mg/kg b.w. per day	29 days, sacrifice 2 h or 24 h* after final treatment	Positive at all doses Positive at the two highest doses	
MN test	Male Wistar rats. Bone marrow cells Peripheral blood**	Oral (gavage)	0, 10, 20 or 100 mg/kg b.w. per day (= maximum tolerated dose)	3 days, sacrifice 24 h after final treatment ** sacrifice day 4	Negative	Rothfuss et al. (2010)
			0, 5, 10 or 20 mg/kg b.w. per day (= maximum tolerated dose)	29 days, sacrifice 24 h after final treatment ** sacrifice day 15 and day 29	Negative	

4841 Table continued overleaf.
4842

4843 **Table 22:** Experimental design and results for selected *in vivo* genotoxicity tests with acrylamide (AA) (continued)

Test	Species	Route of administration	Dose	Exposure time	Result	Reference
Micronucleus test	Male B6C3F$_1$ mice. Peripheral blood cells	Oral (gavage)	0 (water), 0.125, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 16 or 24 mg/kg b.w. per day	28 days	Increase MN-NCE from 4 mg/kg b.w. per day and MN-RET from 6 mg/kg b.w. per day (less than a doubling at high dose)	Zeiger et al. (2009)
Micronucleus test	Male and female Wistar rats. Bone marrow cells	Oral (drinking water)	0 (water), 2 and 5 mg/kg b.w. per day	90 days, sacrifice 24 h after last treatment	Decrease PCE/NCE ratio in treated animals Statistically significant increase MNPCE in treated animals (M: 2.8, 8.0 and 8.4 MNPCE/2000 PCE, F: 2.6, 6.8 and 7.6 MNPCE/2000 PCE in control, low dose and high dose, respectively)	Yener (2013)

4844 h: hour(s); MN: micronucleous; NCE: normochromatic erythrocytes; PBS: phosphate buffered saline; PCE: polychromatic erythrocytes; RET: reticulocytes.

4845 7.3.3.3. Conclusions

4846 AA is not mutagenic in bacterial cells. In mammalian cells *in vitro*, AA is a weak, direct-acting
4847 mutagen, but an effective direct-acting clastogen. It induces also, to a lower extent, aneuploidy,
4848 polyploidy and other mitotic disturbances. With the exception of a positive result in HepG2 cells, AA
4849 did not induce DNA damage as measured by the Comet assay.

4850 In *in vivo* mammalian somatic cells assays, AA appears to be clearly genotoxic but at relatively high
4851 doses, producing positive results particularly in the micronucleus and in the Comet assays. *In vivo*
4852 studies have also shown the induction by AA of mutations at different genetic loci in lymphocytes and
4853 target organs such as liver, lung, testes in mice and bone marrow and thyroid in rats.

4854 AA is clearly positive in a number of different germ cell assays (dominant lethal assays, heritable
4855 translocation and specific locus mutation assays) indicating that it induces heritable genetic damage at
4856 the gene and chromosome level. AA showed stronger germ cell effects in mice after repeated
4857 administration of low doses compared with a single high dose. DNA damage generated in male germ
4858 cells of mice by chronic exposure to AA was observed in the absence of major effects on mouse health
4859 or defects in spermatogenesis.

4860 The metabolism of AA to GA is the crucial prerequisite for the genotoxicity of AA. Therefore, the
4861 mutagenic effects will be higher in cells or tissues with elevated capability of metabolic conversion of
4862 AA to GA. Studies using knockout (CYP2E1-null) and wild-type mice showed that CYP2E1-mediated
4863 oxidation is the predominant pathway leading to the formation of DNA adducts. Studies in wild-type
4864 and CYP2E1 knockout mice have also shown that GA is the active metabolite of AA responsible for
4865 somatic and germ cell mutations and dominant lethality.

4866 The level of DNA adducts produced by AA in rats was generally lower than in mice at similar doses.
4867 These findings are consistent with the greater proficiency of the mouse to produce GA from AA
4868 (Tables 16 and 17).

4869 In situations of limited CYP2E1 activity (as occurs in neonatal mice, mouse lymphoma cells, and
4870 possibly TK6 cells, and Big Blue mouse embryonic fibroblasts), AA can induce gene mutations by a
4871 pathway not involving GA. This may involve the generation of ROS and induction of oxidative DNA
4872 damage. This alternative pathway appears to take place only with very high toxic doses of AA.

4873 Young rats are more susceptible than adult rats to AA-induced testicular genotoxicity (gpt-mutant
4874 frequency and N7-GA-Gua adducts), whereas in the mammary gland and thyroid, adduct levels did
4875 not differ significantly between young and adults.

4876 In contrast to AA, GA is a strong mutagen *in vitro* via a DNA adduct-mediated mechanism. GA-
4877 induced damage is revealed by the Comet assay and significantly amplified in its detection when fpg
4878 sensitive sites are measured. GA induced also clastogenic effects.

4879 *In vivo*, GA is clearly positive in the micronucleus assay in mice and, with lower potency, in rats, with
4880 a predominant chromosome-breaking mechanism instead of chromosome loss. GA is also a potent
4881 mutagen *in vivo*. It interacts with DNA bases, predominantly by forming N7 adducts with guanine and,
4882 to a much lower extent, N3 adducts with adenine. GA induced dominant lethal mutations, similarly to
4883 AA.

4884 Dosing mice or rats with GA generally produced higher DNA adduct levels than observed with AA.

4885 In conclusion, *in vitro* genotoxicity studies indicate that AA is a weak mutagen in mammalian cells
4886 but an effective clastogen. GA is a strong mutagen and a clastogen. It induces mutations via a DNA
4887 adduct mechanism. *In vivo*, AA is clearly genotoxic in somatic and germ cells. AA exerts its
4888 mutagenicity via metabolism by CYP2E1 to GA. At high concentrations AA can also induce gene
4889 mutations by a pathway involving the generation of ROS and oxidative DNA damage.

7.3.4. Long-term toxicity and carcinogenicity

Since a large portion of the available long-term toxicity studies in laboratory animals had a focus on neurotoxic effects, these are dealt with under the previous Section 7.3.2. Thus, this chapter is limited to the carcinogenic effects of AA observed in long-term animal studies.

7.3.4.1. Studies in mice

In mice (A/J, SENCAR, BALB/c, Swiss-ICR and B6C3F$_1$), AA was tested for carcinogenicity in five chronic/subchronic studies.

In a study by Bull et al. (1984a), groups of 16-40 male and female A/J mice were treated with AA by gastric gavage or *i.p.* injection over 8 weeks starting from an age of 8 weeks. The dose levels were 0, 6.25, 12.5 and 25 mg/kg b.w. applied by gavage three times a week, or 0, 1, 3, 10, 30 and 60 mg/kg b.w. applied three times a week by *i.p.* injection. The treatment resulted in a significant increase in lung tumours both in male and female animals, but the strain of mice used shows a high background incidence of lung tumours. In the same study, female SENCAR mice were treated following an initiation-promotion protocol with *i.p.* injection of AA and dermal application of the skin tumour promoter TPA (12-*O*-Tetradecanoylphorbol-13-acetate). This experimental approach resulted in an increase in skin tumours after the combined AA/TPA treatment only. In a second study from the same laboratory (Bull et al., 1984b), female Swiss-ICR mice were treated with AA by oral gavage six times over two weeks. Afterwards, the animals were treated with TPA. In a study by Robinson et al. (1986), female SENCAR, Balb/c, A/J, and Swiss-ICR mice were treated once with 50 mg/kg b.w. AA by *i.p.* injection followed by treatment with TPA over 20 weeks. Only in SENCAR mice, a significant increase in lung adenoma and skin papilloma was found. The CONTAM Panel noted that the data from both studies (Bull et al., 1984b; Robinson et al., 1986) cannot be used for quantitative risk assessment of AA since the effects were likely due, at least in part, to the tumour promoter TPA.

In the study from NTP (2012) (Table 23), B6C3F$_1$ mice received drinking water containing 6.25, 12.5, 25 or 50 mg/L of AA in groups of 50 male and female mice for 2 years (details on the mean amount of AA consumed by the mice are given in Section 7.3.2.1). At the end of the study tissues from more than 40 sites were examined for every animal. In males, the incidence of Harderian gland adenoma and combined Harderian gland adenoma or adenocarcinoma was increased significantly in all AA dose groups. The incidence of lung alveolar/bronchiolar adenoma and combined lung alveolar/bronchiolar adenoma or carcinoma was increased significantly at 12.5 and 50 mg/L, and the incidence of stomach (forestomach) squamous cell papilloma and combined stomach (forestomach) squamous cell papilloma or carcinoma was increased significantly at 25 and 50 mg/L AA. In female B6C3F$_1$ mice, the incidence of Harderian gland adenoma was increased significantly in all dosed groups. The combined incidence of mammary gland adenoacanthoma or adenocarcinoma was increased significantly at 12.5, 25, and 50 mg/L AA, and the incidence of mammary gland adenocarcinoma was increased significantly at 12.5 and 50 mg/L AA. Incidences of lung alveolar/bronchiolar adenoma, combined lung alveolar/bronchiolar adenoma or carcinoma, and malignant mesenchymal skin tumours (fibrosarcoma, hemangiosarcoma, liposarcoma, myxosarcoma, neurofibrosarcoma, or sarcoma) were increased significantly at 25 and 50 mg/L AA. A significant increase was also observed in the incidence of ovary granulosa cell tumour (benign) and mammary gland adenoacanthoma at 50 mg/L AA.

Von Tungeln et al. (2012) examined the tumourigenicity of AA and GA in mice treated neonatally. Male B6C3F$_1$ mice were injected *i.p.* on PND1, 8 and 15 with 0.0, 0.14 or 0.70 mmol AA (0, 10 or 50 mg) or GA (0, 12 or 61 mg) per kg b.w. per day and the tumourigenicity was assessed after 1 year. The only treatment-related neoplasms involved the liver. There was an increased incidence of combined hepatocellular adenoma or carcinoma in the group treated with the high dose GA (8.3 % in the low dose AA and GA, and 4.2 % in the high dose AA and 71.4 % in the high dose GA). In this group, mice had primarily multiple hepatocellular adenoma (61.9 %). In addition, hepatocellular carcinoma was observed only in this group (9.5 %). The CONTAM Panel noted that there was no clear dose-response relationship with respect to AA-induced tumours. Furthermore the effects of AA

were not statistically significant, while there was a significantly increased incidence of combined hepatocellular adenoma or carcinoma in the group treated with the high dose of GA.

Treatment of female CD1 rats (20 animals) with AA in the drinking water (leading to a daily oral dose of 3-4 mg/kg b.w.) over 8 months did not result in tumours in the organs inspected histopathologically (pituitary, thyroid, and adrenal glands, reproductive tract) (Jin et al., 2008).

B6C3F$_1$/Nctr mice received GA in drinking water for 2 years (NTP, 2013, draft report, see Section 7.3.2.1). Male mice showed an increased incidence of Harderian gland, lung, skin and forestomach neoplasms. In female mice, also increased incidences of Harderian gland, lung, mammary gland, forestomach and skin neoplasms were observed. Benign granulose cell tumours of the ovary were also observed, possibly related to treatment with GA (NTP, 2013, draft report).

Table 23: Tumour incidence and statistical analysis results derived from the 2-years NTP carcinogenicity assays with acrylamide (AA) in B6C3F$_1$ mice (NTP, 2012). Only the major tumour sites and/or those showing significant effects at the lower dose are listed.

Tumour	Gender	Dosage (mg/kg b.w. per day)	Incidence
Harderian gland adenoma	female	0	0/45 (0 %)
		1.10	**8/44 (18 %)**
		2.23	**20/48 (42 %)**
		4.65	**32/47 (68 %)**
		9.96	**31/43 (72 %)**
Mammary gland adenocanthoma and adenocarcinoma	female	0	0/47 (0 %)
		1.10	4/46 (9 %)
		2.23	**7/48 (15 %)**
		4.65	4/45 (9 %)
		9.96	**17/42 (41 %)**
Lung alveolar, bronchiolar adenoma	female	0	1/47 (2 %)
		1.10	4/47 (9 %)
		2.23	6/48 (13 %)
		4.65	**11/45 (24 %)**
		9.96	**19/45 (42 %)**
Ovary granulosa cell tumours (benign)	female	0	0/46 (0 %)
		1.10	1/45 (2 %)
		2.23	0/48 (0 %)
		4.65	1/45 (2 %)
		9.96	**5/42 (12 %)**
Skin, various types of sarcoma	female	0	0/48 (0 %)
		1.10	0/46 (0 %)
		2.23	3/48 (6 %)
		4.65	**10/45 (22 %)**
		9.96	**6/43 (14 %)**
Stomach, foretomach squamous cell papilloma	female	0	4/46 (9 %)
		1.10	0/46 (0 %)
		2.23	2/48 (4 %)
		4.65	5/45 (11 %)
		9.96	**8/42 (19 %)**
Harderian gland adenoma and adenocarcinoma	male	0	2/46 (4 %)
		1.04	**13/46 (28 %)**
		2.20	**27/47 (57 %)**
		4.11	**37/47 (77 %)**
		8.93	**39/47 (83 %)**

Table continued overleaf.

Table 23: Tumour incidence and statistical analysis results derived from the 2-years NTP carcinogenicity assays with acrylamide (AA) in $B6C3F_1$ mice (NTP, 2012). Only the major tumour sites and/or those showing significant effects at the lower dose are listed (continued).

Tumour	Gender	Dosage (mg/kg b.w. per day)	Incidence
Lung alveolar, bronchiolar combined adenoma and carcinoma	male	0	6/47 (13 %)
		1.04	6/46 (13 %)
		2.20	**14/47 (30 %)**
		4.11	10/45 (22 %)
		8.93	**20/48 (42 %)**
Stomach squamous combined papilloma or carcinoma	male	0	0/46 (0 %)
		1.04	2/45 (4 %)
		2.20	2/46 (4 %)
		4.11	**7/47 (15 %)**
		8.93	**8/44 (18 %)**

Note: Statistically significant effects are shown in bold.
b.w.: body weight.

7.3.4.2. Studies in rats

In Fischer 344 rats, AA was tested for carcinogenicity in three long-term studies (Table 24).

In a study by Johnson et al. (1986), rats were treated via the drinking water over 2 years starting from an age of 5-6 weeks. The average calculated daily doses were 0, 0.01, 0.1, 0.5, and 2.0 mg/kg b.w. In females, significant increases in the incidence of tumours (malignant and benign) of the mammary gland, clitoral gland, central nervous system, thyroid gland (follicular epithelia), oral cavity, uterus, and pituitary gland were found. All effects were statistically significant at a dose level of 2.0 mg/kg b.w. per day. In males, significant increases in the incidence of tumours (malignant and mostly benign) of the thyroid gland, oral cavity, adrenal gland (pheochromocytoma) and peritesticular mesothelium (processus vaginalis peritonei) were found. All effects were statistically significant at a dose level of 2.0 mg/kg b.w. per day, and the increase in peritesticular tumours at 0.5 mg/kg b.w. per day.

In a study from the same laboratory, published by Friedman et al. (1995), a similar outcome was obtained. The rats were treated in the same way as in the Johnson et al. (1986) study using the same and two additional (1.0 and 3.0 mg/kg b.w. per day) dose levels and the same route of exposure. Female showed significant increases in the incidence of tumours of the thyroid gland (follicular epithelia) and mammary gland. In males the incidence of peritesticular mesothelioma and thyroid tumours was increased. The effects were significant at a dose level of 3.0 mg/kg b.w. per day except for the peritesticular mesothelioma which showed a significant increase at 2.0 mg/kg b.w. per day, and the mammary gland tumours which showed a significant increase at 1.0 mg /kg b.w. per day.

In the NTP (2012) 2-year study, Fischer F344/N male and female rats received drinking water at concentrations of 0.0875, 0.175, 0.35 and 0.70 mM (equivalent to 0.33, 0.66, 1.32 and 2.71 mg/kg b.w. per day for males, and 0.44, 0.88, 1.84 and 4.02 mg/kg b.w. per day for females) (details on the study design are given in Section 7.3.2.2). At the end of the study tissues from more than 40 sites were examined for every animal. The rates of several types of cancer increased in each of the animal studies. Male and female rats receiving AA had increased incidence of thyroid gland and heart tumours; male rats also had increased incidence of cancer in the pancreatic islets and of malignant mesotheliomas, and female rats also had increased incidence of cancers in the clitoral gland, liver, mammary gland, skin, and mouth or tongue.

4990 **Table 24:** Tumour incidence and statistical analysis results derived from male and female F344 rats
4991 from 2 years carcinogenicity assays with acrylamide (AA). Only the major tumour sites and/or those
4992 showing significant effects at the lower dose are listed.

Tumour	Gender	Dosage (mg/kg b.w. per day)	Incidence	Reference
Mammary gland adenoma, fibroadenoma or fibroma	female	0	10/60 (17 %)	Johnson et al. (1986)
		0.01	11/60 (18 %)	
		0.1	9/60 (15 %)	
		0.5	19/58 (33 %)	
		2.0	**23/61 (38 %)[a]**	
Mammary gland fibroadenoma	female	0	5/46 (11 %)	Friedman et al. (1995)
		1.0	**20/94 (21 %)[b]**	
		3.0	**26/95 (27 %)[b]**	
Mammary gland fibroadenoma	female	0	16/48 (33 %)	NTP (2012)
		0.44	18/48 (38 %)	
		0.88	**24/46 (52 %)[c]**	
		1.84	**22/47 (47 %)[c]**	
		4.02	**31/48 (65 %)[c]**	
Thyroid gland follicular cell adenoma or carcinoma	female	0	1/58 (2 %)	Johnson et al. (1986)
		0.01	0/59 (0 %)	
		0.1	1/59 (2 %)	
		0.5	1/58 (2 %)	
		2.0	**5/60 (8 %)[a]**	
Thyroid gland follicular cell adenoma or carcinoma	female	0	1/50 (2 %)	Friedman et al. (1995)
		1.0	10/100 (10 %)	
		3.0	**23/100 (23 %)[b]**	
Thyroid gland follicular cell adenoma or carcinoma	female	0	0/48 (0 %)	NTP (2012)
		0.44	0/48 (0 %)	
		0.88	2/48 (4 %)	
		1.84	3/48 (6 %)	
		4.02	**4/47 (9 %)[c]**	
Thyroid gland follicular cell adenoma	male	0	1/60 (2 %)	Johnson et al. (1986)
		0.01	0/58 (0 %)	
		0.1	2/59 (15 %)	
		0.5	1/59 (2 %)	
		2.0	**7/59 (12 %)[a]**	
Thyroid gland follicular cell adenoma	male	0	2/100 (2 %)	Friedman et al. (1995)
		0.1	9/203 (4 %)	
		0.5	5/101 (5 %)	
		2.0	**12/75 (16 %)[b]**	
Thyroid gland follicular cell adenoma or carcinoma	male	0	1/48 (2 %)	NTP (2012)
		0.33	3/48 (6 %)	
		0.66	4/47 (9 %)	
		1.32	6/48 (13 %)	
		2.71	**9/48 (19 %)[c]**	
Mesothelioma of the testes tunica albuginea	male	0	3/60 (5%)	Johnson et al. (1986)
		0.01	0/60 (0 %)	
		0.1	7/60 (12 %)	
		0.5	**11/60 (18 %)[a]**	
		2.0	**10/60 (17 %)[a]**	
Mesothelioma of the testes tunica	male	0	4/102 (4 %)	Friedman et al. (1995)
		0.1	9/204 (4 %)	
		0.5	8/102 (8 %)	
		2.0	**13/75 (17 %)[b]**	
Mesothelioma of the epididymis or testes tunica vaginalis	male	0	2/48 (4 %)	NTP (2012)
		0.33	3/48 (4 %)	
		0.66	1/48 (2 %)	
		1.32	5/48 (10 %)	
		2.71	**8/48 (17 %)[c]**	

4993 (a): Statistically significantly different from controls at $\alpha = 0.05$ after mortality adjustment as described by Peto (1974).
4994 (b): Statistically significantly different at $p \leq 0.001$ using the method of Peto et al. (1980).
4995 (c): Statistically significantly different from controls using continuity-corrected Poly-3 tests (Bailer and Portier, 1988),
4996 as modified by Bieler and Williams (1993).
4997

In three less-than lifetime studies, rats received initiating agents were treated with AA subsequently (Raju et al., 2011, 2013; Yener et al., 2013a).

Male F344 rats were subcutaneously injected with azoxymethane and received either low fat (7 % corn oil) or high fat (23.9 % corn oil) diet and AA at 0, 5, 10 or 50 mg/kg diet (wt/wt) for 8 weeks (8 rats per group). Irrespective of the dietary fat level, rats with the highest tested dose of AA (50 mg/kg diet) had significantly lower total aberrant crypt foci (ACF) of the colon and lower large ACF compared with their respective controls. A significantly lower number of large ACF was noted in rats treated with 10 mg/kg diet AA exclusively in the high fat group, compared to the high fat control (Raju et al., 2011).

When male F344 rats received AA (2 mg/kg b.w.) and were then treated with azoxymethane (AOM), 20 weeks after AOM treatment the mean tumour size and the total area occupied by tumours in the colon were significantly higher than after AOM only (Raju et al., 2013).

In another study, 14 day-old male rats received azaserine, an initiator of pancreatic tumours, and AA in the drinking water (calculated daily doses: 5 and 10 mg/kg b.w.) for over 16 weeks (Yener et al., 2013a). Animals dosed AA only showed a significant increase in average diameters of atypical acinar cell foci (AACF) of the pancreas, while azaserine/AA treated rats exhibited significant increases in average diameter, total area, and total volume of AACF of the pancreas (Yener et al., 2013a).

For GA, in the NTP (2013, draft report) study where F344/N Nctr rats received GA in drinking water for 2 years (see Section 7.3.2.2), male rats had an increased incidence of testicular, thyroid, heart and oral cavity neoplasms, as well as an increased incidence of leukemia. For females, an increased incidence of mammary gland, thyroid gland, clitorial gland, oral cavity and forestomach neoplasms, as well as leukemia, were observed.

7.3.4.3. Conclusions

AA is carcinogenic in multiple tissues of both male and female mice and rats.

In rats the major tumours produced by AA are adenomas, fibroadenomas and fibromas of the mammary gland, thyroid gland follicular cell adenomas or carcinomas, and testes or epididymis tunica vaginalis mesotheliomas.

In mice, the major tumours produced by AA are Harderian gland adenomas, mammary gland adenoacanthomas and adenocarcinomas, lung alveolar and bronchiolar adenomas, benign ovary granulosa cell tumours, skin sarcomas of various types, and stomach and forestomach squamous cell papillomas in females, and Harderian gland adenomas and adenocarcinomas, lung alveolar and bronchiolar adenomas and carcinomas, and stomach squamous papillomas and carcinomas in males.

GA produced increased incidences of testicular, thyroid, heart, and oral cavity neoplasms and leukemia in male rats and of mammary gland, thyroid gland, clitoral gland, oral cavity, and forestomach neoplasms, and leukemia in female rats. It led to increased incidences of Harderian gland, lung, skin, and forestomach neoplasms in male and of Harderian gland, lung, mammary gland, forestomach, and skin neoplasms in female mice.

These results are in agreement with the fact that AA is efficiently metabolized to GA as a genotoxic and carcinogenic AA metabolite in both sexes of both species. Based upon the concordance of tumour sites between AA and GA it can be concluded that carcinogenic activity of AA is due to its metabolic conversion to GA.

7.3.5. Reproductive and developmental toxicity

7.3.5.1. Reproductive toxicity

The reproductive and developmental toxicity studies on AA in experimental animals have been evaluated before by the SCF (2002), JECFA (FAO/WHO, 2006) and ATSDR (2012). These previous evaluations referred to several studies reporting on the reproductive and developmental toxicity of orally administered AA (Zenick et al., 1986; Sakamoto and Hashimoto, 1986; Smith et al., 1986; Working et al., 1987; Sublet et al., 1989; Chapin et al., 1995; Tyl et al., 2000a,b).

In its 2005 evaluation, JECFA concluded that the overall NOEL for reproductive and developmental effects was 2 mg/kg b.w. per day (FAO/WHO, 2006). In 2010, JECFA indicated that 'no reproductive toxicity studies were identified' since its evaluation in 2005 (FAO/WHO, 2011).

Sublet et al. (1989) reported statistically significantly decreased sperm mobility in Long-Evans rats administered AA by gavage at dose levels of 45 mg/kg b.w. per day for 5 days, but suggested that this effect was not solely responsible for poorer reproductive performance.

Tyl et al. (2000b) found no significant effects on sperm parameters in Long-Evans hooded rats following repeated oral dosing at levels as high as 60 mg/kg b.w. per day and also suggested that indicators of AA induced reproductive toxicity may be at least partly due to impaired mating performance due to AA neurotoxicity.

Histologic indicators of degenerative effects were reported in spermatids of ddY mice administered AA by daily gavage for 5 days at dose levels of 100 or 150 mg/kg b.w. per day (Sakamoto et al., 1988).

Other studies reported evidence of AA-induced testicular atrophy in F344 rats receiving AA in the drinking water for 28 or 90 days at concentrations resulting in estimated AA doses of 19 or 5 mg/kg b.w. per day, respectively (American Cyanamid Company, 1991, as cited in ATSDR, 2012; Burek et al., 1980). Atrophy of the testes and/or seminal vesicles was reported in F344 rats receiving AA at 19 or 25 mg/kg per day from the drinking water for 28 days; this effect was not seen at 12 mg/kg per day (American Cyanamid Company, 1991, as cited in ATSDR, 2012).

Gross and histopathologic examinations of reproductive organs and tissues from male rats receiving AA from the drinking water for up to 2 years at estimated doses as high as 2 mg/kg per day revealed no signs of AA-induced effects (Johnson et al., 1984, as cited in ATSDR, 2012; Johnson et al., 1986; Friedman et al., 1995). Pre-breeding exposure of female mice to AA at a dose level of 18.7 mg/kg b.w. per day (Sakamoto and Hashimoto, 1986) or of female Long-Evans rats at doses up to 14.6 mg/kg b.w. per day (Zenick et al., 1986) did not adversely affect reproductive performance variables such as fertility or implantation when the animals were bred with non-exposed males. Gross and histopathologic examinations of reproductive organs and tissues from female rats receiving AA from the drinking water for up to 2 years at estimated doses as high as 2-3 mg/kg per day revealed no signs of AA-induced effects (Johnson et al., 1984, as cited in ATSDR, 2012; Johnson et al., 1986; Friedman et al., 1995).

In 2003, Tyl and Friedman published a review on the effects of AA on rodent reproductive performance. They reported that at low doses (5 mg/kg b.w. per day) AA decreases litter size with rats being more sensitive than mice. The overview also showed that at higher doses (15-60 mg/kg b.w. per day) male reproductive toxicity was clearly present, since sperm morphology and motility and neurotoxicity were affected resulting in decerased mating frequency. In addition, the authors indicated that AA does not affect female reproduction even in females showing neurotoxicity.

Exon (2006) reported a review of the toxicology of AA and concluded that neurotoxicity of AA can result in behavioural changes that affect reproductive performance. In addition, the author stated that AA may affect kinesin motor proteins that are important in sperm motility and that the mechanism

underlying effects on sperm motility may also be related to direct interaction with sulfhydryl groups on proteins essential to the function of germ cells.

Male C57Bl/6J mice were fed a normal diet or a high-fat diet (60 % of the kilocalories were from lard) from week 5 to week 30 of age. Age-matched vehicle controls from each group, obese and lean mice, were included (Ghanayem et al., 2010). AA-induced reproductive toxicity was assessed in lean or obese males treated with water or 25 mg AA/kg b.w. per day via gavage for 5 days and then mated to control females. Treatment with AA exacerbated male infertility of obese and lean mice. However, this effect was more pronounced in obese mice. Further, females partnered with AA-treated obese mice exhibited a further decrease in the percentage of live fetuses, whereas the percentage of resorptions increased. The authors concluded that diet-induced obesity in mice caused a significant reduction in male fertility and exacerbated AA-induced reproductive toxicity and germ cell mutagenicity.

Wang et al. (2010b) administered AA by gavage to male Sprague-Dawley weanling rats at 0, 5 or 10 mg/kg b.w. per day for 8 weeks. The results indicated that the growth of rats treated with AA was retarded ($P < 0.05$), but relative weights of testes and epididymides compared to body weight were not significantly different ($P > 0.05$). The results also indicate that the epididymal sperm reserves decreased significantly ($P < 0.05$), suggesting partial depletion of germ cells. Mean epididymal sperm concentrations in the 5 and 10 mg/kg b.w. per day dose groups were approximately 24 and 40 % lower, respectively, than those of controls. In addition, histopathologic lesions were also present in the testes of treated rats. The study also reported increased concentrations of Leydig cells and serum testosterone at a dose of 5 mg/kg b.w. per day and a statistically significant approximately 2-fold increase in these concentrations at 10 mg/kg b.w. per day.

Kermani-Alghoraishi et al. (2010) conducted a study on thirty male NMRI mice, aged 8-10 weeks, receiving AA via drinking water for 2 months at an estimated dose of 0, 5 and 10 mg/kg per day. Total sperm motility and progressive motility (fast and slow) in both groups exposed to AA decreased significantly, but no significant change was observed in non-progressive motility. The total motile sperm percentage decreased significantly only in the 10 mg/kg b.w. per day group. Sperm morphology did not significantly change in the experimental groups compared to the controls. In sperm membrane integrity evaluation, functional intact membrane of sperm tail in both AA exposed groups had a significant decrease, but membrane integrity of the sperm head decreased significantly only in the highest dose group. Based on these results, the authors concluded that AA decreased sperm vitality as well as causing abnormal sperm parameters in progressive motility and total motility.

Zhang et al. (2010) investigated the effect of enhanced fat consumption on deficits of spermatogenesis induced by AA in mice. This was investigated because AA and high contents of fat could be found co-existent in many foods processed by high temperature. Forty-eight male Kunming mice were randomly distributed into four groups administered AA at 0, 10 mg/kg b.w. per day and 10 mg/kg b.w. per day and corn oil or pork fat (0.25-0.30 mL), 5 times a week for 10 consecutive weeks. The results obtained revealed that in mice fed diets enriched with corn oil or pork fat, AA induced decreases of spermatogonia and spermatozoa quality were more pronounced. In addition, enhanced consumption of corn oil or pork fat increased the AA induced malondialdehyde production in epididymal sperm and cauda epididymides, and the AA mediated increase in the levels of protein carbonyls in cauda epididymides. Enhanced consumption of corn oil or pork fat also potentiated the AA induced reduction in superoxide dismutase activity in epididymal sperm, corpus, and cauda epididymides, and the reduced activity of glutathione peroxidase in cauda epididymides. The authors concluded that the data suggest that enhanced feeding of corn oil and pork fat potentiates AA-induced oxidative stress in the epididymis and epididymal sperm and subsequent effects on spermatogenesis. The CONTAM Panel noted that this study did not include control groups on corn oil or pork fat diet without AA and that this hampers the interpretation of the results.

NTP (2012) noted degeneration in the seminiferous tubules (decreased number of germinal cells and presence of multinucleated spermatids in the lumen of seminiferous tubules) of male F344/N rats

receiving AA via the drinking water for up to 14 days at an approximate dose of 7.03 mM corresponding to 68 mg/kg b.w. per day. The NOAEL for this effect was 3.52 mM, corresponding to 37 mg/kg b.w. per day. In other males exposed via the food, degeneration in the seminiferous tubules was observed at an approximate dose of 370 mg AA/kg food, corresponding to 52 mg/kg b.w. per day. The NOAEL for this effect was 185 mg AA/kg food, corresponding to 22 mg/kg b.w. per day (NTP, 2012). In similar 14-day studies of male B6C3F$_1$ mice, no histopathologic evidence of reproductive toxicity was observed at AA doses from the drinking water or food as high as 67 and 73 mg/kg b.w. per day, respectively (NTP, 2012).

In the 13-week oral studies in F344/N rats and B6C3F$_1$ mice (see Sections 7.3.2.2 and 7.3.2.1 for details on the doses administered) degeneration of testicular germinal epithelium of both rats and mice was observed. The effect was observed in all male rats given 22.3 and 8.6 mg AA/kg b.w. per day and in five of eight male rats treated with 4.5 mg AA/kg b.w. per day, and in all dose groups of male rats fed diet containing AA (0.5 - 14.2 mg AA/kg b.w. per day), with the incidence increasing with increasing dose. The average severity of the degenerative change was mild to moderate in the 22.3 mg/kg b.w. per day group and minimal-to-mild in the 8.6 and 4.5 mg/kg b.w. per day groups and in the AA diet groups. Moderate hypospermia in the epididymes was also observed in male rats exposed via the diet at 59 mg/kg b.w. per day. In mice degeneration of testicular epithelium was only observed in animals of the high dose group corresponding to 59 (dietary) and 70 (drinking water) mg/kg b.w. per day, respectively. Anestrus was observed in female F344/N rats and B6C3F$_1$ mice at doses of 26 (drinking water) and 64 (dietary) and 83 (drinking water) mg/kg b.w. per day, respectively (NTP, 2012). An increased number of ovarian cysts was observed in female B6C3F$_1$ mice receiving AA from the drinking water for 2 years at doses of 1.1, 4.7 and 10 mg/kg b.w. per day (NTP, 2012). The CONTAM Panel considered the NOAEL for degeneration of testicular germinal epithelium in mice to be 32.8 mg/kg b.w. per day for AA in drinking water and 32.1 mg/kg b.w. per day for AA in diet. In rats, the NOAEL for degeneration of testicular germinal epithelium was 2.1 mg/kg b.w. per day for AA in drinking water and the LOAEL was 0.5 mg/kg b.w. per day for AA in diet. The CONTAM Panel considered the NOAEL for anestrus in rats to be 12.3 mg/kg b.w. per day for AA in drinking water, and in mice to be 31.4 mg/kg b.w. per day for AA in drinking water and 35.1 mg/kg b.w. per day for AA in diet.

Takami et al. (2012) reported a study of F344 rat pups whose mothers were exposed to AA via drinking water during 3 weeks of lactation followed by 9 weeks of exposure of the pups directly via their drinking water to doses of 0, 10, 20 and 40 mg/L (equivalent after weaning to 0, 1.0/1.2, 2.1/2.5 and 4.4/4.9 mg/kg b.w. per day in males and females, respectively). Adverse effects observed included degenerative effects on seminiferous epithelium of the testis and epididymis at an estimated pup dose of 4.4 mg/kg per day. The CONTAM Panel considered the NOAEL in this study to be 2.1 mg/kg b.w. per day.

In order to assess age-dependence of susceptibility to AA-induced neural and testicular toxicity, 3- and 7-week-old male SD rats were given AA at 0, 50, 100, or 200 mg/L in the drinking water for 4 weeks (corresponding to 0, 8.3, 16 and 26 mg AA/kg b.w. per day in youngs and 0, 6.3, 13 and 19 mg AA/kg b.w. per day in adults), and the nervous and male reproductive systems were examined histopathologically (Takahashi et al., 2011). Testicular genotoxicity was evaluated with the Comet assay and the MN test. Suppression of body weight gain was observed in the young groups at the two highest doses. In both young and adult animals, neurotoxicity (gait abnormality, central chromatolysis of ganglion cells in the trigeminal nerves, increase density of axons in the sciatic nerve in youngs, increase in degenerated axons in the sciatic nerve) was evident from 100 mg/L and increased in proportion to AA intake per body weight. Decreases in absolute weights of the testis and epididymides were observed in young and adult rats. In the testis, marked degeneration, loss of or decrease, exfoliation and appearance of multinucleated giant cells, mainly of spermatids, were observed from 100 mg/L limited to young animals. The Comet assay revealed that AA induced significant DNA damage from 100 mg/L in both life stages, while MNs were found only in young rats exposed to 100 mg/L or higher. These results suggest that susceptibility to neurotoxicity might not differ between young and adult rats when exposure levels are adjusted for body weight. Regarding testicular toxicity,

young animals around puberty proved more susceptible than adult animals. The CONTAM Panel derived a NOAEL from this study of 6.3 mg/kg b.w. per day.

Rajeh et al. (2011) studied histopathological effects of AA on testis and epididymis in male Sprague-Dawley rats orally exposed to 0, 5, 15, 30, 45 or 60 mg AA/kg b.w. per day for five consecutive days. AA induced a significant body weight reduction, increase in testis/body weight ratio and a significant reduction in sperm count, in the groups treated with 45 mg and 60 mg/kg b.w. per day. Abnormal sperm shapes were detected in all groups. Histopathological signs of AA toxicity on testes and epididymis included: degeneration of spermatogonia, widening of intercellular junctions and degeneration of peritubular myoid cell. Sertoli cells showed darkening of the nuclei, detachment from the basement membrane, increase in the number and size of lipid droplets in their cytoplasm, failure of sperm release and phagocytosis of some sperms. Leydig cell atrophy was observed which contributed to sperm defects and various abnormal histopathological lesions including apoptosis in rat testis. A possible cause of tail inter segmentation (partial dissolution of fibrous sheath in the principal piece) seen in mature sperm tails was clarified by electron microscope (EM) examination. The authors concluded that AA induced harmful effects on the testis evidenced by degeneration of spermatogenic and Sertoli cells and Leydig cells atrophy in addition to reducing sperm count and appearance of abnormal sperms with the lowest dose level tested of 5 mg/kg b.w. per day. The CONTAM Panel considered this dose level a LOAEL.

Ma et al. (2011) reported adverse effects on sperm parameters in 3-week-old male Sprague-Dawley rats treated with AA at various doses (0, 5, 15 or 30 mg/kg b.w. per day). Most weaning rats in the 15 and 30 mg/kg b.w. per day groups showed decreased body weight, reduced consumption of food and water, and less activity. In addition, the animals of the 30 mg/kg b.w. per day dose group exhibited distinct hind-leg splay, abnormal gait and muscle weakness. Reproductive organ (testis, prostate and seminal vesicle) indexes of the weaning male rats decreased at the two highest dose levels. Levels of follicle-stimulating hormone (FSH) and testosterone in serum increased while luteinizing hormone (LH) in serum decreased. Histopathological lesions (testis: chaotic cells of epithelia, degeneration of all kinds of cells in seminiferous tubules, reduction of the spermatozoa and Leydig cell; epididymis: disarrangement of epithelial structure, degeneration of cells, hyperplasia of connective tissue and significant reduction of sperm), decrease spermatozoal motility, sperm survival rate and sperm count and abnormal sperms were observed in weaning rats after AA treatment. The results point at adverse effects of AA on the reproductive system of weaning male rats.

Jangir et al. (2012) studied the effects of AA toxicity on the male reproductive system and its correlation with histological changes in testes of rats. Male Wistar rats were administered AA via oral gavage (in distilled water) at doses of 0, 10, 15 and 20 mg/kg b.w. per day for 28 days. There was no statistical difference between the mean weights of prostate of different treatment groups. A significant decrease in testes weight was observed in treated groups (12 %, 13 % and 17 %, respectively) compared to control group. When relative weights were compared, no statistically significant difference was recorded. There was a significant dose-related reduction in total sperm counts in caput, corpus, cauda and testes and a significant dose-related increase in dead sperm counts in treated rats. No gross changes were observed in testes and prostate of treated rats. Histopathological changes in treated rats included destruction of seminiferous tubules with detachment of spermatogonial cells observed at periphery of seminiferous tubules. Atrophy of seminiferous tubules was a constant finding. Some sections of testes of high dose treated rats showed vacuolar degenerative changes in germinal epithelium. Less severe lesions were observed at the two lower doses. There was no significant difference among the different groups in lipid peroxidation (although there was a small increase of 5, 11 and 25 % in the low, medium and high dose groups, respectively), and GSH values. There was a significant dose-related increase in values of superoxide dismutase in testes from treated rats. The authors concluded that pathological alterations in testes were responsible for reduced spermatogenesis in rats and suggested that the increase in lipid peroxidation status of testes could be an alternative mechanism of AA toxicity to spermatogonial cells.

Mustafa (2012) treated male albino Sprague-Dawley rats with AA at 50 mg/kg b.w. per day over 10 days. Degeneration of testicular germ cells, numerous multinucleated giant cells with sloughed seminiferous epithelium, and vacuolation in-between the germ cells were reported.

AA did not significantly affect mating performance in female rats, e.g. pregnancy rates, litter size, or survival. However, it significantly depressed pup body weight at birth and weight gain during lactation through post-weaning (Zenick et al., 1986).

Exposure of male F344 rats to 50 mg AA/kg b.w. per day via drinking water for 14 days induced several testicular lesions, including exfoliated germ cells, depletion of germ cells, spermatid retention and apoptosis (Camacho et al., 2012). Both the incidence and the severity of the testicular lesions was high in the rats dosed with 50 mg/kg b.w. per day, but decreased to near control levels in the 10 and 2.5 mg/kg b.w. per day groups, with the exception of the incidence (but not the severity) of the spermatid retention, which remained elevated in all AA groups compared to the control. There was also an increase in the incidence and severity of exfoliated germ cells in the epididymis at the high dose. Testicular absolute weight was decreased in the high dose group compared to control. There was a decrease in Leydig cell total and cytoplasmic volume, with no appreciable change in their nuclear size. Furthermore, the estimated cell count in the high dose group was not significantly different from control (Camacho et al., 2012).

For GA, in the NTP (2013, draft report) study where groups of eight male and eight female mice were administered GA in drinking water for 13-week (see Section 7.3.2.1 for details on the doses administered), degeneration of the germ cells in the testes was observed in seven out of eight male mice give the highest GA dose (3.52 mM, equivalent to 306 mg/L, and corresponding to approximately 45.9 mg/kg b.w. per day) (NTP, 2013, draft report). The NOAEL for testicular germ cell degeneration was 1.41 mM corresponding to 18.3 mg GA/kg b.w. per day. Likewise, for rats (see Section 7.3.2.2 for details of the doses administered), degeneration of the germ cells in testes were observed in all male animals at the three highest doses of GA (0.70, 1.41 and 3.52 mM, equivalent to 61.2, 122 and 306 mg/L, and corresponding to approximately 5.5, 11.0 and 27.5 mg/kg b.w. per day) in three of eight male rats given 0.35 mM GA, and in two of eight male rats given 0.14 mM GA. The severity of the degenerative change was moderate to marked in the 1.41 and 3.52 mM GA groups and mild to minimal in 0.14, 0.35, and 0.70 mM GA groups. In the epididymides, exfoliated degenerating germ cells and cellular debris were observed in all males at the three highest doses and in three males at 0.35 mM, and hypospermia was observed at the three highest doses (NTP, 2013, draft report). The LOAEL for testicular germ cell degeneration was 0.14 mM corresponding to 1.1 mg GA/kg b.w. per day.

In conclusion, several rodent studies have demonstrated adverse effects of AA on male reproductive parameters including reduced sperm counts and effects of sperm and testis morphology with a NOAEL of approximately 2 mg/kg b.w. per day (NTP, 2012; Takami et al., 2012). Minimal to mild degeneration of the testicular germinal epithelium was observed in male rats in the 90-day dietary and drinking water studies. The incidence was dose-related. In the drinking water study, the NOAEL was 2.1 mg/kg b.w. per day, however a low incidence (2/8 animals) was already observed at the lowest doses of 0.5 and 1.4 mg/kg b.w. per day in the dietary study. This lesion was also observed in the 14-day NTP (2012) studies, in other studies which used higher doses of AA and in the 90-day study with GA and at higher doses in mice. However, this lesion was not observed in the 2-year studies with either AA (at doses up to 2 or 2.71 mg/kg b.w. per day (Johnson et al., 1986; Friedman et al., 1995; NTP, 2012) or GA (NTP, 2013, draft report), from which more reliable dose-response would be expected. This lesion is judged to be secondary to primary effects of AA/GA on the Leydig or Sertoli cells (Camacho et al., 2012). The adversity of this lesion at minimal-mild severity is questionable since changes in sperm count or fertility in this low dose range are not observed in the reproductive toxicity studies (Tyl et al., 2000a). Therefore, the CONTAM Panel concluded that this endpoint (i.e. minimal to mild degeneration of the testicular germinal epithelium) should not be used for human risk assessment, and still considered the NOAEL of approximately 2 mg/kg b.w. per day as the relevant one for reproductive toxicity.

5288 7.3.5.2. Developmental toxicity

5289 Developmental toxicity was assessed in the offspring of rat or mouse dams administered AA via the
5290 diet or daily gavage, during gestation and/or lactation. It has been reviewed by SCF (2002), JECFA
5291 (FAO/WHO, 2011) and ATSDR (2012).

5292 Field et al. (1990) studied developmental toxicity of AA in CD rats and CD-1 mice. AA was dosed by
5293 gavage once a day to mice on GD6-17 at dose levels of 0, 3, 15 or 45 mg/kg b.w. per day, and to rats
5294 on GD6-20 at dose levels of 0, 2.5, 7.5 or 15 mg/kg b.w. per day. Fetuses were examined for external,
5295 visceral and skeletal malformations. During treatment, maternal toxicity was observed at the highest
5296 dose levels reflected in reduced body weight in both species and hindlimb splaying in treated mice
5297 only. Weight gain corrected for gravid uterine weight was also reduced in rats at the two highest
5298 doses, and gravid uterine weight was reduced in mice at the two highest doses. Embryo/fetal toxicity
5299 was not observed in rats, but fetal weight was reduced in mice of the 45 mg/kg b.w. per day dose
5300 group. No increased incidence of malformations was observed in either species, however, the
5301 incidence of variations (mainly extra ribs) dose-dependently increased. The authors concluded that the
5302 NOAEL for maternal toxicity in rats was 2.5 mg/kg b.w. per day, that 15 mg/kg b.w. per day
5303 represented a NOAEL for developmental toxicity in rats, and that the NOAEL for maternal and fetal
5304 toxicity in mice was 15 mg/kg b.w. per day. The CONTAM Panel noted that in mice effects were
5305 already observed at this dose level and therefore did not agree with the NOAEL derived for mice by
5306 the authors.

5307 In a study of developmental neurotoxicity, in which Sprague-Dawley rats were given AA orally from
5308 gestational day (GD) 6 until day 10 of lactation, the NOEL for developmental neurotoxicity was
5309 10 mg/kg b.w. per day (Wise et al., 1995).

5310 Husain et al. (1987) reported significantly decreased levels of selected catecholamines (noradrenaline,
5311 dopamine, 5-hydroxytryptamine) in brains of pups of Wistar albino rat dams administered AA at
5312 25 mg/kg per day during lactation. Levels of brain catecholamines were affected in a similar way in rat
5313 pups at 12-21 days of age at the beginning of a 5-day period in which they were administered AA by
5314 gavage at 25 mg/kg per day, whereas these effects were not seen in rat pups that were 60 days of age
5315 at the initiation of dosing

5316 Friedman et al. (1999) reported increased mortality and reduced body weights in pups of Wistar rat
5317 dams dosed at 25 mg/kg per day during lactation, but this effect was accompanied by serious maternal
5318 toxicity.

5319 Tyl et al. (2000a) reported a two-generation reproduction and dominant lethal study in rats. Fischer
5320 344 weanling rats (30 per sex and group) were exposed to AA via their drinking water at 0, 0.5, 2.0 or
5321 5.0 mg/kg b.w. per day for 10 weeks and then mated. Exposure of F0 females continued during
5322 gestation and lactation of F1 litters. F1 weanlings (30 per sex and group) were exposed for 11 weeks
5323 to the same dose levels and then mated to produce the F2 generation. Dose levels of 2.0 and 5.0 mg/kg
5324 b.w. per day resulted in systemic toxicity and increased head tilt and/or foot splay was observed for
5325 rats in all dose groups. Reproductive indices and gestational length were unaffected. Implantations and
5326 live pups per fetus were reduced and survival for PND0 through PND4 was reduced at the highest
5327 dose group. At the highest dose group peripheral nerves in the F1 exhibited axonal fragmentation
5328 and/or swelling. The NOEL for prenatal developmental toxicity was 2.0 mg/kg b.w. per day. The
5329 NOEL for adult systemic toxicity including neurotoxicity was reported to be ≤ 0.5 mg/kg b.w. per day.

5330 Takahashi et al. (2008) evaluate the developmental effects of exposure to AA on the nervous and male
5331 reproductive system using pregnant Sprague-Dawley rats given AA at 0, 50, 100 or 200 mg/L in the
5332 drinking water from GD10 to PND21 and histopathological assessment of offspring was performed at
5333 weaning and postnatal week 11. Mean daily intake of AA by dams during the gestation and lactation
5334 periods was 0, 9.9, 17 and 22 mg/kg b.w. per day. Decreases of food and water consumption and
5335 suppression of body weight gain were observed in the dams at ≥100 mg/L. Maternal neurotoxicity was

evident at 100 mg/L (abnormal gait, central chromatolysis of ganglion cells in the trigeminal nerves (already observed at 50 mg/L), dose-related increases of degenerated axons and myelinated nerves of < 3 µm in diameter, increase of dot-like SYP-immunoreactive structures in cerebellar molecular layer), but at this dose level no neurotoxicity or testicular toxicity was observed in offspring. AA did not affect the gestation period, the number of implantations, or the live birth ratio of the male pups. Significant depression of body weight was observed from PND 2 through weaning from 50 mg/L in males and 100 mg/L in females. At necropsy at weaning, body weights of pups in both sexes were significantly decreased from 100 mg/L and pups in the 100 and 200 mg/L groups showed little milk content in their stomach. Maternal malnutrition was apparent at ≥ 100 mg/L during the lactation period, indicating that poor lactational AA-exposure due to maternal toxicity might account for the lack of AA-induced offspring toxicity except for retarded body growth. After weaning, body weights of male and female pups was still lower as compared to the control from 100 mg/L in a dose-dependent manner.

In another study, Takahashi et al. (2009) gave AA to pregnant Sprague-Dawley rats in the drinking water at 0, 25, 50 or 100 mg/L (mean AA intake 0, 3.72, 7.89 or 14.56 mg/kg b.w. per day) from GD6 through PND21. The 100 mg/L dams exhibited increasing severity of gait abnormalities from PND2 onwards and abnormal gait was observed in 50 mg/L dams from PND18 onwards. Their body weights were suppressed in parallel with the progression of neurotoxic symptoms. Histopathological analysis of dams showed central chromatolysis of ganglion cells in the trigeminal nerves from 50 mg/L. Morphometric assessment of the nervous tissues of dams showed significant increases of degenerated axons and myelinated nerves of <3 µm in diameter at 100 mg/L. No effect on the gestation period, number of implantations, live birth ratio and male pup ratio was observed. At PND21, body weights of dams decreased (without statistical significance). At the highest dose the male and female pups exhibited approximately 42 and 46 % lower mean body weights, respectively, compared to unexposed control pups. No changes suggestive of neurotoxicity or testicular toxicity were observed in the offspring. Free AA was neither detected in the serum of the dams or their offspring, nor in the stomach milk of offspring. The CONTAM Panel derived a NOAEL from this study for both maternal toxicity and development amounting to 25 mg/L, equivalent to 3.72 mg/kg b.w. per day.

Delayed pinnae detachment (a developmental landmark) and deficient negative geotaxis and rotarod performance were reported in F344 rat pups that had been exposed via their mothers (10 mg AA/kg b.w. per day by gavage) during gestation followed by gavage of the pups at the same dose from PND1 through PND22. These effects were not seen at doses ≤ 5 mg/kg b.w. per day (Garey et al., 2005). Decreases in body weight gain (2 - 8 % for females and 5 - 10 % for males on PND22) were observed in pups exposed at doses of 1, 2.5, 5 or 10 mg AA/kg b.w. per day. The CONTAM Panel noted that these decreases are not considered to be biologically relevant. Furthermore, there was no consistent dose-response on body weight gain in the male pups. No effect was observed on fur development or eye opening, on performance of righting reflex, duration of forelimb hang time or open field activity. The NOAEL for maternal toxicity was 10 mg AA/kg b.w. per day based on the absence of effects on b.w., food and water intake, while the NOAEL for developmental toxicity was 5 mg AA/kg b.w. per day.

The effects of daily AA exposure on food-motivated behaviour were studied in Fischer 344 rats by Garey and Paule (2007). Exposures began prenatally on GD6 and continued up to PND85. Plug-positive dams were gavaged with AA (0, 0.1, 0.3, 1.0, 5.0 mg/kg b.w. per day). On PNDs 1-22, pups were gavaged with the same dose their dam had received. At weaning (PND 22), pups were pair-housed with a same-sex littermate and AA exposure continued at 0, 1, 3, 10 and 50 ppm in drinking water. A decreased performance in an operant test of cognitive motivation was observed at 5 mg AA/kg b.w. per day. The NOAEL for developmental toxicity was 1 mg AA/kg b.w. per day.

Garey and Paule (2010) also evaluated the effects of AA on learning task performance in Fischer 344 rats exposed daily beginning prenatally and continuing throughout the lifespan. Dams were gavaged with AA from GD6 onwards at dose levels of 0, 0.1, 0.3, 1.0 or 5.0 mg/kg b.w. per day through parturition. Pups were administered the same dose levels by gavage through weaning until PND22

after which dosing continued via their drinking water. One male and one female per litter (8-9 per treatment group) were tested. AA-exposed rats exhibited altered performance in an incremental repeat acquisition (IRA) task to assess learning ability by 4 months of age. From approximately 1-8 months of age (through ~ PND240), over 52 testing sessions, a significant treatment effect was found on percent task completed (PTC), with a significantly lower PTC for the 5.0 mg/kg b.w. per day group compared to controls. While there was no treatment effect on accuracy, a significant decrease in response rate was seen at 5.0 mg/kg b.w. per day pointing at a NOAEL of 1.0 mg/kg b.w. per day. The CONTAM Panel noted that the data on IRA response, from which the NOAEL was derived, revealed only a reduction at the highest dose level tested which made the data not suitable for dose-response modeling

Allam et al. (2010) studied the effect of prenatal and perinatal AA exposure on the biochemical and morphological changes in the liver of developing albino rats. Pregnant albino rats were given saline (group A) or AA by gastric intubation at a dose of 10 mg/kg b.w. per day, from GD7 till birth (prenatal intoxication, group B) or from GD7 till PND28 (perinatal intoxication, group C). Pups from each group were killed at PND7, 14, 21 and 28. Pups from prenatally and perinatally AA-treated groups showed significant increase in lipid peroxidation with maximum increase in thiobarbituric acid-reactive substances (TBARS) at PND7 in the perinatally intoxicated group and decreasing thereafter with age. The pups from treated animals showed marked decrease in liver GSH at PND7 and 14 compared to controls. Total thiol content was significantly reduced in treated groups compared to the control group. AA treatment produced a significant decrease in peroxidase and superoxide dismutase (SOD) activities. AA treatment significantly increased ALT in both treated groups at all ages of the pups compared to the controls. AP activity was significantly reduced in treated groups at all ages except at PND21 in group B. Total lipids including cholesterol and triglycerides were significantly increased in the serum of treated animals. Sodium and potassium concentrations were increased, but calcium, phosphorus and iron levels were significantly reduced in the serum of treated animals. AA also produced significant electrophoretic changes in serum proteins. The most noticeable change was splitting of β-globulin into β1- and β2-globulins. Light microscopy showed AA-induced fatty deposits (both groups), congested central vein (group C), vacuolisation (both groups) and chromatolysis (group C) in hepatocytes. Ultrastructural studies revealed vacuolated cytoplasm, lipid droplets of variable size and mitochondria with damaged cristae and vacuolisation. The nuclei in AA-treated groups showed marked decrease in the staining of nuclear DNA. The authors concluded that AA affects the liver of the developing rat during gestation and lactation periods. AA-induced structural changes in the liver may be caused by oxidative stress and perturbation of lipid and protein metabolism. Perinatal exposure increased the toxicity of AA compared to the prenatal exposure.

In another study, Allam et al. (2011) examined the effects of AA on the development of external features and cerebellum in albino rats when pregnant females were exposed to 0 or 10 mg AA/kg b.w. per day by gastric intubation, either from GD7 till birth (prenatal intoxicated group); or from GD7 till PND28 (perinatally intoxicated group). Signs of AA toxicity were observed postnatally on the treated mothers represented by ataxia, splayed hind limb, weakness of hind-limb muscles and finally paralysis causing alteration in maternal behavior, so their newborns suffered from bad lactation and consequently malnutrition. At birth, the newborns of all groups were hairless. The time of fur appearing and ear and eye opening was retarded in newborns from treated dams. AA administered either prenatally or perinatally was shown to induce significant retardation in the body weights development of the newborn rats, and to increase thiobarbituric acid-reactive substances (TBARS) and oxidative stress (significant reductions in GSH, total thiols, SOD and peroxidase activities) in the developing cerebellum. AA treatment delayed the proliferation in the granular layer and delayed both cell migration and differentiation. AA treated animals also displayed Purkinje cell loss. Ultrastructural studies of Purkinje cells in the perinatal group showed microvacuolations and cell loss. The authors concluded that prenatal and perinatal exposure to AA caused oxidative stress, resulted in a marked suppression of the antioxidant defence system and induced structural changes in the developing rat cerebellum.

The effects of AA on the development of the medulla oblongata and of oxidative stress during pre- and perinatal maternal AA exposure was studied in newborn rats by the same authors (Allam et al., 2013). Pregnant albino rats were given saline (group A) or AA by gastric intubation at a dose of 10 mg/kg b.w. per day, from GD7 till birth (prenatal intoxication, group B) or from GD7 till PND28 after birth (perinatal intoxication, group C). The pups from each group were killed on PND7, 14, 21 and 28. Signals of AA toxicity were observed postnatally in the treated mothers and were represented by ataxia, splayed hind limbs, weakness of the hind limb muscles, and paralysis, which caused alterations in maternal behavior. Newborns suffered from poor lactation, and consequently, malnutrition, particularly in group C. The newborns of all groups were hairless at birth. The time when fur appeared and ears and eyes opened was delayed in groups B and C. The maternal AA exposure during the gestation and lactation periods produced a pronounced increase in oxidative stress and marked suppression in the antioxidant defense system in the medulla oblongata of newborn rats. The lipid peroxidation level was markedly elevated, whereas the GSH and total thiol content were greatly depleted. Moreover, the antioxidant enzyme activities (SOD and peroxidase) were also depressed in the treated groups. The increase in TBARS observed in the study paralleled the decrease in the GSH concentration in the medulla oblongata of AA-treated newborns. The authors indicated that the enhanced lipid peroxidation and deterioration of the antioxidant defense system that resulted from AA exposure may play a significant role in the pathogenesis and deleterious histological effects on the medulla oblongata of newborns. The pathological cases reflected CNS neuropathy caused by AA. AA affected the medulla oblongata of developed newborn rats if their mothers were exposed to AA during gestation and lactation. These effects, which appeared as histopathological changes within the medulla oblongata, resulted from perturbations of oxidative stress.

Pregnant Fischer 344 dams were given 0.0, 0.1, 0.3, 1.0 or 5.0 mg AA/kg b.w. per day by gavage beginning on GD6 and ending on the day of parturition (Ferguson et al., 2010). Beginning on PND1 and continuing through PND21, all pups/litter were gavaged with the same dose as their dam. There were no AA related effects in offspring on parameters including fur development, pinnae detachment or eye opening. Offspring body weight was somewhat decreased in the 5.0 mg/kg b.w. per day group, particularly in males. AA treatment did not significantly alter righting reflex (PNDs 4-7), slant board (i.e. negative geotaxis) (PNDs 8-10), forelimb hang (PNDs 12-16), and rotarod behavior (PNDs 21-22). Male and female offspring of the 5.0 mg/kg b.w. per day group were 30-49 % less active in the open field at PNDs 19-20. The fact that serum AA levels of GD20 dams and their fetuses were comparable, indicated that AA is able to cross the placental barrier. The authors concluded that these data demonstrate that overt preweaning neurobehavioral effects are apparent in rats exposed to AA pre- and postnatally. A NOAEL of 1.0 mg/kg b.w. per day was identified by the CONTAM Panel. The CONTAM Panel noted that the data on off spring body weight, from which the NOAEL was derived, revealed only a reduction at the highest dose level tested which made the data not suitable for dose-response modeling.

Ogawa et al. (2011) performed immunohistochemical analysis of the offspring of pregnant Sprague-Dawley rats treated with AA at 0, 25, 50 or 100 mg/L in drinking water from GD6 until weaning on PND21 (0, 3.7, 7.9 and 14.6 mg/kg b.w. per day) in the study described above (Takahashi et al., 2009). Offspring were immunohistochemically examined at the end of exposure. Dams in the 100 mg/L group exhibited gait abnormality from PND2, which progressed to a moderate or severe degree at PND21. Body weight in this group was suppressed in parallel with the progression of neurotoxic symptoms. At 50 mg/L, a slightly abnormal gait appeared from PND18. Tendencies for decreased food and water consumption were observed at 100 mg/L during the lactation period. No apparent abnormalities were found on clinical observation in offspring exposed to AA maternally at any dose. Maternally exposed offspring showed decreased body weight at 100 mg/L (nearly 50 %), increased dose-dependently the number of Reelin-immunoreactive cells (a molecule regulating neuronal migration and positioning in the hilus of the hippocampal dentate gyrus) (from 25 mg/L AA) and glutamic acid decarboxylase 67-immunoreactive cells (from 50 mg/L AA), confirming an increase in γ-aminobutyric acid-ergic interneurons. The results revealed decreased apoptosis in the neuroblast-producing subgranular zone of the dentate gyrus of maternally exposed pups at 100 mg/L, and the authors determined the LOAEL to be 25 mg/L (3.72 mg/kg b.w. per day).

Pregnant Sprague-Dawley rats were given drinking water containing AA at 0, 4, 20, 100 mg/L from GD10 to PND21 (Ogawa et al., 2012). There was no observable gait abnormality of dams through to the day 21 after delivery and no significant changes were observed in food intake and water intake consumption during the whole exposure period as compared with the controls. A slight reduction in the absolute liver weight was observed at high dose. No effect was observed on the duration of pregnancy, number of implantation sites, live birth ratio or male pup ratio. At the necropsy on PND 21, statistically significant decreases were found in the body and absolute brain weights of offspring at the high dose that continued to PND77, however, gait abnormalities were not observed. Male offspring were examined immunohistochemically on PND21 and PND77. On PND21, maternal AA-exposure decreased progenitor cell proliferation in the subgranular zone (SGZ) at the two highest dose levels, accompanied with increased density of reelin-producing interneurons and NeuN-expressing mature neurons within the hilus at 100 mg/L. In the SGZ of the 100 mg/L group, cellular populations immunoexpressing doublecortin or dihydropyrimidinase-like 3, were decreased suggesting postmitotic immature granule cells. On PND77, the SGZ cell proliferation and reelin-producing interneuron density recovered, while the hilar mature neurons sustained to increase at the two highest dose levels. The authors concluded that developmental exposure to AA reversibly affects hippocampal neurogenesis targeting the proliferation of type-3 progenitor cells resulting in a decrease in immature granule cells in rats and that a sustained increase in hilar mature neurons could be the signature of the developmental effect of AA. The authors considered the lowest dose level of 4 mg/L (corresponding to 0.36 - 0.89 mg/kg b.w. per day, based on water intake) to be the NOAEL. The authors concluded that while the neurotoxic effect of AA on neurogenesis and following neuronal migration in the dentate gyrus observed from 20 mg/L was subtle and reversible, the sustained increase in mature neurons in the hilus at the later stages after AA-exposure from 20 mg/L was considered to be irreversible. As pointed by the authors, the biological significance of the findings needs to be assessed in relation to functional endpoints (i.e. behavioural alterations). Therefore, the CONTAM Panel did not consider the data suitable for identifying a NOAEL.

El-Sayyad et al. (2011a) investigated the neurotoxic effects of AA on postnatal development. In this study, female rats were treated with AA at a dose of 30 mg/kg b.w. per day during pregnancy, or fed a standard diet (control), and their offspring were examined. Female rats treated with AA gave birth to litters with delayed growth and decreased body and brain weights. Light microscopic studies of the cerebellar cortex of treated animals revealed decreases in Purkinje cells and internal granular layers. Pups born to treated mothers showed different patterns of cell death in Purkinje cells and neurons in the brain. Ultrastructural analysis of Purkinje cells revealed changes in the endoplasmic reticulum, loss of the normal arrangement of polyribosomes, swollen mitochondria with abnormally differentiated cristae, and an abnormal Golgi apparatus. The gastrocnemius muscle in the AA group showed extensive degeneration of myofibrils as evidenced by poorly differentiated A, H, and Z bands. This study reveals that rat fetal exposure to AA via dosing pregnant dams at a dose level of 30 mg/kg b.w. per day, causes cerebellar cortical defects and myodegeneration of the gastrocnemius muscle during the postnatal development of pups.

In another study, El-Sayyad et al. (2011b) investigated the effects of fried potato chips on the development of the retina in albino rats. Pregnant rats (n = 15) were maintained on control diet or from GD6 on a diet formed of potato chips obtained form the market mixed (50:50) with standard diet and their offspring was maintained on the same control and fried potato chips diet till day 7 or 14 post partum. Histological examination of the retina of the exposed offspring revealed many histopathological changes but since the dose of AA in the fried potato chip diet was not quantified, the CONTAM Panel did not considered the study of use for the risk assessment of AA.

El-Sayyad et al. (2011c) also investigated the effects of fried potato chip supplementation on mouse pregnancy and fetal development. Pregnant mice were divided into three groups. Group 1 (n = 20) contained the non-treated control mice, and group 2 (n = 40) consisted of mice treated with AA at a daily dose of 25 mg/kg b.w. per day given orally by stomach tube to pregnant mice from day 6 of gestation until parturition. Group 3 (n = 20) consisted of mice that were given a diet containing fried potato chips which was mixed with the standard diet at a concentration of about 33 % starting from

day 6 of gestation to days 14,16, or 17 of fetal age and at parturition. The standard diet mixed with fried potato chips matched the diet given to the control group and was well balanced for gestating and lactating mice, containing all the ingredients required, including vitamins and minerals. In the pregnant mice, similar histologic abnormalities were found in various tissues (especially liver, kidney, heart muscle, and epiphyseal cartilage of experimental dams) for the AA and the fried potato chips group. AA and fried potato chip exposure increased the rate of abortion and neonatal mortality and decreased the total number, body weight, size, and crown-rump length of the offspring before and after birth. Higher rates of congenital malformations were observed in the fried potato chip-treated group. Ossification of axial and appendicular bones was markedly retarded during fetal development, and some ossified bones were missing in newly born offspring of treated groups. In the fried potato chip-treated neonates the incidence of missing ossification centers was higher than in the AA-treated neonates.

Hułas-Stasiak et al. (2013) reported the effects of maternal AA treatment on ovarian follicle number in newborn guinea pig offspring. 90 day-old pregnant guinea pigs (n = 5 per group) were exposed to 0 (control) or 3 mg/kg b.w. per day beginning on GD32 until parturition via drinking water. After prenatal AA treatment, the pool of primordial and primary follicles was significantly reduced and the number of caspase 3 and TUNEL positive oocytes increased compared to the control group. The authors concluded that the data suggest that prenatal exposure to AA reduced the number of ovarian follicles by inducing follicular atresia mediated by oocyte apoptosis.

The CONTAM Panel concluded that in rats and mice some signs of developmental toxicity (increased incidence of skeletal variations, slightly impaired body weight gain, histological changes in the CNS, and neurobehavioural effects) are observed at exposure levels that are in some cases also associated with maternal toxicity (including neurotoxicity and decreased maternal body weight). The lowest NOAEL reported for developmental toxicity was 1.0 mg/kg b.w. per day from studies in rats exposed gestationally and neonatally (Garey and Paule, 2007, 2010; Ferguson et al., 2010).

7.3.6. Mechanisms and modes of action

7.3.6.1. Chemical reactivity of AA

AA acts as an electrophilic molecule which can bind directly to nucleophilic sites in proteins, nucleic acids, etc. and may act in this way to perturb cellular functions in various cell types including neuronal cells (LoPachin and Gavin, 2012), immune cells (Yener et al., 2013b), and germ cells (Shipp et al., 2006) eventually leading to apoptosis (Park et al., 2010). As an α,ß-unsaturated carbonyl derivative, AA can form Michael-type adducts with nucleophiles via second-order addition reactions to the β-carbon. AA is a soft electrophile which reacts preferentially with soft nucleophiles such as cysteine residues and is less reactive towards harder nucleophiles such as DNA bases, lysine or histidine. With respect to the cysteine side-chain, calculations have revealed that the thiolate ion, representing a particularly soft nucleophile, is likely to be a preferential target for AA (LoPachin and Gavin, 2012).

Reaction of AA with proteins according to the aforementioned mechanism is thought to cause several of the adverse effects of AA. Likewise, AA has been demonstrated to target mitochondrial function in various cell types (Chen et al., 2013a) probably interacting with mitochondrial proteins.

Efforts have been made to characterize target proteins of AA, e.g. in plasma (Feng and Lu, 2011) and dopaminergic cells (Martyniuk et al., 2013). In V79 cells, AA was shown to interact with topoisomerase II (Sciandrello et al., 2010).

The motor protein kinesin is another example for a protein targeted by AA since microtubule-binding of kinesin, an element of microtubule motility in neurons, spermatids etc., was inhibited by 100 µM AA (Sickles et al., 2007), GA being more potent than AA. Kinesin-mediated microtubule motility is can be inhibited by both AA and GA *in vitro* (Friedman et al., 2008).

5589 7.3.6.2. Mode of action of neurotoxicity

5590 The neurotoxic action of AA was suggested to be due to effects on cells of the central and peripheral
5591 nervous system including changes in cellular metabolism (Howland et al., 1980; Brimijoin and
5592 Hammond,1985; Medrano and LoPachin, 1989; Exon, 2006), changes in gene transcription and
5593 protein synthesis (Cavanagh and Nolan, 1982a,b; Cavanagh, 1982; Cavanagh and Gysbers, 1983;
5594 Bisby and Redshaw, 1987; Lin et al., 2000; El-Alfy et al., 2011; Seale et al., 2012), effects on
5595 neurotransmitter levels and turn-over (Dixit et al., 1981; Uphouse and Russell, 1981; Aldous et
5596 al.,1983; Shi et al., 2012), binding to cellular proteins including damage to microtubular and
5597 neurofilamental proteins (Hashimoto and Aldridge, 1970; Tanii and Hashimoto, 1983; Carrington et
5598 al., 1991; Reagan et al., 1994; Gupta and Abou-Donia, 1996, 1997; Lapadula et al., 1989; Xiwen et al.,
5599 1992), changes in ion distribution (Lehning et al., 1998; LoPachin and Lehning, 1994), and axonal
5600 transport (Chretien et al., 1981; Miller and Spencer, 1984; Gold et al., 1985; Moretto and Sabri, 1988;
5601 Logan and McLean, 1988; Harry et al., 1989; Sabri and Spencer, 1990; Martenson et al., 1995; Sickles
5602 et al., 1995,1996; Stone et al., 2001). However, the minimal effects of AA-treatment, by up to a
5603 maximally tolerated dose, on: (i) gene expression related to cholinergic, noradrenergic, dopaminergic,
5604 GABAergic, or glutamatergic neurotransmitter systems; (ii) neurotransmitter levels related to
5605 dopaminergic and seratonergic transmission; and (iii) histological integrity (axonal, dendritic, neuronal
5606 cell body damage or microglial activation) of F344 rat forebrain motor and somatosensory areas of the
5607 brain (striatum, substantia nigra, parietal cortex) serve to emphasize the predominant role of peripheral
5608 neuropathic mechanisms (Bowyer et al., 2009).

5609 AA has been shown to react with certain cysteine residues (Cys) in neuronal proteins such as Cys342
5610 in the presynaptic Na+-dependent dopamine transporter (Barber et al., 2007). Although the cysteine
5611 thiol moiety is basically present in the non-ionized form at intra-cellular pH-values, its position
5612 adjacent to polarizing amino acids in catalytic sites of proteins may lead to thiolate formation.
5613 Cysteine residues in such so-called catalytic triads have been demonstrated to react preferentially with
5614 AA, e.g. in the human erythrocyte glyceraldehyde-3-phosphate dehydrogenase (Thomas et al., 1995).

5615 Thiolates in catalytic triads are typical targets for regulatory nitrosylation by endogenous NO. NO
5616 signaling modulates synaptic transmission by reversibly inhibiting the function of several proteins
5617 involved in the synaptic neurotransmitter vesicle cycle, for example, N-ethylmaleimide (NEM)-
5618 sensitive factor, the dopamine membrane transporter, and the vesicular monoamine transporter (Kiss,
5619 2000; LoPachin and Barber, 2006; Rudkouskaya et al., 2010).

5620 Determination of cysteine adduct levels in the CNS has revealed a progressive increase under AA
5621 exposure, which may explain the accumulative neurotoxicity observed during chronic AA treatment of
5622 animals. The low turnover of axonal proteins, when compared to proteins of other cell types, is likely
5623 to contribute to this observation (LoPachin et al., 2002, 2004, 2006).

5624 There is also indication that GA can exert some neurotoxicity. When administered to rats in the
5625 drinking water over 13 week (NTP, 2013, draft report), the highest dose of 3.52 mM GA (250 mg/L)
5626 caused hind-leg paralysis and low incidence of radiculoneurophathy involving the sciatic nerve and
5627 lumbar spinal cord. This was accompanied at times, by atrophy in the skeletal muscle of the hindlimb
5628 and urinary bladder dilatation.

5629 In neuronal cells, obtained from isolated embryonic stem cells, Sisnaiske et al. (2014) found a
5630 reduction of acetylcholine- and glutamate-induced calcium responses after treatment with AA.

5631 A study by Lee et al. (2014) analysed the effects of AA on rat primary astrocytes and three human
5632 astrocytoma-derived cell lines. Treatment with 1 and 2 mM AA for 24-72 h resulted in decreased cell
5633 viability. Decreases in cell viability could be blocked in most cell types by the caspase inhibitor
5634 Z-DEVD FMK. AA-induced concentration-dependent apoptotic effects were also demonstrated by
5635 increases in the sub-G1 phase and by interruption of mitochondrial membrane.

5636 7.3.6.3. Mode of action of genotoxicity

5637 The electrophilic character of AA in principle enables this compound to react with nucleophilic targets
5638 in nucleic acids (Exon, 2006; Besaratinia and Pfeifer, 2007). AA is slow to react with DNA and only
5639 forms adducts under forced chemical conditions and after extended reaction time (Solomon et al.,
5640 1985). The latter carried out an *in vitro* assay in order to analyze the direct alkylation of
5641 2'-deoxynucleosides and calf thymus DNA following reaction at neutral pH and 37 °C with AA (1.4
5642 M). This resulted in the formation of 2-formamidoethyl and 2-carboxyethyl (CE) adducts via Michael
5643 addition. After 40 days, 1-(2-carboxyethyl)-dAdo (1-CE-dAdo), n6-CE-dAdo, 1-CE-dGuo, 7-(2-
5644 formamidoethyl)-Gua (7-FAE-Gua), 7,9-bis-FAE-Gua, and 3-FAE-dThd were the alkylated 2-
5645 deoxynucleoside adducts isolated at a percentage of 8, 21, 4, 6, 1 or 4, respectively. Following reaction
5646 of AA with calf thymus DNA, the products isolated included 1-CE-dAdo, N6-CE-dAdo, 3-CEdCyd,
5647 1-CE-dGuo, and 7-FAE-Gua at a level of 5.5, 1.4, 2.8, 0.3 or 1.6 nmol/mg DNA.

5648 The relevance of these AA reaction products *in vitro* appears minimal given the extreme reaction
5649 conditions used *in vitro* and the absence of evidence for AA-DNA adducts *in vivo*.

5650 AA has been shown to produce reactive oxygen species *in vitro* that can attack all cellular constituents
5651 and induce oxidative DNA damage (Blasiak et al., 2004; Jiang et al., 2007). Oxidative stress can cause
5652 DNA damage including double-strand breakage. The clastogenicity of AA without metabolic
5653 activation might result from the relatively increased reactive oxygen species (ROS) and/or the
5654 impaired oxidative defense system (Puppel et al., 2005). Jiang et al. (2007) evaluated (i) the generation
5655 of ROS and (ii) the level of oxidative DNA damage by immuno cytochemical analysis of
5656 8-hydroxydeoxyguanosine in human HepG2 cells treated with (i) 5-40 mM AA for 1h or with (ii) 0,
5657 1.25, 2.5, 5, 10 and 20 mM AA for 3h. AA induced significant and dose-related increase in
5658 intracellular generation of ROS from 10 mM onwards. Following AA treatment, the staining intensity
5659 of 8-OHdG increased in a dose-dependent manner (4-fold at 20 mM). The authors concluded that AA
5660 exerts genotoxic effects in HepG2 cells, probably through oxidative DNA damage induced by
5661 intracellular ROS and depletion of GSH. The CONTAM Panel noted that HepG2 cells do not express
5662 CYP2E1, and that the effective AA concentration of 10 mM is extremely high.

5663 The AA metabolite GA is a harder electrophile than AA, being more reactive with hard nucleophiles,
5664 like DNA bases, than AA. It readily reacts with nuclophilic targets such as proteins and nucleic acids
5665 with a broad spectrum of consequences for their integrity, function and for long-term effects such as
5666 cancer. GA is significantly cytotoxic at concentrations above 0.5 mM. When MCF10A cells were pre-
5667 incubated with 100 μM BSO for 24 h to deplete GSH, the increased cytotoxicity showed that
5668 endogenous GSH is crucial for detoxification of GA. The role of antioxidants with distinct modes of
5669 action was also studied in MCF10A cells treated with GA. The results obtained with three
5670 complementary redox modulators suggest that oxidative stress is not involved in GA-induced
5671 cytotoxicity in MCF10A cells. The results with oxidant-sensitive probes obtained in these assays
5672 demonstrate that GA, up to a concentration in the millimolar range (4 mM), does not induce ROS.
5673 Taken together, the data presented in this work consistently suggest that oxidative stress is not the
5674 mode of action of GA in human mammary cells (Bandarra et al., 2013).

5675 It has been suggested that the metabolic formation of GA is the primary pathway responsible for the
5676 genotoxicity of AA in animal experiments (Gamboa da Costa et al., 2003) and in mammalian cells
5677 (Besaratinia and Pfeifer, 2004).

5678 The electrophilic character of GA enables this compound to react with nucleophilic targets in nucleic
5679 acids (Exon, 2006) when it is formed during AA metabolism. *In vivo*, the following studies were
5680 conducted (reviewed in Shipp et al., 2006). Male Sprague-Dawley rats and male BALB/c micewere
5681 given single *i.p.* injections of [^{14}C]-AA at doses of 46 or 53 mg/kg b.w., respectively (Segerbäck et al.,
5682 1995). In addition, an *in vitro* experiment was conducted in which DNA was incubated with [^{14}C]-AA
5683 in the presence of S9 liver mix prepared from non-induced male Sprague-Dawley rats. In both species,
5684 the major adduct formed was N7-GA-Gua, which according to the authors is formed by the reaction of

DNA with the AA metabolite GA. Similar levels of the N7-GA-Gua were found in different organs of the rat, indicating that GA adducts were evenly distributed in the rat. In the mouse, the levels of N7-GA-Gua were not as evenly distributed and the overall levels were higher in organs than those seen in the rat. The authors concluded that the organ-specific carcinogenesis of AA in the rat cannot be explained by selective accumulation of the DNA-reactive metabolites in target organs.

Gamboa da Costa et al. (2003) administered 50 mg/kg b.w. of AA or an equimolar dose of GA by *i.p.* injection to adult male and female mice. N7-GA-Gua and N3-GA-Ade adducts were detected in the liver, kidney, and lung. Approximately 100-fold more N7-GA-Gua adducts were formed than N3-Ga-Ade adducts. More of these DNA adducts were noted following administration of GA compared to AA (1.2- to 1.5-fold higher). However, in neonatal mice, treatment with GA produced 5- to 7-fold the number of these adducts than following administration of AA, which is consistent with a deficiency of CYP450 activity in neonates resulting in less metabolism of AA to GA. According to the authors, these data demonstrated that GA, and not AA, is the DNA-reactive compound likely responsible for the genotoxicity seen in mouse studies.

Adult $B6C3F_1$ mice and adult F344 rats were dosed with single *i.p.* injections of AA at a dose of 50 mg/kg b.w. or GA at 61 mg/kg b.w. (Doerge et al., 2005c). In both rats and mice the major adduct formed was N7-GA-Gua. In mice dosed with AA, N7-GA-Gua was found in all tissues examined, including liver, lung, kidney, leukocytes, and testis, with little variation. DNA adduct levels were significantly higher in males in liver and lung tissue, and significantly higher levels were seen in females in kidney tissue. GA also produced similar levels of N7-GA-Gua adducts in all tissues examined in mice. Similarly, rats dosed with AA had DNA adducts in all tissues examined. In males, DNA adduct levels were found to be significantly higher in testes, leukocytes, and brain tissue than in thyroid. Female rats contained significantly higher adduct levels in leukocytes and mammary-gland tissue as opposed to that of the brain, liver and thyroid. When compared to AA, GA produced higher levels of DNA adducts (160 - 560 %) in all tissues examined in male and female rats. The authors concluded that while the evidence provides some support for a genotoxic mechanism of AA carcinogenicity, other factors beyond the formation of GA and its DNA adducts may be important in determining the organ specificity of tumour formation in chronic bioassays with AA in rodents.

Doerge et al. (2005c) evaluated the formation of DNA adducts in the liver after administration of AA in groups of male and female $B6C3F_1$ mice and F344 rats following a single dose by the gavage route at a dose of 0.1 mg AA/kg b.w. In mice, the major adduct formed was N7-GA-Gua, as in other DNA adduct studies. DNA adduct levels were approximately four times higher than in controls but differed little between males and females, in contrast to the slower production of GA in female mice. However, according to the authors, the small size of the study may have precluded detection of statistically significant differences.When compared to data from a previous study (Gamboa da Costa et al., 2003), the adduct data are in agreement with the conclusion that (relative) metabolic conversion to GA decreases as AA dose increases.

In another study, Sprague-Dawley rats were given either 18 or 54 mg AA/kg b.w. by gavage (Manière et al., 2005). Tissue samples from brain, liver, and testes were collected at 5, 24, 48 and 72 h, and blood samples at 5, 24 and 48 h after dosing. DNA adducts were measured in all tissues sampled. The N3-GA-Ade DNA adduct levels were considerably lower than the N7-GA-Gua (about 50- to 100-fold). The predominant adduct disappeared slowly from the rat organs, remaining at relatively high levels 3 days after treatment with a half-life of 50 to 70 h.

As a consequence of the genotoxic properties, AA is expected to cause mutations in genes critical for the process of carcinogenesis. Choi et al. (2009) reported that co-administration of MNU and AA to rats resulted in a significant increase in codon 12 mutations in the H-ras gene of mammary tumours when compared to tumours in rats treated with MNU only.

5732 7.3.6.4. Modes of action of carcinogenicity

5733 There is strong evidence for a major role of metabolic activation to GA in the genotoxicity of AA,
5734 which is derived from various lines of evidence. Absence of CYP2E1, which converts AA into GA,
5735 led to an almost complete loss of DNA adduct formation (Ghanayem et al., 2005c). Furthermore,
5736 formation of GA-derived DNA adducts was reported after application of AA to rodents (Gamboa da
5737 Costa et al., 2003). Finally, the general patterns of tumor sites in rodents were similar after application
5738 of either AA or GA, indicating that activation of AA to GA is also important in the carcinogenicity
5739 (NTP, 2012, 2013, draft report).

5740 Although GA-mediated DNA damage is considered as the crucial initiating effect leading to AA-
5741 induced carcinogenesis, the level of GA-derived DNA adducts cannot predict the localization and
5742 incidence of tumors with respect to organ specificity. This is particularly evident for the liver which
5743 shows high levels of DNA adducts in AA-treated rodents and, however, do not develop increased rates
5744 of hepatic tumour.

5745 These and other arguments have been brought forward suggesting that other (additional) effects of AA
5746 may be important if not crucial for the carcinogenic mode of action of AA. These are based on
5747 observations of occurrence of mostly benign tumours in AA-treated rats, late age of tumour onset
5748 supported by findings from interim kills that did not demonstrate early tumour response (Johnson et
5749 al., 1986), tumours in a variety of highly hormone-responsive tissues, etc (Maier et al., 2012).

5750 A number of non-genotoxic modes of action of carcinogenicity of AA have been discussed in the
5751 literature. Since tumours originating from endocrine tissues were significantly increased in rat
5752 bioassays, it was argued that AA may act as a carcinogen via adverse effects on endocrine regulation.
5753 It was proposed that AA acts as an agonist at dopamine D1-receptors in rat ovaries thus increasing
5754 prolactin release (reviewed in Shipp et al., 2006). Upon prolactin increase, the corpora lutea increase
5755 gestagen formation which, together with increased prolactin, may stimulate the mammary gland
5756 resulting in increased rates of mammary gland fibroadenoma in female F344 rats.

5757 This mode of action was hypothesized to be active in older rats, while in young adult animals, AA
5758 failed to enhance circulating prolactin levels (Friedman et al., 1999; Khan et al., 1999). It has to be
5759 noted that this hypothesis requires experimental confirmation.

5760 Maier et al. (2012) reviewed linear dose-response modelling of AA tumour data on mammary tumours
5761 in rats. The study compared a linear low-dose response assessment based only on the combined
5762 incidences of adenomas and adenocarcinomas and a mutagenic mode of action and a non-linear
5763 extrapolation using data from tumour promotion potency based on the combined incidence of
5764 adenomas, adenocarcinomas, fibroadenomas and fibromas that occur as a result of 'endocrine
5765 disruption'. The authors concluded that a weight of evidence approach evaluating several hypothesised
5766 modes of action indicated that a non-linear approach would be more appropriate for evaluation of AA-
5767 induced mammary tumours.

5768 Analysing data on AA-related tunica vaginalis mesothelium (TVM) tumours in rats, a tumour which is
5769 almost unknown in humans, and taking into account general biological considerations and modes of
5770 action, Haber at al. (2009) concluded that the overall weight of evidence concerning the mode of
5771 action leads to the conclusion that the most appropriate estimate of human cancer risk based on the rat
5772 TVMs associated with AA exposure is either de minimis or nil. It was concluded that the modes of
5773 action that were most likely driving this tumour response were either not relevant to humans, or would
5774 be properly modelled with a non-linear dose-response leading to quantitative difference in response
5775 between rat and human.

5776 In two cancer studies in F344 rats, increased incidences of tumours of the TVM were found in males
5777 after AA treatment (Johnson et al., 1986; Friedman et al., 1995). In the Johnson et al. study and in a
5778 retrospective examination of the study slides from the Friedman et al. study by Iatropoulos et al.
5779 (1998, as cited by Shipp et al., 2006), a large number of Leydig cell adenomas were found. The

malignant mesotheliomas, classified by Iatropoulos, were only seen in animals that had more than 75 % of their testicular parenchyma replaced by Leydig cell tumours. The CONTAM Panel noted the difficulty of interpreting this hypothesis given the historically high incidences of Leydig cell tumours in untreated 2-year old male F334 rats.

Dourson et al. (2008) evaluated mode of action based formation of thyroid tumours in rats as reported by Johnson et al. (1986) and Friedman et al. (1995) including both mutagenic and thyroid growth stimulation based modes of action. Based on the weight of evidence they concluded that both modes of action may be relevant with the mutagenic mode of action determining the low dose response and growth stimulation dominating the response at higher doses.

Shipp et al. (2006) suggested that enhanced dopamine signalling via AA would trigger down-regulation of LH receptors). The local release of growth-stimulating factors into the testicular vicinity would thus enhance proliferation of the tunica vaginalis eventually resulting in TVM generation.

In a review by Maronpot et al. (2009) on TVM, it was stated that both TVM and Leydig cell tumours are seen most frequently in F344 rats as opposed to other rat strains used in carcinogenicity bioassays.

A large number of publications dealt with the potential protective effects reported for drugs, natural compounds and other chemicals towards biochemical and adverse effects of AA and GA. The protective effects observed are frequently interpreted as counteracting the modes of action of AA and GA (as described above) by antagonizing the electrophilic attack of target molecules/oxidative stress (Kurebayashi and Ohno, 2006; Mehri et al., 2012; Zhang et al., 2013) or by inhibiting CYP2E1 (Taubert et al., 2006). Taken together, these studies confirm the current hypotheses on the modes of action of AA and GA. Table H1 (Appendix H) provides an overview of this type of studies, but does not claim for completeness.

The CONTAM Panel noted a number of other studies on AA, that were either confirmative, performed at extremely high dose levels, or of unclear relevance, which are not described in detail (Begum Sheikh and Kedam, 2010; Céspedes-Camacho et al., 2010; Muthukumar et al., 2011; Zhang et al., 2011; Yener and Dikmenli, 2011; Szewczyk et al., 2012; El-Alfy et al., 2013; Tarskikh et al., 2013; Yerlikaya et al., 2013).

In summary, the CONTAM Panel noted that there is convincing evidence for an electrophilic interaction of AA with proteins resulting in a broad spectrum of cell damage in different tissues. Although a number of hypotheses have been presented on the detailed interaction with target structures, the underlying mechanisms resulting in adverse effects in certain tissues but not in others need further investigation.

The metabolic conversion of AA into GA via CYP2E1 and subsequent binding of GA to DNA is thought to be the major initial event in AA-driven genotoxicity and carcinogenicity. Modifying factors such as tissue-specific biochemical/endocrine events could play a role in targeting certain tissues in AA-driven rodent carcinogenicity. However, these hypotheses need further elucidation and are currently ill-defined although a number have been proposed. In particular, it is unclear if those poorly defined modifying factors in rodents play a role in targeting the carcinogenicity of GA to certain organs in humans. The validity of rat-specific modes of action in endocrine-responsive tissues is questioned by the tumour target tissues for AA in lifetime mouse exposure (e.g. lung, Harderian grand). Furthermore, the concordance between tumour target tissues in AA- vs. GA-treated rats and mice is further evidence for a specific role of GA in the carcinogenic process in both rats and mice.

7.3.6.5. Endocrine/reproductive toxicity

In this chapter, the mode of action of both endocrine and reproductive toxicities of AA are presented and discussed together because of the overlap between adverse effects on endocrine organs, some of them having a pivotal role in reproduction.

In Fischer rats, AA had been reported to lead to a depression of serum testosterone and prolactin (Uphouse et al., 1982; Ali et al., 1983).

Female Fischer 344 rats were given AA at doses of 2 mg/kg b.w. per day and 15 mg/kg b.w. per day for 2 or 7 days by gavage. Twenty-four hours after the last dose, plasma thyroxine (T4), thyroid stimulating hormone (TSH), prolactin (PRL), and pituitary TSH and PRL were unchanged vs. untreated animals. In the 7-day study, there was a slight dose-dependent increase in plasma T4 and a slight dose-dependent decrease in plasma TSH. In the thyroid gland a significant decrease in the colloid area and a significant increase in the follicular cell height were noted (Khan et al., 1999).

Bowyer et al. (2008) treated male Fischer 344 rats over 14 days with doses of 2.5, 10 and 50 mg/kg b.w. per day. There were no significant changes in mRNA levels in hypothalamus or pituitary for thyrotropin-releasing hormone (TRH), TSH, thyroid hormone receptor alpha and beta, as well 10 other hormones or releasing factors, mRNA levels in thyroid for thyroglobulin, thyroid peroxidase, sodium iodide symporter, or type I deiodinases, serum TSH or triiodothyronine (T3) levels (T4 was decreased at high dose only), dopamine, serotonine and metabolites levels in the hypothalamus and pituitary or in cell proliferation (Mki67 mRNA and Ki-67 protein levels) in thyroid or pituitary.

Ma et al. (2011) investigated the reproductive toxicity of AA in 3-week-old weaning male Sprague-Dawley rats treated with 0, 5, 15 or 30 mg/kg b.w. per day. Levels of follicle-stimulating hormone and testosterone in serum increased while luteinizing hormone in serum decreased. Histopathological lesions and abnormal sperms were found in weaning rats after AA treatment, effects on spermatozoidal motility and sperm survival rate being significant at all AA doses tested.

Hamdy et al. (2012) treated adult male Sprague-Dawley rats orally with AA with doses of 5, 10 or 15 mg/kg b.w. per day for 8 weeks. The results indicated that the plasma carcino-embryonic antigen (CEA) and malondialdehyde (MDA) levels were higher, but free and total testosterone, T3 and T4 and corticosterone levels were lower in rats treated with AA than that in control rats. The authors concluded that this study provides evidence of endocrine disturbance to the testis, thyroid and adrenal glands, which are also the organs in which AA has been shown to cause tumours in experimental animals.

Hashimoto et al. (1981) reported that AA produced testicular atrophy in mice with degeneration of the epithelial cells of the seminiferous tubules, the interstitial cells being normal.

In a study by Sakamoto et al. (1988) male mice received 100 or 150 mg AA/kg b.w. One day after treatment, degeneration of round spermatids, especially in the Golgi phase (stage I-III), was found by histological examination of the testis.

A number of studies suggested that DNA damage may be involved in the adverse effects of AA on spermatogenesis in mice. Sega et al. (1990) found that 7.8 and 125 mg AA/kg b.w. applied to male mice resulted in DNA binding and increased UDS in testis or early spermatocytes, respectively. Increased incidence of micronuclei was found in early spermatids in male rats treated with a single dose of 100 or a fractionated dosing of 4 x 50 mg AA/kg b.w. (Xiao and Tates, 1994). AA treatment of male mice with single acute doses of 75 or 125 mg/kg b.w. or with five daily injections of 50 mg/kg b.w. resulted in significant increases of structural chromosomal aberrations in late spermatids/spermatozoa (Pacchierotti et al., 1994).

In AA-treated male Sprague-Dawley rats (0, 5, 15, 30, 45 and 60 mg/kg b.w. per day for 5 consecutive days by oral gavage), 5 mg/kg b.w. reduced the sperm concentration in the Cauda epididymis and led to sperm degeneration, 30 mg/kg b.w. led to a decrease in serum testosterone and to a reduction of Leydig cell viability (Yang et al., 2005).

Wang et al. (2010b) treated male Sprague-Dawley rats of 21 days of age with 0, 5 and 10 mg/kg b.w. per day for 8 consecutive weeks. Relative weights of testes and epididymides compared to body

weight were not significantly different. The epididymal sperm reserves decreased significantly. In addition, histopathologic lesions were also present in the testes of treated rats. Furthermore, distinct changes in expression patterns of testicular soluble guanylate caclase (sGC) heterodimers were observed in AA-treated rats.

Wang et al. (2010a) treated male Big Blue transgenic mice with 1.4 or 7.0 mM of AA or GA in the drinking water for up to 4 weeks. Testicular cII mutant frequency (MF) was increased significantly 3 weeks after the last treatment in mice treated with either the low or high exposure concentrations of AA and GA. There was no significant difference in the cII MFs between AA and GA at the low exposure concentration. The mutation spectra differed significantly between testes and livers and also differed significantly between the two tissues following treatment with either AA or GA. The authors concluded that AA possesses mutagenic effects on testes by virtue of its metabolism to GA, possibly targeting spermatogonial stem cells, but possibly via different pathways when compared to mutations in the liver.

In a study by Takahashi et al. (2011), 3- and 7-week-old male SD rats were given AA at 0, 50, 100 or 200 mg/kg in the drinking water for 4 weeks. In the testis, marked degeneration and exfoliation, mainly of spermatids, were observed at > 100 mg/kg limited to young animals. The Comet assay revealed AA to significantly induce DNA damage at > 100 mg/kg in both life stages, while micronuclei were found only in young rats at > 100 mg/kg. The authors suggested that young animals around puberty were more susceptible than adult animals, possibly due to their lower level of testicular GST activity than that in adult animals.

Camacho et al. (2012) evaluated the effects of a 14 day exposure to AA administered through the drinking water on reproductive tissues and the hypothalamic-pituitary-testes (HPG) axis in male F344 rats. The doses were approximately 2.5, 10 and 50 mg/kg b.w. per day. Serum levels of testosterone were significantly decreased by 10 and 50 mg/kg b.w. per day. Serum levels of LH and the % area of LH-staining in the pituitary were significantly elevated by 10 and 50 mg/kg b.w. per day. Serum FSH was decreased at the highest dose, while serum levels of progesterone and estradiol were unchanged. Histopathological inspection revealed several types of testicular lesions at the highest dose, while spermatid retention was observed at all AA doses investigated including the lowest dose of 2.5 mg/kg b.w. per day. Exposure to 50 mg/kg b.w. per day significantly decreased the DNA labeling index (proliferation) of mesothelial cells of testis, epididymis, and along the combined length of the serosal surfaces. The authors concluded that the absence of evidence for increased proliferation of the peri-testicular mesothelium (Ki-67 immunoreactivity) does not support hormonal dysregulation as a contributing factor to the predisposition of this tissue to the carcinogenic effects of AA.

Friedman et al. (2008) suggested that inhibition of nuclear kinesin is responsible for AA-induced clastogenicity and aneuploidy in isolated rat testicular cells. Two kinesin motors, KIFC5A and KRP2, which are responsible for spindle assembly and disassembly of kinetochore microtubules, were inhibited by AA.

In germ cell studies, analysis of DNA, total sperm head and sperm protamine alkylation suggest AA/GA binding to cysteine sulfhydryl groups in sperm protamine. In mid- to late-spermatid stages, chromosomal histones are replaced by protamines that are relatively rich in arginine and cysteine. Alkylation of free sulfhydryl groups of cysteine in the 'immature' protamine of late spermatids and early spermatozoa may perturb normal chromatin condensation (Shipp et al., 2006).

Taking together the findings in rodents, AA acts as an agent which affects a variety of endocrine and reproductive functions, most notably of male reproduction. In the range of 1-10 mg AA/kg b.w. per day and above, sperm functionality was perturbed and sperm reservoirs in the Cauda epidymidis decreased. At the same dose levels, genotoxicity of AA was seen in several types of testicular cells.

Furthermore, interactions of AA with endocrine/paracrine functions such as Leydig cell toxicity and changes in serum hormones such as testosterone, prolactin, thyroid hormone etc. were reported by

some authors to occur in rodents. These effects were not found by others. One study found enhanced DNA damage in the Comet assay in early male germ cells at a much lower dose level, i.e. at 1.1 µg/kg b.w. per day in male mice.

In summary, AA has been shown to cause adverse effects on the male fertility in rodents, which may occur probably by both damage of proteins by interaction with AA and by damage of nucleic acids (and proteins) by the metabolite GA. Further effects related to systemic or paracrine changes in hormone or growth factor production or release as a mechanism of action for carcinogenicity and/or endocrine toxicity were not reported consistently in the literature.

7.3.6.6. Accompanying effects

In addition to the primary effects described in the previous sub-chapters, AA treatment leads, probably as secondary events, to a broad spectrum of changes in metabolism and gene expression in muscle (Seale et al., 2012) or in the liver (Mei et al., 2008b; Lee et al., 2012; Al-Azkawi et al., 2013) of rodents.

Furthermore, AA-mediated electrophilic stress was suggested to result in the induction of hepatic glutathione S-transferases (Begum Sheikh and Kedam, 2010; Ehlers et al., 2013). COX-2, AP-1 and NF kappa B (Lim et al., 2011) and casein kinase 2 (Lee et al., 2010) are among the genes/proteins reported to be activated by AA in certain cell types. Gene expression changes in non-tumourigenic mammary cells such as changes in inducible NO synthetase and COX-2 were suggested to contribute to AA carcinogenicity (Lyn-Cook et al., 2011).

Soluble guanylate cyclase (sGC) belongs to the NO-regulated enzymes, playing an important role in spermatogenesis, sperm motility, tight junctions in neuronal blood-brain barrier, etc. Wang et al. (2010b) reported that distinct changes in expression patterns of testicular sGC heterodimers were observed in AA-treated rats.

7.4. Observations in humans

7.4.1. Epidemiological studies: cancer

The association between AA exposure and human cancer risk has been studied first in occupational studies. A few retrospective cohort studies have published several papers on the risk of cancer in workers who had been exposed occupationally to AA. Following the discovery in 2002 that AA is present in many foods, there was a great need for epidemiological data. Since then, the association of dietary AA intake and cancer risk has been studied in several case-control and prospective cohort studies. Different methods have been used to measure exposure and to adjust for confounding by, e.g. smoking. In these paragraphs the epidemiological evidence is summarized and discussed.

7.4.1.1. Occupational studies and cancer

The association of occupational exposure to AA has been reported in five publications from two retrospective cohort studies. The first cohort included 371 workers exposed to AA in a US factory (Sobel et al., 1986). This cohort has been updated in a publication with additionally exposed workers and follow-up was extended with 19 years (Swaen et al., 2007). The second cohort included more than 8 800 workers from three AA factories in the US and one in The Netherlands and results have been published in three articles with different follow-up periods (Collins et al., 1989; Marsh et al., 1999, 2007).

The first cohort study (Swaen et al., 2007) included 696 workers (41 women and 655 men) who had worked in an AA facility between 1955 and 1997 and were followed-up until 2001. Exposure information was available as personal air samples. Workers employed in operations were estimated to have been exposed to 0.25 mg/m^3 before 1970 and to 0.05 mg/m^3 after 1970, and workers employed in administration or maintenance were estimated to have been exposed to 0.125 mg/m^3 before 1970 and

to 0.02 mg/m^3 after 1970. Mean duration of employment in an AA job was 42 months and the mean estimated cumulative exposure score was 4.6 mg/m^3 months per worker.

There were 43 deaths from cancer in the cohort compared to 45.4 expected (Standardised Mortality Ratio, SMR 0.95; 95 % Confidence Intervals, 95 % CI 0.69-1.28). SMRs were not statistically significant increased or decreased for most subtypes of cancer, although SMRs were increased for rectal cancer (SMR 2.43; 2 observed deaths and 0.8 expected deaths), pancreatic cancer (SMR 2.22; 5 observed and 2.3 expected deaths), and kidney cancer (SMR 2.45; 3 observed and 1.2 expected deaths). For all deaths because of a malignancy, the SMR was higher in workers with a low cumulative exposure (< 1 mg/m^3: SMR, 1.05; 95 % CI 0.64-1.61) than in workers with a high exposure (> 1 mg/m^3: SMR, 0.88; 95 % CI 0.56-1.32). In both rectal and pancreatic cancer, SMRs were also higher in workers with a low than in workers with a high cumulative exposure. Only for kidney cancer the SRM for kidney cancer was higher in the high-exposure group.

The second cohort (Marsh et al., 2007) included workers from three US plants and one Dutch plant. The most recent update of this study describes the mortality follow-up of 8 852 male workers who had worked between 1925 and 1973 in one of the four plants. Follow-up was updated until 2002 (US plants) or 2004 (Dutch plant). AA exposure was estimated using the job history of every worker and job- and time-specific exposure estimates. Workers were considered exposed to AA if their cumulative exposure score was larger than 0.001 mg/m^3-years.

In the follow-up until 1994 in that cohort (Marsh et al., 1999) a statistically significant increased risk of pancreatic cancer was observed for high cumulative AA exposure (SMR 2.26; 95 % CI 1.03-4.29).

In workers from the Dutch plant of the cohort (follow-up until 2004; Marsh et al., 2007), 21 cancer deaths were observed as compared to 44.3 expected deaths (SMR 0.47, 95 % CI 0.29-0.73). The SMR for lung cancer was 0.54 (95 % CI 0.25-1.02), while there were no deaths from pancreatic and kidney cancer versus 1.9 and 1.3 deaths expected, respectively. Among workers in the US plants exposed to AA (Marsh et al., 2007), the SMRs (follow-up until 2002) were 0.99 (95 % CI 0.86-1.12) for all malignant neoplasms, 1.41 (95 % CI 0.81-2.29) for pancreatic cancer, 1.14 (95 % CI 0.92-1.40) for lung cancer, and 1.27 (95 % CI 0.55-2.50) for kidney cancer. There was no consistent evidence for these cancers of a dose-response relationship.

Both cohort studies compared the observed number of cases in the cohort with expected numbers based on national (or regional) mortality rates. This could have caused bias in both directions. Workers could have had better diagnoses thus increasing their cancer risk (Swaen et al., 2007). But it is also possible that the workers are healthier than the general population and experience lower cancer mortality rates (known as the healthy worker effect).

In conclusion, two epidemiological studies of occupational exposure to AA do not indicate an increased risk of cancer. There were indications of an increased risk of pancreatic cancer in both cohorts, although in one cohort the risk attenuated and was not statistically significant after longer follow-up. In the other cohort, the increased risk was not statistically significant and the excess risk was highest in workers with a low cumulative exposure to AA which is not in accordance with a dose-response relationship.

7.4.1.2. Dietary studies and cancer

The following section includes a summary of epidemiological studies which analyzed the association between AA exposure through diet and the incidence or mortality from cancer. The section first provides a general description of the studies, including in particular the cancer sites considered and the instrument used to evaluate AA exposure (food frequency questionnaires, FFQs, national and international databases of AA content in foods, ad hoc analyses of AA food content, or measures of Hb adducts), and the validity/reproducibility of information on dietary AA, if any. Such information is also summarized in Table J1 (Appendix J). Subsequently, the major results on AA exposure through diet and cancer risk are described (and also summarized in Appendix J, Tables J2 to J6) by cancer

sites, i.e. cancers of reproductive organs (breast, endometrial, ovarian, Table J2), cancers of the gastro-intestinal tract (oesophageal, stomach, colorectal, pancreatic, Table J3), cancers of the urinary tract (prostate, renal cell, Table J4), cancers of the respiratory tract (oral and pharyngeal, laryngeal, lung, Table J5), and other cancers (brain, thyroid, lymphatic,Table J6). Moreover, a paragraph summarizes the results and conclusions of a meta-analysis and various reviews of epidemiological studies on dietary AA and cancer risk.

A final conclusive section summarises the overall evidence of the role of dietary AA on cancer incidence and mortality and briefly discusses the strengths, limitations and drawbacks of the epidemiological studies considered.

7.4.1.2.1. Studies of dietary exposure to AA

At least 34 publications, based on 16 original studies, considered cancer risk in relation to AA exposure through diet (**T**able J1). Eleven of those studies had a cohort design, four case-control design, and one was a nested case-control study within a cohort. Six studies were conducted in Sweden, six in other European countries and four in the US.

The first study was published in 2003 (Mucci et al., 2003a) and updated during the same year (Mucci et al., 2003b) adding further data on coffee. This was a population-based case-control study from Sweden, including 591 colorectal, 263 bladder, and 133 kidney cancer cases, and 538 healthy controls. AA intake was calculated for each subject by multiplying data from a semi-quantitative FFQ by the AA level ranking of 12 foods, derived from the Swedish National Food Agency (SNFA). Mean AA intake was 27.5 µg per day (i.e. 0.39 µg/kg b.w. per day) among controls.

The second study (Mucci et al., 2004) was a Swedish population-based case-control study of renal cell cancer, including 379 cases and 353 controls. AA intake was estimated from information on 11 AA-rich food items included in the FFQ, and the corresponding AA content obtained from the SNFA and the US Food and Drug Administration (US-FDA). Mean AA intake was 27.6 µg per day (i.e. 0.39 µg/kg b.w. per day) among controls.

The Women's Lifestyle and Health Cohort (Mucci et al., 2005) examined the association between AA intake and breast cancer in 43 404 Swedish women followed-up between 1991 and 2002 for a total of 490 000 person-years and including 667 incident breast cancer cases. The study used a semi-quantitative FFQ, including information on about 10 AA-rich foods and derived AA food contents from the SNFA (2002). Mean AA intake in the study population was 25.9 µg per day (i.e. 0.37 µg/kg b.w. per day).

Another Swedish prospective study, the Swedish Mammography Cohort (SMC) (Mucci et al., 2006; Larsson et al., 2009a,b,c), included about 61 500 women enrolled in a screening program between 1987 and 1990, that were followed for cancer outcome up to the end of 2007 (except for colorectal cancer, that ended in 2003). Cancer incidence was obtained through linkage of the cohort to Swedish Cancer registries, and four sites were investigated in relation to AA intake: colorectum (741 cases), breast (2 952 cases), endometrium (687 cases), and ovary (368 cases). Two FFQs were used to collect dietary information, a 67-item FFQ at baseline and a 96-item FFQ in a second interview in 1997. Dietary AA was estimated by combining data of 21 of food items (15 for colorectal cancer) to their AA content, obtained from a Swedish study (Bergström et al., 1991) and from the SNFA (2002). Cumulative average AA intake was considered in order to account for dietary changes during follow-up and to better represent long-term dietary intake. Mean intake of AA was 24.6 ± 7.6 µg per day (i.e. 0.38 ± 0.17 µg/kg b.w. per day). The validity of the baseline FFQ was assessed on 129 women randomly chosen in the cohort, by comparing information collected through the FFQ with that collected through four 1-week dietary records. The correlation coefficients were 0.6 for coffee, 0.5 for whole grain bread, and 0.6 and breakfast cereals/muesli.

A multicentric network of hospital-based case-control studies conducted in Southern Europe between 1991 and 2002 considered dietary AA and cancer risk. These data are published in three papers: the

first one (Pelucchi et al., 2006) referred to cancer of the oral cavity and pharynx (749 cases), esophagus (395 cases), colorectum (2 280 cases), larynx (527 cases), breast (2 900 cases), ovary (1 031 cases), and prostate (1 294 cases). The second one to renal cell cancer (767 cases) (Pelucchi et al., 2007) and the third one (Pelucchi et al., 2011a) to pancreatic cancer (326 cases). In all studies, dietary information was based on a 78-item FFQ. Dietary AA intake was obtained linking information on nine food items included in the FFQ, and data on AA content of foods obtained from the WHO and the Swiss Federal Office of Public Health (SFOPH). The average AA intake among different control groups ranged between 23.3 and 37 µg per day (i.e. between 0.33 and 0.48 µg/kg b.w. per day). Reproducibility of dietary information was evaluated by comparing the FFQ administered twice at an interval of 3-10 months to 452 volunteers. The correlation coefficients were between 0.52 and 0.75 for main AA-containing foods.

The Netherlands Cohort Study (NLCS) considered the relation between dietary AA and several neoplasms using a case-cohort approach (Hogervorst et al., 2007, 2008a,b, 2009a,b; Schouten et al., 2009; Pedersen et al., 2010; Bongers et al., 2012; Hogervorst et al., 2014). This prospective study included 58 279 men and 62 573 women aged 55-69 years at baseline, followed between 1986 and 1997-2002. The following cancer sites were examined: oral cavity (101 with data allowing analyses on AA intake), oro-pharynx (83), esophagus (216 cases), stomach (563 cases), colorectum (2 190 cases), pancreas (349 cases), larynx (180), lung (1 895 cases), breast (2 225 cases), endometrium (221 cases), ovary (195 cases), prostate (2 246 cases), bladder (1 210 cases), renal cell (339 cases), brain (216 cases), thyroid (66 cases), and lymphatic malignancies (1 233 cases). The NLCS included a FFQ with 150 food items, 16 of which were reported to contain AA. To estimate AA intake, data were taken from the Dutch Food and Consumer Product Safety Authority and from *ad hoc* analyses conducted by this authority for the NLCS to estimate the AA exposure. Mean AA intake in the overall population was 21.8 ± 12.0 µg per day, i.e. 0.30 ± 0.18 µg/kg b.w. per day (22.6 ± 12.2 µg per day in men and 21.0 ± 11.9 µg per day in women, i.e. 0.29 ± 0.16 and 0.32 ± 0.19 µg/kg b.w. per day, respectively). Validity of the dietary information was assessed on 109 random subjects of the cohort comparing information from a FFQ completed 2 years after the baseline one with reference information collected through dietary record kept over three 3-days periods, 4-5 months apart (Goldbohm et al., 1994). The correlation was 0.74 for potatoes, 0.80 for bread, 0.65 for cakes and cookie. Similarly, correlations were between 0.70 and 0.75 for carbohydrates, fiber, and energy intake. Reproducibility was estimated on 400 random subjects comparing the FFQ information at baseline and that collected in five repeated measurements (Goldbohm et al., 1995). Correlation coefficients were 0.66-0.71 for carbohydrates and fiber.

Within the Cohort of Swedish Men (Larsson et al., 2009d,e), AA intake was analysed in relation to colorectal and prostate cancer. The cohort included 45 306 men enrolled in 1997 and follow-up until the end of 2007, for a total of over 400 000 person-years. During this period, 676 incident colorectal cancer cases and 2 696 incident prostate cancer cases were identified through linkage to Swedish Cancer registries. AA intake was estimated using the same methods of the SMC study. Mean AA intake in the study population was 36.1 ± 9.6 µg per day (i.e. 0.52 ± 0.14 µg/kg b.w. per day).

The Cancer of the Prostate in Sweden (CAPS) study (Wilson et al., 2009a), included 1 499 incident, prostate cancer cases and 1 130 male controls. The self-administered FFQ included 261 food items, 18 of which were reported to contain AA. To estimate total AA intake of each subject, information collected from the FFQ was combined to data of the SNFA (2002). Mean intake of AA was 44.5 ± 14.5 µg per day among controls (i.e. 0.56 ± 0.20 µg/kg b.w. per day).

The Nurses' Health Study II (NHS-II) was the first non-European study that considered the issue of AA and cancer risk (Wilson et al., 2009b). This prospective study included 90 628 premenopausal women enrolled in 1991. Until 2005, it collected almost 950 000 person-years of follow-up, and recorded 1 179 cases of breast cancer. Subjects' dietary habits were assessed through a 130-item FFQ completed every four years of investigation. For 42 food items, data on AA content were available from the US-FDA or the SNFA. Cumulative average AA intake was computed, to account for dietary changes during follow-up and to better represent long-term dietary intake. The mean of the middle

quintile of AA intake was 20.2 µg per day (i.e. 0.32 µg/kg b.w. per day). FFQ information was compared with 28-day diet records from a subset of 173 women within the NHS (Salvini et al., 1989; Wilson et al., 2009b). The correlation between the two measures of AA intake was 0.60 for potato crisps, 0.73 for French fries, 0.78 for coffee, and 0.79 for breakfast cereals.

Further information on the association between AA and female hormonal cancers were provided by the NHS (Wilson et al., 2010), a prospective study on 88 672 women enrolled in 1980. Between 1980 and 2006, 6 301 cases of breast, 484 of endometrial and 416 of ovarian cancer were identified. Dietary habits were investigated using a 61-item FFQ, which was expanded to 116 items in 1984. AA food content was estimated as in the companion NHS II study (Wilson et al., 2009b). Median intake of AA was 16 µg per day (i.e. 0.24 µg/kg b.w. per day)

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study in Finland (Hirvonen et al., 2010), a cohort investigation including 27 111 male smokers recruited in 1985-1988, investigated AA in relation to various cancer sites. After an average 10.2 year of follow-up, 1 703 cases of lung cancer, 799 of prostate, 365 of urothelial cancers, 316 of colorectal, 224 of stomach, 192 of pancreatic, 184 of renal cell and 175 of lymphomas were recorded. Diet was assesed using a self-administered modified diet-history method with 276 items. AA intake was estimated mainly using published Finnish data (Eerola et al., 2007) and by chemical analyses conducted particularly for the present study on the basis on 26 foods. In order to be eligible for the ATBC study, participants had to have smoked at least five cigarettes per day at study entry. Restriction to never- or former-smokers was therefore not possible and it is not possible to rule out the possibility of residual confounding by smoking. Median intake of AA was 36.7 µg per day (i.e. 0.52 µg/kg b.w. per day). Reproducibility of dietary information was tested by comparing information collected at interview and that provided in a second questionnaire filled at the end of the pilot study. Validity was tested comparing information colleted at interview with that from 24 days food records (Pietinen et al., 1988; Hirvonen et al., 2010). The correlation coefficient of dietary AA was 0.73 for reproducibility and 0.43 for validity.

The UK Women's Cohort Study included 33 731 women enrolled in 1995-1998 followed-up to for a median of 11 years for breast cancer occurrence (1 084 cases) (Burley et al., 2010). A validated 217-item FFQ, adopted in the UK arm of the EPIC study, was used to assess dietary information. Estimates of AA intake were computed on the basis of 24 foods using EU estimates of AA content of food (EC, 2006). Mean dietary AA was 15 µg per day (i.e. 0.23 µg/kg b.w. per day). To test for reproducibility of dietary information, the FFQ was repeated in a random subsample of 1 859 women 5 years after the first questionnaire was filled in. The correlation coefficient for dietary AA was 0.61, with similar values across all dietary sources of AA.

A Swedish population-based case-control study was conducted between 1995 and 1997 on 189 cases of oesophageal adenocarcinoma, 262 gastroesophageal junctional adenocarcinomas, and 167 cases of squamous cell oesophageal cancer. Habitual diet 20 years before interview was assessed using a validated FFQ with 63 items (Lin et al., 2011). AA intake was derived from nine foods on the basis of data from the SNFA (2002). Mean dietary AA was 36.3 ± 14.3 µg per day among controls (i.e. 0.52 ± 0.20 µg/kg b.w. per day).

The Health Professionals' Follow-up study (HPFS, Wilson et al., 2012) analyzed the association between AA and prostate cancer. This prospective cohort study included 47 869 US men interviewed for the first time in 1986 and followed-up to 2006 for a total of 5 025 prostate cancers (642 lethal). Dietary habits and AA intake was assessed as in the companion NHS and NHS II studies (Wilson et al., 2009a). The median of the middle quintile of AA intake was 20.8 µg per day (i.e. 0.30 µg/kg b.w. per day).

In the EPIC study, a large prospective study enrolling over 500 000 men and women from 10 European countries between 1992 and 1998, the relation between AA intake and the risk of pancreatic cancer was investigated on 865 adenocarcinomas of the exocrine pancreas identified after a mean follow-up of 11 years (Obón-Santacana et al., 2013; Luján-Barroso et al., 2014). In this study,

food and alcohol consumption was assessed at cohort enrolment by country-specific validated FFQs. Information on AA levels was obtained from an EU database (JRC-IRMM), further integrated with additional sources. The main determinants of dietary AA intake in the EPIC cohort were eight foods. Mean dietary AA was 26.2 ± 14.8 µg per day (i.e. 0.38 ± 0.21 µg/kg b.w. per day) in the overall cohort, 31.9 ± 16.9 µg per day (i.e. 0.40 µg/kg b.w. per day) in men and 23.8 ± 13.1 µg per day (i.e. 0.37 µg/kg b.w. per day) in women. Comparing dietary estimates of AA from FFQ and a random sample of 510 subjects from 9 European countries with a 24-hour dietary recall interview, gave a correlation coefficient of 0.17, while comparison with AA adducts in Hb gave a correlation coefficient of 0.08 (Ferrari et al., 2013).

Four epidemiological studies examined the relation between AA and cancer risk using biomarkers of AA exposure, the Danish Diet, Cancer and Health study (Olesen et al., 2008), the CAPS study (Wilson et al., 2009a), the NHS and NHS II prospective studies (Xie et al., 2013) and a Danish study which analysed survival from breast cancer (Olsen et al., 2012).

The Danish Diet, Cancer and Health is a prospective study conducted in Denmark between 1993 and 1997 (Olesen et al., 2008) on 24 697 post-menopausal women followed-up until the end of 2000. A nested case-control study was conducted on 374 breast cancer cases and 374 controls. Levels of AA and GA were estimated from Hb adducts: median concentrations were 47 pmol/g Hb for AA-Hb and 26 pmol/g Hb for GA-Hb adducts in controls, with similar values in cases. In both cases and controls, smokers had 3-3.5-fold higher AA and GA adduct levels as compared with non-smokers. Within the same cohort (Olsen et al., 2012), the mortality of 420 breast cases was investigated in relation to AA-Hb and GA-Hb. In this population, median AA and GA-Hb adducts was 57 pmol/g Hb among non-smokers and 187 pmol/g Hb among smokers.

The CAPS study (Wilson et al., 2009a) measured AA adducts to Hb in blood samples of a subsample of 170 cases and 161 controls. In this study, mean AA-Hb adduct levels were 53.7 pmol/g Hb among controls. GA-Hb adducts were not measured. After adjustment for selected covariates, including energy intake, the partial correlation between AA as estimated from FFQ and AA adducts to Hb was 0.25.

AA exposure was also evaluated using red blood cell AA and GA Hb adducts among women from the NHS and NHS II prospective studies (Xie et al., 2013). The association was then evaluated on 263 ovarian cancer cases. In this study, median values of total adducts were 113.9 pmol/g Hb in controls; corresponding AA adducts and GA adducts were 62.2 and 51.1 pmol/g Hb. Among a sample of 296 non-smoking women, the correlation between AA as estimated from the FFQ in 1999 and the sum of AA and GA Hb-adducts was 0.34 (Wilson et al., 2009c; Xie et al., 2013).

7.4.1.2.2. Cancers of reproductive organs (Appendix J, Table J2)

Breast cancer

At least nine studies provided information on dietary AA and breast cancer risk (Mucci et al., 2005; Pelucchi et al., 2006; Hogervorst et al., 2007; Olesen et al., 2008; Larsson et al., 2009a; Wilson et al., 2009b, 2010; Burley et al., 2010; Pedersen et al., 2010). Moreover, one study (Olsen et al., 2012) analyzed survival from breast cancer in relation to pre-diagnostic AA-Hb and GA-Hb.

The Swedish Women's Lifestyle and Health Cohort study (Mucci et al., 2005) reported a relative risk (RR) of breast cancer of 1.19 (95 % CI 0.91-1.55) for the highest (≥ 34 µg per day, i.e. ≥ 0.49 µg/kg b.w. per day) versus lowest quintile (≤ 17 µg per day, i.e. ≤ 0.24 µg/kg b.w. per day) of AA intake, with no evidence of a dose-risk relationship.

In a case-control study from Italy and Switzerland (Pelucchi et al., 2006), the RRs of breast cancer were 1.01 (95 % CI 0.85-1.20), 1.01 (95 % CI 0.85-1.20), 1.09 (95 % CI 0.92-1.31) and 1.06 (95 % CI 0.88-1.28) for subsequent quintiles (up to ≥ 34 µg per day, i.e. ≥ 0.49 µg/kg b.w. per day) of AA

6207 intakes as compared with the first quintile (< 11 µg per day, i.e. < 0.16 µg/kg b.w. per day), with no
6208 significant linear trend in risk.

6209 The NLCS did not find an association between dietary AA and breast cancer (Hogervorst et al., 2007).
6210 The RR was 0.93 (95 % CI 0.73–1.19) for the highest quintile of intake (~ 37 µg per day, i.e.
6211 ~ 0.53 µg/kg b.w. per day) versus the lowest (~ 10 µg per day, i.e. ~ 0.14 µg/kg b.w. per day) and
6212 0.99 (95 % CI 0.92-1.06) for an increase in AA intake of 10 µg per day (i.e. ~ 0.14 µg/kg b.w. per
6213 day). In never-smokers, the RRs were only slightly higher (RR 1.10, 95 % CI 0.80-1.52 and 1.01,
6214 95 % CI 0.93-1.11, respectively).

6215 In the nested case-control study within the Danish Diet, Cancer and Health, which examined the
6216 relation between AA and cancer risk using biomarkers of AA exposure (Olesen et al., 2008), the RR of
6217 breast cancer for a 10-fold increase in concentration was 1.05 (95 % CI 0.66–1.69) for AA-Hb and
6218 0.88 (95 % CI 0.51-1.52) for GA-Hb. No association emerged for either adduct according to estrogen
6219 receptor (ER) status of breast cases. After careful adjustment for smoking, including past smoking,
6220 amount and duration, the RRs increased to 1.9 (95 % CI 0.9-4.0) for a 10-fold increase in
6221 concentration of AA-Hb and to 1.3 (95% CI 0.6-2.8) for GA-Hb. The corresponding estimates for
6222 ER+ cases were 2.7 (95 % CI 1.1-6.6) and 1.5 (95 % CI 0.6-3.8), respectively. Inclusion of both types
6223 of adducts in the same models resulted in a decrease of the risk estimate for GA-Hb adducts, while
6224 that for AA-Hb adduct remained virtually unchanged.

6225 In a survival analysis among 420 women with breast cancer from the Danish Diet, Cancer and Health
6226 (Olsen et al., 2012), higher concentration of AA-Hb and GA-Hb were associated with a higher hazard
6227 ratio (HR) of breast cancer specific mortality (HR 1.21, 95 % CI 0.98-1.50 and 1.63, 95 % CI
6228 1.06-2.51, respectively, for 25 pmol/g globin). Higher risks were observed in ER+ women.

6229 The SMC study (Larsson et al., 2009a) reported a multivariate RR for breast cancers of 0.91 (95 % CI
6230 0.80 -1.02) for high (≥ 29 µg per day, i.e. ≥ 0.41 µg/kg b.w. per day) versus low (< 20 µg per day, i.e.
6231 < 0.29 µg/kg b.w. per day) long-term AA intake (p for trend 0.06). Similar RRs were found according
6232 to ER and progesterone receptor (PR) status and smoking habit.

6233 The US NHS II found no relation between dietary AA and breast cancer risk among premenopausal
6234 women (Wilson et al., 2009b), the multivariate RRs for the fifth (~ 38 µg per day, i.e. ~ 0.58 µg/kg
6235 b.w. per day) versus first (~ 11 µg per day, i.e. ~ 0.17 µg/kg b.w. per day) quintile of intake being
6236 0.92 (95 % CI 0.76-1.11) for all breast cancers. Corresponding RRs were 0.82 (95 % CI 0.64-1.05) for
6237 never-smokers, 1.11 (95 % CI 0.85-1.46) for ER+/PR+ breast cancer, and 0.90 (95 % CI 0.57-1.43)
6238 for ER−/PR− breast cancer. No significant difference was found in strata of other covariates analyzed,
6239 including age, BMI and alcohol drinking.

6240 An update (Pedersen et al., 2010) of the NLCS cohort with the follow-up extended from 1997
6241 (Hogervorst et al., 2007) to December 1999 reported no association for overall breast cancer (RR for
6242 the highest (~ 37 µg per day, i.e. ~ 0.57 µg/kg b.w. per day) quintile as compared to the lowest
6243 (~ 10 µg per day, i.e. ~ 0.14 µg/kg b.w. per day) 0.92, 95 % CI 0.73-1.15). Similarly, no associations
6244 were found in ER or PR-negative cancers and in never-smoking women. However, non significant
6245 increased risks were found for ER+ (RR 1.31, for the highest versus the lowest quintile, p for trend
6246 0.26), PR+ (RR 1.47, p for trend 0.14) and ER+PR+ (RR 1.43, p for trend 0.16) cancers in never-
6247 smoking women.

6248 In the US NHS (Wilson et al., 2010), no association was found between AA and breast cancer overall
6249 (RR 0.95, 95 % CI 0.87-1.03, for the highest versus the lowest quintile of intake, i.e. for ~ 25 versus
6250 ~ 9 µg per day, i.e. for ~ 0.36 versus ~ 0.13 µg/kg b.w. per day) or according to ER or PR status.
6251 Comparable results were observed in strata of smoking, menopausal status, and BMI.

6252 The UK Women's Cohort Study (Burley et al., 2010) reported no overall association between AA
6253 intake and breast cancer (RR 1.16, 95 % CI 0.88-1.52 for ~ 32 versus ~ 6 µg per day, i.e. ~ 0.46 versus

6254 ~ 0.09 µg/kg b.w. per day, and 1.08, 95 % CI 0.98-1.18, per 10 µg per day, i.e. ~ 0.14 µg/kg b.w. per
6255 day). The risk was higher in pre-menopausal women (RR 1.18, 95 % CI 1.05-1.34, p for trend 0.008),
6256 while no association was found in post-menopausal ones (RR 1.00). Similar RR findings were
6257 reported in never-smokers.

6258 **Endometrial cancer**

6259 The risk of endometrial cancer was examined in three cohort studies, the NLCS (Hogervorst et al.,
6260 2007), the SMC (Larsson et al., 2009b), and the NHS (Wilson et al., 2010).

6261 In the NLCS study of postmenopausal women (Hogervorst et al., 2007), the multivariate RRs of
6262 endometrial cancer were 0.95 (95 % CI 0.59-1.54), 0.94 (95 % CI 0.56-1.56), 1.21 (95 % CI
6263 0.74-1.98), and 1.29 (95 % CI 0.81-2.07) for subsequent quintiles (fifth quintile, ~ 37 µg per day, i.e.
6264 ~ 0.5 µg/kg b.w. per day) of AA intake as compared to the first one (~ 10 µg/day, i.e. ~ 0.14 µg/b.w.
6265 per day). To eliminate the possibility of residual confounding by smoking, analyses were also carried
6266 out in never-smokers (150 cases). RRs were higher in never-smokers, the RRs being 1.16 (95 % CI
6267 0.63-2.15), 1.35 (95 % CI 0.73-2.51), 1.30 (95 % CI 0.69-2.46), and 1.99 (95 % CI 1.12-3.52, p for
6268 trend = 0.03), for subsequent quintiles of AA intake as compared to the first one, and 1.12 (95 % CI
6269 0.95-1.33) for an increase in AA intake of 10 µg per day, i.e. ~ 0.49 µg/kg b.w. per day.

6270 A Swedish cohort study (Larsson et al., 2009b) reported multivariate RRs for endometrial cancer of
6271 1.10 (95 % CI 0.89-1.36), 1.08 (95 % CI 0.88-1.34), and 0.96 (95 % CI 0.76-1.21) for increasing
6272 quartiles of AA intake (last quartile ≥ 29 µg per day, i.e. ≥ 0.41 µg/kg b.w. per day) as compared to the
6273 first one (< 20 µg per day, i.e. < 0.29 µg/kg b.w. per day), with no meaningful differences across
6274 menopausal status. In a subanalysis using only 273 cases interviewed in 1997 (using a more extensive
6275 FFQ), corresponding the RRs were 1.08 (95 % CI 0.76-1.53), 1.20 (95 % CI 0.85-1.69) and
6276 1.12 (95 % CI 0.79-1.59) in all women, and 1.31 (95 % CI 0.85-2.04), 1.30 (95 % CI 0.83-2.02) and
6277 1.20 (95 % CI 0.76-1.90) in never-smokers.

6278 In the US NHS (Wilson et al., 2010), an increased risk of endometrial cancer was found for high AA
6279 intake (RR 1.41, 95 % CI 1.01-1.97 for ~ 25 µg per day versus ~ 9 µg per day, i.e. for ~ 0.36 versus
6280 ~ 0.13 µg/kg b.w. per day, p for trend = 0.03). Comparable results were observed in strata of smoking
6281 and menopausal status, while a stronger increased risk was found in non overweight/obese women
6282 (RR 2.51, 95 % CI 1.32-4.77).

6283 **Ovarian cancer**

6284 Data on ovarian cancer were available from the same cohort studies of endometrial cancer (Hogervorst
6285 et al., 2007; Larsson et al., 2009c; Wilson et al., 2010), the case-control study from Italy and
6286 Switzerland (Pelucchi et al., 2006), and the study on the NHS and the NHS II which measured AA and
6287 GA adducts (Xie et al., 2013).

6288 In the study from Italy and Switzerland (Pelucchi et al., 2006), the RRs of ovarian cancer were
6289 1.03 (95 % CI 0.79-1.34), 1.09 (95 % CI 0.83-1.44), 1.01 (95 % CI 0.76-1.34), and 0.97 (95 % CI
6290 0.73-1.31) for subsequent quintiles of AA intake (last quintile ≥ 32 µg per day, i.e. ≥ 0.46 µg/kg b.w.
6291 per day) as compared with the lowest one (< 10 µg per day, i.e. < 0.14 µg/kg b.w. per day).

6292 The NLCS (Hogervorst et al., 2007) reported RRs of 1.22 (95 % CI 0.73-2.01), 1.12 (95 % CI
6293 0.65-1.92), 1.28 (95 % CI 0.77-2.13), and 1.78 (95 % CI 1.10-2.88) for increasing quintiles of intake
6294 (fifth quintile ~ 37 µg/day, i.e. ~ 0.53 µg/kg b.w. per day) as compared with the first one (~ 10 µg per
6295 day, i.e. ~ 0.14 µg/kg b.w. per day, p for trend 0.02). The RR was 1.11 (95 % CI 0.99-1.25) for an
6296 increment of 10 µg per day (i.e. 0.14 µg/kg b.w. per day) of dietary AA. Analyses in never-smokers
6297 were carried out to eliminate the possibility of residual confounding by smoking. As for endometrial
6298 cancer, the RR were somewhat higher in never-smokers, with RRs of 1.60 (95 % CI 0.85-3.02),

6299 1.64 (95 % CI 0.84-3.19), 1.86 (95 % CI 1.00-3.48), and 2.22 (95 % CI 1.20-4.08, p for trend = 0.01),
6300 and of 1.17 (95 % CI 1.01-1.36) increased risk for an increment of 10 µg/day of dietary AA.

6301 In the SMC study (Larsson et al., 2009c), no association emerged with ovarian cancer on the whole
6302 database (RRs 0.91, 95 % CI 0.68-1.21, 0.97, 95 % CI 0.73-1.29, and 0.86, 95 % CI 0.63-1.16, for
6303 increasing quartiles of long-term AA intake, up to ≥ 29 µg per day, i.e. ≥ 0.41 µg/kg b.w. per day, as
6304 compared to the lowest one, < 20 µg per day, i.e. < 0.29 µg/kg b.w. per day) nor in the subgroup that
6305 participated in the second interview in 1997 (corresponding RRs of 1.09, 1.03, and 1.17, respectively).
6306 In the latter subgroup, the RR for the highest versus the lowest quartile of intake was 0.97 (95 % CI
6307 0.49-1.93) in never-smokers.

6308 The US NHS (Wilson et al., 2010) found that the risk of ovarian cancer increased (though not
6309 significantly) for increasing AA intake (RR 1.25, 95 % CI 0.88-1.77 for the highest (~ 26 µg per day,
6310 i.e. ~ 0.42 µg/kg b.w. per day) versus the lowest (~ 9 µg per day, ~ 0.13 µg/kg b.w. per day) quintile
6311 of intake, p for trend 0.12), and a significantly for serous tumours (RR 1.58, 95 % CI 0.99-2.52, p for
6312 trend 0.04). Comparable results were observed in strata of smoking and menopausal status, while a
6313 significant increased risk was found in women of normal weight (RR 1.84, 95 % CI 1.14-2.97, p for
6314 trend 0.01).

6315 In the analysis of the NHS and NHS II cohorts (Xie et al., 2013) where AA exposure was measured
6316 using Hb-adducts, a non significant reduced risk was reported for the highest (> 134 pmol/g Hb)
6317 versus the lowest tertile (< 99 pmol/g Hb) of exposure of total adducts (RR 0.79, 95 % CI 0.50-1.24).
6318 Results were consistent when AA or GA adducts were considered separately. Moreover, similar
6319 results were reported in non-smokers and according to tumour histology subtypes.

6320 7.4.1.2.3. Cancers of the gastro-intestinal tract (Appendix J, Table J3)

6321 **Oesophageal cancer**

6322 The association between AA and oesophageal cancer was analyzed in two case-control studies
6323 (Pelucchi et al., 2006; Lin et al., 2011) and one cohort study (Hogervorst et al., 2008a).

6324 In the case-control study from Italy and Switzerland (Pelucchi et al., 2006), the RRs of oesophageal
6325 cancer for increasing quintiles of AA exposure (from < 13 to ≥ 40 µg per day, i.e. from 0.19 to
6326 ≥ 0.57 µg/kg b.w. per day) were 1.16 (95 % CI 0.75-1.81), 1.20 (95 % CI 0.75-1.93), 0.74 (95 % CI
6327 0.44-1.24) and 1.10 (95 % CI 0.65-1.86), with non-significant trend in risk.

6328 The NLCS study (Hogervorst et al., 2008a) found no association between AA intake and oesophageal
6329 cancer, the RR being 0.83 (95 % CI 0.54-1.30) for the highest (~ 40-42 µg per day, i.e.
6330 ~ 0.57-0.60 µg/kg b.w. per day) versus the lowest (~9-10 µg/day i.e. ~ 0.13-0.14 µg/b.w. per day)
6331 level of exposure. The RRs for an increase in AA intake of 10 µg/day, i.e. 0.14 µg/kg b.w. per day,
6332 were 0.96 (95 % CI 0.85-1.09) for all oesophageal cancers, 1.00 (95 % CI 0.85-1.17) for oesophageal
6333 adenocarcinoma, and 0.95 (95 % CI 0.78-1.16) for oesophageal squamous cell carcinoma. The results
6334 were not meaningfully different in never-/ex-smokers. A significant effect modification by obesity
6335 was found, with RR of all oesophageal cancers in obese subjects of 1.55 (95 % CI 1.08-2.21) for an
6336 increment of 10 µg/day of AA, although the estimate was based on a small number of obese cases
6337 (n = 20).

6338 In a Swedish case-control study (Lin et al., 2011), a significant increased risk of oesophageal cancer
6339 was observed for the highest quartile of AA intake (≥ 44 µg per day, i.e. ≥ 0.63 µg/kg b.w. per day) as
6340 compared to the lowest (< 27 µg per day, i.e. < 0.39 µg/kg b.w. per day, RR 1.23, 95 % CI 1.02-1.75),
6341 although with no significant trend in risk. Comparable results were found for various tumour subtypes,
6342 i.e. oesophageal adenocarcinoma, gastroesophageal junction adenocarcinoma, oesophageal squamous
6343 cell carcinoma, and gastrooesophageal junction. Stronger associations were observed in overweight

6344 subjects (RR 1.88, 1.06-3.34 for all neoplasms) and in non-smokers (RR 1.46, 95 % CI 0.96-2.21 for
6345 all neoplasms) only.

6346 The EPIC cohort (Luján-Barroso et al., 2014) observed increased risks of oesophageal cancer for the
6347 middle quartiles of AA intake, but there was no evidence of a dose-response trend. Multivariable
6348 hazard ratios were 1.75 (95 % CI 1.12-2.74), 1.66 (95 % CI 1.05-2.61) and 1.41 (95 % CI 0.86-2.71)
6349 for subsequent quartile of intake (up to ≥ 34 µg per day or ≥ 0.49 µg/kg b.w. per day) vs the lowest
6350 one (< 15.7 µg per day or < 0.22 µg/kg b.w. per day). Estimates by histological subgroups
6351 (adenocarcinoma and squamous cell carcinoma) as well as in never-smokers/quitters since ≥ 20 years
6352 were comparable with the overall ones, although they were not statistically significant due to lower
6353 power. When energy-adjusted AA intake was used most risk estimates were attenuated.

6354 **Stomach cancer**

6355 Two studies investigated AA exposure in relation to gastric cancer (Hogervorst et al., 2008a; Hirvonen
6356 et al., 2010).

6357 The NLCS (Hogervorst et al., 2008a) found no relationship between dietary AA and stomach cancer
6358 (RR 1.06, 95 % CI 0.78-1.45 for the highest (~ 40-42 µg per day, i.e. ~ 0.57-0.60 µg/kg b.w. per day)
6359 versus the lowest (~ 9-10 µg per day, i.e. ~ 0.13-0.14 µg/kg b.w. per day) intake, 1.02, 95 % CI
6360 0.94-1.10, for an increase in intake of 10 µg per day). No significant association was also found for
6361 gastric cardia adenocarcinoma (RR 1.05, 95 % CI 0.91-1.20) and noncardia gastric cancer (RR 0.99,
6362 95 % CI 0.89-1.11) nor in never-/former-smokers (RR 1.09, 95 % CI 0.98-1.22).

6363 No association was also found in the ATBC study on male smokers (Hirvonen et al., 2010), the RR for
6364 the highest (~ 56 µg per day, i.e. ~ 0.80 µg/kg b.w. per day) as compared to the lowest (~ 22 µg per
6365 day, i.e. ~ 0.31 µg/kg b.w. per day) quintile of AA intake being 0.96 (95 % CI 0.60-1.53).

6366 **Colorectal cancer**

6367 Six studies investigated AA exposure in relation to colorectal cancer (Mucci et al., 2003a, 2006;
6368 Pelucchi et al., 2006; Hogervorst et al., 2008a; Larsson et al., 2009d; Hirvonen et al., 2010).

6369 A Swedish case-control study (Mucci et al., 2003a) found a decreased colorectal cancer risk (RR 0.6,
6370 95 % CI 0.4-1.0, for the highest versus the lowest quartile of ranked AA intake, p for trend 0.01).

6371 The SMC study (Mucci et al., 2006) reported a multivariate RR of 0.9 (95 % CI 0.7-1.3) for women in
6372 the highest (≥ 31 µg per day, i.e. ≥ 0.44 µg/kg b.w. per day) versus lowest (< 16 µg per day, i.e.
6373 < 0.23 µg/kg b.w. per day) quintile of AA intake. The corresponding RRs were 0.9 (95 % CI 0.6-1.4)
6374 for colon (504 cases) and 1.0 (95 % CI 0.6-1.8) for rectal (237 cases) cancer. Results were consistent
6375 across strata of age and BMI.

6376 In the case-control study from Italy and Switzerland (Pelucchi et al., 2006), no association was found
6377 between dietary AA and large bowel cancer (RRs for the highest, > 40 µg per day, i.e. > 0.87 µg/kg
6378 b.w. per day, versus the lowest, < 12 µg per day, i.e. < 0.17 µg/kg b.w. per day, quintile of intake 0.97,
6379 95 % CI 0.80-1.18, for colorectal; 0.98, 95 % CI 0.78-1.23, for colon; and 0.96, 95 % CI 0.73-1.26, for
6380 rectal cancer).

6381 In the NLCS study (Hogervorst et al., 2008a), the RR for the highest level of intake (~ 40-42 µg per
6382 day, i.e. ~ 0.57-0.60 µg/kg b.w. per day) versus the lowest one (~ 9-10 µg per day, ~0.13-0.14 µg/kg
6383 b.w. per day) was 1.00 (95 % CI 0.84-1.20). The RRs for an increment of 10 µg/day of AA were
6384 1.00 (95 % CI 0.96-1.06) for colorectal, 1.03 (95 % CI 0.98–1.09) for colon, and 0.97 (95 % CI
6385 0.89-1.05) for rectal cancers. In never-smokers, corresponding RRs were 1.03 (95 % CI 0.94-1.12),
6386 1.04 (95 % CI 0.94-1.14), and 1.02 (95 % CI 0.86-1.20).

Within the NLCS, a case-cohort analysis was conducted on 733 colorectal cancer cases followed-up for 7.3 years for whom mutations in Kristen-ras (KRAS) and adenomatous polyposis coli (APC) genes were measured (Hogervorst et al., 2014). This analyses showed AA intake was associated with a significant increased colorectal cancer risk (HR for the fourth versus the first quartile of AA intake 2.12, 95 % CI 1.16-3.87, p for trend 0.01) in men with an activating KRAS mutation, but not in those with an APC mutation (HR = 1.16, 95 % CI 0.67-2.02). On the other hand, AA intake was associated with a non significant reduced colorectal cancer risk (HR for the fourth versus the first quartile of AA intake 0.61, 95 % CI 0.30-1.20, p for trend 0.01) in women with an activating KRAS mutation, and with a significant reduced risk in those with an APC mutation (HR = 0.47, 95 % CI 0.23-0.94, p for trend 0.02).

In the cohort of Swedish Men (Larsson et al., 2009d), the multivariate RRs for the highest (≥ 42 µg per day, i.e. ≥ 0.60 µg/kg b.w. per day) versus the lowest (< 30 µg per day, i.e. < 0.43 µg/kg b.w. per day) quartile of dietary AA were 0.95 (95 % CI 0.74-1.20) for colorectal, 0.97 (95 % CI 0.71-1.31) for colon, and 0.91 (95 % CI 0.62-1.32) for rectal cancer. The results were comparable in never-, past and current smokers.

The ATBC study on male smokers (Hirvonen et al., 2010) reported an RR of 0.93 (95 % CI 0.65-1.34) for the highest (~ 56 µg per day, i.e. ~ 0.80 µg/kg b.w. per day) as compared to the lowest (~ 22 µg per day, i.e. ~ 0.80 µg/kg b.w. per day) quintile of AA intake for colorectal cancer.

Pancreatic cancer

Four studies considered AA from diet in relation to pancreatic cancer risk (Hogervorst et al., 2008a; Hirvonen et al., 2010; Pelucchi et al., 2011a; Obón-Santacana et al., 2013).

The NLCS database (Hogervorst et al., 2008a) found no overall association. For subsequent quintiles of AA intake (up to ~ 40-42 µg per day, i.e. 0.57-0.60 µg/kg b.w. per day) as compared to the first one (~ 9-10 µg per day, i.e. 0.57-0.60 µg/kg b.w. per day), the RRs of pancreatic cancer were 1.02 (95 % CI 0.72-1.44), 0.96 (95 % CI 0.66-1.38), 0.87 (95 % CI 0.60-1.27) and 0.98 (95 % CI 0.68-1.40). The RRs in never-/former-smokers were 0.72 (95 % CI 0.43-1.20), 1.07 (95 % CI 0.65-1.76), 0.73 (95 % CI 0.43-1.26) and 0.80 (95 % CI 0.48-1.32). There was significant effect modification by obesity, the RR being higher in obese subjects (RR 1.59, 95 % CI 0.87-1.59, for an increase of 10 µg/day, based on 14 cases).

The ATBC study on male smokers (Hirvonen et al., 2010) found no meaningful association between AA intake and pancreatic cancer, with a RR of 1.00 (95 % CI 0.62-1.62) for the highest (~ 56 µg per day, i.e. ~ 0.80 µg/kg b.w. per day) as compared to the lowest (~ 22 µg per day, i.e. ~ 0.31 µg/kg b.w. per day) quintile of AA intake.

In the Italian case-control study (Pelucchi et al., 2011a), the RRs for subsequent quintiles of AA intake as compared to the lowest one were 1.48 (95 % CI 0.88-2.50), 1.57 (95 % CI 0.91-2.69), 1.70 (95 % CI 0.98-2.96), and 1.49 (95 % CI 0.83-2.70).

The EPIC cohort (Obón-Santacana et al., 2013) found no association between AA intake and pancreatic cancer (RR 0.77, 95 % CI 0.58-1.04, for ≥ 37 µg per day, i.e. ≥ 0.53 µg/kg b.w. per day, versus < 14 µg per day, i.e. < 0.20 µg/kg b.w. per day, and 0.95, 95 % CI 0.89-1.01, for 10 µg per day, i.e. 0.14 µg/kg b.w. per day). Consistent results were found across strata of smoking. However, a significant inverse association was found in obese subjects (RR 0.73, 95 % CI 0.61-0.88) defined by BMI, but not by waist or hip circumference or their ratio.

6429 7.4.1.2.4. Cancers of the urinary tract (Appendix J, Table J4)

6430 **Prostate cancer**

6431 The relation between dietary AA and prostate cancer was examined in four cohorts (Hogervorst et al.,
6432 2008b; Larsson et al., 2009e; Hirvonen et al., 2010; Wilson et al., 2012) and two case-control studies
6433 (Pelucchi et al., 2006; Wilson et al., 2009a) .

6434 In the Italian case-control study (Pelucchi et al., 2006), no significant association between AA and
6435 prostate cancer was found, the RR for the highest (≥ 36 µg per day, i.e. ≥ 0.51 µg/kg b.w. per day)
6436 versus the lowest (< 12 µg per day, i.e. < 0.14 µg/kg b.w. per day) quintile of dietary AA being
6437 0.92 (95 % CI 0.69-1.23).

6438 Similarly, in the NLCS (Hogervorst et al., 2008b), the RRs were 1.06 (95 % CI 0.87-1.30) for the
6439 highest (~ 41 µg per day, i.e. ~ 0.59 µg/kg b.w. per day) versus lowest (~ 10 µg per day, i.e.
6440 ~ 0.14 µg/kg b.w. per day) level of AA intake and 1.01 (95 % CI 0.96-1.07) for an increment of
6441 10 µg/day. Comparable results were reported in never-/former-smokers, while a non significant
6442 inverse association was reported for advanced cancer in never-smokers.

6443 In the Cohort of Swedish Men (Larsson et al., 2009e), the RR was 0.88 (95 % CI 0.70-1.09) for high
6444 (~ 43 µg per day, i.e. ~ 0.61 µg/kg b.w. per day) versus low (~ 28 µg per day, i.e. ~ 0.40 µg/kg b.w.
6445 per day) AA intake. No association was found according to smoking habit or progression status of
6446 prostate cancer.

6447 In the CAPS study (Wilson et al., 2009a), the RRs of prostate cancer were 0.97 (95% CI 0.75-1.27) for
6448 the highest (~ 56 µg per day, i.e. ~ 0.80 µg/kg b.w. per day) versus lowest (~ 32 µg per day, i.e. ~ 0.46
6449 µg/kg b.w. per day) quintile of dietary AA and 0.99 (95 % CI 0.94-1.05) for an increment of
6450 10 µg/day. In the same study, the RR for the upper quartile (≥ 56 pmol/g globin) of AA-Hb adduct as
6451 compared with the lowest one (< 33 pmol/g globin) was 0.93 (95 % CI 0.47-1.85), and the RR for a
6452 10-unit increment of AA-Hb was 1.00 (95 % CI 0.86-1.16). No significant associations were found by
6453 stage, grade or prostate-specific antigen (PSA) level.

6454 The ATBC study on male smokers (Hirvonen et al., 2010) reported no association, with an RR of
6455 1.05 (95 % CI 0.83-1.32) for the highest (~ 56 µg per day, i.e. ~ 0.80 µg/kg b.w. per day) quintile of
6456 AA intake versus the lowest (~ 22 µg per day, i.e. ~ 0.31 µg/kg b.w. per day).

6457 The US HPFS (Wilson et al., 2012) gave a multivariate RR of prostate cancer for the highest (~ 35 µg
6458 per day, i.e. ~ 0.50 µg/kg b.w. per day) versus the lowest (~ 12 µg per day, i.e. ~ 0.17 µg/kg b.w. per
6459 day) quintile of AA intake of 1.02 (95 % 0.92-1.13). Comparable results were reported in non smokers
6460 and in men who had PSA tests. Moreover, no associations were found in lethal, advanced or localized
6461 cancers nor in high and low-grade cancers.

6462 **Bladder cancer**

6463 Three studies considered the association between AA exposure through diet and bladder cancer risk
6464 (Mucci et al., 2003a, Hogervorst et al., 2008b, Hirvonen et al., 2010).

6465 The Swedish case-control study (Mucci et al., 2003a) reported no association between AA intake and
6466 bladder cancer, with a RR of 0.8, 95 % CI 0.5-1.5, for the highest versus the lowest intake. Similar
6467 results were observed in nonsmokers (RR 0.7) and current smokers (RR 1.0).

6468 The NLCS study (Hogervorst et al., 2008b) showed no relation between AA intake and bladder cancer
6469 (RR 0.91, 95 % CI 0.73-1.15, for the highest, ~ 41 µg per day, i.e. ~ 0.59 µg/kg b.w. per day, versus
6470 the lowest intake, ~ 10 µg per day, i.e. ~ 0.14 µg/kg b.w. per day) in the overall dataset, while found a
6471 significant inverse relation in never-smokers (RR 0.55, 95 % CI 0.33-0.93).

In the ATBC study on male smokers (Hirvonen et al., 2010), no association was observed between AA intake and urothelial cancers, the RR being 1.99 (95 % CI 0.71-1.39) for the highest quintile of AA intake (~ 56 µg per day, i.e. ~ 0.80 µg/kg b.w. per day) versus the lowest (~ 22 µg per day, i.e. ~ 0.31 µg/kg b.w. per day).

Renal cell cancer

Two cohort (Hogervorst et al., 2008b; Hirvonen et al., 2010) and three case-control (Mucci et al., 2003a, 2004; Pelucchi et al., 2007) studies provided information on the relation of AA with kidney cancer.

In the Swedish case-control study (Mucci et al., 2003a), the RRs for kidney cancer were 1.0 (95 % CI 0.6-1.9), 1.1 (95 % CI 0.6-2.0), and 0.8 (95 % CI 0.4-1.7) for subsequent quartiles of AA intake. Considering coffee in the estimate of AA intake, the RRs of kidney cancer slightly decreased (Mucci et al., 2003b).

Another Swedish case-control study (Mucci et al., 2004) reported a RR for kidney cancer of 1.1 (95 % CI 0.7-1.8) for the highest (> 32 µg per day, i.e. ≥ 0.49 µg/kg b.w. per day) versus the lowest (< 20 µg per day, i.e. < 0.29 µg/kg b.w. per day) quartile of intake, with no trend in risk. Results were not different across strata of smoking.

The Italian case-control study (Pelucchi et al., 2007) reported RRs of 1.21 (95 % CI 0.94-1.57), 1.14 (95 % CI 0.86-1.51), and 1.20 (95 % CI 0.88-1.63) for subsequent quartiles of dietary AA (highest quartile > 44 µg per day, i.e. > 0.63 µg/kg b.w. per day) as compared with the lowest one (< 20 µg per day, i.e. < 0.29 µg/kg b.w. per day). The continuous RR for an increase of 18.1 µg per day, i.e. 0.26 µg/kg b.w. per day, (one standard deviation) of AA was 1.05 (95 % CI 0.94-1.16).

Among the cohort studies, the NLCS study (Hogervorst et al., 2008b) showed an association between AA and kidney cancer, with RRs of 1.25 (95 % CI 0.86-1.83), 1.48 (95 % CI 1.02-2.15), 1.23 (95 % CI 0.83-1.81), and 1.59 (95 % CI 1.09-2.30) for subsequent quintiles of AA intake (highest quintile ~ 41 µg per day, i.e. ~ 0.59 µg/kg b.w. per day) as compared with the lowest one (~ 10 µg per day, ~ 0.14 µg/kg b.w. per day, for trend 0.04). The continuous RR for an increment of 10 µg/day of AA was 1.10 (95 % CI 1.01-1.21). Results were consistent in the two sexes and in never-smokers.

In the ATBC study on male smokers (Hirvonen et al., 2010), the RRs of kidney cancer were 1.25 (95 % CI 0.86-1.83), 1.65 (95 % CI 1.02-2.67), 1.47 (95 % CI 0.89-2.41) and 1.28 (95 % CI 0.76-2.15) for subsequent quintiles (highest quintile ~ 56 µg per day, i.e. ~ 0.80 µg/kg b.w. per day), compared with the lowest (~ 22 µg per day, i.e. ~ 0.31 µg/kg b.w. per day) quintile of AA intake, with no significant trend in risk (p 0.12). Moreover, because the ATBC study only included smokers, residual confounding by smoking cannot be ruled out completely.

7.4.1.2.5. Cancers of the respiratory tract (Appendix J, Table J5)

Oral and pharyngeal cancer

The case-control study conducted in Italy and Switzerland found no association between dietary AA and cancer of the oral cavity/pharynx (Pelucchi et al., 2006), with multivariate RRs of 1.10 (95 % CI 0.78-1.57), 1.27 (95 % CI 0.89-1.81), 1.04 (95 % CI 0.72-1.51) and 1.12 (95 % CI 0.76-1.66) for subsequent quintiles of AA intake (highest quintile ≥ 40 µg per day, i.e. ≥ 0.57 µg/kg b.w. per day) as compared with the lowest one (< 12 µg per day, i.e. < 0.17 µg/kg b.w. per day).

In the NLCS study (Schouten et al., 2009), the RRs for the third tertile (~ 40-42 µg per day, i.e. ~ 0.57-0.60 µg/kg b.w. per day) of AA intake as compared to the first one (~ 9-10 µg per day, i.e. ~ 0.13-0.14 µg/kg b.w. per day) were 0.72 (95 % CI 0.36-1.42) for oral cavity, and 0.61 (95 % CI 0.33-1.12) for oro-hypopharyngeal cancer. RRs for an increment of 10 µg per day (i.e. ~ 0.14 µg/kg b.w. per day) were 0.90 (95 % CI 0.73-1.10) and 0.74 (95 % CI 0.53-1.03) for the two neoplasms,

6517 respectively. A significant association was reported for oral cancer in non-smoking women (RR 1.28,
6518 95 % CI 1.01-1.62 per 10 µg per day), although the result was based on 21 cases only.

6519 **Laryngeal cancer**

6520 The same two studies considered the association between AA and laryngeal cancer risk (Pelucchi et
6521 al., 2006; Schouten et al., 2009).

6522 In the case-control study from Italy and Switzerland (Pelucchi et al., 2006), the RRs for laryngeal
6523 cancer were 1.04 (95 % CI 0.70-1.57), 0.85 (95 % CI 0.56-1.29), 0.89 (95 % CI 0.59-1.36), and 1.23
6524 (95 % CI 0.80-1.90) for subsequent quintiles of AA intake (highest quintile ≥ 38 µg per day, i.e.
6525 ≥ 0.54 µg/kg b.w. per day) as compared to the first one (< 13 µg per day, i.e. < 0.19 µg/kg b.w. per
6526 day). None of the estimates was significant, and no significant trend in risk was found.

6527 In the NLCS study (Schouten et al., 2009), no association was observed between AA and laryngeal
6528 cancer, the RR being 0.93 (95 % CI 0.54-1.58) for the highest (~ 37 µg per day, i.e. ~ 0.53 µg/kg b.w.
6529 per day) versus the lowest (~ 10 µg per day, i.e. ~ 0.14 µg/kg b.w. per day) quintile of AA and 1.05
6530 (95 % CI 0.91-1.21) for an increment of 10 µg per day.

6531 **Lung cancer**

6532 The NLCS (Hogervorst et al., 2009a) and ATBC (Hirvonen et al., 2010) cohort studies considered AA
6533 in relation to lung cancer.

6534 In the NLCS study (Hogervorst et al., 2009a), no association was observed between AA intake and
6535 lung cancer in men (RR 1.03, 95 % CI 0.77-1.39, for the highest (~ 42 µg per day, i.e. ~ 0.60 µg/kg
6536 b.w. per day) versus the lowest (~ 10 µg per day, i.e. ~ 0.14 µg/kg b.w. per day) quintile of AA
6537 intake), while an inverse relation was observed in women (RR 0.45, 95 % CI 0.27-0.76, for ~ 40 µg
6538 per day, i.e. ~ 0.57 µg/kg b.w. per day, versus ~ 9 µg per day, i.e. ~ 0.13 µg/kg b.w. per day), p for
6539 trend 0.01), particularly for adenocarcinoma. RRs were somewhat higher in male never-smokers (RR
6540 2.18, 95 % CI 0.61-7.82), but these results were based on a small number of subjects.

6541 In the ATBC cohort (Hirvonen et al., 2010), a significant RR for lung cancer of 1.18 (95 % 1.01-1.38)
6542 was reported for the highest (~ 56 µg per day, i.e. ~ 0.80 µg/kg b.w. per day) quintile of AA intake as
6543 compared to the lowest (~ 22 µg per day, i.e. ~ 0.31 µg/b.w. per day,), although in the absence of a
6544 significant trend in risk. However, because the ATBC study only included smokers, residual
6545 confounding by smoking cannot be ruled out completely.

6546 7.4.1.2.6. Other cancers (Appendix J, Table J6)

6547 **Brain cancer**

6548 Only the NLCS study (Hogervorst et al., 2009b) investigated the relation between AA intake and brain
6549 cancer. No evidence of an association was found (RR 0.87, 95 % CI 0.54-1.41, for ~ 40-42 µg per day,
6550 i.e. ~ 0.57-0.60 µg/kg b.w. per day, versus ~ 9-10 µg per day, i.e. ~ 0.13-0.14 µg/b.w. per day, of AA
6551 intake) both in the total dataset and in never-smokers.

6552 **Thyroid cancer**

6553 The association between AA intake and thyroid cancer was considered only in the NLCS study
6554 (Schouten et al., 2009), which reported RRs of 1.14 (95 % CI 0.58-2.26) and 1.33 (95 % CI 0.70-2.53)
6555 for the second and third tertile (~ 40-42 µg per day, i.e. ~ 0.57-0.60 µg/kg b.w. per day) of intake,
6556 respectively versus the first (~ 9-10 µg per day, i.e., ~ 0.13-0.14 µg/kg b.w. per day). The RR was
6557 1.03 (95 % CI 0.82-1.27) for a 10 µg/day increment of AA. No significant associations were found
6558 also in non-smokers.

Lymphatic/myeloid malignancies

Data on AA and lymphatic/myeloid neoplasms were provided by the ATBC (Hirvonen et al., 2010) and the NLCS (Bongers et al., 2012) cohort studies.

In the ATBC study on male smokers (Hirvonen et al., 2010), no meaningful association was reported with lymphomas, the multivariate RR for the highest (~ 56 µg per day, i.e. ~ 0.80 µg/kg b.w. per day) quintile of AA intake versus the lowest (~ 22 µg per day, i.e. ~ 0.31 µg/kg b.w. per day) being 1.10 (95 % CI 0.67-1.80).

Similarly, in the NLCS study (Bongers et al., 2012) no significant associations were observed for most malignancies investigated, including among others multiple myeloma (RR for the highest as compared to the lowest quintile of AA intake 1.54, 95 % CI 0.92-2.58 in men and 0.93, 95 % CI 0.50-1.73 in women), diffuse large-cell lymphoma (RR 1.06, 95 % CI 0.61-1.38, in men and 1.38, 95 % CI 0.63-3.02, in women), and chronic lymphocytic leukemia (RR for an increment of 10 µg per day 0.88, 95 % CI 0.74-1.09 in men and 0.83, 95 % CI 0.64-1.09 in women). For multiple myeloma a significant trend in risk was found for increasing levels of AA intake in men only. For all lymphatic malignancies considered, no significant associations were found in never-smokers.

7.4.1.2.6. Results from meta-analysis studies and reviews

Pelucchi et al. (2011b) conducted a systematic review and meta-analysis of 25 studies on dietary AA intake and cancer risk published up to June 2009. These included data on oesophageal (2 studies), colorectal (5 studies), breast (5 studies), endometrial (2 studies), ovarian (3 studies), prostate (4 studies), bladder (2 studies) and kidney (4 studies) cancer. The authors concluded that for none of the cancer sites studied RRs of the meta-analysis were significant increased. Only for kidney cancer a modest significant increased risk was reported. The CONTAM Panel noted that the current scientific opinion considers additional studies that were not included in the meta-analysis by Pelucchi et al. (2011b).

A few review papers also considered the epidemiologic evidence on dietary AA and cancer risk up to 2009-2011 but did not provide a quantification of cancer risk (Mucci and Wilson, 2008; Hogervorst et al., 2010; Lipworth et al., 2012; Pelicioli et al., 2014).

7.4.1.2.7. Discussion and conclusions

In the epidemiological studies available to date, AA intake was not associated with an increased risk of common cancers, including those of the GI or respiratory tract, breast, prostate and bladder. This is consistent with the conclusion of a systematic review and meta-analysis of studies published up to 2009 (Pelucchi et al., 2011b). A few studies suggested an increased risk for renal cell, endometrial and ovarian cancer (for the two latter particularly in never-smokers), but the evidence is limited and inconsistent. An updated report from the World Cancer Research Fund also concluded that data on AA and endometrial cancer are too limited to draw any conclusion (World Cancer Research Fund[35]). Moreover, one study suggested a worse survival in non-smoking breast cancer women with a high pre-diagnostic exposure to AA but more studies are necessary to confirm this result.

Although a hormonal mechanism of AA has been hypothesized to explain the observed associations with cancers of the female genital tract (Hogervorst et al., 2007, 2010), there is at the moment only suggestive evidence. A study among 687 postmenopausal and 1 300 premenopausal women from the Nurses' Health Studies did not show conclusive associations between AA intake and serum levels of sex hormones, thus not supporting the biological plausibility to the hormonal hypothesis. However, some associations between AA intake and sex hormones in subgroups of women were observed (Hogervorst et al., 2013). Statistically significant positive associations were observed between AA

[35] World Cancer Research Fund/American Institute for Cancer Research. Continuous Update Project Report. Food, Nutrition, Physical Activity, and the Prevention of Endometrial Cancer. 2013. Available at http://www.dietandcancerreport.org

intake and levels of luteal oestradiol and free oestradiol levels in normal weight premenopausal women. In postmenopausal women, statistically significant inverse associations were observed between AA intake and oestrone, free oestradiol and prolactin levels in normal weight women[36], while statistically significant positive associations were observed between AA intake and testosterone and androstenedione levels in overweight women.

Among the limitations of the epidemiological studies considered is possible confounding, since the observed associations with AA may be due (partly or totally) to the effects of other independent factors associated with both AA and the neoplasm of interest. However, in most studies, estimates for the association between AA exposure and cancer risk were adjusted for age, sex, education, and various other risk factors for the cancers considered, including tobacco smoking, body mass index, alcohol drinking, energy intake, and hormonal and reproductive factors for female cancers. Moreover, most epidemiological studies have conducted subgroup analyses in never- or non-smokers. This is a particularly important issue, since exposure to AA in humans is not restricted to the diet, but is also possible through smoking (see Section 6.4). Indeed, cigarette smoking is a much more important source of AA than diet, smokers having on average four times higher levels of AA-Hb adducts than non-smokers (Schettgen et al., 2004b). Thus, smoking may bias (or obscure) the association between AA through diet and cancer risk. For this reason, restriction to never-smokers is the most thorough way to control possible confounding by smoking.

It is also important to consider possible limitations of the methodologies used to estimate AA intake, i.e. the self-reported FFQ used to assess the subject's food intake and databases of AA content in foods. In particular,

(i) the FFQ used to assess food intake in many cases were not specifically designed to estimate AA intake and may not have been able to capture the high variation on AA content of foods. This applies in particular to the earlier studies that reported on the association between AA exposure and cancer, which had shorter FFQs and used incomplete AA databases;

(ii) Most FFQ did not include specific questions on cooking methods, which have been shown to influence AA content of foods;

(iii) The sources of AA contents of foods are often referring to other populations and calendar periods than those under investigation, thus reducing the specificity of country-specific estimates. Nevertheless, there was an about 2-fold difference in the estimates of AA intake between various populations;

(iv) The ability to measure subject's intake of AA using a single value based on the mean of several samples of the same foods has also been questioned. A study using 39 24-h duplicate diets which compared AA exposure estimated by using a questionnaire and the mean AA values for food items from the national AA database to the AA levels as measured by direct chemical analyses (Konings et al., 2010), reported a Spearman correlation of 0.82 between the two measures, thus indicating that a single mean – instead of the actual – AA value may well classify subjects according to their AA exposure in epidemiological studies using questionnaires. Despite the good correlation, the two highest measured levels (regarded as outliers), were more than five-fold the estimated intake from the questionnaire. In one case the fries, prepared at home, contained apparently more than the average AA level, in the other case no clear explanation was found. This study therefore shows also the difficulties in human studies measuring AA intake by questionnaires, in particular for food products prepared at home.

[36] BMI Normal Range: 18.5 - 24.99 kg/m^2. WHO (1995). Physical status: the use and interpretation of anthropometry. WHO Technical report Series 854.

(v) Although a few studies have shown that information on dietary AA collected through FFQs had satisfactory validity (and reproducibility) of information on dietary AA, multiple-day dietary records may also be subject to measurement errors. All such limitations may have introduced some misclassifications of AA exposure. Such misclassifications, however, are likely to be non-differential (i.e. similar in cases and non-cases) with respect to cancer outcome, and thus tend to bias the estimates towards the null.

A few studies compared AA levels estimated from FFQs to biomarkers of AA exposure (AA-Hb and GA-Hb) and reported low correlations (Wilson et al., 2009c, 2010; Ferrari et al., 2013). However, this is not surprising since FFQs and Hb-adducts measure different aspects of AA exposure, i.e. intake a few years before (usually one or two) for the former, and exposure, absorption and metabolism over the previous 4 months for the latter. Moreover, biomarkers represent AA exposure from other sources too, including in particular tobacco smoking. For prostate cancer, the only study that used biomarkers of AA exposure did not observe an association (Wilson et al, 2009a), in agreement with the result of prostate cancer studies using FFQs for the assessment of AA intake. Similarly, for ovarian cancer, Xie et al. (2013) did not observe an association using biomarkers, as shown in most studies using FFQs. For overall breast cancer, most studies using FFQs showed no association, while two studies using biomarkers for AA assessment for breast cancer risk (Olesen et al., 2008), and breast cancer survival (Pedersen et al., 2012), showed positive associations.

The association between AA exposure and cancer risk has been studied in occupational and dietary studies, as summarized in previous paragraphs. The (cumulative) exposure to AA from occupational and dietary exposure is, however, difficult to compare quantitatively. As compared to dietary studies, occupational studies are likely to measure AA exposure more accurately. Moreover, workers have been exposed to higher doses of AA (particularly in the past, Swaen et al., 2007), although individual occupational exposure may be variable and limited in time. Accordingly, various studies which have measured AA- and GA-Hb adducts in different populations (as biomarkers for AA exposure) showed that occupational exposures to AA have been considerably higher than in the general population, either in non-smoking or smoking groups. In the study by Moorman et al. (2012), the average adduct level in AA production workers was 220 pmol/g Hb (range: 29 - 1 884 pmol/g Hb). AA adduct levels in the general population have been reported to be consistently lower, both in never-smokers (median: 43 pmol/g Hb, 5^{th} - 95^{th} percentile: 24 - 88 pmol/g Hb) and in smokers (median: 121 pmol/g Hb, 5^{th}-95^{th} percentile: 44 - 285 pmol/g Hb) (Vesper et al., 2008), which is in agreement with other studies for the general population (Schettgen et al., 2003; Olesen et al., 2008; Kütting et al., 2009). Occupational studies, with temporarily higher AA exposures, have not shown consistent increased risk for cancer.

7.4.2. Epidemiological studies: pre-natal exposure

7.4.2.1. Reproductive/developmental consequences

In a Danish study, Hb-AA and Hb-GA adduct levels were measured in 87 maternal blood and 219 cord blood samples (von Stedingk et al., 2011). The correlation between cord and maternal blood were 0.69 ($p < 0.001$) and 0.78 ($p < 0.001$) for AA and GA, respectively. This study showed that AA from food consumed by pregnant women passes the placenta. Furthermore, *in vitro* studies showed that rates of adduct formation are lower in the cord blood probably because of structural differences between fetal and adult Hb. The authors therefore conclude that the fetus is exposed to the same AA and GA doses as the mother (von Stedingk et al., 2011).

In a prospective mother child study with 1 101 singleton pregnant women from Denmark, England, Greece, Norway and Spain it was investigated whether AA and GA exposures are associated with the development of the child during pregnancy (Pedersen et al., 2012). AA and GA adduct levels were measured in cord blood. Both AA and GA adduct levels were significantly associated with a reduced birth weight and head circumference. The difference in birth weight of newborn children for the highest quartile versus the lowest quartile of AA adducts was -132 g (95 % CI: -207, -56) and the

corresponding difference for head circumference was -0.33 cm (95 % CI: -0.61, -0.06). Results were similar in children from non-smoking mothers, and remained statistically significant after adjustment for factors that are associated with reduced birth weight. The authors also showed that a food score for AA was associated with AA and GA adduct levels in the cord blood of 801 children of non-smoking mothers. A one-unit change in this AA food score was associated with an increase of 0.68 pmol/g in AA Hg adduct cord blood levels (95 % CI: 0.30-1.06). The AA food score was also associated with reduced birth weight, although not statistically significant.

A Norwegian Mother and Child Study assessed the association between prenatal dietary AA intake and indicators of fetal growth (Duarte-Salles et al., 2013). The study included 50 561 women (including 46 420 non-smokers) and AA exposure was estimated using a FFQ. In a subset of 79 non-smoking women, the FFQ estimated dietary AA intake was validated by comparison to Hb adduct measurements. The correlation with AA and GA adducts was 0.24 and 0.48, respectively. Fetal growth was measured by determining whether an infant was small for gestational age (birth weight below the 10^{th} percentile according to week of gestational age and parity). Children of non-smoking mothers with a high dietary AA intake were at increased risk of being small for gestational age, the adjusted odds ratio (OR) being 1.13 (95 % CI, 1.03-1.23) for children of women with the highest quartile of AA intake versus those in the lowest quartile of intake. The point estimate was similar in children of smoking women, although not statistically significant because of low power. Birth weight was inversely associated with mother's AA intake: children of mothers with the highest quartile of dietary AA intake had a multivariable adjusted coefficient of -25.7 g (95 % CI: -35.9, -15.4) compared to the lowest quartile. This coefficient was similar in non-smoking women.

Epidemiological studies on the association between dietary AA intake and fetal growth are still limited. However, both prospective cohort studies suggested that a high dietary AA intake during pregnancy was associated with a lower fetal growth. The findings were similar in smoking and non-smoking women. In one of the two studies, AA exposure was measured using Hb adducts, and the association between AA exposure and birth weight was present as well. In both studies the possibility of residual confounding by cigarette smoking was adequately addressed by stratified analyses according to smoking status.

Two studies have studied the associations between dietary AA and birth weight, both suggesting an inverse association. However, such an association can be attributed to other unidentified exposures correlated with AA intake and there is no clear biological explanation of this association. Therefore, it cannot be established whether the association between dietary AA and birth weight is causal. More epidemiological studies are needed to confirm such a relationship in other populations, as well as experimental studies that may identify possible mechanisms. However, the CONTAM Panel noted that in rats and mice some signs of developmental toxicity (increased incidence of skeletal variations and slightly impaired body weight gain) are only observed at exposure levels that are also associated with maternal toxicity, and that these effects are considered likely to be secondary to maternal toxicity. These dose levels are extremely high compared to those considered in the human studies of Pedersen et al. (2012) and of Duarte-Salles et al. (2013). The CONTAM Panel therefore concluded that there are yet too many uncertainties to conduct a risk assessment based on these human data.

7.4.2.2. Effects on gene expression

In a substudy of the Norwegian Mother and Child Cohort (the BraMat cohort), umbilical blood cord samples of 45 male and 66 female newborn children were investigated (Hochstenbach et al., 2012). Eighty-four percent of the mothers were non-smokers. AA-Hb and GA-Hb adducts were measured, and mean values were 16.5 (± 6.6) pmol/g Hg and 10.0 (± 4.0) pmol/g Hb, respectively, with no major differences between male and female newborn children. Gene expression analysis using microarray was conducted to investigate whether AA and GA-Hb adduct levels were associated with alterations of gene expression. GA-Hb adduct levels were not associated with altered gene expression, while AA-Hb adduct levels were associated with activation of the Wnt-signaling pathway in males, but not in females. The deregulation of this pathway has been reported in different malignancies (Polakis, 2012).

The CONTAM Panel noted that the association was observed with an intermediate biomarker and the functional implications of the findings are unknown.

7.4.3. Epidemiological studies: neurological alterations

AA exposure can cause neurological symptoms in humans following skin absorption, inhalation, and ingestion. The variety of reported symptoms suggests possible involvement of the peripheral and the central nervous system, as well as the autonomic nervous system. Reported symptoms include muscular weakness, paraesthesia, numbness in hands, feet, lower legs and arms, and unsteadiness (WHO, 1985).

Several dozens of cases of AA poisoning have been reported in the literature. Acute and high exposures to AA more often result in early central nervous system involvement, while long-term exposure to low levels of AA are associated with peripheral neuropathy (WHO, 1985).

Duration of exposure was associated with the number of neurological symptoms in a small study with 15 exposed workers by Takahashi et al. (1971). He et al. (1989) studied neurotoxicological effects of AA exposure in 71 exposed workers (45 males and 26 females) aged between 17 and 41. A referent group of 51 workers (33 males and 18 females) from the same town were examined as well. Production of AA started in May 1984 and symptoms of workers exposed to AA were investigated up to October 1985. The atmospheric concentration of AA had reached a maximum of 5.56 to 9.02 mg/m^3 between March and June 1985 and decreased to an average of 0.0324 mg/m^3 after a renovation of the workplace. An AA level of 410 mg/L was measured in the water in which three of the workers washed their hands. In October 1985, three cases had severe AA poisoning (involvement of the cerebellum with polyneuropathy), six moderate poisoning and 43 mild poisoning.

The AA exposed workers reported significantly more frequent symptoms like skin peeling from the hands, numbness in the hands and feet, lassitude, sleepiness, muscle weakness, clumsiness of the hands, anorexia, unsteady gait, coldness of hands and feet, difficulty in grasping and stumbling and falling. Electromyographic investigation revealed a decrease in the sensory action potential amplitude, prolonged duration of motor units and increased polyphasic potentials. The results of this study are difficult to interpret, however, because of different exposure routes and poor control of confounding by e.g. smoking.

Myers and Macun (1991) investigated AA related neuropathy in a cohort of 66 people who had worked in a South African factory. The mean duration of AA exposure was two years. AA exposure levels ranged from 0.07 to 2.50 times the NIOSH recommended exposure limit (REL) of 0.3 mg/m^3. Workers were classified as being exposed to AA when exposure levels exceeded the REL (n = 22) and as unexposed when exposure levels were below REL (n = 41). The exposed group showed an increased prevalence of neurological symptoms, signs and reflexes, but only abnormal sensation symptoms were statistically significant increased. Overall prevalence of AA-related symptoms was 66.7 % among exposed and 14.3 among unexposed workers ($p < 0.05$). Bachmann et al. (1992) conducted a follow-up study in the same South African factory (Myers and Macun, 1991). Workers were classified, based on job titles, into a high-dose group (average exposure 0.33 mg/m^3 during on average 5.2 years) and a low-dose group (average exposure 0.02 mg/m^3 during on average 5.8 years). Numbness of hands and feet, weakness of arms and legs, sweating hands, and pain in arms and legs were statistically significantly more frequently reported in the high-dose group.

Calleman et al. (1994) studied neurological health effects in 41 workers exposed to AA and 105 unexposed healthy adults. Workers were exposed from 1 month to 11.5 years to AA in a Chinese factory. Of the exposed workers, 13 were synthesis workers (mean air concentration 1.07 mg/m^3 of AA), 12 were polymerisation workers (mean air concentration 3.27 mg/m^3 AA), and 16 were exposed otherwise. Several biomarkers of exposure were measured, e.g. free AA in plasma, MAs in urine (measured as S-(carboxyethyl)cysteine), and AA-Hb adducts (Table 25). Neurotoxicity was measured by calculating a weighted sum score of symptoms of neuropathy (14 items; maximum 50 points). The

neurotoxicity index predicted a clinical diagnosis of peripheral neuropathy. Average neurotoxicity scores were 22.2 ± 7.1 and 7.4 ± 5.3 in workers with and without peripheral neuropathy, respectively. AA adducts, acrylonitrile adducts and accumulated AA dose, were positively associated with the neurotoxicity index (Table 26).

In a cohort of 210 tunnel workers, who had been exposed during about two months, to a chemical-grouting agent containing AA and N-methylol AA, AA-Hb adducts were measured as well as health effects (Hagmar et al., 2001). Fourty-seven workers had Hb-adduct levels of AA within the normal background range (0.02-0.07 nmol/g globin) and 74 workers had Hb-adduct levels of AA exceeding 0.30 nmol/g globin. Repeated sampling from five workers within five months showed a decrease consistent with the 120-days lifespan of human erythrocytes. Self-reported exposure was positively associated with the measured levels of Hb-adduct ($p < 0.0001$). There were statistically significant associations between Hb-adduct levels and prevalence of peripheral nervous symptoms (Table 27).

Table 25: Mean and standard deviation of acrylamide (AA) biomarkers and neurological health effects in 41 exposed workers and 105 unexposed controls according to job title (adapted from Calleman et al., 1994)

Group	Free AA µmol/l*	MAs µmol/24 h	AA Val nmol/g Hb	AN Val nmol/g Hb	Hand VU	Foot VU	$AccD_{AA}$ mM/hour	Neurotox. index
Controls (n = 10)	0.92	3.0 ± 1.8	0.0 ± 0.0	0.23 ± 0.18	1.6 ± 0.3	3.0 ± 0.9	0.0 ± 0.0	0.0 ± 0.0
Packaging (n = 5)	2.2	93 ± 72	3.9 ± 2.5	19.1 ± 5.7	2.5 ± 1.3	5.5 ± 3.2	8.1 ± 6.6	8.9 ± 9.1
Polymerisation (n = 12)	1.3	58 ± 75	7.7 ± 3.4	19.1 ± 12.9	2.1 ± 1.0	4.3 ± 2.5	27.0 ± 23.9	10.0 ± 5.8
Ambulatory (n = 6)	2.0	53 ± 35	9.5 ± 7.3	16.3 ± 3.7	2.4 ± 1.0	5.0 ± 1.8	37.6 ± 21.9	11.3 ± 9.8
Synthesis (n = 13)	1.8 ± 0.8	64 ± 46	13.4 ± 9.8	19.5 ± 7.6	2.9 ± 1.3	7.2 ± 5.1	68.3 ± 64.2	19.2 ± 10.6

MAs: Mercapturic acid (S-(2 carboxyethyl)cysteine/24 hours in hydrolyzed urine); Val: valine; AN: acrylonitrile; VU: vibration units; $AccD_{AA}$: Accumulated *in vivo* dose of AA throughout the duration of employment.

Table 26: Correlations between acrylamide (AA) biomarkers of exposures and neurotoxicity index (adapted from Calleman et al., 1994)

	Correlation coefficient with neurotoxicity index	*p* value
Free AA	0.15	0.31
Urinary mercapturic acid	0.42	< 0.01
Hb adducts with AA	0.67	< 0.001
Hb adducts with acrylonitrile	0.69	< 0.001
Accumulated *in vivo* AA dose	0.60	< 0.001

Table 27: Prevalences (%) of work-related symptoms according to levels of Hb-adducts of AA among 210 tunnel workers (adapted from Hagmar et al., 2001).

Symptoms	AA-Hb adducts (nmol/g globin)				p-value
	< 0.08 (n = 47)	0.08-0.29 (n = 89)	0.30-1.00 (n = 36)	> 1.00 (n = 38)	
Numbness or tingling in hands	13	13	31	29	0.04
Numbness or tingling in feet or legs	4	11	25	37	< 0.001
Leg cramps	6	7	6	26	0.003
Increased hand or foot sweating	2	3	17	11	0.02
Skin peeling in hands	6	2	8	24	0.001
Irritation of the eyes	14	23	47	47	< 0.001
Irritation of the nose	14	21	36	53	< 0.001
Irritation of the throat	10	23	47	47	< 0.001
Coughing	10	11	31	50	< 0.001
Dyspnea or wheezing	2	9	17	24	0.02
Irritation of the skin	14	18	31	39	0.02
Headache	14	33	31	63	< 0.001
Nausea	5	13	14	47	< 0.001
Dizziness	7	13	31	24	0.02

Fifty symptomatic workers had been referred for further neurophysiological examinations and 29 had objective findings in the clinical examination. These 29 workers had adduct levels of at least 0.30 nmol/g globin. Of these 29 workers, two showed neurographic signs of polyneuropathy, eight had a slight impairment of nerve conduction or amplitude, nine had increased sensory thresholds, and the remaining nine subjects had no neurophysiological abnormalities. Almost all workers recovered 18 months after exposure had been stopped.

Kjuus et al. (2004) investigated toxic effects on the peripheral nerves in 74 Norwegian tunnel workers of whom 24 had been exposed to AA and *N*-methyloacrylamide during an average of 19.4 months. Mean AA adduct concentration (measured 2-5 months after cessation of the exposure) was 82 pmol/g Hb (range 33-85) for non-smoking exposed workers (n = 11) and 33 pmol/g Hb for non-smoking referents (n = 6). Neurological symptoms (paresthesia, pain and weakness of hands and feet) were reported more often during exposure than 16 months after exposure, although the differences were not statistically significant. Neurophysiological measurements showed a statistically significant reduction in mean sensory nerve conduction value of the ulnar nerve in exposed workers, and a prolonged distal delay in the ulnar nerve. Most effects were reversible, with a change to normal after one year.

Goffeng et al. (2008a) compared 44 workers exposed to AA in the past (2-10 years before), 24 workers recently exposed to AA (16 months before) and 49 workers who were not exposed to AA. Exposure to AA was measured using questionnaires. In this cross-sectional study, slight but persistent subclinical nervous system effects on the sural nerve as well as signs of retinal and optic nerve impairment were shown.

In the same cross-sectional study, Goffeng et al. (2008b) investigated colour vision and light sensitivity in 44 workers who had been exposed to AA and *N*-methylolacrylamide (NMA) 2-10 years before the study, as well as in 44 controls. Exact levels of exposure were not available. Based on questionnaire information, a sum score of intensity and time of exposure was calculated. A slightly, although statistically significant, reduced light sensitivity and colour discrimination was observed in exposed workers compared to non-exposed workers. However, there was no dose-response relationship between exposure intensity and light sensitivity and colour discrimination.

Finally, Goffeng et al. (2011) in the same 44 exposed and 49 non-exposed workers, investigated whether self-reported symptoms and neuropsychological test results were different during work and at the time of examination. The retrospectively self-reported prevalence of symptoms like paresthesia in hands and legs and leg cramps during work periods were higher in the exposed workers. Current self-reported symptoms like impaired memory and concentration were also higher in exposed workers. No association was observed between neuropsychological test results and estimated AA exposure.

In summary, occupational exposure to AA in humans has been shown to cause toxicity in the peripheral and central nervous system. The first reports were published forty years ago, but most reports had either a poor characterisation of exposure, had a cross-sectional design and/or included a limited number of persons only. However, the available evidence indicates that exposure to AA is positively associated to prevalence of peripheral nervous system symptoms, although in most cases symptoms were reversible.

7.5. Considerations of critical effects and possibilities for derivation of a health based guidance value

7.5.1. Considerations of critical effects

A large body of evidence has been published that demonstrates that AA exposure by oral and parenteral routes can result in peripheral neuropathy in humans (Hagmar et al., 2001) and experimental animals (Spencer and Schaumberg, 1974; Tilson, 1981; Sickles et al., 2002; Tyl and Friedman, 2003; LoPachin, 2004; Beland et al., 2013). Peripheral neuropathy in rats is manifested as hindlimb weakness, foot splay, and gait abnormalities. Peripheral nerve degeneration was also observed in the three long-term carcinogenicity studies of AA (Johnson at al., 1986; Friedman et al., 1995; NTP, 2012). In addition, studies in experimental animals have documented effects on male reproduction, developmental toxicity and carcinogenicity as critical effects (Garey and Paule, 2007, 2010; Fergusson et al., 2010; NTP, 2012; Takami et al., 2012).

From all data available, the CONTAM Panel identified four possible critical endpoints for AA toxicity, i.e. neurotoxicity, effects on male reproduction, developmental toxicity and carcinogenicity.

7.5.2. Dose-response assessment

The data from human studies were not adequate for dose-response assessment. Therefore, the CONTAM Panel performed BMD analysis on the data on neurotoxicity and carcinogenicity in experimental animals. The BMD approach is a scientifically more advanced method to the NOAEL approach for deriving a reference point, since it makes extended use of available dose-response data and it provides a quantification of the uncertainties in the dose-response data, overall resulting in a more consistent reference point (EFSA, 2009b). Details on the BMD analysis are given in Appendix K, and Table 28 provides a summary of the BMD and $BMDL_{10}$ values obtained.

For the evaluation of the data on neurotoxicity, the CONTAM Panel decided to use the data from the 2-year NTP study in rats (NTP, 2012) instead of the data from Burek et al. (1980) as used, e.g. by JECFA (FAO/WHO, 2006, 2011). This was based on the following considerations: (i) the NTP (2012) study is a 2-year study as compared to a 90-day study performed by Burek et al. (1980), (ii) the Burek et al. (1980) data did not show a clear dose-response, (iii) the Burek et al. (1980) data were based on only 3 rats per group obtaining 150 dependent data points from each rat, and (iv) the determination of the NOAEL was not based on a statistical analysis of the data.

The CONTAM Panel also considered the data on sciatic nerve degeneration in male and female F344 rats exposed to AA via drinking water for 106 weeks (Friedman et al., 1995) and on tibial nerve degeneration in male and female F344 rats exposed to AA via drinking water for 2 years (Johnson et al., 1986). Dose-response analysis of these data by Doerge et al. (2008) provided $BMDL_{10}$ values of 0.65 mg/kg b.w. per day for males and 0.60 mg/kg b.w. per day for females from the data from Johnson et al. (1986), and of 0.37 mg/kg b.w. per day for males and 0.90 mg/kg b.w. per day for

6893 females from the data from Friedman et al. (1995). The Panel noted, however, that the data from these
6894 studies as well as the data for peripheral nerve (sciatic) axonal degeneration observed in female F344
6895 rats exposed to AA in drinking water for 2 years (NTP, 2012) did not reveal a clear dose-response
6896 since only the value in the highest dose group was increased, and for the Friedman et al. (1995) and
6897 Johnson et al. (1986) study without statistical significance. Therefore the Panel concluded that the
6898 most suitable dose-response was observed for the data on peripheral nerve (sciatic) axonal
6899 degeneration in male F344 rats exposed to AA in drinking water for 2 years (NTP, 2012).

6900 Based on these considerations, it was concluded that the most adequate endpoint for neurotoxicity was
6901 the incidence of peripheral nerve (sciatic) axonal degeneration observed in male F344 rats exposed to
6902 AA in drinking water for 2 years (NTP, 2012) (Table 20). For this endpoint, the CONTAM Panel
6903 derived a $BMDL_{10}$ value of 0.43 mg/kg b.w. per day (the lowest $BMDL_{10}$ obtained) as the reference
6904 point for non-neoplastic effects.

6905 Rodent studies have demonstrated adverse effects of AA on male reproductive parameters, particularly
6906 reduced sperm counts and effects on sperm and testis morphology. The data on effects of AA on male
6907 reproduction were not suitable for dose-response modelling but several studies demonstrated adverse
6908 effects of AA on these male reproductive parameters with NOAEL values of approximately 2 mg/kg
6909 b.w. per day (Takami et al., 2012). Based on the fact that this NOAEL of approximately 2 mg/kg b.w.
6910 per day is higher than the $BMDL_{10}$ value of 0.43 mg/kg b.w. per day obtained for neurotoxicity, the
6911 CONTAM Panel concluded that using the $BMDL_{10}$ for neurotoxicity as the reference point is
6912 conservative when considering possible effects of AA on male reproductive parameters.

6913 The lowest NOAEL reported for developmental toxicity was 1.0 mg/kg b.w. per day from studies in
6914 rats exposed gestationally and neonatally (Ferguson et al., 2010; Garey and Paule, 2010). The
6915 CONTAM Panel considered the data not suitable for dose-response modelling. Based on the fact that
6916 this NOAEL of 1 mg/kg b.w. per day is higher than the $BMDL_{10}$ value of 0.43 mg/kg b.w. per day
6917 obtained for neurotoxicity, the CONTAM Panel concluded that using the $BMDL_{10}$ for neurotoxicity as
6918 the reference point is conservative when considering possible effects of AA on developmental toxicity.

6919 For neoplastic effects, the CONTAM Panel selected as reference point the value of 0.17 mg/kg b.w.
6920 per day derived as the lowest $BMDL_{10}$ from data on incidences of Harderian gland adenomas and
6921 adenocarcinomas in male $B6C3F_1$ mice exposed to AA for 2 years (NTP, 2012) (see Table 23).

6922 The CONTAM Panel considered that even though the Harderian gland is not present in humans, this
6923 rodent organ represents a sensitive endpoint for detecting compounds that are both genotoxic and
6924 carcinogenic (Cohen, 2004; Maronpot et al., 2004; Edler et al., 2013). Harderian gland tumours and
6925 tumours in other rodent organs including the lung in mice, the brain in rats, and the mammary gland
6926 and forestomach in both species are prone to tumour formation upon exposure to epoxides or epoxide-
6927 forming carcinogens (Melnick, 2002), such as AA. Furthermore, tumour formation at the tunica
6928 vaginalis testis in male rats observed with AA, is also in agreement with findings reported for
6929 carcinogenic epoxides such as glycidol (Irwin et al., 1996) and ethylene oxide (Lynch et al., 1984).

6930 Therefore, the CONTAM Panel concluded that the results on the Harderian gland in mice cannot be
6931 disregarded in the risk assessment of AA. This is also supported by the fact that the benchmark dose
6932 ranges for Harderian gland and mammary gland tumours in female rats are overlapping.

6933

Table 28: BMD_{10} and $BMDL_{10}$ values for critical effects of AA on neurotoxicity and carcinogenicity. For details of the dose-response assessment see Appendix K.

Critical endpoint	BMD_{10} mg/kg b.w. per day	$BMDL_{10}$ mg/kg b.w. per day	Reference	Appendix K
Neurotoxicity				
Peripheral nerve (sciatic) axonal degeneration in male F344 rats	0.61	0.43	NTP (2012)	Table K1
Carcinogenicity				
Mesothelioma of the testes tunica in male F344 rats	1.32	0.51	Friedman et al. (1995)	Table K3
Mesothelioma of the epididymis or testes tunica vaginalis in male F344 rats	2.25	1.13	NTP (2012)	Table K4
Various types of sarcomas in female $B6C3F_1$ mice	2.80	1.56	NTP (2012)	Table K5
Lung tumours in male $B6C3F_1$ mice	2.25	1.13	NTP (2012)	Table K6
Harderian gland adenomas in female $B6C3F_1$ mice	0.47	0.28	NTP (2012)	Table K10
Harderian gland adenomas and adenocarcinomas in male $B6C3F_1$ mice	0.37	0.17	NTP (2012)	Table K11
Mammary gland fibroadenomas in female F344 rats	0.55	0.30	NTP (2012)	Table K12

7.5.3. Possibilities for derivation of a health based guidance value

The fact that AA and also its metabolite GA are positive in a variety of genotoxicity tests indicates that AA is of concern with respect to genotoxicity. Therefore, the CONTAM Panel considered it inappropriate to establish a TDI.

Risk characterisation for non-neoplastic effects was performed using the margin of exposure (MOE) approach and the $BMDL_{10}$ value of 0.43 mg/kg b.w. per day for the most relevant and sensitive endpoint for neurotoxicity, i.e. the incidence of peripheral nerve (sciatic) axonal degeneration observed in F344 male rats exposed to AA in drinking water for 2 years (NTP, 2012).

For risk characterisation for neoplastic effects, the MOE approach for compounds that are both genotoxic and carcinogenic is considered appropriate, using as the reference point the $BMDL_{10}$ of 0.17 mg/kg b.w. per day, i.e. the lowest $BMDL_{10}$ from data on incidences of Harderian gland adenomas and adenocarcinomas in male $B6C3F_1$ mice exposed to AA for 2 years (NTP, 2012).

8. Risk characterisation

Non-neoplastic effects

The CONTAM Panel evaluated the risks of neurotoxicity, which is considered to be due to parent AA rather than to a metabolite, using the $BMDL_{10}$ value of 0.43 mg/kg b.w. per day derived from the incidence of peripheral nerve (sciatic) axonal degeneration observed in male F344 rats exposed to AA in drinking water for 2 years (NTP, 2012) as the reference point.

Data on human exposure levels to AA across surveys and age groups are presented in Table 8 and show mean exposure values that range from 0.3 (minimum LB) to 1.9 µg/kg b.w. per day (maximum UB). The 95th percentile exposure levels range from 0.5 (minimum LB) to 3.4 µg/kg b.w. per day

(maximum UB). Using the $BMDL_{10}$ value for neurotoxicity and these data for exposure to AA, the MOE values range from 1 433 (minimum LB) to 226 (maximum UB) for the mean exposure, and from 717 (minimum LB) to 126 (maximum UB) for the 95th percentile exposure estimates across surveys and age groups (Table 29).

Table 29: Margins of exposure (MOE) values for neurotoxicity of acrylamide (AA) across age groups and surveys

Age group	Mean		P95	
	Minimum LB	Maximum UB	Minimum LB	Maximum UB
Infants (N[(a)] = 4 / 3)	860	253	307	154
Toddlers (N = 8 / 5)	391	226	187	126
Other children (N = 17 /17)	478	269	307	134
Adolescents (N = 17 / 16)	1 075	478	478	215
Adults (N = 16 / 16)	1 075	717	614	307
Elderly (N = 11 / 11)	1 433	860	717	430
Very elderly (N = 9 / 8)	1 433	717	717	430

N: number of surveys; LB: lower bound; UB: upper bound.
(a): Number of surveys used to derive the minimum/median/maximum mean exposure levels/number of surveys used to derive the minimum/median/maximum 95th percentile exposure levels.

Usually for non-genotoxic compounds, unless there are major gaps in the toxicological database, a MOE of 100 is considered sufficient to conclude that there is no health concern. This MOE covers uncertainties and variability with regard to both kinetic and dynamic differences between experimental animals and humans (factor 4 × 2.5 = 10), and within the human population (factor 3.2 × 3.2 = 10) (EFSA SC, 2012a).

It is of interest to note that the PBPK models allow derivation of compound-specific adjustment factors for AA that could replace default uncertainty factor for toxicokinetics as it relates to use of the $BMDL_{10}$ for AA-induced axonal degeneration in male F334 rats. Given that the standard MOE value of 100 includes a default uncertainty factor of 4 for toxicokinetics (EFSA SC, 2012a), the CONTAM Panel considered whether the use of a compound-specific adjustment factor (i.e. HED) would influence the risk characterisation for AA-induced neurotoxicity. The three PBPK modelling studies in male rats reported that the dose of AA required to produce an equivalent AUC for AA was 4- to 6-fold higher than in a human (mean value = 5, see Table 19). This approach would substitute an HED (5 at the mean) for the default uncertainty factor for interspecies differences in toxicokinetics (4-fold). This would result in an adjusted MOE of 125 (100 / 4 × 5) obtained from substituting the mean AA HED of 5 for the default uncertainty factor of 4.

All MOEs obtained for the current exposure to AA across surveys and age groups (Table 29) are above the adjusted MOE of 125, which is considered sufficient to conclude that there is no health concern for neurotoxicity. However, for the 95th percentile UB exposure estimates, the CONTAM Panel noted that the maxiumum UB MOEs of 126 and 134 for toddlers and other children, respectively, are close to the adjusted MOE of 125.

Neoplastic effects

The reference point for risk characterisation of the neoplastic effects of AA derived by the CONTAM Panel is the $BMDL_{10}$ of 0.17 mg/kg b.w. per day derived as the lowest $BMDL_{10}$ from data on incidences of Harderian gland adenomas and adenocarcinomas in male $B6C3F_1$ mice exposed to AA for 2 years (NTP, 2012).

Comparison of the data on human exposure levels to AA across surveys and age groups reported above (Table 8) to this $BMDL_{10}$ of 0.17 mg/kg b.w. per day, reveals MOE values (Table 30) that range

from 567 (minimum LB) to 89 (maximum UB) for the mean exposure estimates, and from 283 (minimum LB) to 50 (maximum UB) for the 95th percentile exposure estimates across surveys and age groups.

Table 30: Margins of exposure (MOE) values for neoplastic effects of AA across age groups and surveys

Age group	Mean		P95	
	Minimum LB	Maximum UB	Minimum LB	Maximum UB
Infants (N[(a)] = 4/3)	340	100	121	61
Toddlers (N = 8/5)	155	89	74	50
Other children (N = 17/17)	189	106	121	53
Adolescents (N = 17/16)	425	189	189	85
Adults (N = 16/16)	425	283	243	121
Elderly (N = 11/11)	567	340	283	170
Very elderly (N = 9/8)	567	283	283	170

N: number of surveys; LB: lower bound; UB: upper bound.
(a): Number of surveys used to derive the minimum/median/maximum mean exposure levels/number of surveys used to derive the minimum/median/maximum 95th percentile exposure levels.

The EFSA Scientific Committee concluded that, for substances that are both genotoxic and carcinogenic, an MOE of 10 000 or higher, based on a $BMDL_{10}$ from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view (EFSA, 2005b; EFSA SC, 2012a). Since the calculated MOE values are all lower than 10 000, the CONTAM Panel concluded that the MOEs across surveys and age groups indicate a concern with respect to neoplastic effects.

As noted above for AA-induced neurotoxicity, it is also possible to use the PBPK models to derive compound-specific adjustment factors that could replace default uncertainty factors for toxicokinetics of GA, the metabolite responsible for genotoxicity and carcinogenicity of AA. The MOE of 10 000 covers uncertainties and variability with regard to both kinetic and dynamic differences between experimental animals and humans (factor 4 × 2.5 = 10), and within the human population (factor 3.2 × 3.2 = 10), as well as uncertainties related to the carcinogenic process (EFSA, 2005b). Given that the value of 10 000 includes a default uncertainty factor of 10 for interspecies variation, which includes a factor of 4 for toxicokinetics (EFSA, 2005b), the CONTAM Panel considered whether information on inter-species difference in toxicokinetics would modify the risk characterisation. PBPK modelling revealed that the dose of AA required to produce an equivalent AUC for GA in male and female mice was 0.5-0.7-fold lower, respectively, than in humans (see Table 19, Young et al. (2007)), which underscores the proficiency of mice in converting AA to GA (see Table 18). This approach could support elimination of the default uncertainty factor for interspecies differences in toxicokinetics (4-fold). The CONTAM Panel concluded that even if this inter-species difference is taken into account, and although the human studies have not demonstrated AA to be a human carcinogen, the MOEs across dietary surveys and age groups indicate a concern with respect to neoplastic effects. The CONTAM Panel also noted that the MOEs for those tumour sites other than Harderian gland with the lowest $BMDL_{10}$ values are also below 10 000 and thus also indicate a concern.

The CONTAM Panel noted that AA is a germ cell mutagen and that there are at present no established procedures for risk assessment using this endpoint.

9. Uncertainty

The evaluation of the inherent uncertainties in the risk assessment on AA has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary

Exposure Assessment (EFSA, 2006). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' (WHO/IPCS, 2008) has been considered. According to the guidance provided by the EFSA opinion (2006) the following sources of uncertainties have been considered: Assessment objectives, exposure scenario, exposure model, and model input (parameters).

9.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference.

9.2. Exposure scenarios/Exposure models

A total of 7 448 samples submitted by 24 European countries and 35 971 samples submitted by 6 food associations were used in the exposure assessment. The amount of occurrence data submitted differs considerably depending on food product and reporting data provider. While the data from the food associations widely reflect a representative overview on the AA levels in the respective products, there is some uncertainty whether the results submitted by the European countries cover possible regional differences. Regarding the data provided by the European countries, 70 % of the samples for which the information is available are indicated to be taken from targeted sampling design. In general, the analytical methods for the determination of AA in food are adapted to the indicative values set by the EU Commission and not necessarily to follow lower background levels. Therefore, the use of the upper bound approach (UB) for left-censored data in combination with the targeted sampling may have lead to bias towards an over estimation of the AA levels used as input of the exposure model/scenarios. Some food groups, not covered by any occurrence data submitted, could not be taken into account in the exposure assessment. However, these foods are not known to contain considerable AA concentrations, so the absence of data for these foods is unlikely to contribute to any underestimation of exposure as those food products that are known to potentially contain AA are covered.

The food consumption habits reported in the Comprehensive Database are not described in a manner fully compatible with the exposure assessment to heat-induced contaminants. The consumption data have been mostly reported at the level of raw commodities such as potato, without systematic information on how the potatoes were cooked before consumption. Consumption of coffee was also most often reported without any detail on the kind of coffee beverage consumed. Such cases, representing 11 % of the total consumption events of potato products (potato crisps and other snacks excluded) and 40 % of the total consumption events of coffee beverages, were handled by assumptions. The kind of coffee beverage most frequently consumed in the corresponding survey and age group was attributed to the unspecified coffee beverage. The unspecified potato was assumed to be consumed as fried or not fried according to the quantity of oil/fat consumed during the same meal. The impact of such assumption was assessed in a sensitivity analysis. When considering the unspecified potato as not fried, this could result in exposure levels up to 33 % lower compared to the baseline scenario depending on the consumption survey and age group. When considering the unspecified potato as fried, this could result in exposure levels up to 200 % higher compared to the baseline exposure scenario. This adds to the uncertainty of the total dietary exposure to AA.

Second, the consumers' preferences regarding the degree of potato frying and bread toasting, the coffee and potato crisps brand, as well as the place of consumption of potato fried products are not reported in the Comprehensive Database. Some exposure scenarios were set in order to address specific consumer's behaviours and preferences. Scenarios on the brand loyalty for potato crisps and on bread toasting resulted in variation less than 5 % in the final estimates of the total exposure to AA. Scenarios on the brand loyalty for coffee products and on the potato frying resulted in variation up to respectively 15 % and 80 % in the final estimates of the total exposure to AA.

Finally, it should also be noted that all available occurrence data have been used altogether in order to produce a single 'European' estimate without taking into account possible variability throughout Europe. As a consequence, the exposure estimates at population group level may either be over- or underestimated.

9.3. Model input (parameters)

Laboratories may use any appropriate method of analysis provided it can be demonstrated in a traceable manner that they fulfil the requirements according to ISO 17025. As some harmonised performance criteria, especially regarding LODs, are laid down, and taking into account that the analytical results were generated with MS based methods in accredited laboratories, this add only slightly to the uncertainty of the analytical results.

9.4. Other uncertainties

It has not been demonstrated that exposure to AA in humans *in vivo* is able to generate DNA adducts presumably due to analytical limitations for detection.

Epidemiological studies have used FFQs to estimate AA exposure in individuals. FFQs in most cases were not designed specifically to measure AA intake and may not have been able to capture the high variation on AA content of foods. As a consequence, the ability of FFQs to measure AA intake accurately is probably low, as shown in the few validation studies performed, and the uncertainties in the measurement of AA intake in epidemiological studies may thus hamper the corresponding risk estimates.

Epidemiological studies did not indicate an increased risk of cancer in workers occupationally exposed to AA. Similarly, they did not provide consistent evidence for carcinogenicity of dietary intake of AA in humans. It remains therefore unclear if AA, although being carcinogenic in rodents, is a human carcinogen.

Although GA-mediated DNA damage is considered as the crucial initiating effect leading to AA-induced carcinogenesis, the level of GA-derived DNA adducts cannot predict the localization and incidence of tumors with respect to organ specificity. This is particularly evident for the liver which shows high levels of DNA adducts in AA-treated rodents and, however, do not develop increased rates of hepatic tumour. Furthermore, it appears unclear if the target organs affected by the carcinogenic action of AA in rodents are also targets for tumour formation in humans. In general, it cannot be expected that the patterns of target organs for a given carcinogen are similar between rodents and humans. Likewise, the general uncertainty originating from this fact is not considered to be increased by the use of data on the carcinogenic action of AA on the Harderian gland of rodents, an organ not found in the human body.

Dose-response analysis has revealed that increases in Harderian gland tumour frequency was the most sensitive tumour responses towards AA in rodents. It remains uncertain if there is/are human organ(s) as sensitive to AA carcinogenicity as the Harderian gland.

AA is a germ cell mutagen but there are at present no accepted procedures for using AA doses relevant for human dietary exposure in the risk characterisation.

The toxic effects of AA might be influenced by other dietary components (e.g. ethanol, garlic, other Maillard reaction products).

9.5. Summary of uncertainties

In Table 31 a summary of the uncertainty evaluation is presented, highlighting the main sources of uncertainty and indicating an estimate of whether the respective source of uncertainty might have led to an over- or underestimation of the dietary exposure or the resulting risk**.**

7125 **Table 31:** Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of
7126 exposure of AA in food

Sources of uncertainty	Direction
Lack of representativeness of the occurrence data for certain food commodities	+/-[(a)]
Use of lower/upperbound estimation	-/+
Heterogeneity of the data regarding the mode of preparation of the products before analysis	+/-
Limited number of samples available in some food groups	-
Lack of information in the consumption data on the way potato and coffee are prepared	+/-
Long-term (chronic) exposure assessed from few days of consumption without removing the within-individual variability	+
Use of the Harderian gland as the target tissue	+
Relatively wide variation in the outcomes of the BMD modelling	+/-
Lack of support from the human occupational studies for the major critical effects, except for neurotoxicity	-
Inconsistency in the human studies of associations between AA dietary exposure and cancer	+/-

7127 (a): + = uncertainty with potential to cause over-estimation of exposure/risk; - = uncertainty with potential to cause under-
7128 estimation of exposure/risk.
7129
7130 The CONTAM Panel considered that the impact of the uncertainties on the risk assessment of human
7131 exposure to AA through consumption of food is moderate.

7132

7133 ## CONCLUSIONS AND RECOMMENDATIONS

7134 ### CONCLUSIONS

7135 ***Background***

7136 • Acrylamide (AA) is produced for a wide variety of industrial applications. In view of the
7137 known toxic effects of AA, including genotoxicity and carcinogenicity, concerns about human
7138 exposure to AA arose in 2002 when it was discovered that it is formed when certain foods are
7139 prepared at high temperatures.

7140 • AA forms predominantly from free asparagine and reducing sugars during high temperature
7141 processing, such as frying, roasting and baking.

7142 • An important initiative to reduce AA in various food categories is the development of the
7143 FoodDrinkEurope 'Acrylamide toolbox'. The aim of the toolbox is to provide national and
7144 local authorities, manufacturers and other relevant bodies, with brief descriptions of
7145 intervention steps which may prevent and reduce formation of AA in specific manufacturing
7146 processes and products.

7147 ***Sampling and methods of analysis***

7148 • A detailed description of sampling time, point and procedure is of special importance for the
7149 interpretation of analytical results as seasonality, storage and processing of the respective food
7150 product may have a substantial impact on their AA levels.

7151 • The analytical determination of AA in food products is most frequently performed by high
7152 performance liquid chromatographic (HPLC) or gas chromatographic (GC) separation
7153 methods with mass spectrometric detection (MS), either in selected ion monitoring (SIM) or
7154 by tandem mass spectrometry (MS/MS) in multiple reaction mode (MRM).

- Isotope labelled standards of AA are readily commercially available, either as AA-D3, AA-D5, 13C1-AA or as 13C3-AA.

- Certified reference materials (CRMs) containing AA in various food products are commercially available.

- Several proficiency tests and interlaboratory studies comprising AA in various food products were performed. In general, there was no evident trend in performance or bias in results obtained with GC-MS or HPLC-MS based methods.

Occurrence

- Occurrence data on AA in food generated with analytical methods based on GC or HPLC, collected since 2010 and representing products available in the European market were considered. This represented a total of 7 448 results reported by 24 European countries and 35 971 results reported by 6 food associations.

- Data provided by food associations and those provided by European countries gave overall consistent and complementary information. The Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that the datasets could be combined to perform the exposure assessment of the European population.

- AA was found at the highest levels in 'Coffee and coffee substitutes' (average MB levels of 578 µg/kg) followed by 'Potato crisps and snacks' (average MB level of 389 µg/kg) and 'Potato fried products' (average medium bound (MB) level of 308 µg/kg). Lower levels were found in 'Processed cereal-based baby foods' (average MB level of 73 µg/kg), 'Soft bread' (average MB level of 42 µg/kg) and 'Baby foods, other than cereal-based' (average MB level of 24 µg/kg).

- 'Potato fried products' as well as 'Potato crisps and snacks' made from fresh potato contained higher AA levels than those made from potato dough.

- Higher AA levels were observed in 'Crisp bread' than in 'Soft bread'. In both 'Crisp bread' and 'Soft bread', higher AA levels were observed in products mainly made from rye compared to products mainly made from wheat.

- Mean and 95th percentile AA levels were higher in 'Bran and whole grains breakfast cereals' than in 'Wheat and rye based breakfast cereals' and in 'Maize, oat, spelt, barley and rice based breakfast cereals'.

- 'Gingerbread' contained higher AA levels than 'Crackers' and 'Biscuits and wafers'.

- 'Roasted coffee' contained lower AA levels than 'Instant coffee'. A roasting effect was also observed, light roasting being associated with higher levels than medium and dark roasting. 'Coffee substitutes, based on chicory' contained higher AA levels than 'Coffee substitutes, based on cereals'.

- Higher AA levels were observed in 'Processed cereal-based baby foods' than in 'Baby foods, other than cereal based'. Within this last category, 'Baby foods, containing prunes' contained higher levels than 'Baby foods, not containing prunes'.

Human dietary exposure

- Infants, toddlers and other children were the most exposed groups. Depending on the survey and age group, chronic dietary exposure of children was estimated to be on average between

0.5 and 1.9 µg/kg b.w. per day and the 95th percentile between 1.4 and 3.4 µg/kg b.w. per day. Chronic dietary exposure of adolescents, adults, elderly and very elderly was estimated to be on average between 0.3 and 0.9 µg/kg b.w. per day and the 95th percentile between 0.6 and 2.0 µg/kg b.w. per day depending on the survey and age group.

- The main contributor to the total exposure of infants was the 'Baby foods, other than processed cereal-based' followed by 'Other products based on potatoes' and 'Processed cereal-based baby foods'. The main contributor to the total exposure of toddlers, other children and adolescents was 'Potato fried products' representing up to half the total exposure, followed by 'Soft bread', 'Biscuits, crackers, crisp bread', 'Other products based on cereals' and 'Other products based on potatoes'. These foods groups were also the main contributors to the total exposure of adults, elderly and very elderly together with 'Coffee and coffee substitutes'.

- A sensitivity analysis was conducted in order to assess the influence of specific behaviours (brand loyalty, places of consumption, home-cooking habits) on the total dietary exposure to AA. Scenarios on the brand loyalty for potato crisps and coffee products resulted in variations of less than 5 and 15 %, respectively, of the total dietary exposure to AA. In scenarios on home-cooking behaviours, degree of bread toasting resulted in variations of less than 5 %, while for conditions of potato frying the total dietary exposure to AA could be increased up to 80 %.

- Substantial uncertainties were associated with the exposure assessment regarding the mode of preparation of 'Potato fried products', present in both the consumption and occurrence datasets.

Hazard identification and characterisation

Toxicokinetics

- AA is extensively absorbed from the gastrointestinal tract in humans and experimental animals.

- In experimental animals it has been shown that AA is rapidly distributed into the tissues.

- AA crosses the placenta and is transferred to a small extent into human milk.

- AA is metabolised to glycidamide (GA), which is a reactive epoxide and is widely distributed into the tissues. The main enzyme involved in the AA epoxidation is CYP2E1.

- Mice are more proficient in converting AA into GA than either rats or humans.

- Both AA and GA are conjugated with glutathione (GSH), primarily mediated by glutathione-S-transferases, and the GSH adducts are subsequently converted to mercapturic acids (MA). This reaction is considered a detoxification pathway. The MAs of AA and GA represent the major metabolites and are excreted in urine. They can be used as biomarkers of AA exposure.

- AA and GA can react with proteins to form covalent adducts, e.g. with haemoglobin (Hb). The Hb adducts represent important biomarkers of AA exposure.

- Covalent adducts of AA with DNA have been generated in chemical reactions, but have never been detected in vivo or in vitro in animal or human tissues. In contrast, covalent DNA adducts of GA have been amply demonstrated in vitro and in experimental animals. These are used as biomarkers of AA exposure.

- Physiologically based pharmacokinetic (PBPK) models allow derivation of human-equivalent doses (HEDs), which could be used to convert external doses of AA that produce critical effects in animal studies to the human external doses required to produce equivalent area under the curve (AUC) values for either AA or GA, depending on the toxic endpoint used.

- The HEDs derived from equivalent AA-AUCs in rats and mice suggest that endpoints related to AA-mediated effects (e.g. neurotoxicity) require 4- to 6-fold higher doses in rats when compared to humans, based on inter-species differences in toxicokinetics. However, 0.5- to 0.7-fold lower doses of AA would be required in mice to produce equivalent GA-AUCs for genotoxicity-related endpoints when compared to humans.

Biomarkers of exposure/effects

- The three main types of biomarkers for internal exposure to AA and GA are: (i) urinary mercapturic acids (MAs), (ii) Hb adducts of AA and GA and (iii) DNA adducts of GA. They reflect different timescales for the detection of the exposure. There are correlations both between these types of biomarkers, and between them and exposure to AA.

- The N7-guanine adduct derived from GA (N7-GA-Gua) is the most abundant DNA adduct following AA exposure.

- GA-DNA adducts in experimental animals are found at similar levels in various tissues of the body, although CYP2E1 is primarily located in the liver.

Toxicity

- Toxicological studies with AA have been conducted in rats, mice, monkeys, cats and dogs, using various dosing protocols and routes of exposure.

- The major non-neoplastic findings were adverse effects on the peripheral nervous system, such as hind-limb strength, rotorod performance or histopathological changes in nerves and nervous system structures. The results are consistent with the notion that AA causes neurotoxic effects in various mammalian species in a similar dose range.

- Rodent studies have demonstrated adverse effects of AA on male reproductive parameters, particularly reduced sperm counts and effects on sperm and testis morphology.

- Rat and mouse studies have shown some signs of developmental toxicity (increased incidence of skeletal variations, slightly impaired body weight gain, histological changes in the central nervous system, and neurobehavioural effects) at exposure levels that in some cases are also associated with maternal toxicity.

Genotoxicity

- *In vitro* genotoxicity studies indicate that AA is a weak mutagen in mammalian cells but an effective clastogen.

- GA is a strong mutagen and a clastogen. It induces mutations via a DNA adduct mechanism.

- *In vivo*, AA is clearly genotoxic in somatic and germ cells.

- AA exerts its mutagenicity via metabolism by CYP2E1 to GA. AA can also induce gene mutations by a pathway involving the generation of reactive oxygen species (ROS) and oxidative DNA damage.

Carcinogenicity

- AA is carcinogenic in multiple tissues from both male and female mice and rats.

- In rats, the major tumours produced by AA are: adenomas, fibroadenomas and fibromas of the mammary gland, thyroid gland follicular cell adenomas or carcinomas, and testes or epididymis tunica vaginalis mesotheliomas.

- In mice, the major tumours produced by AA are: Harderian gland adenomas, mammary gland adenoacanthomas and adenocarcinomas, lung alveolar and bronchiolar adenomas, benign ovary granulosa cell tumours, skin sarcomas, and stomach and forestomach squamous cell papillomas in females, and Harderian gland adenomas and adenocarcinomas, lung alveolar and bronchiolar adenomas and carcinomas, and stomach squamous papillomas and carcinomas in males.

- A similar spectrum of tumours is observed when equimolar concentrations of GA were administered in drinking water to rats and mice, which is consistent with GA being the proximate carcinogenic metabolite of AA.

Mode of action

- AA is an electrophilic molecule, which can undergo Michael addition-type reactions with nucleophilic target molecules. In particular, activated thiolate moieties in cysteine residues of enzymes and other functional proteins, e.g. in neuronal cells or spermatocytes, have been described as targets. The neurotoxic properties of AA are considered to originate mainly from this type of reactivity.

- AA shows some reactivity towards nucleic acids, whereas reports on the formation of DNA adducts in vivo suggest that GA is mainly if not exclusively responsible for the formation of DNA adducts in AA-treated animals.

- Evidence from the available studies in the literature on hormonal and endocrine effects of AA is equivocal. This is particularly true for changes in hormone levels in AA-treated animals which were reported in some studies. Mechanistic hypotheses on local endocrine effects of AA which may explain tumour formation in certain hormone- or paracrine-regulated target tissues lack experimental proof.

Observations in humans

- Two cohort studies considered occupational exposure to AA and did not indicate an increased cancer risk.

- Associations between AA exposure through diet and cancer risk have been analysed in at least 34 publications, based on 16 epidemiological studies on several cancer sites. For most cancer sites there is no consistent indication for an association between AA exposure and increased risk. With respect to renal cell, endometrial and ovarian cancer a few studies have reported positive associations with AA intake, although the overall evidence is limited and inconsistent.

- Two studies reported an inverse relation between AA exposure (measured by levels of AA and GA adducts) and birth weight and other markers of foetal growth. It has not been established whether the association between dietary AA exposure and these outcomes is causal.

- Studies among workers occupationally exposed to AA showed an increased risk of neurological alterations, including mostly the peripheral, but also the central nervous system.

Consideration of critical effects and dose-response modelling

- From all data available, the CONTAM Panel identified four posible critical endpoints for AA toxicity, i.e. neurotoxicity, effects on male reproduction, developmental toxicity and carcinogenicity.

- The data from human studies were not adequate for dose-response assessment.

- The CONTAM Panel performed benchmark dose (BMD) analyses on data for neurotoxicity and on the tumour incidences of AA in rats and mice.

- For the adverse effects of AA on male reproductive parameters in rodents, particularly reduced sperm counts and effects on sperm and testis morphology, the no-observed-adverse-effect level (NOAELs) were about 2 mg/kg b.w. per day.

- The lowest NOAEL reported for developmental toxicity was 1 mg/kg b.w. per day from studies in rats exposed gestationally and neonatally.

- The CONTAM Panel selected the value of 0.43 mg/kg b.w. per day derived as the lowest $BMDL_{10}$ from the data data on incidences of peripheral nerve (sciatic) axonal degeneration in male F344 rats exposed to AA in drinking water for two years as the reference point for non-neoplastic effects.

- Based on the fact that this $BMDL_{10}$ value of 0.43 mg/kg b.w. per day obtained for neurotoxicity is lower than the NOAEL of approximately 2.0 mg/kg b.w. per day for adverse effects on male reproductive parameters and of 1.0 mg/kg b.w. per day for developmental toxicity, the CONTAM Panel concluded that using the $BMDL_{10}$ for neurotoxicity as the reference point is conservative when considering possible non-neoplastic effects of AA.

- For neoplastic effects, the CONTAM Panel selected as the reference point the value of 0.17 mg/kg b.w. per day derived as the lowest $BMDL_{10}$ from data on incidences of Harderian gland adenomas and adenocarcinomas in male $B6C3F_1$ mice exposed to AA for two years.

- The CONTAM Panel noted that the Harderian gland is an organ absent in humans, but that in rodents this organ is a sensitive target tissue to detect compounds that are both genotoxic and carcinogenic. Taking into account that target tissues for tumour formation by a given genotoxic carcinogen may differ between rodents and humans, the CONTAM Panel considered the most sensitive target tissue in rodent bioassays, the Harderian gland, a conservative endpoint for assessment of the risk for neoplastic effects of AA in humans.

Risk characterisation

- The CONTAM Panel calculated margin of exposure (MOE) values for the neurotoxic effects (ranging from 1 433 (minimum lower bound (LB)) to 226 (maximum upper bound (UB)) for the mean exposure, and from 717 (minimum LB) to 126 (maximum UB) for the 95th percentile exposure estimates across surveys and age groups). Taking into account differences between species and within the human population, the Panel concluded that the MOEs across surveys and age groups are not of concern. However, the Panel noted that the MOEs for the 95th percentile UB exposure estimates for toddlers and other children are close to the value that might be of concern for neurotoxicity.

- Comparison of the reference point for risk characterisation of the neoplastic effects of AA to the estimates of human dietary exposure to AA across surveys and age groups reveals MOEs that range from 567 (minimum LB) to 89 (maximum UB) for the mean exposure estimates, and from 283 (minimum LB) to 50 (maximum UB) for the 95th percentile exposure estimates.

The EFSA Scientific Committee concluded that for substances that are both genotoxic and carcinogenic, an MOE of 10 000 or higher, based on a $BMDL_{10}$ from an animal study, and taking into account overall uncertainties in the interpretation, would be of low concern from a public health point of view. Since the MOEs are all considerably lower than 10 000, the CONTAM Panel concluded that although the available human studies have not demonstrated AA to be a human carcinogen, the MOEs based on the current levels of dietary exposure to AA across surveys and age groups indicate a concern with respect to neoplastic effects.

- The CONTAM Panel noted that AA is a germ cell mutagen and that there are at present no established procedures for risk assessment using this endpoint.

RECOMMENDATIONS

- The reporting of AA occurrence data should be improved regarding the mode of preparation of the products before analysis.

- Duplicate diet studies are recommended in order to improve exposure assessment, since they provide a more accurate indication of AA levels in food as prepared and consumed at home.

- Data on urinary metabolites levels from individuals participating in the duplicate diet studies should be generated for the purpose of validation of the biomarkers.

- Further epidemiological studies are required to confirm or refute the inverse relation between dietary AA intake and birth weight and other markers of foetal growth observed in two studies.

- Improved approaches for the detection and risk assessment of germ cell mutagens should be developed, and applied to AA and GA.

DOCUMENTATION PROVIDED TO EFSA

1. FoodDrinkEurope comments to EFSA's full Risk asseement of acrylamide in foods. May 2013. Submitted by FoodDrinkEurope under the Consultation on acrylamide with the EFSA Stakeholder Consultative Platform.

2. Some of the work undertaken by BEUC members on acrylamide. May 2013. Submitted by BEUC, The European Consumer Organisation, under the Consultation on acrylamide with the EFSA Stakeholder Consultative Platform.

- *Kartoffelchips - Wenn ich nur aufhören könnt'!*. Konsument (2003), 10, 22-24.
- *Acrylamid in Kartoffelchips - Knistern, Knabbern, Kalorien*. Konsument (2006), 5, 31-33.
- *Lebkuchen - Alarmsignal für Österreich*. Konsument (2008), 12, 14-16.
- *Knabbergebäck - Pringles und Kelly's im Abseits*. Konsument (2008), 6, 23-25.
- *Frites - Rien ne vaut une frite maison*. Test-achats (2008), 526, 18-21.
- *Löskaffee - Wem's schmeckt…* Konsument (2009), 8, 18-19.
- *Aifryer: une friteuse sans huile, mais…* Test-Achats (2011)[37].
- *Chips - Pringles fällt aus der Rolle*. Konsument (2012), 6, 32-33.
- *Patatas fritas. Mejores grasas que antes*. OCU-Compra Maestra (2012), 371, 13-16.
- *Bem frito sem risco*. Proteste (2013), 343, 16-19.

[37] http://www.test-achats.be/electromenager/petit-electromenager/en-direct/airfryer-une-friteuse-sans-huile-mais

7398 • *Freidoras*. OCU-Compra Maestra (2013), 377, 46-48.

7399 • *Acrylamide - Bak ze niet te bruin*. Gezondgids (2013), 8-11.

7400

7401 **REFERENCES**

7402 Abramsson-Zetterberg L, 2003. The dose-response relationship at very low doses of acrylamide is
7403 linear in the flow cytometer-based mouse micronucleous assay. Mutation Research/Genetic
7404 Toxicology and environmental Mutagenesis, 535, 215-222.

7405 ACGIH (American Conference of Governmental Industrial Hygienists), 2011. Acylamide. In:
7406 Threshold limit values for chemical substances and physical agents and biological exposure
7407 indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists. As cited in
7408 ATSDR, 2012.

7409 Adams A, Hamdani S, Van Lancker F, Mejri S and De Kimpe N, 2010. Stability of acrylamide in
7410 model systems and its reactivity with selected nucleophiles. Food Research International, 43, 1517-
7411 1522.

7412 Adler I-D, 1990. Clastogenic effects of acrylamide in different germ cell stages of male mice, in:
7413 Biology of Mammalian Germ Cell Mutagenesis. Eds Allen JW, Bridges BA, Lyon MF, Moses MJ,
7414 and Russell LB. Banbury Report 34, Cold Spring Harbor Press, Cold Spring Harbor, 1990, 115-
7415 131. As cited in Favor and Shelby (2005).

7416 Adler ID, Zouh R and Schmid E, 1993. Perturbation of cell division by acrylamide *in vitro* and *in vivo*.
7417 Mutation Research, 301, 249–254.

7418 Adler ID, Reitmeir P, Schmoller R and Schrieverschwemmer G, 1994. Dose-response for heritable
7419 translocations induced by acrylamide in spermatids of mice. Mutation Research-Fundamental and
7420 Molecular Mechanisms of Mutagenesis, 309, 285-291.

7421 Adler ID, Baumgartner A, Gonda H, Friedman MA and Skerhut M, 2000. 1-Aminobenzotriazole
7422 inhibits acrylamide-induced dominant lethal effects in spermatids of male mice. Mutagenesis, 15,
7423 133-136.

7424 Adler ID, Gonda H, de Angelis MH, Jentsch I, Otten IS and Speicher MR, 2004. Heritable
7425 translocations induced by dermal exposure of male mice to acrylamide. Cytogenetic and Genome
7426 Research, 104, 271-276.

7427 Afssa (Agence Française de Sécurité Sanitaire des Aliments), 2003, Acrylamide: point d'information
7428 n°2. Afssa – Saisine n° 2002-SA-0300. Maisons-Alfort, le 21 février 2003.

7429 Afssa (Agence Française de Sécurité Sanitaire des Aliments), 2005. Acrylamide: point d'information
7430 n°2. Afssa – Saisine n° 2002-SA-0300. Maisons-Alfort, le 13 mai 2005.

7431 Ahmed HH, Elmegeed GA, El-Sayed E-SM, Abd-Elhalim MM, Shousha WG and Shafic RW, 2010.
7432 Potent neuroprotective role of novel melatonin derivatives for management of central neuropathy
7433 induced by acrylamide in rats. European Journal of Medicinal Chemistry, 45, 5452-5459.

7434 Albishri HM and El-Hady DA, 2014. Eco-friendly ionic liquid based ultrasonic assisted selective
7435 extraction coupled with a simple liquid chromatography for the reliable determination of
7436 acrylamide in food samples. Talanta, 118, 129-136.

7437 Aldous CN, Farr CH and Shrama RP, 1983. Evaluation of acrylamide treatment on levels of major
7438 brain biogenic-amines, their turnover rates, and metabolites. Fundamental and Applied Toxicology,
7439 3, 182-1286.

7440 Ali SF, Hong JS, Wilson WE, Uphouse LL and Bondy SC, 1983. Effect of acrylamide on
7441 neurotransmitter metabolism and neuropeptide levels in several brain-regions and upon circulating
7442 hormones. Archives of Toxicology, 52, 35-43.

7443 Allam AA, El-Ghareeb AW, Abdul-Hamid M, El Bakery A, Gad M and Sabri M, 2010. Effect of
7444 prenatal and perinatal acrylamide on the biochemical and morphological changes in liver of
7445 developing albino rat. Archives of Toxicology, 84, 129-141.

7446 Allam A, El-Gharee AA, Abdul-Hamid M, Baikry A and Sabri MI, 2011. Prenatal and perinatal
7447 acrylamide disrupts the development of cerebellum in rat: Biochemical and morphological studies.
7448 Toxicology and Industrial Health, 27, 291-306.

7449 Allam A, El-Gareeb A, Abdul-Hamid M, El-Bakry A and Ajarem J, 2013. Effect of acrylamide on the
7450 development of medulla oblongata in albino rat: biochemical and morphological studies. African
7451 Journal of Pharmacy and Pharmacology, 7, 1320-1331.

7452 Altaeva AA, Sycheva LP and Belyaeva NN, 2011. Mutagenic Activity of Acrylamide in the Rat
7453 Thyroid Cells under Conditions of a Subacute Experiment. Bulletin of Experimental Biology and
7454 Medicine, 152, 275-277.

7455 Alturfan AA, Tozan-Beceren A, Sehrili AO, Demiralp E, Sener G and Omurtag GZ, 2012a.
7456 Resveratrol ameliorates oxidative DNA damage and protects against acrylamide-induced oxidative
7457 stress in rats. Molecular Biology Reports, 39, 4589-4596.

7458 Alturfan EI, Beceren A, Sehirli AO, Demiralp ZE, Sener G and Omurtag GZ, 2012b. Protective effect
7459 of N-acetyl-L-cysteine against acrylamide-induced oxidative stress in rats. Turkish Journal of
7460 Veterinary & Animal Sciences, 36, 438-445.

7461 Alzahrani HAS, 2011. Protective effect of L-carnitine against acrylamide-induced DNA damage in
7462 somatic and germ cells of mice. Saudi Journal of Biological Sciences, 18, 29-36.

7463 Al-Azkawi AS, Al-Bahry SN, Mahmoud IY and Barry MJ, 2013. Effect of acrylamide on liver
7464 proteins expression in mice. Journal of Food Research, 2, 132-142.

7465 American Cyanamid Company, 1979. A fetal toxicity study of acrylamide in rats. Submitted to the
7466 U.S. Environmental Protection Agency under TSCA Section 8D. EPA878211679. OTS206055. As
7467 cited in ATSDR (2012).

7468 Annola K, Keski-Rahkonen P, Vahakangas K and Lehtonen M, 2008a. Simultaneous determination of
7469 acrylamide, its metabolite glycidamide and antipyrine in human placental perfusion fluid and
7470 placental tissue by liquid chromatography-electrospray tandem mass spectrometry. Journal of
7471 Chromatography B, 876, 191-197.

7472 Annola K, Karttunen V, Keski-Rahkonen P, Myllynen P, Segerbäck D, Heinonen S and Vahakangas
7473 K, 2008b. Transplacental transfer of acrylamide and glycidamide are comparable to that of
7474 antipyrine in perfused human placenta. Toxicology Letters, 182, 50-56.

7475 Anses (French agency for food, environmental and occupational health and safety), 2013. Note
7476 d'appui scientifique et technique de l'Agence nationale de sécurité sanitaire de l'alimentation de
7477 l'encironement et du travail relatif à «l'Etude de l'alimentation total français». Demande n 2006-
7478 SA-0361.

7479 Ao L, Liu S-X, Yang M-S, Fong C-C, An H and Cao J, 2008. Acrylamide-induced molecular mutation
7480 spectra at HPRT locus in human promyelocytic leukaemia HL-60 and NB4 cell lines. Mutagenesis,
7481 23, 309-315.

7482 Ao L and Cao J, 2012. Genotoxicity of acrylamide and glycidamide: areview of the studies by HPRT
7483 gene and TK gene mutation assays. Genes and Environment, 31, 8.

7484 Arvanitoyannis IS and Dionisopoulou N, 2014. Acrylamide: formation, occurrence in food products,
7485 detection methods, and legislation. Critical Reviews in Food Science and Nutrition, 54, 708-733.

7486 ATSDR (Agency for Toxic Substances and Disease Registry), 2012. Toxicological profile of
7487 acrylamide. U.S. Department of Health and Human Services. Public Health Service. December
7488 2012. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp203.pdf

7489 Aureli F, Di Pasquale M, Lucchetti D, Aureli P and Coni E, 2007. An absorption study of dietary
7490 administered acrylamide in swine. Food and Chemical Toxicology, 45, 1202-1209.

7491 Bachmann M, Myers JE and Bezuidenhout BN, 1992. Acrylamide Monomer and Peripheral
7492 Neuropathy in Chemical Workers. American Journal of Industrial Medicine, 21, 217-222.

7493 Bailer AJ and Portier CJ, 1988. Effects of treatment-induced mortality and tumor-induced mortality on
7494 tests for carcinogenicity in small samples. Biometrics 44, 417-431.

7495 Bandarra S, Fernandes AS, Magro I, Guerreiro PS, Pingarilho M, Churchwell MI, Gil OM, Batinic-
7496 Haberle I, Goncalves S, Rueff J, Miranda JP, Marques MM, Beland FA, Castro M, Gaspar JF and
7497 Oliveira NG, 2013. Mechanistic insights into the cytotoxicity and genotoxicity induced by
7498 glycidamide in human mammary cells. Mutagenesis, 28, 721-729.

7499 Bandarra S, Fernandes AS, Magro I, Guerreiro PS, Pingarilho M, Churchwell MI, Gil OM, Batinic-
7500 Haberle I, Goncalves S, Rueff J, Miranda JP, Marques MM, Beland FA, Castro M, Gaspar JF and
7501 Oliveira NG, 2014. *Erratum*. Mechanistic insights into the cytotoxicity and genotoxicity induced
7502 by glycidamide in human mammary cells. Mutagenesis, 29, 97.

7503 Barber DS, Hunt JR, Ehrich MF, Lehning EJ and LoPachin RM, 2001. Metabolism, toxicokinetics and
7504 hemoglobin adduct formation in rats following subacute and subchronic acrylamide dosing.
7505 Neurotoxicology, 22, 341-353.

7506 Barber DS, Stevens S and LoPachin RM, 2007. Proteomic analysis of rat striatal synaptosomes during
7507 acrylamide intoxication at a low dose rate. Toxicological Sciences, 100, 156-167.

7508 Basile A, Ferranti P, Moccaldi R, Spagnoli G and Sannolo N, 2008. Proteomic approach for the
7509 analysis of acrylamide-hemoglobin adducts Perspectives for biological monitoring. Journal of
7510 Chromatography A, 1215, 74-81.

7511 Baum M, Fauth E, Fritzen S, Herrmann A, Mertes P, Merz K, Rudolphi M, Zankl H and Eisenbrand
7512 G, 2005. Acrylamide and glycidamide: genotoxic effects in V79-cells and human blood. Mutation
7513 Research, 580, 61-69.

7514 Baum M, Loeppky RN, Thielen S and Eisenbrand G, 2008. Genotoxicity of glycidamide in
7515 comparison to 3-N-nitroso-oxazolidin-2-one. Journal of Agricultural and Food Chemistry, 56,
7516 5989-5993.

7517 Becalski A, Brady B, Feng S, Gauthier BR and Zhao T, 2011. Formation of acrylamide at
7518 temperatures lower than 100 °C: the case of prunes and a model study. Food Additives and
7519 Contaminants 28, 726-730.

7520 Begum Sheikh R and Kedam T, 2010. Effect of acrylamide on chick embryonic liver glutathione S-
7521 transferases. Mediterranean Journal of Nutrition and Metabolism, 3, 31-38.

7522 Beland FA, 2010. Technical report for experiment No. 2150.05 and 2150.07. Genotoxicity and
7523 carcinogenicity of acrylamide and its metabolite, glycidamide, in rodents: Two year chronic study
7524 of acrylamide in B6C3F1 mice and F334 rats. Unpublished study. Submitted to FAO/WHO by the
7525 United States National Center for Toxicological Research, Jefferson, AK. As cited by FAO/WHO
7526 (2011).

7527 Beland FA, Benso RW, Mellick PW, Kovatch RM, Roberts DW, Fang J-L and Doerge DR, 2003.
7528 Effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F$_1$ mice. Food and
7529 Chemical Toxicology, 43, 1-19.

7530 Beland FA, Mellick PW, Olson GR, Mendoza MC, Marques MM and Doerge DR, 2013.
7531 Carcinogenicity of acrylamide in B6C3F(1) mice and F344/N rats from a 2-year drinking water
7532 exposure. Food and Chemical Toxicology, 51, 149-159.

7533 Bent GA, Maragh P and Dasgupta T, 2012. Acrylamide in Caribbean foods - Residual levels and their
7534 relation to reducing sugar and asparagine content. Food Chemistry, 133, 451-457.

7535 Berger FI, Feld J, Bertow D, Eisenbrand G, Fricker G, Gerhardt N, Merz KH, Richling E and Baum
7536 M, 2011. Biological effects of acrylamide after daily ingestion of various foods in comparison to
7537 water: a study in rats. Molecular Nutrition and Food Research, 55, 387-399.

7538 Bergmark E, Calleman CJ and Costa LG, 1991. Formation of hemoglobin adducts of acrylamide and
7539 its epoxide metabolite glycidamide in the rat. Toxicology and Applied Pharmacology, 111, 352-
7540 363.

7541 Bergmark E, 1997. Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers,
7542 smokers and nonsmokers. Chemical Research in Toxicology, 10, 78-84.

7543 Bergström L, Kylberg E, Hagman U, Eriksson H and Bruce Å, 1991. The food composition database
7544 KOST: the National Food Administration's Information System for nutritive values of food. Vår
7545 Föda, 43, 439-447.

7546 Besaratinia A and Pfeifer GP, 2003. Weak yet distinct mutagenicity of acrylamide in mammalian
7547 cells. Journal of the National Cancer Instute, 95, 889-896.

7548 Besaratinia A and Pfeifer GP, 2004. Genotoxicity of acrylamide and glycidamide. Journal of the
7549 National Cancer Insture, 96, 1023-1029.

7550 Besaratinia A and Pfeifer GP, 2007. A review of mechanisms of acrylamide carcinogenicity.
7551 Carcinogenesis, 28, 519-528.

7552 Bethke PC and Bussan AJ, 2013. Acrylamide in Processed Potato Products. American Journal of
7553 Potato Research, 90, 403-424.

7554 BfR (Bundesinsitute für Risikobewertung), 2011. Acrylamid in Lebensmitteln. Stellungnahme Nr.
7555 043/2011 des BfR vom 29. Juni 2011. Available at: www.bfr.de

7556 Biedermann M, Biedermann-Brem S, Noti A, Grob K, Egli P, and Mändli H, 2002. Two GC-MS
7557 methods for the analysis of acrylamide in foods. Mitteilungen aus Lebensmitteluntersuchung und
7558 Hygiene, 93, 638-652.

7559 Biedermann-Brem S, Noti A, Grob K, Imhof D, Bazzocco D and Pfefferle A, 2003. How much
7560 reducing sugar may potatoes contain to avoid excessive acrylamide formation during roasting and
7561 baking? European Food Research and Technology, 217, 369-373.

7562 Bieler GS and Williams RL, 1993. Ratio estimates, the delta method, and quantal response tests for
7563 increased carcinogenicity. Biometrics 49, 793-801.

7564 Bisby MA and Redshaw JD, 1987. Acrylamide neuropathy: changes in the composition of proteins of
7565 fast axonal transport resemble those observed in regenerating axons. Journal of Neurochemistry,
7566 48, 924-928.

7567 Bjellas T, Janak K, Lundanes E, Kronberg L and Becher G, 2005. Determination and quantification of
7568 urinary metabolites after dietary exposure to acrylamide. Xenobiotica, 35, 1003-1018.

7569 Bjellaas T, Olstorn HB, Becher G, Alexander J, Knutsen SH and Paulsen JE, 2007a. Urinary
7570 metabolites as biomarkers of acrylamide exposure in mice following dietary crisp bread
7571 administration or subcutaneous injection. Toxicological Sciences, 100, 374-380.

7572 Bjellaas T, Stolen LH, Haugen M, Paulsen JE, Alexander J, Lundanes E and Becher G, 2007b.
7573 Urinary acrylamide metabolites as biomarkers for short-term dietary exposure to acrylamide. Food
7574 Chemistry and Toxicology, 45, 1020-1026.

7575 Bjellaas T, Olesen PT, Frandsen H, Haugen M, Stolen LH, Paulsen JE, Alexander J, Lundanes E and
7576 Becher G, 2007c. Comparison of estimated dietary intake of acrylamide with hemoglobin adducts
7577 of acrylamide and glycidamide. Toxicological Sciences, 98, 110-117.

7578 Blasiak J, Gloc E, Wozniak K and Czechowska A, 2004. Genotoxicity of acrylamide in human
7579 lymphocytes. Chemico-Biological Interactions, 149, 137-149.

7580 Blumenthal GM, Abdel-Rahman AA, Wilmarth KR, Friedman MA and Abou-Donia MB, 1995.
7581 Toxicokinetics of a single 50 mg/kg oral dose of [2,3-14C]acrylamide in White Leghorn hens.
7582 Fundamental and Applied Toxicology, 27, 149-153.

7583 Boettcher MI and Angerer J, 2005. Determination of the major mercapturic acids of acrylamide and
7584 glycidamide in human urine by LC-ESI-MS/MS. Journal of Chromatography B – Analytical
7585 Technologies in the Biomedical and Life Sciences, 824, 283-294.

7586 Boettcher MI, Schettgen T, Kütting B, Pischetsrieder M and Angerer J, 2005. Mercapturic acids of
7587 acrylamide and glycidarnide as biomarkers of the internal exposure to acrylamide in the general
7588 population. Mutation Research-Genetic Toxicology and Environmental Mutagenesis, 580, 167-176

7589 Boettcher MI, Bolt HM, Drexler H and Angerer J, 2006a. Excretion of mercapturic acids of
7590 acrylamide and glycidamide in human urine after single oral administration of deuterium-labelled
7591 acrylamide. Archives of Toxicology, 80, 55-61.

7592 Boettcher MI, Bolt HM and Angerer J, 2006b. Acrylamide exposure via the diet: influence of fasting
7593 on urinary mercapturic acid metabolite excretion in humans. Archives of Toxicology, 80, 817-819.

7594 Bolger PM, Leblanc J-C and Setzer RW, 2010. Application of the Margin of Exposure (MoE)
7595 approach to substances in food that are genotoxic and carcinogenic EXAMPLE: Acrylamide (CAS
7596 No. 79-06-1). Food and Chemical Toxicology, 48, S25-S33.

7597 Bolt HM, Roos PH and Thier R, 2003. The cytochrome P-450 isoenzyme CYP2E1 in the biological
7598 processing of industrial chemicals: consequences for occupational and environmental medicine.
7599 International Archives of Occupational and Environmental Health, 76, 174-185.

7600 Bongers ML, Hogervorst JG, Schouten LJ, Goldbohm RA, Schouten HC and van den Brandt PA,
7601 2012. Dietary acrylamide intake and the risk of lymphatic malignancies: the Netherlands Cohort
7602 Study on diet and cancer. PLoS One, 7, e38016.

7603 Bowyer JF, Latendresse JR, Delongchamp RR, Muskhelishvili L, Warbritton AR, Thomas M, Tareke
7604 E, McDaniel LP and Doerge DR, 2008. The effects of subchronic acrylamide exposure on gene
7605 expression, neurochemistry, hormones, and histopathology in the hypothalamus-pituitary-thyroid
7606 axis of male Fischer 344 rats. Toxicology and Applied Pharmacology, 230, 208-215.

7607 Bowyer JF, Latendresse JR, Delongchamp RR, Warbritton AR, Thomas M, Divine B and Doerge DR,
7608 2009. The mRNA expression and histological integrity in rat forebrain motor and sensory regions
7609 are minimally affected by acrylamide exposure through drinking water. Toxicology and Applied
7610 Pharmacology, 240, 401-411.

7611 Brady JF, Ishizaki H, Fukuto JM, Lin MC, Fadel A, Gapac JM and Yang CS, 1991. Inhibition of
7612 cytochrome P-450 2E1 by diallyl sulfide and its metabolites. Chemical Research in Toxicology, 4,
7613 642-647.

7614 Brantsæter AL, Haugen M, de Mul A, Bjellaas T, Becher G, Van Klaveren J, Alexander J and Meltzer
7615 HM, 2008. Exploration of different methods to assess dietary acrylamide exposure in pregnant
7616 women participating in the Norwegian Mother and Child Cohort Study (MoBa). Food and
7617 Chemical Toxicology, 46, 2808-2814.

7618 Brimijoin WS and Hammond PI, 1985. Acrylamide neuropathy in the rat: effects on energy
7619 metabolism in sciatic nerve. Mayo Clinic Proceedings, 60, 3-8.

7620 Brisson B, Ayotte P, Normandin L, Gaudreau E, Bienvenu J-F, Fennell TR, Blanchet C, Phaneuf D,
7621 Lapointe C, Bonvalot Y, Gagne M, Courteau M, Snyder RW and Bouchard M, 2014. Relation
7622 between dietary acrylamide exposure and biomarkers of internal dose in Canadian teenagers.
7623 Journal of Exposure Science and Environmental Epidemiology, 24, 215-221.

7624 Bull RJ, Robinson M, Laurie RD, Stoner GD, Greisiger E, Meier JR and Stober J, 1984a.
7625 Carcinogenic effects of acrylamide in Sencar and A/J mice. Cancer Research, 44, 107-111.

7626 Bull RJ, Robinson M and Stober JA, 1984b. Carcinogenic activity of acrylamide in the skin and lung
7627 of Swiss-ICR mice. Cancer Letters, 24, 209-212.

7628 Burek JD, Albee RR, Beyer JE, Bell TJ, Carreon RM, Morden DC, Wade CE, Hermann EA and
7629 Gorzinski SJ, 1980. Subchronic toxicity of acrylamide administered to rats in the drinking water
7630 followed by up to 144 days of recovery. Journal of Environmental Pathology and Toxicology, 4,
7631 157-182.

7632 Burley VJ, Greenwood DC, Hepworth SJ, Fraser LK, de Kok TM, van Breda SG, Kyrtopoulos SA,
7633 Botsivali M, Kleinjans J, McKinney PA and Cade JE, 2010. Dietary acrylamide intake and risk of
7634 breast cancer in the UK women's cohort. British Journal of Cancer, 103, 1749-1754.

7635 Butterworth BE, Eldridge SR, Sprankle CS, Working PK, Bentley KS and Hurtt ME, 1992. Tissue-
7636 specific genotoxic effects of acrylamide and acrylonitrile. Environmental and Molecular
7637 Mutagenesis, 20, 148-155.

7638 Calleman CJ, 1996. The metabolism and pharmacokinetics of acrylamide: implications for
7639 mechanisms of toxicity and human risk estimation. Drug Metabolism Reviews, 28, 527-590.

7640 Calleman CJ, Bergmark E and Costa LG, 1990. Acrylamide is metabolized to glycidamide in the rat:
7641 evidence from hemoglobin adduct formation. Chemical Research in Toxicology, 3, 406-412.

7642 Calleman CJ, Stern LG, Bergmark E and Costa LG, 1992. Linear versus nonlinear models for
7643 hemoglobin adduct formation by acrylamide and its metabolite glycidamide - implications for risk-
7644 estimation. Cancer Epidemiology Biomarkers & Prevention, 1, 361-368.

7645 Calleman CJ, Wu Y, He F, Tian G, Bergmark E, Zhang S, Deng H, Wang Y, Crofton KM, Fennell T
7646 and Costa LG, 1994. Relationships between biomarkers of exposure and neurological effects in a
7647 group of workers exposed to acrylamide. Toxicology and Applied Pharmacology, 126, 361-371.

7648 Camacho L, Latendresse JR, Muskhelishvili L, Patton R, Bowyer JF, Thomas M and Doerge DR,
7649 2012. Effects of acrylamide exposure on serum hormones, gene expression, cell proliferation, and
7650 histopathology in male reproductive tissues of Fischer 344 rats. Toxicology Letters, 211, 135-143.

7651 Can NO and Arli G, 2014. Analysis of acrylamide in traditional and nontraditional foods in Turkey
7652 using HPLC-DAD with SPE cleanup. Journal of Liquid Chromatography and Related
7653 Technologies, 37, 850-863.

7654 Carere A, 2006. Genotoxicity and carcinogenicity of acrylamide: a critical review. Annali dell'Istituto
7655 Superiore di Sanità, 42, 144-155.

7656 Carlson GP and Weaver PM, 1985. Distribution and binding of [14C]acrylamide to macromolecules in
7657 SENCAR and BALB/c mice following oral and topical administration. Toxicology and Applied
7658 Pharmacology, 79, 307-313.

7659 Carrington CD, Lapadula DM, Dulak L, Friedman M and Abou-Donia MB, 1991. *In vivo* binding of
7660 [14C]acrylamide to proteins in the mouse nervous system. Neurochemistry International, 18, 191-
7661 197.

7662 Casado FJ, Montaño A, Spitzner D and Carle R, 2013. Investigations into acrylamide precursors in
7663 sterilized table olives: Evidence of a peptic fraction being responsible for acrylamide formation.
7664 Food Chemistry, 141, 1158-1165.

7665 Cavanagh JB and Gysbers MF, 1983. Ultrastructural features of the Purkinje cell damage caused by
7666 acrylamide in the rat: a new phenomenon in cellular neuropathology. Journal of Neurocytology, 12,
7667 413-437.

7668 Cavanagh JB and Nolan CC, 1982a. Selective loss of Purkinje-cells from the rat cerebellum caused by
7669 acrylamide and the responses of beta-glucuronidase and beta-galactosidase. Acta
7670 Neuropathologica, 58, 210-214.

7671 Cavanagh JB and Nolan CC, 1982b. The effects of acrylamide on beta-glucuronidase and acid-
7672 phosphatase activities in rat sciatic-nerve above and below a ligature. Neuropathology and Applied
7673 Neurobiology, 8, 465-476.

7674 Cavanagh JB, 1982. The pathokinetics of acrylamide intoxication: a reassessment of the problem.
7675 Neuropathology and Applied Neurobiology, 8, 315-336.

7676 Cengiz MF and Gündüz CPB, 2013. Acrylamide exposure among Turkish toddlers from selected
7677 cereal-based baby food samples. Food and Chemical Toxicology, 60, 514-519.

7678 Céspedes-Camacho IF, Manso JA, Pérez-Prior MT, Gómez-Bombarelli R, González-Párez M, Calle E
7679 and Casado J, 2010. Reactivity of acrylamide as an alkylating agent: a kinetic approach. Journal of
7680 Physical Organic Chemistry, 23, 171-175.

7681 Chan-Hon-Tong A, Charles MA, Forhan A, Heude B and Sirot V, 2013. Exposure to food
7682 contaminants during pregnancy. Science of the Total Environment, 458, 27-35.

7683 Chapin RE, Fail PA, George JD, Grizzle TB, Heindel JJ, Harry GJ, Collins BJ and Teague J, 1995.
7684 The reproductive and neural toxicities of acrylamide and three analogues in Swiss mice, evaluated
7685 using the continuous breeding protocol. Fundamental and Applied Toxicology, 27, 9-24.

7686 Chen Y-H, Xia E-Q, Xu X-R, Ling W-H, Li S, Wu S, Deng G-F, Zou Z-F, Zhou J and Li H-B, 2012.
7687 Evaluation of acrylamide in food from China by a LC/MS/MS Method. International Journal of
7688 Environmental Research and Public Health, 9, 4150-4158.

7689 Chen JH, Yang CH, Wang YS, Lee JG, Cheng CH and Chou CC, 2013a. Acrylamide-induced
7690 mitochondria collapse and apoptosis in human astrocytoma cells. Food Chemistry and Toxicology,
7691 51, 446-452.

7692 Chen W, Feng L, Shen Y, Su H, Li Y, Zhuang J, Zhang L and Zheng X, 2013b. Myricitrin inhibits
7693 acrylamide-mediated cytotoxicity in human Caco-2 cells by preventing oxidative stress. BioMed
7694 Research International, 2013, 724183-Article ID 724183.

7695 Chevolleau S, Jacques C, Canlet C, Tulliez J and Debrauwer L, 2007. Analysis of hemoglobin adducts
7696 of acrylamide and glycidamide by liquid chromatography-electrospray ionization tandem mass
7697 spectrometry, as exposure biomarkers in French population. Journal of Chromatography A, 1167,
7698 125-134.

7699 Choi Y-M, Imai T, Hasumura M, Watanabe N, Ushijima T, Hirose M and Nishikawa A, 2009.
7700 Increased H-ras mutation frequency in mammary tumors of rats initiated with N-methyl-N-
7701 nitrosourea (MNU) and treated with acrylamide. Journal of Toxicological Sciences, 34, 407-412.

7702 Chretien M, Patey G, Souyri F, and Droz B, 1981. Acrylamide-induced neuropathy and impairment of
7703 axonal-transport of proteins .2. Abnormal accumulations of smooth endoplasmic-reticulum as sites
7704 of focal retention of fast transported proteins - electron-microscope autoradiographic study. Brain
7705 Research, 205, 15-28.

7706 Claeys W, Baert K, Mestdagh F, Vercammen J, Daenens P, De Meulenaer B, Maghuin-Rogister G and
7707 Huyghebaert A, 2010. Assessment of the acrylamide intake of the Belgian population and the effect
7708 of mitigation strategies. Food Additives and Contaminants Part a-Chemistry Analysis Control
7709 Exposure and Risk Assessment, 27, 1199-1207.

7710 Clarke DB, Kelly J and Wilson LA, 2002. Assessment of performance of laboratories in determining
7711 acrylamide in crispbread. Journal of the AOAC International, 85, 1370-1373.

7712 Claus A, Weisz GM, Kammerer DR, Carle R and Schieber A, 2005. A method for the determination
7713 of acrylamide in bakery products using ion trap LC-ESI-MS/MS. Molecular Nutrition and Food
7714 Research, 49, 918-925.

7715 Claus A, Weisz GM, Schieber A and Carle R, 2006. Pyrolytic acrylamide formation from purified
7716 wheat gluten and gluten-supplemented wheat bread rolls. Molecular Nutrition and Food Research,
7717 50, 87-93.

7718 Cohen SM, 2004. Human carcinogenic risk evaluation: An alternative approach to the two-year rodent
7719 bioassay. Toxicological Sciences, 80, 225-229.

7720 Collins JJ, Swaen GM, Marsh GM, Utidjian HM, Caporossi JC and Lucas LJ, 1989. Mortality patterns
7721 among workers exposed to acrylamide. Journal of Occupational Medicine, 31, 614-617.

7722 COM (UK Committee on Mutagenicity of Chemicals in Food, Consumers Products and the
7723 Environment), 2009. Statement on the Genotoxicity of Acrylamide. COM/09/S1. Available at:
7724 http://www.iacom.org.uk/statements/documents/COM09S1Acrylamide.pdf

7725 Costa LG, Deng H, Gregotti C, Manzo L, Faustman EM, Bergmark E and Calleman CJ, 1992.
7726 Comparative studies on the neuro- and reproductive toxicity of acrylamide and its epoxide
7727 metabolite glycidamide in the rat. Neurotoxicology, 13, 219-224.

7728 Dearfield KL, Abernathy CO, Ottley MS, Brantner JH and Hayes PF, 1988. Acrylamide: its
7729 metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity. Mutation
7730 Research, 195, 45-77.

7731 Dearfield KL, Douglas GR, Ehling UH, Moore MM, Sega GA and Brusick DJ, 1995. Acrylamide: a
7732 review of its genotoxicity and an assessment of heritable genetic risk. Mutation
7733 Research/Fundamental and Molecular Mechanisms of Mutagenesis, 330, 71-99.

7734 Delgado-Andrade C, Morales FJ, Seiquer I and Pilar Navarro M, 2010. Maillard reaction products
7735 profile and intake from Spanish typical dishes. Food Research International, 43, 1304-1311.

7736 Delgado-Andrade C, Mesias M, Morales FJ, Seiquer I and Pilar Navarro M, 2012. Assessment of
7737 acrylamide intake of Spanish boys aged 11-14 years consuming a traditional and balanced diet.
7738 Lwt-Food Science and Technology, 46, 16-22.

7739 DeWoskin RD, Sweeney LM, Teeguarden JG, Sams 2nd R and Vandenberg J, 2013. Comparison of
7740 PBTK model and biomarker based estimates of the internal dosimetry of acrylamide. Food and
7741 Chemical Toxicology, 58, 506-521.

7742 Diekmann J, Wittig A and Stalbbert R, 2008. Gas chromatographic-mass spectrometric analysis of
7743 acrylamide and acetamide in cigarette mainstream smoke after on-column injection. Journal of
7744 Chromatographic Science, 46, 659-663.

7745 Dixit R, Husain R, Mukhtar H and Seth PK, 1981. Effect of acrylamide on biogenic amine levels,
7746 monoamine oxidase, and cathepsin D activity of rat brain. Environmental Research, 26, 168-173.

7747 Dobrowolski P, Huet P, Karlsson P, Eriksson S, Tomaszewska E, Gawron A and Pierzynowski SG,
7748 2012. Potato fiber protects the small intestinal wall against the toxic influence of acrylamide.
7749 Nutrition, 28, 428-435.

7750 Dobrzynska MM, 2007. Assessment of DNA damage in multiple organs from mice exposed to X-rays
7751 or acrylamide or a combination of both using the comet assay. In Vivo, 21, 657-662.

7752 Doerge DR, Young JF, McDaniel LP, Twaddle NC and Churchwell MI, 2005a. Toxicokinetics of
7753 acrylamide and glycidamide in Fischer 344 rats. Toxicology and Applied Pharmacology, 208, 199-
7754 209.

7755 Doerge DR, Young JF, McDaniel LP, Twaddle NC and Churchwell MI, 2005b. Toxicokinetics of
7756 acrylamide and glycidamide in B6C3F1 mice. Toxicology and Applied Pharmacology, 202, 258-
7757 267.

7758 Doerge DR, da Costa GG, McDaniel LP, Churchwell MI, Twaddle NC and Beland FA, 2005c. DNA
7759 adducts derived from administration of acrylamide and glycidamide to mice and rats. Mutation
7760 Research, 580, 131-141.

7761 Doerge DR, Twaddle NC, Boettcher MI, McDaniel LP and Angerer J, 2007. Urinary excretion of
7762 acrylamide and metabolites in Fischer 344 rats and B6C3F(1) mice administered a single dose of
7763 acrylamide. Toxicology Letters, 169, 34-42.

7764 Doerge DR, Young JF, Chen JJ, DiNovi MJ and Henry SH, 2008. Using dietary exposure and
7765 physiologically based pharmacokinetic/pharmacodynamic modeling in human risk extrapolations
7766 for acrylamide toxicity. Journal of Agricultural and Food Chemistry, 56, 6031-6038.

7767 Doroshyenko O, Fuhr U, Kunz D, Frank D, Kinzig M, Jetter A, Reith Y, Lazar A, Taubert D,
7768 Kirchheiner J, Baum M, Eisenbrand G, Berger FI, Bertow D, Berkessel A, Sorgel F, Schomig E
7769 and Tomalik-Scharte D, 2009. In vivo role of cytochrome P450 2E1 and glutathione-S-transferase
7770 activity for acrylamide toxicokinetics in humans. Cancer Epidemiology, Biomarkers & Prevention,
7771 18, 433-443.

7772 Dourson M, Hertzberg R, Allen B, Haber L, Parker A, Kroner O, Maier A and Kohrman M, 2008.
7773 Evidence-based dose-response assessment for thyroid tumorigenesis from acrylamide. Regulatory
7774 Toxicology and Pharmacology, 52, 264-289.

7775 DTU (National Food Institute, Technical University of Denmark), 2013. Chemical contaminants 2004-
7776 2011. Food monitoring 2004-2011. National Food Institute, Technical University of Denmark.
7777 Available at: www.food.dtu.dk

7778 Duale N, Bjellaas T, Alexander J, Becher G, Haugen M, Paulsen JE, Frandsen H, Olesen PT and
7779 Brunborg G, 2009. Biomarkers of human exposure to acrylamide and relation to polymorphisms in
7780 metabolizing genes. Toxicological Sciences, 108, 90-99.

7781 Duarte-Salles T, von Stedingk H, Granum B, Gutzkow KB, Rydberg P, Tornqvist M, Mendez MA,
7782 Brunborg G, Brantsæter AL, Meltzer HM, Alexander J and Haugen M, 2013. Dietary acrylamide
7783 intake during pregnancy and fetal growth-results from the Norwegian mother and child cohort
7784 study (MoBa). Environmental Health Perspectives, 121, 374-379.

7785 Edler L, Hart A, Greaves P, Carthew P, Coulet M, Boobis A, Williams GM and Smith B, 2013.
7786 Selection of appropriate tumour data sets for Benchmark Dose Modelling (BMD) and derivation of
7787 a Margin of Exposure (MoE) for substances that are genotoxic and carcinogenic: Considerations of
7788 biological relevance of tumour type, data quality and uncertainty assessment. Food and Chemical
7789 toxicology, in press.

7790 Eerola S, Hollebekkers K, Hallikainen A and Peltonen K, 2007. Acrylamide levels in Finnish
7791 foodstuffs analysed with liquid chromatography tandem mass spectrometry. Molecular Nutrition
7792 and Food Research, 51, 239-247.

7793 EFSA (European Food Safety Authority), 2005a. Statement of the Scientific Panel on Contaminants in
7794 the Food Chain to a summary report on acrylamide in food of the 64th meeting of the Joint
7795 FAO/WHO Expert Committee on Food Additives. Adopted on 19 April 2005.

7796 EFSA (European Food Safety Authority), 2005b. Opinion of the Scientific Committee on a request
7797 from EFSA related to a harmonised approach for Risk Assessment of substances which are both
7798 Genotoxic and Carcinogenic. The EFSA Journal 2005, 282, 1-31.

7799 EFSA (European Food Safety Authority), 2006. Guidance of the Scientific Committee on a request
7800 from EFSA related to Uncertainties in Dietary Exposure Assessment. The EFSA Journal 2006,
7801 438, 1-54.

7802 EFSA (European Food Safety Authority), 2008. EFSA's 11th Scientific Colloquium - Acrylamide
7803 carcinogenicity - New evidence in relation to dietary exposure 22 and 23 May 2008, Tabiano (PR),
7804 Italy.

7805 EFSA (European Food Safety Authority), 2009a. Results on the monitoring of acrylamide levels in
7806 food. The EFSA Journal 2009, 285r, 1-26.

7807 EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on a request
7808 from EFSA on the use of the benchmark dose approach in risk assessment. The EFSA Journal
7809 2009, 1150, 1-72.

7810 EFSA (European Food Safety Authority), 2009c. Guidance of the Scientific Committee on
7811 transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General
7812 principles. The EFSA Journal 2009, 1051, 1-22.

EFSA (European Food Safety Authority), 2010. Results on acrylamide levels in food from monitoring year 2008. Scientific report of EFSA. EFSA Journal 2010;8(5):1599, 31 pp. doi:10.2903/j.efsa.2010.1599

EFSA (European Food Safety Authority), 2011a. Results on acrylamide levels in food from monitoring years 2007- 2009. EFSA Journal 2011;9(4):2133, 48 pp. doi:10.2903/j.efsa.2011.2133

EFSA (European Food Safety Authority), 2011b. Report on the development of a food classification and description system for exposure assessment and guidance on its implementation and use. EFSA Journal 2011;9(12):2489, 84 pp. doi:10.2903/j.efsa.2011.2489

EFSA (European Food Safety Authority), 2011c. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp. doi:10.2903/j.efsa.2011.2097

EFSA (European Food Safety Authority), 2011d. Use of BMDS and PROAST software packages by EFSA Scientific Panels and Units for applying the Benchmark Dose (BMD) approach in risk. EFSA Supporting Publications 2011, EN-113, 190 pp.

EFSA (European Food Safety Authority), 2011e. Overview of the procedures currently used at EFSA for the assessment of dietary exposure to different chemical substances. EFSA Journal 2011;9(12):2490, 33 pp. doi:10.2903/j.efsa.2011.2490

EFSA (European Food Safety Authority), 2012a. Update on acrylamide levels in food from monitoring years 2007 to 2010. EFSA Journal 2012;10(10):2938, 38 pp. doi:10.2903/j.efsa.2012.2938

EFSA (European Food Safety Authority), 2012b. Minimum Criteria for the acceptance of *in vivo* alkaline Comet Assay Reports. EFSA Journal 2012;10(11):2977, 12 pp. doi:10.2903/j.efsa.2012.2977

EFSA SC (EFSA Scientific Committee), 2012a. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. doi:10.2903/j.efsa.2012.2579

EFSA SC (EFSA Scientific Committee), 2012b. Scientific Opinion on Risk Assessment Terminology. EFSA Journal 2012;10(5):2664, 43 pp. doi:10.2903/j.efsa.2012.2664

Ehlers A, Lenze D, Broll H, Zagon J, Hummel M and Lampen A, 2013. Dose dependent molecular effects of acrylamide and glycidamide in human cancer cell lines and human primary hepatocytes. Toxicology Letters, 217, 111-120.

Ehling UH and Neuhäuser-Klaus A, 1992. Reevaluation of the induction of specific-locus mutations in spermatogonia of the mouse by acrylamide. Mutation Research, 283, 185-191.

Elbashir AA, Omar MMA, Ibrahim WAW, Schmitz OJ and Aboul-Enein HY, 2014. Acrylamide analysis in food by liquid chromatographic and gas chromatographic methods. Critical Reviews in Analytical Chemistry, 44, 107-141.

Elmore JS, Mottram DS, Muttucumaru N, Dodson AT, Parry MAJ and Halford NG, 2007. Changes in free amino acids and sugars in potatoes due to sulfate fertilization and the effect on acrylamide formation. Journal of Agricultuural and Food Chemistry, 55, 5363-5366.

El-Alfy AT, Seale S, Feng Q, Mark M, Baerson S and Agarwal A, 2011. Behavioral and transcriptional effects of acrylamide-induced neurotoxicity in rat pups. Faseb Journal, 25.

El-Alfy AT, Dunker A, Brown J, Cooper B, Dudek M and Ostil R, 2013. Tissue specific regulation of kappa opioid receptors and Nr4a2 expression by acrylamide. Faseb Journal, 27.

El-Bohi KM, Moustafa GG, El sharkawi NI and Sabik LME, 2011. Genotoxic effects of acrylamide in adult male albino rats liver. Journal of Americal Science, 7, 1097-1108.

El-Halim SSA and Mohamed MM, 2012. Garlic powder attenuates acrylamide-induced oxidative damage in multiple organs in rat. Journal of Applied Sciences Research, 168-173.

El-Kholy TA, Khalifa NA, Alghamidi AK and Badereldin AM, 2012. A Trail of Using Green Tea for Competing Toxicity of Acrylamide on Liver Function. Life Science Journal-Acta Zhengzhou University Overseas Edition, 9, 3690-3695.

El-Sayyad HI, El-Gammal HL, Habak LA, Abdel-Galil HM, Fernando A, Gaur RL and Ouhtit A, 2011a. Structural and ultrastructural evidence of neurotoxic effects of fried potato chips on rat postnatal development. Nutrition, 27, 1066-1075.

El-Sayyad HI, Sakr SA, Badawy GM and Afify HS, 2011b. Hazardous effects of fried potato chips on the development of retina in albino rats. Asian Pacific Journal of Tropical Biomedicine, 1, 253-260.

El-Sayyad HI, Abou-Egla MH, El-Sayyad FI, El-Ghawet HA, Gaur RL, Fernando A, Raj MHG and Ouhtit A, 2011c. Effects of fried potato chip supplementation on mouse pregnancy and fetal development. Nutrition, 27, 343-350.

Emmert B, Bünger J, Keuch K, Müller M, Emmert S, Hallier E and Westphal GA, 2006. Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent *S. typhimurium* strain YG7108pin3ERb$_5$. Toxicology, 228, 66-76.

Eskin TA, Lapham LW, Maurissen JP and Merigan WH, 1985. Acrylamide effects on the macaque visual system. II. Retinogeniculate morphology. Investigative Ophthalmology and Visual Science, 26, 317-329.

EU (European Union), 2000. European Union Risk Assessment Report. Acrylamide. CAS No: 79-06-1. EINECS No: 201-173-7. European Chemicals Bureau. Volume 24.

Exon JH, 2006. A review of the toxicology of acrylamide. Journal of toxicology and environmental health. Part B, Critical reviews, 9, 397-412.

FAO/WHO (Food and Agricultural Organisation/World health Organisation), 2002. FAO/WHO Consultation on the Health Implications of Acrylamide in Food Geneva, 25-27 June 2002. Summary Report. Available at: http://www.who.int/foodsafety/publications/chem/acrylamide_june2002/en/

FAO/WHO (Joint FAO/WHO Expert Committee on Food Additives), 2006. Evaluation of certain Food Contaminants. Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives (Rome, 8–17 February 2005). WHO Technical Reports Series 930.

FAO/WHO (Joint FAO/WHO Expert Committee on Food Additives), 2011. Evaluation of certain Food Contaminants. Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives (Rome, 16–25 February 2010). WHO Technical Reports Series 959.

Favor J and Shelby MD, 2005. Transmitted mutational events induced in mouse germ cells following acrylamide or glycidamide exposure. Mutation Research-Genetic Toxicology and Environmental Mutagenesis, 580, 21-30.

FEHD (Food and Environmental Hygiene Department), 2012. Dietary Exposure to Acrylamide of Hong Kong Adult PopulationCentre for Food Safety. Risk Assessment Studies Report No. 43. Food and Environmental Hygiene Department. The Government of the Hong Kong Special Administrative Region. Available at http://www.cfs.gov.hk/english/programme/programme_rafs/programme_rafs_fc_01_25.html

Feng CH and Lu CY, 2011. Modification of major plasma proteins by acrylamide and glycidamide: Preliminary screening by nano liquid chromatography with tandem mass spectrometry. Analytica Chimica Acta, 684, 89-95.

Fennell TR, Snyder RW, Krol WL and Sumner SCJ, 2003. Comparison of the hemoglobin adducts formed by administration of N-methylolacrylamide and acrylamide to rats. Toxicological Sciences, 71, 164-175.

Fennell TR and Friedman MA, 2005. Comparison of acrylamide metabolism in humans and rodents. In: Chemistry and Safety of Acrylamide in Food. Eds Friedman M, Mottram D, 109-116.

7907 Fennell TR, Sumner SC, Snyder RW, Burgess J, Spicer R, Bridson WE and Friedman MA, 2005.
7908 Metabolism and hemoglobin adduct formation of acrylamide in humans. Toxicological Sciences,
7909 85, 447-459.

7910 Fennell TR, Sumner SC, Snyder RW, Burgess J and Friedman MA, 2006. Kinetics of elimination of
7911 urinary metabolites of acrylamide in humans. Toxicological Sciences, 93, 256-267.

7912 Ferguson SA, Garey J, Smith ME, Twaddle NC, Doerge DR and Paule M, 2010. Preweaning
7913 behaviors, developmental landmarks, and acrylamide and glycidamide levels after pre- and
7914 postnatal acrylamide treatment in rats. Neurotoxicology and Teratology, 32, 373-382.

7915 Ferrari P, Freisling H, Duell EJ, Kaaks R, Lujan-Barroso L, Clavel-Chapelon F, Boutron-Ruault M-C,
7916 Nailler L, Polidoro S, Mattiello A, Palli D, Tumino R, Grioni S, Knuppel S, Tjonneland A, Olsen
7917 A, Overvad K, Orfanos P, Katsoulis M, Trichopoulou A, Quiros JR, Ardanaz E, Huerta JM,
7918 Etxezarreta PA, Sanchez MJ, Crowe F, Khaw K-T, Wareham NJ, Ocke M, Bueno-de-Mesquita B,
7919 Peeters PHM, Ericson U, Wirfält E, Hallmans G, Johansson I, Engeset D, Nicolas G, Gallo V,
7920 Norat T, Riboli E and Slimani N, 2013. Challenges in estimating the validity of dietary acrylamide
7921 measurements. European Journal of Nutrition, 52, 1503-1512.

7922 Field EA, Proce CJ, Sleet RB, Marr MC, Schwetz BA and Morrisey RE, 1990. Developmental toxicity
7923 evaluation of acrylamide in rats and mice. Fundamental and Applied Toxicology, 14, 502-512.

7924 Fohgelberg P, Rosen J, Hellenas KE and Abramsson-Zetterberg L, 2005. The acrylamide intake via
7925 some common baby food for children in Sweden during their first year of life - an improved
7926 method for analysis of acrylamide. Food and Chemical Toxicology, 43, 951-959.

7927 Freisling H, Moskal A, Ferrari P, Nicolas G, Knaze V, Clavel-Chapelon F, Boutron-Ruault M-C,
7928 Nailler L, Teucher B, Grote VA, Boeing H, Clemens M, Tjonneland A, Olsen A, Overvad K,
7929 Quiros JR, Duell EJ, Sanchez M-J, Amiano P, Chirlaque M-D, Barricarte A, Khaw K-T, Wareham
7930 NJ, Crowe FL, Gallo V, Oikonomou E, Naska A, Trichopoulou A, Palli D, Agnoli C, Tumino R,
7931 Polidoro S, Mattiello A, Bueno-de-Mesquita HB, Ocke MC, Peeters PHM, Wirfält E, Ericson U,
7932 Bergdahl IA, Johansson I, Hjartaker A, Engeset D, Skeie G, Riboli E and Slimani N, 2013. Dietary
7933 acrylamide intake of adults in the European Prospective Investigation into Cancer and Nutrition
7934 differs greatly according to geographical region. European Journal of Nutrition, 52, 1369-1380.

7935 Friedman MA, Dulak LH and Stedham MA, 1995. A lifetime oncogenicity study in rats with
7936 acrylamide. Fundamental and Applied Toxicology, 27, 95-105

7937 Friedman MA, Tyl RW, Marr MC, Myers CB, Gerling FS and Ross WP, 1999. Effects of lactational
7938 administration of acrylamide on rat dams and offspring. Reproductive Toxicology, 13, 511-520.

7939 Friedman MA, Zeiger E, Marroni DE and Sickles DW, 2008. Inhibition of rat testicular nuclear
7940 kinesins (krp2; KIFC5A) by acrylamide as a basis for establishing a genotoxicity threshold. Journal
7941 of Agricultural and Food Chemistry, 56, 6024-6030.

7942 FSANZ (Food Standards Australia New Zealand), 2014. 24th Australian Total Diet Study. Phase 1.
7943 Food Standards Autralia New Zealand. Published April 2014.

7944 Fuhr U, Boettcher MI, Kinzig-Schippers M, Weyer A, Jetter A, Lazar A, Taubert D, Tomalik-Scharte
7945 D, Pournara P, Jakob V, Harlfinger S, Klaassen T, Berkessel A, Angerer J, Sorgel F and Schomig
7946 E, 2006. Toxicokinetics of acrylamide in humans after ingestion of a defined dose in a test meal to
7947 improve risk assessment for acrylamide carcinogenicity. Cancer Epidemiol Biomarkers Prev, 15,
7948 266-271.

7949 Fullerton PM and Barnes JM, 1966. Peripheral neuropathy in rats produced by acrylamide. British
7950 Journal of Industrial Medicine, 23, 210-221.

7951 Fujiki M, Asada J and Shimizu T, 1982. Studies on analytical method of acrylamide monomer and
7952 accumulation into fish. NTIS/AD P004 743. As reported by EU (2000).

7953 Gamboa da Costa GG, Churchwell MI, Hamilton LP, Von Tungeln LS, Beland FA, Marques MM and
7954 Doerge DR, 2003. DNA adduct formation from acrylamide via conversion to glycidamide in adult
7955 and neonatal mice. Chemical Research in Toxicology, 16, 1328-1337.

7956 Galdo VC, Massart C, Jin L, Vanvooren V, Caillet-Fauquet P, Andry G, Lothaire P, Dequanter D,
7957 Friedman M and Van Sande J, 2006. Acrylamide, an in vivo thyroid carcinogenic agent, induces
7958 DNA damage in rat thyroid cell lines and primary cultures. Molecular and Cellular Endocrinology,
7959 257-8, 6-14.

7960 Garey J and Paule MG, 2007. Effects of chronic low-dose acrylamide exposure on progressive ratio
7961 performance in adolescent rats. NeuroToxicology, 28, 998-1002.

7962 Garey J and Paule MG, 2010. Effects of chronic oral acrylamide exposure on incremental repeated
7963 acquisition (learning) task performance in Fischer 344 rats. Neurotoxicology and Teratology, 32,
7964 220-225.

7965 Garey J, Ferguson SA and Paule MG, 2005. Developmental and behavioral effects of acrylamide in
7966 Fischer 344 rats. Neurotoxicology and Teratology, 27, 553-563.

7967 Gargas ML, Kirman CR, Sweeney LM and Tardiff RG, 2009. Acrylamide: Consideration of species
7968 differences and nonlinear processes in estimating risk and safety for human ingestion. Food and
7969 Chemical Toxicology, 47, 760-768.

7970 GEMS/Food-EURO, 1995. Reliable Evaluation of Low-Level Contamination of Food. Report of the
7971 Workshop held in Kulmbach, Federal Republic of Germany, 26-27 May 1995, 47 pp.

7972 Generoso WM, Sega GA, Lockhart AM, Hughes LA, Cain KT, Cacheiro NLA, Shelby MD, 1996.
7973 Dominant lethal mutations, heritable translocations, and unscheduled DNA synthesis induced in
7974 male mouse germ cells by glycidamide, a metabolite of acrylamide. Mutation Research, 371, 175-
7975 183.

7976 Ghanayem BI, McDaniel LP, Churchwell MI, Twaddle NC, Snyder R, Fennell TR and Doerge DR,
7977 2005a. Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA
7978 and hemoglobin adducts. Toxicological Sciences, 88, 311-318.

7979 Ghanayem BI, Witt KL, El-Hadri L, Hoffler U, Kissling GE, Shelby MD and Bishop JB, 2005b.
7980 Comparison of germ cell mutagenicity in male CYP2E1-null and wild-type mice treated with
7981 acrylamide: evidence supporting a glycidamide-mediated effect. Biology of Reproduction, 72, 157-
7982 163.

7983 Ghanayem BI, Witt KL, Kissling GE, Tice RR and Recio L, 2005c. Absence of acrylamide-induced
7984 genotoxicity in CYP2E1-null mice: evidence consistent with a glycidamide-mediated effect.
7985 Mutation Research, 578, 284-297.

7986 Ghanayem BI, Bai R, Kissling GE, Travlos G and Hoffler U, 2010. Diet-induced obesity in male mice
7987 is associated with reduced fertility and potentiation of acrylamide-induced reproductive toxicity.
7988 Biology of Reproduction, 82, 96-104.

7989 Ghareeb DA, Khalil AA, Elbassoumy AM, Hussien HM and Abo-Sraiaa MM, 2010. Ameliorated
7990 effects of garlic (Allium sativum) on biomarkers of subchronic acrylamide hepatotoxicity and brain
7991 toxicity in rats. Toxicological and Environmental Chemistry, 92, 1357-1372.

7992 Gilbert SG and Maurissen JP, 1982. Assessment of the effects of acrylamide, methylmercury and 2,5-
7993 hexanedione on motor functions in mice. Journal of Toxicology and Environmental Health, 10, 31-
7994 41.

7995 Göbel A and Kliemant A, 2007. The German minimization concept for acrylamide. Food Additives
7996 and Contaminants, 24, 82-90..

7997 Goffeng LO, Heier MS, Kjuus H, Sjöholm H, Sørensen KA, Skaug V, 2008a. Nerve conduction,
7998 visual evoked responses and electroretinography in tunnel workers previously exposed to
7999 acrylamide and N-methylolacrylamide containing grouting agents. Neurotoxicology and
8000 Teratology, 30, 186-194.

8001 Goffeng LO, Kjuus H, Heier MS, Alvestrand M, Ulvestad B, Skaug V, 2008b. Colour vision and light
8002 sensitivity in tunnel workers previously exposed to acrylamide and N-methylolacrylamide
8003 containing grouting agents. Neurotoxicology, 29, 31-39.

8004 Goffeng LO, Alvestrand M, Ulvestad B, Sorensen KA, Skaug V and Kjuus H, 2011. Self-reported
8005 symptoms and neuropsychological function among tunnel workers previously exposed to
8006 acrylamide and N-methylolacrylamide. Scandinavian Journal of Work, Environment and Health,
8007 37, 136-146.

8008 Gold BG, Griffin JW and Price DL, 1985. Slow axonal-transport in acrylamide neuropathy - different
8009 abnormalities produced by single-dose and continuous administration. Journal of Neuroscience, 5,
8010 1755-1768.

8011 Gold BG, Voda J, Yu X and Gordon H, 2004. The uimmunosup`pressant FK506 elicits a neuronal
8012 heat shock response and protects against acrylamide neurophathy. Experimental Neurology, 187,
8013 160-170.

8014 Goldbohm RA, van den Brandt PA, Brants HAM, van't Veer P, Sturmans MAlF and Hermus RJJ,
8015 1994. Validation of a dietary questionnaire used in largesscale prospective cohort study on diet and
8016 cancer. European Journal of Clinical Nutrition, 48, 253-265.

8017 Goldbohm RA, van't Veer P, van den Brandt PA, van't Hof MA, Brants HAM, Sturmans F and
8018 Hermus RJJ, 1995. Reproducibility of a food frequency questionnaire and stability of dietary habits
8019 deremined from five anually repeated measurements. European Journal of Clinical Nutrition, 49,
8020 420-429.

8021 Granvogl M, Jezussek M, Koehler P and Schieberle P, 2004. Quantitation of 3-aminopropionamide in
8022 potatoes – A minor but potent precursor in acrylamide formation. Journal of Agricultural and Food
8023 Chemistry, 52, 4751-4757.

8024 Granvogl M, Koehler P, Latzer L and Schieberle P, 2008. Development of a Stable Isotope Dilution
8025 Assay for the Quantitation of Glycidamide and Its Application to Foods and Model Systems.
8026 Journal of Agricultural and Food Chemistry, 56, 6087-6092.

8027 Griciute L, Castegnaro M and Bereziat JC, 1981. Influence of ethyl-alcohol on carcinogenesis with N-
8028 nitrosodimethylamine. Cancer Letters, 13, 345-352.

8029 Gupta RP and Abou-Donia MB, 1996. Alterations in the neutral proteinase activities of central and
8030 peripheral nervous systems of acrylamide-, carbon disulfide-, or 2,5-hexanedione-treated rats.
8031 Molecular and Chemical Neuropathology, 29, 53-66.

8032 Gupta RP and Abou-Donia MB, 1997. Acrylamide and carbon disulfide treatments increase the rate of
8033 rat brain tubulin polymerization. Molecular and Chemical Neuropathology, 30, 223-237.

8034 Haber LT, Maier A, Kroner OL and Kohrman MJ, 2009. Evaluation of human relevance and mode of
8035 action for tunica vaginalis mesotheliomas resulting from oral exposure to acrylamide. Regulatory
8036 Toxicology and Pharmacology, 53, 134-149.

8037 Habermann CE, 2004. Acrylamide. In: Kirk-Othmer (Ed.), Encyclopedia of Chemical Technology,
8038 vol. 1, fifth ed.. Wiley-Interscience, Hoboken, NJ, pp. 288–304. As cited in Beland et al. (2013).

8039 Hagmar L, Törnqvist M, Nordander C, Rosén I, Bruze M, Kautiainen A, Magnusson AL, Malmberg
8040 B, Aprea P, Granath F and Axmon A, 2001. Health effects of occupational exposure to acrylamide
8041 using hemoglobin adducts as biomarkers of internal dose. Scandinavian Journal of Work
8042 Environment and Health, 27, 219-226.

8043 Hagmar L, Wirfält E, Paulsson B and Törnqvist M, 2005. Differences in hemoglobin adduct levels of
8044 acrylamide in the general population with respect to dietary intake, smoking habits and gender.
8045 Mutation Research-Genetic Toxicology and Environmental Mutagenesis, 580, 157-165.

8046 Halford NG, Curtis TY, Muttucumaru N, Postles J, Elmore JS and Mottram DS, 2012a. The
8047 acrylamide problem: a plant and agronomic science issue. Journal of Experimental Botany, 63,
8048 2841-2851.

8049 Halford NG, Muttucumaru N, Powers SJ, Gillatt PN, Hartley L, Elmore JS and Mottram DS, 2012b.
8050 Concentrations of Free Amino Acids and Sugars in Nine Potato Varieties: Effects of Storage and
8051 Relationship with Acrylamide Formation. Journal of Agricultural and Food Chemistry, 60, 12044-
8052 12055.

8053 Hamdy SM, Bakeer HM, Eskander EF and Sayed ON, 2012. Effect of acrylamide on some hormones
8054 and endocrine tissues in male rats. Human and Experimental Toxicology, 31, 483-491.

8055 Hansen SH, Olsen AK, Soderlund EJ and Brunborg G, 2010. In vitro investigations of glycidamide-
8056 induced DNA lesions in mouse male germ cells and in mouse and human lymphocytes. Mutation
8057 Research, 696, 55-61.

8058 Harry GJ, Goodrum JF, Bouldin TW, Toews AD and Morell P, 1989. Acrylamide-induced increases
8059 in deposition of axonally transported glycoproteins in rat sciatic nerve. Journal of Neurochemistry,
8060 52, 1240-1247.

8061 Hartmann EC, Boettcher MI, Schettgen T, Fromme H, Drexler H and Angerer J, 2008. Hemoglobin
8062 adducts and mercapturic acid excretion of acrylamide and glycidamide in one study population.
8063 Journal of Agricultural and Food Chemistry, 56, 6061-6068.

8064 Hartmann EC, Boettcher MI, Bolt HM, Drexler H and Angerer J, 2009. N-Acetyl-S-(1-carbamoyl-2-
8065 hydroxy-ethyl)-L-cysteine (iso-GAMA) a further product of human metabolism of acrylamide:
8066 comparison with the simultaneously excreted other mercaptuic acids. Archives of Toxicology, 83,
8067 731-734.

8068 Hartmann EC, Latzin JM, Schindler BK, Koch HM and Angerer J, 2011. Excretion of 2,3-dihydroxy-
8069 propionamide (OH-PA), the hydrolysis product of glycidamide, in human urine after single oral
8070 dose of deuterium-labeled acrylamide. Archives Toxicology, 85, 601-606.

8071 Hashimoto K and Aldridge WN, 1970. Biochemical studies on acrylamide, a neurotoxic agent.
8072 Biochemical Pharmacology, 19, 2591-2604.

8073 Hashimoto K and Ando K, 1973. Alteration of amino acid incorporation into proteins of the nervous
8074 system in vitro after administration of acrylamide to rats. Biochemical Pharmacology, 22, 1057-66.

8075 Hashimoto K and Tanii H, 1985. Mutagenicity of acrylamide and its analogues in *Salmonella*
8076 *typhimurium*. Mutation Research/Genetic Toxicology, 158, 129-133.

8077 Hashimoto K, Sakamoto J and Tanii H, 1981. Neurotoxicity of acrylamide and related compounds and
8078 their effects on male gonads in mice. Archives of Toxicology, 47, 179-189.

8079 Hasseeb MM, Al-Hizab FA and Hamouda MA-H, 2013. impacts of grape seed oil supplementation
8080 against the acrylamide induced lesions in male genital organs of rats. Pakistan Veterinary Journal,
8081 33, 282-286.

8082 He FS, Zhang SL, Wang HL, Li G, Zhang ZM, Li FL, Dong XM and Hu FR, 1989. Neurological and
8083 Electroneuromyographic Assessment of the Adverse-Effects of Acrylamide on Occupationally
8084 Exposed Workers. Scandinavian Journal of Work Environment and Health, 15, 125-129.

8085 Health Canada, 2012. Health Canada's Revised Exposure Assessment of Acrylamide in Food. Bureau
8086 of Chemical Safety. Food Directorate. Health Products and Food Branch. August 2012. Available
8087 at: http://www.hc-sc.gc.ca/fn-an/securit/chem-chim/food-aliment/acrylamide/rev-eval-exposure-
8088 exposition-eng.php

8089 Hersch MI, McLeod JG, Satchell PM, Early RG and Sullivan CE, 1989. Breathing pattern, lung
8090 inflation reflex and airway tone in acrylamide neuropathy. Respiration Physiology, 76, 257-276.

8091 Heudorf U, Hartmann E and Angerer J, 2009. Acrylamide in children--exposure assessment via
8092 urinary acrylamide metabolites as biomarkers. International Journal Hygene and Environmental
8093 Health, 212, 135-141.

8094 Hirvonen T, Kontto J, Jestoi M, Valsta L, Peltonen K, Pietinen P, Virtanen SM, Sinkko H, Kronberg-
8095 Kippilä C, Albanes D and Virtamo J, 2010. Dietary acrylamide intake and the risk of cancer among
8096 Finnish male smokers. Cancer Causes Control, 21, 2223-2229.

8097 Hirvonen T, Jestoi M, Tapanainen H, Valsta L, Virtanen SM, Sinkko H, Kronberg-Kippila C, Kontto
8098 J, Virtamo J, Simell O and Peltonen K, 2011. Dietary acrylamide exposure among Finnish adults
8099 and children: the potential effect of reduction measures. Food Additives and Contaminants Part a-
8100 Chemistry Analysis Control Exposure and Risk Assessment, 28, 1483-1491.

8101 Hochstenbach K, van Leeuwen DM, Gmuender H, Gottschalk RW, Lovik M, Granum B, Nygaard U,
8102 Namork E, Kirsch-Volders M, Decordier I, Loock KV, Besselink H, Tornqvist M, von Stedingk H,
8103 Rydberg P, Kleinjans JCS, van Loveren H and van Delft JHM, 2012. Global Gene Expression
8104 Analysis in Cord Blood Reveals Gender-Specific Differences in Response to Carcinogenic
8105 Exposure In Utero. Cancer Epidemiology Biomarkers and Prevention, 21, 1756-1767.

8106 Hodge JE, 1953. Chemistry of the browning reaction in model systems. Journal of Agricultural and
8107 Food Chemistry, 1, 928-943.

8108 Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA and van den Brandt PA, 2007. A prospective
8109 study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. Cancer
8110 Epidemiology, Biomarkers and Prevention, 16, 2304-2313.

8111 Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA, 2008a. Dietary
8112 acrylamide intake is not associated with gastrointestinal cancer risk. Journal of Nutrition, 138,
8113 2229-2236.

8114 Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA, 2008b. Dietary
8115 acrylamide intake and the risk of renal cell, bladder, and prostate cancer. Americal Journal of
8116 Clinical Nutrition, 87, 1428-1438.

8117 Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA, 2009a. Lung cancer risk
8118 in relation to dietary acrylamide intake. J Natl Cancer Inst, 101, 651-662.

8119 Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA, 2009b. Dietary
8120 acrylamide intake and brain cancer risk. Cancer Epidemiology, Biomarkers and Prevention, 18,
8121 1663-1666.

8122 Hogervorst JG, Baars BJ, Schouten LJ, Konings EJ, Goldbohm RA and van den Brandt PA, 2010. The
8123 carcinogenicity of dietary acrylamide intake: a comparative discussion of epidemiological and
8124 experimental animal research.Critical Reviews in Toxicology, 40, 485-512.

8125 Hogervorst JGF, Fortner RT, Mucci LA, Tworoger SS, Eliassen AH, Hankinson SE and Wilson KM,
8126 2013. Associations between dietary acrylamide intake and plasma sex hormone levels. Cancer
8127 Epidemiology Biomarkers and Prevention, 22, 2024-2036.

8128 Hogervorst JGF, de Bruijn-Geraets D, Schouten LJ, van Engeland M, de Kok TMCM, Goldbohm A,
8129 van den Brandt P and Weijenberg MP, 2014. Dietary acrylamide intake and the risk of colorectal
8130 cancer with specific mutations in KRAS and APC. Carcinogenesis, 35, 1032-1038.

8131 Hoorn AJW, Custer LL, Myhr BC, Brusick D, Gossen J and Vijg J, 1993. Detection of Chemical
8132 Mutagens Using Muta(R) Mouse - a Transgenic Mouse Model. Mutagenesis, 8, 7-10.

8133 Howland RD, Vyas IL, Lowndes HE and Argentieri TM, 1980. The etiology of toxic peripheral
8134 neuropathies – *in vitro* effects of acrylamide and 2,5-hexanedione on brain enolase and other
8135 glycolytic-enzymes. Brain Research, 202, 131-142.

8136 Huang Y-F, Chen M-L, Liou S-H, Chen M-F, Uang S-N and Wu K-Y, 2011a. Association of
8137 CYP2E1, GST and mEH genetic polymorphisms with urinary acrylamide metabolites in workers
8138 exposed to acrylamide. Toxicology Letters, 203, 118-126.

8139 Huang YF, Wu KY, Liou SH, Uang SN, Chen CC, Shih WC, Lee SC, Huang CCJ and Chen ML,
8140 2011b. Biological monitoring for occupational acrylamide exposure from acrylamide production
8141 workers. International Archives of Occupational and Environmental Health, 84, 303-313.

8142 Huang YF, Chiang SY, Liou SH, Chen ML, Chen MF, Uang SN and Wu KY, 2012. The modifying
8143 effect of CYP2E1, GST, and mEH genotypes on the formation of hemoglobin adducts of
8144 acrylamide and glycidamide in workers exposed to acrylamide. Toxicology Letters, 215, 92-99.

8145 Hughes E, Newton D, Harling R and Begg S, 1994. Validation of neurotoxicity screen with reference
8146 to motor and locomotor functions. Huntingdon Cambridgeshire: Huntingdon Research Centre. Ltd.
8147 As cited in FAO/WHO, 2006.

8148 Hułas-Stasiak M, Dobrowolski P, Tomaszewska E and Kostro K, 2013. Maternal acrylamide treatment
8149 reduces ovarian follicle number in newborn guinea pig offspring. Reproductive Toxicology, 42,
8150 125-131.

8151 Husain R, Dixit R, Das M and Seth PK, 1987. Neurotoxicity of acrylamide in developing rat brain:
8152 changes in the levels of brain biogenic amines and activities of monoamine oxidase and
8153 acetylcholine esterase. Industrial Health, 25-19-28.

8154 Huybrechts I, Sioen I, Boon PE, Ruprich J, Lafay L, Turrini A, Amiano P, Hirvonen T, De Neve M,
8155 Arcella D, Moschandreas J, Westerlund A, Ribas-Barba L, Hilbig A, Papoutsou S, Christensen T,
8156 Oltarzewski M, Virtanen S, Rehurkova I, Azpiri M, Sette S, Kersting M, Walkiewicz A, Serra-
8157 Majem L, Volatier JL, Trolle E, Tornaritis M, Busk L, Kafatos A, Fabiansson S, De Henauw S and
8158 Van Klaveren J, 2011. Dietary Exposure Assessments for Children in Europe (the EXPOCHI
8159 project): rationale, methods and design. Archives of Public Health, 69(4), [12 pp].

8160 IARC (International Agency for Research on Cancer), 1994. IARC Monographs on the Evaluations of
8161 Carcinogenic Risks to Humans. Volume 60. Some Industrial Chemicals. Acrylamide. Summary of
8162 data reported and Evaluation. Last updated: 13 April 1999.

8163 Iatropoulos M, Lebish I and Wang C, 1998. Microscopic evaluation of proliferative mesothelial
8164 lesions diagnosed previously as mesothelioma of the tunica vaginalis testis. Sponsored by CYTEC
8165 Industries. West Paterson, NJ. Unpublished. As cited by Shipp et al. (2006).

8166 Ikeda GJ, Miller E, Sapienza PP, Michel TC, King MT, Turner VA, Blumenthal H, Jackson WE, 3rd
8167 and Levin S, 1983. Distribution of 14C-labelled acrylamide and betaine in foetuses of rats, rabbits,
8168 beagle dogs and miniature pigs. Food and Chemical Toxicology, 21, 49-58.

8169 Ikeda GJ, Miller E, Sapienza PP, Michel TC, King MT and Sager AO, 1985. Maternal-foetal
8170 distribution studies in late pregnancy. II. Distribution of [1-14C]acrylamide in tissues of beagle
8171 dogs and miniature pigs. Food and Chemical Toxicology, 23, 757-761.

8172 Ikeda GJ, Miller E, Sapienza PP, Michel TC and Inskeep PB, 1987. Comparative tissue distribution
8173 and excretion of [1-14C]acrylamide in beagle dogs and miniature pigs. Food and Chemical
8174 Toxicology, 25, 871-875.

8175 Imai T and Kitahashi T, 2014. A 13-week toxicity study of acrylamide administered in drinking water
8176 to hamsters. Journal of Applied Toxicology, 34, 57-65.

8177 IPCS (International Programme on Chemical Safety), 1999. Acrylamide. International Programme on
8178 Chemical Safety. Poisons Information Monograph 652. Available at
8179 http://www.inchem.org/documents/pims/chemical/pim652.htm

8180 Irwin RD, Eustis SL, Stefanski S and Haseman JK, 1996. Carcinogenicity of glycidol in F344 rats and
8181 B6C3F$_1$ mice. Journal of Applied Toxicology, 16, 2001-2009.

8182 Jackson LS and Al-Taher F, 2005. Effects of consumer food preparation on acrylamide formation. In:
8183 Chemistry and Safety of Acrylamide in Food (Ed: Friedman and Mottram), 447-465.
8184 Springer+Business Media Inc.

8185 Jangir BL, Jaya R, Santosh R, Manoj P, Arun B and Nitin K, 2012. Effect of acrylamide toxicity on
8186 male reproductive system of Wistar rats. Indian Journal of Veterinary Pathology, 36, 37-40.

8187 Ji K, Kang S, Lee G, Lee S, Jo A, Kwak K, Kim D, Kho D, Lee S, Kim S, Kim S, Hiuang Y-F, Wu K-
8188 Y and Choi K, 2013. Urinary levels of N-acetyl-S-(2-carbamoylethyl)-cysteine (AAMA), an

8189 acrylamide metabolite, in Korean children and their association with food consumption. Science of
8190 the Total Environment, 456-457, 17-23.

8191 Jiang L, Cao J, An Y, Geng C, Qu S, Jiang L and Zhong L, 2007. Genotoxicity of acrylamide in
8192 human hepatoma G2 (HepG2) cells. Toxicology in Vitro, 21, 1486-1492.

8193 Jin L, Chico-Galdo V, Massart C, Gervy C, De Maertelaere V, Friedman M and Van Sande J, 2008.
8194 Acrylamide does not induce tumorigenesis or major defects in mice in vivo. Journal of
8195 Endocrinology, 198, 301-307.

8196 Johansson F, Lundell T, Rydberg P, Erixon K and Jenssen D, 2005. Mutagenicity and DNA repair of
8197 glycidamide-induced adducts in mammalian cells. Mutation Research-Genetic Toxicology and
8198 Environmental Mutagenesis, 580, 81-89.

8199 Johnson KA, Beyer JE, Bell TJ, et al. 1984. Acrylamide: A two-year drinking water chronic toxicity
8200 oncogenicity study in Fischer 344 rats. American Cyanamid Company. Dow Chemical U.S.A.
8201 Nalco Chemical Company. The Standard Oil Company. Submitted to the U.S. Environmental
8202 Protection Agency under TSCA Section 4. OTS0507273. As cited in ATSDR (2012).

8203 Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA and Mast RW, 1986.
8204 Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking-water of
8205 Fischer 344 rats. Toxicology and Applied Pharmacology, 85, 154-168

8206 Kadry AM, Friedman MA and Abdel-Rahman MS, 1999. Pharmacokinetics of acrylamide after oral
8207 administration in male rats. Environmental Toxicology and Pharmacology, 7, 127-133.

8208 Katic J, Cemeli E, Baumgartner A, Laubenthal J, Bassano I, Stolevik SB, Granum B, Namork E,
8209 Nygaard UC, Lovik M, van Leeuwen D, Vande Loock K, Anderson D, Fucic A and Decordier I,
8210 2010. Evaluation of the genotoxicity of 10 selected dietary/environmental compounds with the in
8211 vitro micronucleus cytokinesis-block assay in an interlaboratory comparison. Food and Chemical
8212 Toxicology, 48, 2612-2623.

8213 Katz JM, Winter CK, Buttrey SE and Fadel JG, 2012. Comparison of acrylamide intake from Western
8214 and guideline based diets using probabilistic techniques and linear programming. Food and
8215 Chemical Toxicology, 50, 877-883.

8216 Kellert M, Scholz K, Wagner S, Dekant W and Volkel W, 2006. Quantitation of mercapturic acids
8217 from acrylamide and glycidamide in human urine using a column switching tool with two trap
8218 columns and electrospray tandem mass spectrometry. Journal of Chromatography A, 1131, 58-66.

8219 Keramat J, LeBail A, Prost C and Soltanizadeh N, 2011. Acrylamide in foods: Chemistry and analysis.
8220 A review. Food Bioprocess Technol, 4, 340-363.

8221 Kermani-Alghoraishi M, Anvari M, Talebi AR, Amini-Rad O, Ghahramani R and Miresmaili SM,
8222 2010. The effects of acrylamide on sperm parameters and membrane integrity of epididymal
8223 spermatozoa in mice. European Journal of Obstetrics and Gynecology and Reproductive Biology,
8224 153, 52-55.

8225 Kersting M, Alexy U, Sichert-Hellert W, Manz F and Schöch G, 1998. Measured consumption of
8226 commercial infant food products in German infants: results from the DONALD study. Dortmund
8227 Nutritional and Anthropometrical Longitudinally Designed. Journal of Pediatric Gastroenterology
8228 and Nutrition, 27, 547-552.

8229 Khan MA, Davis CA, Foley GL, Friedman MA and Hansen LG, 1999. Changes in thyroid gland
8230 morphology after acute acrylamide exposure. Toxicological Sciences, 47, 151-157.

8231 Khan MR, Afzaal M, Saeed N and Shabbir M, 2011. Protective potential of methanol extract of
8232 *Digera muricata* on acrylamide induced hepatotoxicity in rats. African Journal of Biotechnology,
8233 10, 8456-8464.

8234 Kienzle E, Ranz D, Thielen C, Jezussek M and Schieberle P, 2005. Carry over (transfer) of feed-borne
8235 acrylamide into eggs, muscle, serum, and faeces - a pilot study with Japanese quails (*Coturnix*
8236 *coturnix japonica*). Journal of Animal Physiology and Animal Nutrition, 89, 79-83.

8237 Kim B, Park S, Lee I, Lim Y, Hwang E and So H-Y, 2010. Development of a certified reference
8238 material for the determination of acrylamide in potato chips. Analytical and Bioanalytical
8239 Chemistry, 398, 1035-1042.

8240 Kirman CR, Gargas ML, Deskin R, Tonner-Navarro L and Andersen ME, 2003. A physiologically
8241 based pharmacokinetic model for acrylamide and its metabolite, glycidamide, in the rat. Journal of
8242 Toxicology and Environmental Health A, 66, 253-274.

8243 Kjuus H, Goffeng LO, Heier MS, Sjoholm H, Ovrebo S, Skaug V, Paulsson B, Tornqvist M and
8244 Brudal S, 2004. Effects on the peripheral nervous system of tunnel workers exposed to acrylamide
8245 and N-methylolacrylamide. Scandinavian Journal of Work Environment and Health, 30, 21-29.

8246 Kiss JP, 2000. Role of nitric oxide in the regulation of monoaminergic neurotransmission. Brain
8247 Research Bulletin, 52, 459-466.

8248 Klaffke H, Fauhl C, Mathar W, Palavinskas R, Wittkowski R, Wenzl T and Anklam E, 2005. Results
8249 from two interlaboratory comparison tests organized in Germany and the EU Level for analysis of
8250 acrylamide in food. Journal of the AOAC International, 88, 292-298.

8251 Konings EJM, Hogervorst JGF, van Rooij L, Schouten LJ, Sizoo EA, van Egmond HP, Goldbohm RA
8252 and van den Brandt PA, 2010. Validation of a database on acrylamide for use in epidemiological
8253 studies. European Journal of Clinical Nutrition, 64, 534-540.

8254 Kopp EK, Sieber M, Kellert M and Dekant W, 2008. Rapid and Sensitive HILIC-ESI-MS/MS
8255 quantitation of polar metabolites of acrylamide in human urine using column switching with an
8256 online trap column. Journal of Agricultural and Food Chemistry, 56, 9828-9834.

8257 Kopp EK and Dekant W, 2009. Toxicokinetics of acrylamide in rats and humans following single oral
8258 administration of low doses. Toxicology and Applied Pharmacology, 235, 135-142.

8259 Kotova N, Juren T, Myohanen K, Cornelius M, Abramsson-Zetterberg L, Backman J, Menzel U,
8260 Rydberg P, Kronberg L, Vahakangas K and Segerbäck D, 2011. (3)(2)P-HPLC analysis of N1-(2-
8261 carboxy-2-hydroxyethyl)deoxyadenosine: a DNA adduct of the acrylamide-derived epoxide
8262 glycidamide. Toxicology Letters, 207, 18-24.

8263 Koyama N, Sakamoto H, Sakuraba M, Koizumi T, Takashima Y, Hayashi M, Matsufuji H, Yamagata
8264 K, Masuda S, Kinae N and Honma M, 2006. Genotoxicity of acrylamide and glycidamide in
8265 human lymphoblastoid TK6 cells. Mutation Research-Genetic Toxicology and Environmental
8266 Mutagenesis, 603, 151-158.

8267 Koyama N, Yasui M, Kimura A, Takami S, Suzuki T, Masumura K, Nohmi T, Masuda S, Kinae N,
8268 Matsuda T, Imai T and Honma M, 2011a. Acrylamide genotoxicity in young versus adult gpt delta
8269 male rats. Mutagenesis, 26, 545-549.

8270 Koyama N, Yasui M, Oda Y, Suzuki S, Satoh T, Suzuki T, Matsuda T, Masuda S, Kinae N and
8271 Honma M, 2011b. Genotoxicity of acrylamide in vitro: Acrylamide is not metabolically activated
8272 in standard in vitro systems. Environmental and Molecular Mutagenesis, 52, 11-19.

8273 Kraus D, Rokitta D, Fuhr U and Tomalik-Scharte D, 2013. The role of human cytochrome P450
8274 enzymes in metabolism of acrylamide in vitro. Toxicology Mechanism and Methods, 23, 346-351.

8275 Kumar D, Singh BP and Kumar P, 2004. An overview of the factors affecting sugar content of
8276 potatoes. Annals of Applied Biology, 145, 247-256.

8277 Kurebayashi H and Ohno Y, 2006. Metabolism of acrylamide to glycidamide and their cytotoxicity in
8278 isolated rat hepatocytes: protective effects of GSH precursors. Archives of Toxicology, 80, 820-
8279 828

8280 Kütting B, Uter W and Drexler H, 2008. The association between self-reported acrylamide intake and
8281 hemoglobin adducts as biomarkers of exposure. Cancer Causes Control, 19, 273-281.

8282 Kütting B, Schettgen T, Schwegler U, Fromme H, Uter W, Angerer J and Drexler H, 2009.
8283 Acrylamide as environmental noxious agent A health risk assessment for the general population

based on the internal acrylamide burden. International Journal of Hygene and Environmental Health, 212, 470-480.

Lakshmi D, Gopinath K, Jayanthy G, Anjum S, Prakash D and Sudhandiran G, 2012. Ameliorating Effect of Fish Oil on Acrylamide Induced Oxidative Stress and Neuronal Apoptosis in Cerebral Cortex. Neurochemical Research, 37, 1859-1867.

Lamy E, Voelkel Y, Roos PH, Kassie F and Mersch-Sundermann V, 2008. Ethanol enhanced the genotoxicity of acrylamide in human, metabolically competent HepG2 cells by CYP2E1 induction and glutathione depletion. International Journal of Hygiene and Environmental Health, 211, 74-81

Lantz I, Ternité R, Wilkens J, Hoenicke K, Guenther H, van der Stegen G, 2006. Studies on acrylamide levels in roasting, storage and brewing of coffee. Molecular Nutrition and Food Research, 50, 1039-1046.

Lapadula DM, Bowe M; Carrington CD, Dulak K, Friedman M and Aboudonia MB, 1989. *Invitro* binding of [C-14] acrylamide to neurofilament and microtubule proteins of rats. Brain Research, 481, 157-161.

Larsson SC, Akesson A and Wolk A, 2009a. Long-term dietary acrylamide intake and breast cancer risk in a prospective cohort of Swedish women. American Journal of Epidemiology, 169, 376-381.

Larsson SC, Håkansson N, Akesson A and Wolk A, 2009b. Long-term dietary acrylamide intake and risk of endometrial cancer in a prospective cohort of Swedish women. International Journal of Cancer, 124, 1196-1199.

Larsson SC, Akesson A and Wolk A, 2009c. Long-term dietary acrylamide intake and risk of epithelial ovarian cancer in a prospective cohort of Swedish women. Cancer Epidemiology, Biomarkers and Prevention, 18, 994-997.

Larsson SC, Akesson A, Bergkvist L and Wolk A, 2009d. Dietary acrylamide intake and risk of colorectal cancer in a prospective cohort of men. European Journal of Cancer, 45, 513-516.

Larsson SC, Akesson A and Wolk A, 2009e. Dietary acrylamide intake and prostate cancer risk in a prospective cohort of Swedish men. Cancer Epidemiol Biomarkers Prevention, 18, 1939-1941.

Latzin JM, Schindler BK, Weiss T, Angerer J and Koch HM, 2012. Determination of 2,3-dihydroxypropionamide, an oxidative metabolite of acrylamide, in human urine by gas chromatography coupled with mass spectrometry. Analytical and Bioanalytical Chemistry, 402, 2431-2438.

Lee HR, Cho SJ, Park HJ, Kim KH, Rhee DK and Pyo S, 2010. The inhibitory effect of acrylamide on NCAM expression in human neuroblastoma cells: involvement of CK2/Ikaros signaling pathway. Toxicol In Vitro, 24, 1946-1952.

Lee T, Manjanatha MG, Aidoo A, Moland CL, Branham WS, Fuscoe JC, Ali AA and Desai VG, 2012. Expression analysis of hepatic mitochondria-related genes in mice exposed to acrylamide and glycidamide. Journal of Toxicology and Environmental Health A, 75, 324-339.

Lee J-G, Wang Y-S and Chou C-C, 2014. Acrylamide-induced apoptosis in rat primary astrocytes and human astrocytoma cell lines. Toxicology in Vitro, 28, 568-570.

Lehning EJ, Persaud A, Dyer KR, Jortner BS and LoPachin RM, 1998. Biochemical and morphologic characterization of acrylamide peripheral neuropathy. Toxicology and Applied Pharmacology, 151, 211-221.

Lim TG, Lee BK, Kwon JY, Jung SK and Lee KW, 2011. Acrylamide up-regulates cyclooxygenase-2 expression through the MEK/ERK signaling pathway in mouse epidermal cells. Food Chemistry and Toxicology, 49, 1249-1254.

Lim PK, Jinap S, Sanny M, Tan Cp and Khatib A, 2014. The influence of deep frying using various vegetable oils in acrylamide formation sweet potato (*Ipomoea batatas* L. Lam) chips. Journal of Food Science, 79, 115-121.

8331 Lin WW, Friedman MA, Wang XF and Abou-Donia MB, 2000. Acrylamide-regulated neurofilament
8332 expression in rat pheochromocytoma cells. Brain Research, 852, 297-304.

8333 Lin Y, Lagergren J and Lu Y, 2011. Dietary acrylamide intake and risk of esophageal cancer in a
8334 population-based case-control study in Sweden. International Journal Cancer, 128, 676-81.

8335 Lin C-Y, Lee H-L, Chen Y-C, Lien G-W, Lin L-Y, Wen L-L, Liao C-C, Chien K-L, Sung F-C, Chen
8336 P-C and Su T-C, 2013. Positive association between urinary levels of 8-hydroxydeoxyguanosine
8337 and the acrylamide metabolite N-acetyl-S-(propionamide)-cysteine in adolescents and young
8338 adults. Journal of Hazardous Materials, 261, 372-377.

8339 Lipworth L, Sonderman JS, Tarone RE and McLaughlin JK, 2012. Review of epidemiologic studies of
8340 dietary acrylamide intake and the risk of cancer. European Journal of Cancer Prevention, 21, 375-
8341 386.

8342 Logan MJ and McLean WG, 1988. A comparison of the effects of acrylamide and experimental
8343 diabetes on the retrograde axonal transport of proteins in the rat sciatic nerve: analysis by two-
8344 dimensional polyacrylamide gel electrophoresis. Journal of Neurochemistry, 50, 183-189.

8345 LoPachin RM and Barber, 2006. Synaptic cysteine sulfhydryl groups as targets of electrophilic
8346 neurotoxicants. Toxicological Sciences, 94, 240-255.

8347 LoPachin RM and Lehning EJ, 1994. Acrylamide-induced distal axon degeneration – a proposed
8348 mechanism of action. Neurotoxicology, 15, 247-259.

8349 LoPachin RM, Ross JF and Lehning EJ, 2002. Nerve terminals as the primary site of acrylamide
8350 action: A hypothesis. Neurotoxicology, 23, 43-59.

8351 LoPachin RM, 2004. The changing view of acrylamide neurotoxicity. Neurotoxicology, 25, 617-630.

8352 LoPachin RM, Schwarcz AI, Gaughan CL, Mansukhani S and Das S, 2004. *In vivo* and *in vitro* effects
8353 of acrylamide on synaptosomal neurotransmitter uptake and release. Neurotoxicology, 25, 349-363.

8354 LoPachin RM, Barber DS, He D and Das S, 2006. Acrylamide inhibits dopamine uptake in rat striatal
8355 synaptic vesicles. Toxicological Sciences, 89, 224-234.

8356 LoPachin RM and Gavin T, 2012. Molecular Mechanism of Acrylamide Neurotoxicity: Lessons
8357 Learned from Organic Chemistry. Environmental Health Perspectives, 120, 1650-1657.

8358 Lujan-Barroso L, González CA, Slimani N, Obón-Santacana M, Ferrari P, Freisling H, Overvad K,
8359 Clavel-Chapelon F, Boutron-Ruault M-C, Racine A, Katzke V, Kühn T, Tjonneland A, Olsen A,
8360 Quirós JR, Sánchez-Cantalejo E, Amiano P, Navarro C, Barricarte A, Khaw K-T, Wareham N,
8361 Travis RC, Trichopoulou A, Bamia C, Benetou V, Saieva C, Grioni S, Tumino R, Vineis P,
8362 Mattiello A, Bueno-de-Mesquita HB, Siersema PD, Numans ME, Peeters PH, Ericson U, Wirfält
8363 E, Sund M, Johansson M, Weiderpass E, Skeie G, Riboli E, Boeing H and Duell EJ, 2014. Dietary
8364 intake of acrylamide and esophageal cancer risk in the European Prospective Investigation into
8365 Cancer and Nutrition cohort. Cancer Causes and Control, 25, 639-646..

8366 Lynch DW, Lewis TR, Moorman WJ, Burg JR, Groth DH, Khan A, Ackerman LJ and Cockrell BY,
8367 1984. Carcinogenic and toxicologic effects of inhaled ethylene oxide and propylene oxide in F344
8368 rats. Toxicology and Applied Pharmacology, 76, 69-84.

8369 Lyn-Cook LE, Jr., Tareke E, Word B, Starlard-Davenport A, Lyn-Cook BD and Hammons GJ, 2011.
8370 Food contaminant acrylamide increases expression of Cox-2 and nitric oxide synthase in breast
8371 epithelial cells. Toxicology and Industrial Health, 27, 11-18.

8372 Ma YX, Shi J, Zheng MG, Liu J, Tian SM, He XH, Zhang DX, Li GY and Zhu JY, 2011.
8373 Toxicological effects of acrylamide on the reproductive system of weaning male rats. Toxicology
8374 and Industrial Health, 27, 617-627.

8375 MAF (Ministry of Agriculture and Forestry), 2012. Acrylamide in New Zealand food and updated
8376 exposure assessment. MAF Technical Paper No: 2011/19.

8377 Maillard LC, 1912. Action des acides aminés sur les sucres: formation des mélanoidines par voie
8378 méthodique. Compte-rendu de l'Académie des Sciences, 154, 66-68.

8379 Maier A, Kohrman-Vincent M, Hertzberg R, Allen B, Haber LT and Dourson M, 2012. Critical
8380 review of dose-response options for F344 rat mammary tumors for acrylamide - additional insights
8381 based on mode of action. Food and Chemical Toxicology, 50, 1763-1775.

8382 Manière I, Godard T, Doerge DR, Churchwell MI, Guffroy M, Laurentie M and Poul JM, 2005. DNA
8383 damage and DNA adduct formation in rat tissues following oral administration of acrylamide.
8384 Mutation Research-Genetic Toxicology and Environmental Mutagenesis, 580, 119-129.

8385 Manjanatha MG, Aidoo A, Shelton SD, Bishop ME, McDaniel LP, Lyn-Cook LE and Doerge DR,
8386 2006. Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to
8387 male and female Big Blue mice. Environmental and Molecular Mutagenesis, 47, 6-17.

8388 Manson J, Brabec MJ, Buelke-Sam J, Carlson GP, Chapin RE, Favor JB, Fischer LJ, Hattis D, Lees
8389 PSJ, Perreault-Darney S, Rutledge J, Smith TJ, Tice RR and Working P, 2005. NTP-CERHR
8390 expert panel report on the reproductive and developmental toxicity of acrylamide. Birth Defects
8391 Research Part B-Developmental and Reproductive Toxicology, 74, 17-113.

8392 Marchetti F, Lowe X, Bishop J and Wyrobek J, 1997. Induction of chromosomal aberrations in mouse
8393 zygotes by acrylamide treatment of male germ cells and their correlation with dominant lethality
8394 and heritable translocations. Environmental and Molecular Mutagenesis, 30, 410-417.

8395 Marchetti F, Bishop JB, Lowe X, Generoso WM, Hozier J and Wyrobek AJ, 2001. Etoposide induces
8396 heritable chromosomal aberrations and aneuploidy during male meiosis in the mouse. Proc. Natl.
8397 Acad. Sci. U. S. A. 98, 3952-3957.

8398 Marchetti F, Bishop JB, Cosentino L, Moore D II and Wyrobek AJ, 2004. Paternally transmitted
8399 chromosomal aberrations in mouse zygotes determine their embryonic fate. Biol. Reprod. 70, 616-
8400 624.

8401 Marchetti F, Essers J, Kanaar R and Wyrobek AJ, 2007. Disruption of maternal DNA repair increases
8402 sperm-derived chromosomal aberrations. Proc. Natl. Acad. Sci. U. S. A. 104, 17725-17729.

8403 Marchetti F, Bishop J, Xiu L and Wyrobek AJ, 2009. Chromosomal Mosaicism in Mouse Two-Cell
8404 Embryos after Paternal Exposure to Acrylamide. Toxicological Sciences, 107, 194-205.

8405 Marlowe C, Clark MJ, Mast RW, Friedman MA and Waddell WJ, 1986. The distribution of
8406 [14C]acrylamide in male and pregnant Swiss-Webster mice studied by whole-body
8407 autoradiography. Toxicology and Applied Pharmacology, 86, 457-465.

8408 Maronpot RR, Flake G and Huff J, 2004. Relevance of animal carcinogenesis findings to human
8409 cancer predictions and prevention. Toxicologic Pathology, 32, 40-48.

8410 Maronpot RR, Zeiger E, McConnell EE, Kolenda-Roberts H, Wall H and Friedman MA, 2009.
8411 Induction of tunica vaginalis mesotheliomas in rats by xenobiotics. Critical Reviews in Toxicology,
8412 39, 512-537.

8413 Marsh GM, Lucas LJ, Youk AO and Schall LC, 1999. Mortality patterns among workers exposed to
8414 acrylamide: 1994 follow up. Occupational and Environmental Medicine, 56, 181-190.

8415 Marsh GM, Youk AO, Buchanich JM, Kant IJ and Swaen G, 2007. Mortality patterns among workers
8416 exposed to acrylamide: updated follow up. Journal of Occupational and Environmental Medicine,
8417 49, 82-95.

8418 Martenson CH, Odom A, Sheetz MP abd Graham DG, 1995. The effect of acrylamide and other
8419 sulfhydryl alkylators on the ability of dynein and kinesin to translocate microtubules in-vitro.
8420 Toxicology and Applied Pharmacology, 133, 73-81.

8421 Martins C, Oliveira NG, Pingarilho M, da Costa GG, Martins V, Marques MM, Beland FA,
8422 Churchwell MI, Doerge DR, Rueff J and Gaspar JF, 2007. Cytogenetic damage induced by

acrylamide and glycidamide in mammalian cells: Correlation with specific glycidamide-DNA adducts. Toxicological Sciences, 95, 383-390.

Martyniuk CJ, Feswick A, Fang B, Koomen JM, Barber DS, Gavin T and LoPachin RM, 2013. Protein targets of acrylamide adduct formation in cultured rat dopaminergic cells. Toxicology Letters, 219, 279-287.

Matthäus, 2002. BAGKF, Bundesanstalt für Getreide- Kartoffel und Fettforschung. Available at: http://www.bfr.bund.de/cm/343/acrylamidgehalte_von_im_backofen_zubereiteten_pommes_frites_und_von_reibekuchen.pdf

Maurissen JP, Weiss B and Davis HT, 1983. Somatosensory thresholds in monkeys exposed to acrylamide. Toxicology and Applied Pharmacology, 71, 266-279.

Maurissen JP, Weiss B and Cox C, 1990. Vibration sensitivity recovery after a second course of acrylamide intoxication. Fundamental and Applied Toxicology, 15, 93-98.

McCollister D, Oyen F and Rowe V, 1964. Toxicology of acrylamide. Toxicology and Applied Pharmacology, 6, 172-181.

Medrano CJ and LoPachin RM, 1989. Effects of acrylamide and 2,5-hexanedione on brain mitochondrial respiration. Neurotoxicology, 10, 249-255.

Mehri S, Abnous K, Mousavi SH, Shariaty VM and Hosseinzadeh H, 2012. Neuroprotective effect of crocin on acrylamide-induced cytotoxicity in PC12 cells. Cellular and Molecular Neurobiology, 32, 227-235.

Mehri S, Karami HV, Hassani FV and Hosseinzadeh H, 2014. Chrysin reduced acrylamide-induced neurotoxicity in both in vitro and in vivo assessments. Iranian Biomedical Journal, 18, 101-106.

Mei N, Hu J, Churchwell MI, Guo L, Moore MM, Doerge DR and Chen T, 2008a. Genotoxic effects of acrylamide and glycidamide in mouse lymphoma cells. Food and Chemical Toxicology, 46, 628-636.

Mei N, Guo L, Tseng J, Dial SL, Liao W and Manjanatha MG, 2008b. Gene expression changes associated with xenobiotic metabolism pathways in mice exposed to acrylamide. Environ Mol Mutagen, 49, 741-745.

Mei N, McDaniel LP, Dobrovolsky VN, Guo X, Shaddock JG, Mittelstaedt RA, Azuma M, Shelton SD, McGarrity LJ, Doerge DR and Heflich RH, 2010. The genotoxicity of acrylamide and glycidamide in big blue rats. Toxicological Sciences, 115, 412-421.

Melnick RL, 2002. Carcinogenicity and mechanistic insights on the behavior of epoxides and epoxide-forming chemicals. Annals of the New York Academy of Sciences, 982, 177-189

Merigan WH, Barkdoll E and Maurissen JP, 1982. Acrylamide-induced visual impairment in primates. Toxicology and Applied Pharmacology, 62, 342-345.

Merigan WH, Barkdoll E, Maurissen JP, Eskin TA and Lapham LW, 1985. Acrylamide effects on the macaque visual system. I. Psychophysics and electrophysiology. Investigative Opthalmology and Visual Science. 26, 30-36.

Merten C, Ferrari P, Bakker M, Boss A, Hearty A, Leclercq C, Lindtner O, Tlustos C, Verger P, Volatier JL, Arcella D, 2011. Methodological characteristics of the national dietary surveys carried out in the European Union as included in the European Food Safety Authority (EFSA) Comprehensive European Food Consumption Database. Food Additives and Contaminants: Part A, 28, 975-995.

Mestdagh F, De Wilde T, Castelein P, Németh O, Van Peteghem C and De Meulenaer B, 2008. Impact of the reducing sugars on the relationship between acrylamide and Maillard browning in French fries. European Food Research and Technology, 227, 69-76.

Michalak J, Gujska E and Klepacka J, 2011. The effect of domestic preparation of some potato products on acrylamide content. Plant Foods for Human Nutrition, 66, 307-312..

8470 Michalak J, Gujska E and Kuncewicz A, 2013. RP-HPLC-DAD studies on acrylamide in cereal-based
8471 baby foods. Journal of Food Composition and Analysis, 32, 68-73.

8472 Miller MJ, Carter DE and Sipes IG, 1982. Pharmacokinetics of acrylamide in Fisher-344 rats.
8473 Toxicology and Applied Pharmacology, 63, 36-44.

8474 Miller MS and Spencer PS, 1984. Single doses of acrylamide reduce retrograde transport velocity.
8475 Journal of Neurochemistry, 43, 1401-1408.

8476 Mohareb RM, Ahmed HH, Elmegeed GA, Abd-Elhalim MM and Shafic RW, 2011. Development of
8477 new indole-derived neuroprotective agents. Bioorganic and Medicinal Chemistry, 19, 2966-2974.

8478 Mojska H, Gielecinska I, Szponar L and Oltarzewski M, 2010. Estimation of the dietary acrylamide
8479 exposure of the Polish population. Food and Chemical Toxicology, 48, 2090-2096.

8480 Mojska H, Gielecinska I and Stos K, 2012. Determination of acrylamide level in commercial baby
8481 foods and an assessment of infant dietary exposure. Food and Chemical Toxicology, 50, 2722-
8482 2728.

8483 Moldoveanu SC and Gerardi AR, 2011. Acrylamide analysis in tobacco, alternative tobacco products,
8484 and cigarette smoke. Journal of Chromatographic Science, 49, 234-242.

8485 Monks TJ, Anders MW, Dekant W, Stevens JL, Lau SS and Van Bladeren PJ, 1990. Contemporary
8486 issues in toxicology. Glutathione conjugate mediated toxicities. Toxicology and Applied
8487 Pharmacology, 106, 1-19.

8488 Moorman WJ, Reutman SS, Shaw PB, Blade LM, Marlow D, Vesper H, Clark JC and Schrader SM,
8489 2012. Occupational exposure to acrylamide in closed system production plants: air levels and
8490 biomonitoring. Journal of Toxicology and Environmental Health A, 75, 100-111.

8491 Moretto A and Sabri MI, 1988. Progressive deficits in retrograde axon transport precede degeneration
8492 of motor axons in acrylamide neuropathy. Brain Research, 440, 18-24.

8493 Mose T, Mathiesen L, Karttunen V, Nielsen JKS, Sieppi E, Kummu M, Mørk TA, Myöhäanen K,
8494 Partanen H, Vähäkangas K, Knudsen LE and Myllynen P, 2012. Meta-analysis of data from human
8495 ex vivo placental perfusion studies on genotoxic and immunotoxic agents within the integrated
8496 European project NewGeneris. Placenta, 33, 433-439.

8497 Mottram DS, Wedzicha BL and Dodson AT, 2002. Food chemistry: Acrylamide is formed in the
8498 Maillard reaction. Nature, 419, 448-449.

8499 Motwani HV and Törnqvist M, 2011. Quantitative analysis by liquid chromatography–tandem mass
8500 spectrometry of glycidamide using the cob(I)alamin trapping method: Validation and application to
8501 in vitro metabolism of acrylamide. Journal of Chromatography A, 1218, 4389-4394.

8502 Mucci LA, Dickman PW, Steineck G, Adami HO and Augustsson K, 2003a. Dietary acrylamide and
8503 cancer of the large bowel, kidney, and bladder: absence of an association in a population-based
8504 study in Sweden. British Journal of Cancer, 88, 84-89.

8505 Mucci LA, Dickman PW, Steineck G, Adami HO and Augustsson K, 2003b. Reply: Dietary
8506 acrylamide and cancer risk: additional data on coffee. British Journal of Cancer, 89, 775-777.

8507 Mucci LA, Lindblad P, Steineck G and Adami HO, 2004. Dietary acrylamide and risk of renal cell
8508 cancer. International Journal of Cancer, 109, 774-776.

8509 Mucci LA, Sandin S, Bälter K, Adami HO, Magnusson C, Weiderpass E, 2005. Acrylamide intake and
8510 breast cancer risk in Swedish women. JAMA, 293, 1326-1327.

8511 Mucci LA, Adami HO and Wolk A, 2006. Prospective study of dietary acrylamide and risk of
8512 colorectal cancer among women. International Journal of Cancer, 118, 169-173.

8513 Mucci LA and Wilson KA, 2008. Acrylamide intake through diet and human cancer risk. Journal of
8514 Agricultural and Food Chemistry, 56, 6013-6019.

8515 Mustafa HN, 2012. Effect of acrylamide on testis of albino rats Ultrastructure and DNA cytometry
8516 study. Saudi Medical Journal, 33, 722-731.

8517 Muthukumar K, Gurusamy P, Rajasingh S and Karunakaran C, 2011. Theoretical description of
8518 cytotoxic potential of glycidamide, an epoxide metabolite of acrylamide. Computational and
8519 Theoretical Chemistry, 964, 7-11.

8520 Muttucumaru N, Powers SJ, Elmore JS, Mottram DS, Halford NG, 2013. Effects of nitrogen and
8521 sulfur fertilization on free amino acids, sugars, and acrylamide-forming potential in potato. Journal
8522 of Agricultural and Food Chemistry, 61, 6734-6742.

8523 Myers JE and Macun I, 1991. Acrylamide Neuropathy in a South-African Factory - an Epidemiologic
8524 Investigation. American Journal of Industrial Medicine, 19, 487-493.

8525 Newton D, Hughes E, Harling R, Gopinath C and Beg S, 1992. A neurotoxicity screen in rats
8526 following treatment with acrylamide, carbaryl or p,p'-DDT. Huntingdon, Cambridgeshire:
8527 Huntingdon Research Centre Ltd. As cited in JECFA (2006).

8528 NICNAS (National Industrial Chemicals Notification and Assessment Scheme), 2002. Acrylamide.
8529 Priority Existing Chemical. Assessment Report No. 23. Comonwealth of Australia, May 2002.

8530 Nishimura M, Yaguti H, Yoshitsugu H, Naito S and Satoh T, 2003. Tissue distribution of mRNA
8531 expression of human cytochrome p450 isoforms assessed by high-sensitivity real-time reverse
8532 transcription PCR. Yakugaku Zasshi-Journal of the Pharmaceutical Society of Japan, 123, 369-375.

8533 Nixon BJ, Stanger SJ, Nixon B and Roman SD, 2012. Chronic exposure to acrylamide induces DNA
8534 damage in male germ cells of mice. Toxicological Sciences, 129, 135-145.

8535 Nixon BJ, Stanger SJ, Nixon B and Roman SD, 2013. *Erratum*. Chronic exposure to acrylamide
8536 induces DNA damage in male germ cells of mice. Toxicological Sciences, 132, 250.

8537 Nixon BJ, Katen AL, Stanger SJ, Schjenke JR, Nixon B and Roman SD, 2014. Mouse spermatocytes
8538 express CYP2E1 and respond to acrylamide exposure. PLOS One, 9, e94904.

8539 Normandin L, Bouchard M, Ayotte P, Blanchet C, Becalski A, Bonvalot Y, Phaneuf D, Lapointe C,
8540 Gagné M and Courteau M, 2013. Dietary exposure to acrylamide in adolescents from a Canadian
8541 urban center. Food and Chemical Toxicology, 57, 75-83.

8542 Noti A, Biedermann-Brem S, Biedermann M, Grob K, Albisser P, and Realini P, 2003. Storage of
8543 potatoes at low temperatures should be avoided to prevent increased acrylamide formation during
8544 frying or roasting. Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 94, 167-180.

8545 NTP (National Toxicology Program), 2011. Report on Carcinogens. 12th Edition. U.S. Department of
8546 Health and Human Services. Public Health Service National Toxicology Program.

8547 NTP (National Toxicology Program), 2012. NTP Technical Report on the Toxicology and
8548 Carcinogenesis Studies of Acrylamide (CAS No. 79-06-1) in F344/N rats and B6C3F1 mice (feed
8549 and drinking water studies). NTP TR 575. NIH Publication No. 12-5917. National Institutes of
8550 Health. Public Health Service. U.S. Department of Health and Human Services. July 2012.

8551 NTP (National Toxicology Program), 2013, draft report. NTP Technical report on the toxicology and
8552 carcinogenesis studies of glycidamide (CAS No. 5694-00-8) in F344/N Nctr rats and B6C3F$_1$/Nctr
8553 mice (drinking water study). Draft report. Peer-reviewed by the NTP Technical Reports Peer
8554 Review Panel on October 29, 2013. Available at
8555 http://ntp.niehs.nih.gov/ntp/about_ntp/trpanel/2013/october/draft_tr-588.pdf

8556 Nurullahoglu-Atalik E, Okudan N, Belviranli M, Esen H, Yener Y and Celik I, 2013. Responses of
8557 acrylamide-treated rat bladders. Bratislava Medical Journal-Bratislavske Lekarske Listy, 114, 7-11.

8558 Obón-Santacana M, Slimani N, Lujan-Barroso L, Travier N, Hallmans G, Freisling H, Ferrari P,
8559 Boutron-Ruault MC, Racine A, Clavel F, Saieva C, Pala V, Tumino R, Mattiello A, Vineis P,
8560 Argüelles M, Ardanaz E, Amiano P, Navarro C, Sánchez MJ, Molina Montes E, Key T, Khaw KT,
8561 Wareham N, Peeters PH, Trichopoulou A, Bamia C, Trichopoulos D, Boeing H, Kaaks R, Katzke

8562 V, Ye W, Sund M, Ericson U, Wirfält E, Overvad K, Tjønneland A, Olsen A, Skeie G, Asli LA,
8563 Weiderpass E, Riboli E, Bueno-de-Mesquita HB and Duell EJ, 2013. Dietary intake of acrylamide
8564 and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition
8565 (EPIC) cohort. Annals of Oncology, 24, 2645-2651.

8566 Ogawa B, Ohishi T, Wang L, Takahashi M, Taniai E, Hayashi H, Mitsumori K and Shibutani M,
8567 2011. Disruptive neuronal development by acrylamide in the hippocampal dentate hilus after
8568 developmental exposure in rats. Archives of Toxicology, 85, 987-994.

8569 Ogawa B, Wang L, Ohishi T, Taniai E, Akane H, Suzuki K, Mitsumori K and Shibutani M, 2012.
8570 Reversible aberration of neurogenesis targeting late-stage progenitor cells in the hippocampal
8571 dentate gyrus of rat offspring after maternal exposure to acrylamide. Archives of Toxicology, 86,
8572 779-790.

8573 Olesen PT, Olsen A, Frandsen H, Frederiksen K, Overvad K, Tjønneland A, 2008. Acrylamide
8574 exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer
8575 and Health Study. International Journal of Cancer, 122, 2094-2100.

8576 Olsen A, Christensen J, Outzen M, Olesen PT, Frandsen H, Overvad K and Halkjær J, 2012. Pre-
8577 diagnostic acrylamide exposure and survival after breast cancer among postmenopausal Danish
8578 women. Toxicology, 296, 67-72.

8579 Oliveira NG, Pingarilho M, Martins C, Fernandes AS, Vaz S, Martins V, Rueff J and Gaspar JF, 2009.
8580 Cytotoxicity and chromosomal aberrations induced by acrylamide in V79 cells: role of glutathione
8581 modulators. Mutation Research, 676, 87-92.

8582 Oracz J, Nebesny E and Żyżelewicz D, 2011. New trends in quantification of acrylamide in food
8583 products. Talanta, 86, 23-34.

8584 Outzen M, Egeberg R, Dragsted L, Christensen J, Olesen PT, Frandsen H, Overvad K, Tjønneland A,
8585 Olsen A, 2011. Dietary determinants for Hb-acrylamide and Hb-glycidamide adducts in Danish
8586 non-smoking women. British Journal of Nutrition, 105, 1381-1387.

8587 Overton CL, Hudder A and Novak FR, 2008. The CYP2E subfamily, in Cytochrome P450: Role in the
8588 Metabolism and Toxicity of Drugs and Other Xenobiotics (Ioannides C ed) pp 276-308, Royal
8589 Society of Chemistry, Cambridge, UK.

8590 Owen LM, Castle L, Kelly J, Wilson L and Lloyd AS, 2005. Acrylamide analysis: assessment of
8591 results from six rounds of Food Analysis Performance Assessments Scheme (FAPAS) proficiency
8592 testing. Journal of the AOAC International, 88, 285-291.

8593 Ozer MS, Kola O, Altan A, Duran H and Zorlugenc B, 2012. Acrylamide content of some Turkish
8594 traditional desserts. Journal of Food Agriculture and Environment, 10, 74-77.

8595 Pabst K, Mathar W, Palavinskas R, Meisel H, Bluthgen A and Klaffke H, 2005. Acrylamide-
8596 occurrence in mixed concentrate feed for dairy cows and carry-over into milk. Food Additives and
8597 Contaminants, 22, 210-213.

8598 Pacchierotti F, Tiveron C, D'Archivio M, Bassani B, Cordelli E, Leter G and Spanò M, 1994.
8599 Acrylamide-induced chromosomal damage in male mouse germ cells detected by cytogenetic
8600 analysis of one-cell zygotes. Mutation Research/Fundamental and Molecular Mechanisms of
8601 Mutagenesis, 309, 273-284.

8602 Park HR, Kim MS, Kim SJ, Park M, Kong KH, Kim HS, Kwack SJ, Kang TS, Kim SH, Kim HS and
8603 Lee J, 2010. Acrylamide induces cell death in neural progenitor cells and impairs hippocampal
8604 neurogenesis. Toxicology Letters, 193, 86-93.

8605 Paulsson B, Grawe J and Tornqvist M, 2002. Hemoglobin adducts and micronucleus frequencies in
8606 mouse and rat after acrylamide or N-methylolacrylamide treatment. Mutation Research-Genetic
8607 Toxicology and Environmental Mutagenesis, 516, 101-111.

8608 Paulsson B, Kotova N, Grawe J, henderson A, Granath F, Golding B and Törnqvist M, 2003.
8609 Induction of micronuclei in mouse and rat by glycidamide, genotoxic metabolite of acrylamide.
8610 Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 535, 15-24.

8611 Pedersen GS, Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA, 2010.
8612 Dietary acrylamide intake and estrogen and progesterone receptor-defined postmenopausal breast
8613 cancer risk. Breast Cancer Res Treat, 122, 199-210.

8614 Pedersen M, von Stedingk H, Botsivali M, Agramunt S, Alexander J, Brunborg G, Chatzi L, Fleming
8615 S, Fthenou E, Granum B, Gutzkow KB, Hardie LJ, Knudsen LE, Kyrtopoulos SA, Mendez MA,
8616 Merlo DF, Nielsen JK, Rydberg P, Segerbäck D, Sunyer J, Wright J, Törnqvist M, Kleinjans JC
8617 and Kogevinas M, 2012. NewGeneris Consortium. Birth weight, head circumference, and prenatal
8618 exposure to acrylamide from maternal diet: the European prospective mother-child study
8619 (NewGeneris). Environmental Health Perspectives, 120, 1739-1745.
8620

8621 Pelicioli BR, Marchand ÁV and Dubois JM, 2014. Risks of dietary acrylamide exposure: A systematic
8622 review. Food Chemistry, 157, 310-322.

8623 Pelucchi C, Galeone C, Levi F, Negri E, Franceschi S, Talamini R, Bosetti C, Giacosa A and La
8624 Vecchia C, 2006. Dietary acrylamide and human cancer. International Journal of Cancer, 118, 467-
8625 471.

8626 Pelucchi C, Galeone C, Dal Maso L, Talamini R, Montella M, Ramazzotti V, Negri E, Franceschi S,
8627 La Vecchia C, 2007. Dietary acrylamide and renal cell cancer. International Journal of Cancer, 120,
8628 1376-1377.

8629 Pelucchi C, Galeone C, Talamini R, Negri E, Polesel J, Serraino D and La Vecchia C, 2011a. Dietary
8630 acrylamide and pancreatic cancer risk in an Italian case - control study. Annals of Oncology, 22,
8631 1910-1915.

8632 Pelucchi C, La Vecchia C, Bosetti C, Boyle P and Boffetta P, 2011b. Exposure to acrylamide and
8633 human cancer - a review and meta-analysis of epidemiologic studies. Annals of Oncology, 22,
8634 1487-1499.

8635 Pérez HL and Osterman-Golkar S, 2003. A sensitive gas chromatographic-tandem mass spectrometric
8636 method for detection of alkylating agents in water: Application to acrylamide in drinking water,
8637 coffee and snuff. Analyst, 128, 1033-1036.

8638 Petersen DW, Kleinow KM, Kraska RC and Lech JJ, 1985. Uptake, disposition, and elimination of
8639 acrylamide in rainbow-trout. Toxicology and Applied Pharmacology, 80, 58-65.

8640 Peto R, 1974. Guidelines on the analysis of tumour rates and death rates in experimental animals.
8641 British Journal of Cancer, 29, 201-105.

8642 Peto R, Pike M, Day N, Gray R, Lee P, Parish S, Peto J, Richards S and Wahrendorf J, 1980.
8643 Guidelines for simple sensitive significance tests for carcinogenic effects in long-term animal
8644 experiments, annex to long-term and short-term screening assays for carcinogens: A critical
8645 appraisal. IARC Monographs, Supplement 2, 311.426.

8646 Phillips DH and Venitt S, 2012. DNA and protein adducts in human tissues resulting from exposure to
8647 tobacco smoke. International Journal on Cancer, 131, 2733-2753.

8648 Pietinen P, Hartman AM, Haapa E, Rasanen L, Haapakoski J, Palmgren J, Albanes D, Virtamo J and
8649 Huttunen JK, 1988. Reproducibility and validity of dietary assessment instruments .1. a self-
8650 administered food use questionnaire with a portion size picture booklet. American Journal of
8651 Epidemiology, 128, 655-666.

8652 Pingarilho M, Oliveira NG, Martins C, Fernandes AS, de Lima JP, Rueff J and Gaspar JF, 2012.
8653 Genetic polymorphisms in detoxification and DNA repair genes and susceptibility to glycidamide-
8654 induced DNA damage. Journal of Toxicology and Environmental Health A, 75, 920-933.

8655 Pingarilho M, Oliveira NG, Martins C, Gomes BC, Fernandes AS, Martins V, Labilloy A, de Lima JP,
8656 Rueff J and Gaspar JF, 2013. Induction of sister chromatid exchange by acrylamide and
8657 glycidamide in human lymphocytes: Role of polymorphisms in detoxification and DNA-repair
8658 genes in the genotoxicity of glycidamide. Mutation Research, 752, 1-7.

8659 Polakis P, 2012. Wnt signalin in cancer. Cold Spring Harbor Perspectives in Biology, 2012;4:a008052

8660 Post EJ and McLeod JG, 1977. Acrylamide autonomic neuropathy in the cat. I. Neurophysiological
8661 and histological studies. Journal of Neurological Sciences, 33, 353-374.

8662 Powers SJ, Mottram DS, Curtis A and Halford NG, 2013. Acrylamide concentrations in potato crisps
8663 in Europe from 2002 to 2011. Food Additives and Contaminants-Part A, 30, 1493-1500.

8664 Prasad SN and Muralidhara, 2013. Neuroprotective efficacy of eugenol and isoeugenol in acrylamide-
8665 induced neuropathy in rats: behavioral and biochemical evidence. Neurochemical Research, 38,
8666 330-345.

8667 Preston A, Fodey T, Douglas A and Elliott CT, 2009. Monoclonal antibody development for
8668 acrylamide-adducted human haemoglobin; a biomarker of dietary acrylamide exposure. Journal
8669 Immunol Methods, 341, 19-29.

8670 Puppel N, Tjaden Z, Fueller F and Marko D, 2005. DNA strand breaking capacity of acrylamide and
8671 grlycidamide in mammalian cells. Mutation Research-Genetic Toxicology and Environmental
8672 Mutagenesis, 580, 71-80.

8673 Rahangadale S, Kurkure N, Prajapati B, Hedaoo V and Bhandarkar AG, 2012. Neuroprotective effect
8674 of vitamin e supplementation in wistar rat treated with acrylamide. Toxicology International, 19, 1-
8675 8.

8676 Rajeh N, Al Saggaf S, Ayuob N and ElAssouli S, 2011. Characterization of Acrylamide Mediated
8677 Testicular Toxicity in Rat: Light and Electron Microscopic Study. Kuwait Medical Journal, 43,
8678 196-205.

8679 Raju J, Sondagar C, Roberts J, Aziz SA, Caldwell D, Vavasour E and Mehta R, 2011. Dietary
8680 acrylamide does not increase colon aberrant crypt foci formation in male F344 rats. Food and
8681 Chemical Toxicology, 49, 1373-1380.

8682 Raju J, Roberts J, Sondagar C, Kapal K, Aziz SA, Caldwell D and Mehta R, 2013. Negligible colon
8683 cancer risk from food-borne acrylamide exposure in male F344 rats and nude (nu/nu) mice-bearing
8684 human colon tumor xenografts. PLoS One, 8, e73916.

8685 Rawi SM MM, Fahmy SR, EL-Abied SA., 2012. Hazardous effects of acrylamide on immature male
8686 and female rats. African Journal of Pharmacy and Pharmacology, 6, 20.

8687 Raymer JH, Sparacino CM, Velez GR, Padilla S, Macphail RC and Crofton KM, 1993. Determination
8688 of acrylamide in rat serum and sciatic-nerve by gas-chromatography electron-capture detection.
8689 Journal of Chromatography-Biomedical Applications, 619, 223-234.

8690 Reagan KE, Wilmarth KR, Friedman M and Abou-Donia MB, 1994. Acrylamide increases in vitro
8691 calcium and calmodulin-dependent kinase-mediated phosphorylation of rat brain and spinal cord
8692 neurofilament proteins. Neurochemistry International, 25, 133-143.

8693 Recio L, Hobbs C, Caspary W and Witt KL, 2010. Dose-response assessment of four genotoxic
8694 chemicals in a combined mouse and rat micronucleus (MN) and Comet assay protocol. The Journal
8695 of the Toxicological Sciences, 35, 149-162.

8696 Rice JM, 2005. The carcinogenicity of acrylamide. Mutation Research-Genetic Toxicology and
8697 Environmental Mutagenesis, 580, 3-20.

8698 Robinson M, Bull RJ, Knutsen GL, Shields RP and Stober J, 1986. A combined carcinogen bioassay
8699 utilizing both the lung adenoma and skin papilloma protocols. Environmental Health Perspectives,
8700 68, 141-145.

8701 Rodríguez-Ramiro I, Martin MA, Ramos S, Bravo L and Goya L, 2011a. Olive oil hydroxytyrosol
8702 reduces toxicity evoked by acrylamide in human Caco-2 cells by preventing oxidative stress.
8703 Toxicology, 288, 43-48.

8704 Rodríguez-Ramiro I, Ramos S, Bravo L, Goya L and Martin MA, 2011b. Procyanidin B2 and a cocoa
8705 polyphenolic extract inhibit acrylamide-induced apoptosis in human Caco-2 cells by preventing
8706 oxidative stress and activation of JNK pathway. Journal of Nutritional Biochemistry, 22, 1186-
8707 1194.

8708 Rothfuss A, O'Donovan M, De Boeck M, Brault D, Czich A, Custer L, Hamada S, Plappert-Helbig U,
8709 Hayashi M, Howe J, Kraynak AR, van der Leede BJ, Nakajima M, Priestley C, Thybaud V, Saigo
8710 K, Sawant S, Shi J, Storer R, Struwe M, Vock E and Galloway S, 2010. Collaborative study on
8711 fifteen compounds in the rat-liver Comet assay integrated into 2- and 4-week repeat-dose studies.
8712 Mutation Research, 702, 40-69.

8713 Rudkouskaya A, Sim V, Shah AA, Feustel PJ, Jourd'heuil D and Mongin AA, 2010. Long-lasting
8714 inhibition of presynaptic metabolism and neurotransmitter release by protein S-nitrosylation. Free
8715 Radical Biology and Medicine, 49, 757-769.

8716 Russell LB, Hunsicker PR, Cacheiro NLA and Generoso WM, 1991. Induction of specific-locus
8717 mutations in male germ-cells of the mouse by acrylamide monomer. Mutation Research, 262, 101-
8718 107.

8719 Rydberg P, Eriksson S, Tareke E, Karlsson P, Ehrenberg L and Tornqvist M, 2003. Investigations of
8720 factors that influence the acrylamide content of heated foodstuffs. Journal of Agricultural and Food
8721 Chemistry, 51, 7012-7018.

8722 Sabri MI and Spencer PS, 1990. Acrylamide impairs fast and slow axonal transport in rat optic system.
8723 Neurochemical Research, 15, 603-608.

8724 Sadek K, 2012. Antioxidant and immunostimulant effect of carica papaya linn. Aqueous extract in
8725 acrylamide intoxicated rats. Acta informatica medica: AIM : journal of the Society for Medical
8726 Informatics of Bosnia & Herzegovina : casopis Drustva za medicinsku informatiku BiH, 20, 180-
8727 185.

8728 Sakamoto J and Hashimoto K, 1986. Reproductive toxicity of acrylamide and related compounds in
8729 mice – effects on fertility and sperm morphology. Archives of Toxicology, 59, 201-205.

8730 Sakamoto J, Kurosaka Y and Hashimoto K, 1988. Histological changes of acrylamide-induced
8731 testicular lesions in mice. Experimental and Molecular Pathology, 48, 324-334.

8732 Salvini S, Hunter DJ, Sampson L, Stampfer MJ, Colditz GA, Rosner B and Willett WC, 1989. Food-
8733 based validation of a dietary questionnaire - the effects of week-to-week variation in food-
8734 consumption. International Journal of Epidemiology, 18, 858-867.

8735 Sánchez J, Cabrer JM, Rosselló CA, Palou A and Picó C, 2008. Formation of hemoglobin adducts of
8736 acrylamide after its ingestion in rats is dependent on age and sex. Journal of Agricultural and Food
8737 Chemistry, 56, 5096-5101.

8738 Sanganyado E, Parekh CT and Eriksson S, 2011. Analysis of acrylamide in traditional foodstuffs in
8739 Zimbabwe. African Journal of Food Science, 5, 910-913.

8740 Sanny M, Luning PA, Jinap S, Bakker EJ and Boekel MAJS, 2013. Effect of frying instructions for
8741 food handlers on AA concentration in French fries: an explorative study. Journal of Food
8742 Protection 76, 462-472.

8743 Satchell PM and McLeod JG, 1981. Megaesophagus due to acrylamide neuropathy. Journal of
8744 Neurology, Neurosurgery, and Psychiatry, 44, 906-913.

8745 SCF (Scientific Committee on Food), 2002. Opinion of the Scientific Committee on Food on new
8746 findings regarding the presence of acrylamide in food. Opinion expressed on 3 July 2002.
8747 SCF/CS/CNTM/CONT/4 Final. Available at: http://ec.europa.eu/food/fs/sc/scf/out131_en.pdf

8748 Schabacker J, Schwend T and Wink M, 2004. Reduction of acrylamide uptake by dietary proteins in a
8749 caco-2 gut model. Journal of Agricultural and Food Chemistry, 52, 4021-4025.

8750 Schettgen T, Weiss T, Drexler H and Angerer J, 2003. A first approach to estimate the internal
8751 exposure to acrylamidein smoking and non-smoking adults from Germany. International Journal of
8752 Hygene and Environmental Health, 206, 9-14.

8753 Schettgen T, Kütting B, Hornig M, Beckmann MW, Weiss T, Drexler H and Angerer J, 2004a. Trans-
8754 placental exposure of neonates to acrylamide - a pilot study. International Archives of Occupational
8755 and Environmental Health, 77, 213-216.

8756 Schettgen T, Rossbach B, Kütting B, Letzel S, Drexler H and Angerer J, 2004b. Determination of
8757 haemoglobin adducts of acrylamide and glycidamide in smoking and non-smoking persons of the
8758 general population. International Journal of Hygene and Environmental Health, 207, 531-539.

8759 Schettgen T, Musiol A and Kraus T, 2008. Simultaneous determination of mercapturic acids derived
8760 from ethylene oxide (HEMA), propylene oxide (2-HPMA), acrolein (3-HPMA), acrylamide
8761 (AAMA) and N,N-dimethylformamide (AMCC) in human urine using liquid
8762 chromatography/tandem mass spectrometry. Rapid Communications in Mass Spectrometry, 22,
8763 2629-2638.

8764 Schettgen T, Mueller J, Fromme H and Angerer J, 2010. Simultaneous quantification of haemoglobin
8765 adducts of ethylene oxide, propylene oxide, acrylonitrile, acrylamide and glycidamide in human
8766 blood by isotope-dilution GC/NCI-MS/MS. Journal of Chromatography B-Analytical Technologies
8767 in the Biomedical and Life Sciences, 878, 2467-2473.

8768 Schouten LJ, Hogervorst JG, Konings EJ, Goldbohm RA, van den Brandt PA, 2009. Dietary
8769 acrylamide intake and the risk of head-neck and thyroid cancers: results from the Netherlands
8770 Cohort Study. American Journal of Epidemiology, 170, 873-884.

8771 Schulze GE and Boysen BG, 1991. A neurotoxicity screening battery for use in safety evaluation:
8772 effects of acrylamide and 3',3'-iminodipropionitrile. Fundamental and Applied Toxicology, 16,
8773 602-615.

8774 Schwend T, Schabacker J, Wetterauer B and Wink M, 2008. Uptake of S-(3-amino-3-oxopropyl)-
8775 cysteine by Caco-2 cells. Zeitschrift für Naturforschung, 63, 913-918.

8776 Sciandrello G, Mauro M, Caradonna F, Catanzaro I, Saverini M and Barbata G, 2010. Acrylamide
8777 catalytically inhibits topoisomerase II in V79 cells. Toxicology in Vitro, 24, 830-834.

8778 Seale SM, Feng Q, Agarwal AK and El-Alfy AT, 2012. Neurobehavioral and transcriptional effects of
8779 acrylamide in juvenile rats. Pharmacology, Biochemistry and Behavior, 101, 77-84.

8780 Sega GA, 1991. Adducts in sperm protamine and DNA vs. mutation frequency. Prog. Clin. Biol. Res.
8781 372, 521-530.

8782 Sega GA, Alcota RP, Tancongco CP and Brimer PA, 1989. Acrylamide binding to the DNA and
8783 protamine of spermiogenic stages in the mouse and its relationship to genetic damage. Mutation
8784 Research, 216, 221-230.

8785 Sega GA, Generoso EE and Brimer PA, 1990. acrylamide exposure induces a delayed unscheduled
8786 DNA-synthesis in germ-cells of male-mice that is correlated with the temporal pattern of adduct
8787 formation in testis DNA. Environmental and Molecular Mutagenesis, 16, 137-142.

8788 Segerbäck D, Calleman CJ, Schroeder JL, Costa LG and Faustman EM, 1995. Formation of N-7-(2-
8789 Carbamoyl-2-Hydroxyethyl)Guanine in DNA of the mouse and the rat following intraperitoneal
8790 administration of [C-14] acrylamide. Carcinogenesis, 16, 1161-1165.

8791 Sen A, Ozgun O, Arinc E and Arslan S, 2012. Diverse action of acrylamide on cytochrome P450 and
8792 glutathione S-transferase isozyme activities, mRNA levels and protein levels in human
8793 hepatocarcinoma cells. Cell Biology and Toxicology, 28, 175-186.

8794 Settels E, Bernauer U, Palavinskas R, Klaffke HS, Gundert-Remy U and Appel KE, 2008. Human
8795 CYP2E1 mediates the formation of glycidamide from acrylamide. Archives of Toxicology, 82,
8796 717-727.

8797 SFOPH (Swiss Federal Office of Public Health), 2002. Assessment of Acrylamide Intake by Duplicate
8798 Diet Study. Bern, Switzerland: Swiss Federal Office of Public Health; 2002.

8799 Shelby MD, Cain KT, Còrnett CV and Generoso WM, 1987. Acrylamide: induction of heritable
8800 translocations in male mice. Environmental Mutagenesis, 9, 363-368.

8801 Shi J, Ma YX, Zheng MG, Ruan ZG, Liu J, Tian SM, Zhang DX, He XH and Li GY, 2012. Effect of
8802 sub-acute exposure to acrylamide on GABAergic neurons and astrocytes in weaning rat
8803 cerebellum. Toxicology and Industrial Health, 28, 10-20.

8804 Shinomol GK, Raghunath N, Bharath MMS and Muralidhara, 2013. Prophylaxis with Bacopa
8805 monnieri attenuates acrylamide induced neurotoxicity and oxidative damage via elevated
8806 antioxidant function. Central Nervous System Agents in Medicinal Chemistry, 13, 3-12.

8807 Shipp A, Lawrence G, Gentry R, McDonald T, Bartow H, Bounds J, Macdonald N, Clewell H, Allen
8808 B and Van Landingham C, 2006. Acrylamide: review of toxicity data and dose-response analyses
8809 for cancer and noncancer effects. Critical Reviews in Toxicology, 36, 481-608.

8810 Shiraishi Y, 1978. Chromosome aberrations induced by monomeric acrylamide in bone marrow and
8811 germ cells of mice. Mutation Research, 57, 313-324.

8812 Siahkoohi S, Anvari M, Tafti MA and Hosseini-Sharifabad M, 2014. The effects of vitamin e on the
8813 liver integrity of mice fed with acrylamide diet. Iranian Journal of Pathology, 9, 89-98.

8814 Sickles DW, Welter DA and Friedman MA, 1995. Acrylamide arrests mitosis and prevents
8815 chromosome migration in the absence of changes in spindle microtubules. Journal of Toxicology
8816 and Environmental Health, 44, 73-86.

8817 Sickles DW, Brady ST, Testino A, Friedman MA and Wrenn RW, 1996. Direct effect of the
8818 neurotoxicant acrylamide on kinesin-based microtubule motility. Journal of Neuroscience
8819 Research, 46, 7-17.

8820 Sickles DW, Stone JD and Friedman MA, 2002. Fast axonal transport: A site of acrylamide
8821 neurotoxicity? Neurotoxicology, 23, 223-251.

8822 Sickles DW, Sperry AO, Testino A and Friedman M, 2007. Acrylamide effects on kinesin-related
8823 proteins of the mitotic/meiotic spindle. Toxicology and Applied Pharmacology, 222, 111-121.

8824 Sirot V, Hommet F, Tard A and Leblanc JC, 2012. Dietary acrylamide exposure of the French
8825 population: results of the second French Total Diet Study. Food and Chemical Toxicology, 50,
8826 889-894.

8827 Sisnaiske J, Hausherr V, Krug AK, Zimmer B, Hengstler JG, Leist M and van Thriel, 2014.
8828 Acrylamide alters neurotransmitter induced calcium responses 3 in murine ESC-derived and
8829 primary neurons. Neurotoxicology, in press.

8830 Smith MK, Zenick H, Preston RJ, et al, 1986. Dominant lethal effects of subchronic acrylamide
8831 administration in the male Long-Evans rat. Mutation Research, 173, 273-277.

8832 Smith CJ, Perfetti TR, Rumple MA, Rodgman A and Doolittle DJ, 2000. "IARC Group 2A
8833 carcinogens" reported in cigarette mainstream smoke. Food and Chemical Toxicology, 38, 371-
8834 383.

8835 SNFA (Swedish National Food Agency), 2002. Acrylamide in Food. Uppsala, Sweden: Swedish
8836 National Food Administration; 2002.

8837 Sobel W, Bond GG, Parsons TW and Brenner FE, 1986. Acrylamide cohort mortality study. Journal of
8838 Industrial Medicine, 43, 785-788.

8839 Solomon JJ, Fedyk J, Mukai F and Segal A, 1985. Direct alkylation of 2'-Deoxynucleosides and DNA
8840 following invitro reaction with acrylamide. Cancer Research, 45, 3465-3470.

8841 Song J, Zhao M, Liu X, Zhu Y, Hu X and Chen F, 2013. Protection of cyanidin-3-glucoside against
8842 oxidative stress induced by acrylamide in human MDA-MB-231 cells. Food and Chemical
8843 Toxicology, 58, 306-310.

8844 Sörgel F, Weissenbacher R, Kinzig-Schippers M, Hofmann A, Illauer M, Skott A and Landersdorfer
8845 C, 2002. Acrylamide: increased concentrations in homemade food and first evidence of its variable
8846 absorption from food, variable metabolism and placental and breast milk transfer in humans.
8847 Chemotherapy, 48, 267-274.

8848 Spencer PS and Schaumburg HH, 1974. A review of acrylamide neurotoxicity. Part II. Experimental
8849 animal neurotoxicity and pathologic mechanisms. The Canadian journal of neurological sciences.
8850 Le Journal Canadien des Sciences Neurologiques, 1, 152-169.

8851 Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, Robert MC and Riediker S, 2002. Food
8852 chemistry: Acrylamide from Maillard reaction products. Nature, 419, 449-450.

8853 Stadler R and Scholz G, 2004. Acrylamide: An update on current knowledge in analysis, levels in
8854 food, mechanisms of formation, and potential strategies of control. Nutrition Reviews, 62, 449-467.

8855 Stone JD, Peterson AP, Eyer J, Oblak TG and Sickles DW, 2001. Neurofilaments are nonessential to
8856 the pathogenesis of toxicant-induced axonal degeneration. The Journal of Neuroscience, 21, 2278-
8857 2287.

8858 Sublet VH, Zenick H and Smith MK, 1989. Factors associated with reduced fertility and implantation
8859 rates in females mated to acrylamide-treated rats. Toxicology, 55, 53-67.

8860 Sumner SC, MacNeela JP and Fennell TR, 1992. Characterization and quantitation of urinary
8861 metabolites of [1,2,3-13C]acrylamide in rats and mice using 13C nuclear magnetic resonance
8862 spectroscopy. Chemical Reseach in Toxicology, 5, 81-89.

8863 Sumner SCJ, Selvaraj L, Nauhaus SK and Fennell TR, 1997. Urinary metabolites from F344 rats and
8864 B6C3F1 mice coadministered acrylamide and acrylonitrile for 1 or 5 days. Chemical Research in
8865 Toxicology, 10, 1152-1160.

8866 Sumner SC, Fennell TR, Moore TA, Chanas B, Gonzalez F and Ghanayem BI, 1999. Role of
8867 cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. Chemical
8868 Reseach in Toxicology, 12, 1110-1116.

8869 Sumner SC, Williams CC, Snyder RW, Krol WL, Asgharian B and Fennell TR, 2003. Acrylamide: a
8870 comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal,
8871 oral, or inhalation exposure. Toxicological Sciences, 75, 260-270.

8872 Sun J, Schnackenberg LK, Pence L, Bhattacharyya S, Doerge DR, Bowyer JF and Beger RD, 2010.
8873 Metabolomic analysis of urine from rats chronically dosed with acrylamide using NMR and
8874 LC/MS. Metabolomics, 6, 550-563.

8875 Surdyk N, Rosen J, Andersson R and Aman P. 2004. Effects of asparagine, fructose and baking
8876 conditions on acrylamide in yeast-leavened wheat bread. Journal of Agricultural and Food
8877 Chemistry, 52, 2047-2051.

8878 Swaen GM, Haidar S, Burns CJ, Bodner K, Parsons T, Collins JJ and Baase C, 2007. Mortality study
8879 update of acrylamide workers. Occupational and Environmental Medicine, 64, 396-401.

8880 Sweeney LM, Kirman CR, Gargas ML, Carson ML and Tardiff RG, 2010. Development of a
8881 physiologically-based toxicokinetic model of acrylamide and glycidamide in rats and humans.
8882 Food and Chemical Toxicology, 48, 668-685.

8883 Szewczyk L, Ulanska J, Dubiel M, Osyczka AM and Tylko G, 2012. The effect of acrylamide and
8884 nitric oxide donors on human mesenchymal progenitor cells. Toxicology in Vitro, 26, 897-906.

8885 Takahashi M, Ohara T and Hashimoto K, 1971. Electrophysiological study of nerve injuries in
8886 workers handling acrylamide. International Arch. Arbeitsmed, 28, 1-11.

8887 Takahashi M, Shibutani M, Inoue K, Fujimoto H, Hirose M and Nishikawa A, 2008. Pathological
8888 assessment of the nervous and male reproductive systems of rat offspring exposed maternally to
8889 acrylamide during the gestation and lactation periods - a preliminary study.The Journal of
8890 Toxicological Sciences, 33, 11-24.

8891 Takahashi M, Shibutani M, Nakahigashi J, Sakaguchi N, Inoue K, Morikawa T, Yoshida M and
8892 Nishikawa A, 2009. Limited lactational transfer of acrylamide to rat offspring on maternal oral
8893 administration during the gestation and lactation periods. Archives of Toxicology, 83, 785-793.

8894 Takahashi M, Inoue K, Koyama N, Yoshida M, Irie K, Morikawa T, Shibutani M, Honma M and
8895 Nishikawa A, 2011. Life stage-related differences in susceptibility to acrylamide-induced neural
8896 and testicular toxicity. Archives of Toxicology, 85, 1109-1120.

8897 Takami S, Imai T, Cho Y-M, Ogawa K, Hirose M and Nishikawa A, 2012. Juvenile rats do not exhibit
8898 elevated sensitivity to acrylamide toxicity after oral administration for 12 weeks. Journal of
8899 Applied Toxicology, 32, 959-967.

8900 Tanii H and Hashimoto K, 1983 Neurotoxicity of acrylamide and related compounds in rats. Effects of
8901 rotarod performance, morphology of nerves and neurotubulin. Archives of Toxicology, 54, 203-
8902 213.

8903 Tardiff RG, Gargas ML, Kirman CR, Carson ML and Sweeney LM, 2010. Estimation of safe dietary
8904 intake levels of acrylamide for humans. Food and Chemical Toxicology, 48, 658-667.

8905 Tareke E, Rydberg P, Karlsson P, Eriksson S and Törnqvist M, 2000. Acrylamide: A cooking
8906 carcinogen? Chemical Research in Toxicology, 13, 517-522.

8907 Tareke E, Rydberg P, Karlsson P, Eriksson S and Törnqvist M, 2002. Analysis of acrylamide, a
8908 carcinogen formed in heated foodstuffs. Journal of Agricultural and Food Chemistry, 50, 4998-
8909 5006.

8910 Tareke E, Twaddle NC, McDaniel LP, Churchwell MI, Young JF and Doerge DR, 2006. Relationships
8911 between biomarkers of exposure and toxicokinetics in Fischer 344 rats and B6C3F1 mice
8912 administered single doses of acrylamide and glycidamide and multiple doses of acrylamide.
8913 Toxicology and Applied Pharmacology, 217, 63-75.

8914 Tareke E, Lyn-Cook B, Robinson B and Ali SF, 2008. Acrylamide: a dietary carcinogen formed *in*
8915 *vivo*? Journal of Agricultural and Food Chemistry, 56, 6020-6023.

8916 Tareke E, Heinze TM, Gamboa da Costa G and Ali S, 2009. Acrylamide formed at physiological
8917 temperature as a result of asparagine oxidation. Journal of Agricultural and Food Chemistry, 57,
8918 9730-9733.

8919 Tarskikh MM, Klimatskaya LG and Kolesnikov SI, 2013. Pathogenesis of neurotoxicity of acrylates
8920 acrylonitrile and acrylamide: from cell to organism. Bulletin of Experimental Biology and
8921 Medicine, 155, 451-453.

8922 Taubert D, Glöckner R, Müller D and Schömig E, 2006. The garlic ingredient diallyl sulfide inhibits
8923 cytochrome P4502E1 dependent bioactivation of acrylamide to glycidamide. Toxicology Letters,
8924 164, 1-5

8925 Tekkeli SEK, Önal C and Önal, A, 2012. A review of current methods for the determination of
8926 acrylamide in food products. Food Analytical Methods, 5, 29-39.

8927 Thielen S, Baum M, Hoffmann M, Loeppky RN and Eisenbrand G, 2006. Genotoxicity of
8928 glycidamide in comparison to (+/-)-anti-benzo a pyrene-7,8-dihydrodiol-9,10-epoxide and alpha-
8929 acetoxy-N-nitroso-diethanolamine in human blood and in mammalian V79-cells. Molecular
8930 Nutrition and Food Research, 50, 430-436.

8931 Tilson HA, 1981. The neurotoxicity of acrylamide – an overview. Neurobehavioral Toxicology and
8932 Teratology, 3, 445-461.

8933 Tilson HA and Cabe PA, 1979. Effects of acrylamide given acutely or in repeated doses on fore- and
8934 hindlimb function in rats. Toxicology and Applied Pharmacology, 47, 252-260.

8935 Tilson HA, Cabe PA and Spencer PS, 1979. Acrylamide neurotoxicity in rats – a correlated neuro-
8936 behavioral and pathological-study. Neurotoxicology, 1, 89-104.

8937 Thomas JA, Poland B and Honzatko R, 1995. Protein sulfhydryls and their role in the antioxidant
8938 function of protein S-thiolation. Archives of Biochemistry and Biophysics, 319, 1-9.

8939 Toker A, Yerlikaya FH, Yener Y and Toy H, 2013. Serum homocysteine, arginine, citrulline and
8940 asymmetric dimethyl arginine levels, and histopathologic examination of the abdominal aorta in
8941 rats exposed to acrylamide. Biotechnic and Histochemistry, 88, 103-108.

8942 Törnqvist M, Mowrer J, Jensen S and Ehrenberg L, 1986. Monitoring of Environmental Cancer
8943 Initiators through Hemoglobin Adducts by a Modified Edman Degradation Method. Analytical
8944 Biochemistry, 154, 255-266.

8945 Tran NL, Barraj LM, Murphy MM and Bi X, 2010. Dietary acrylamide exposure and hemoglobin
8946 adducts-National Health and Nutrition Examination Survey (2003-04). Food and Chemical
8947 Toxicology, 48, 3098-3108.

8948 Truong VD, Pascua YT, Reynolds R, Thompson RL, Palazoğlu TK, Atac Mogol B, Gökmen V, 2014.
8949 Processing Treatments for Mitigating Acrylamide Formation in Sweetpotato French Fries. Journal
8950 of Agricultural and Food Chemistry, 62, 310–316.

8951 Twaddle NC, McDaniel LP, Gamboa da Costa G, Churchwell MI, Beland FA and Doerge DR, 2004.
8952 Determination of acrylamide and glycidamide serum toxicokinetics in B6C3F1 mice using LC-
8953 ES/MS/MS. Cancer Letters, 207, 9-17.

8954 Tyl RW and Friedman MS, 2003. Effects of acrylamide on rodent reproductive performance.
8955 Reproductive Toxicology, 17, 1-13.

8956 Tyl RW, Friedman MA, Losco PE, Fisher LC, Johnson KA, Strother DE an Wolf CH, 2000a. Rat two-
8957 generation reproduction and dominant lethal study of acrylamide in drinking water. Reproductive
8958 Toxicology, 14, 385-401.

8959 Tyl RW, Marr MC, Myers CB, Ross WP and Friedman MA, 2000b. Relationship between acrylamide
8960 reproductive and neurotoxicity in male rats. Reproductive Toxicology, 14, 147-157.

8961 Uphouse LL and Russell M, 1981. Rapid effects of acrylamide on spiroperidol and serotonin binding
8962 in neural tissue. Neurobehavioral Toxicology and Teratology, 3, 281-284.

8963 Uphouse LL, Nemeroff CB, Mason G, Prange AJ and Bondy SC, 1982. Interactions between handling
8964 and acrylamide on endocrine responses in rats. Neurotoxicology, 3, 121-125.

8965 Urban M, Kavvadias D, Riedel K, Scherer G and Tricker AR, 2006. Urinary mercapturic acids and a
8966 hemoglobin adduct for the dosimetry of acrylamide exposure in smokers and nonsmokers.
8967 Inhalation Toxicology, 18, 831-839.

8968 US-EPA (United States-Environmental Protection Agency), 2010. Toxicological review of acrylamide
8969 (CAS No. 79-06-1). In Support of Summary Information on the Integrated Risk Information
8970 System (IRIS). March 2010. EPA/635/R-07/009F. Available at
8971 http://www.epa.gov/iris/toxreviews/0286tr.pdf

8972 US-EPA (United States-Environmental Protection Agency), 2011. Recommended use of body
8973 weight3/4 as a default method in derivation of the oral reference dose.
8974 http://www.epa.gov/raf/publications/interspecies-extrapolation.htm

8975 Van Bladeren PJ, 2000. Glutathione conjugation as a bioactivation reaction. Chemico-Biological
8976 Interactions, 129, 61-76.

8977 Vesper HW, Ospina M, Meyers T, Ingham L, Smith A, Gray JG and Myers GL, 2006. Automated
8978 method for measuring globin adducts of acrylamide and glycidamide at optimized Edman reaction
8979 conditions. Rapid Communications in Mass Spectrometry, 20, 959-964.

8980 Vesper HW, Slimani N, Hallmans G, Tjonneland A, Agudo A, Benetou V, Bingham S, Boeing H,
8981 Boutron-Ruault MC, Bueno-de-Mesquita HB, Chirlaque D, Clavel-Chapelon F, Crowe F, Drogan
8982 D, Ferrari P, Johansson I, Kaaks R, Linseisen J, Lund E, Manjer J, Mattiello A, Palli D, Peeters
8983 PH, Rinaldi S, Skeie G, Trichopoulou A, Vineis P, Wirfält E, Overvad K and Stromberg U, 2008.
8984 Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European
8985 Prospective Investigation into Cancer and Nutrition (EPIC) Study. Journal of Agricultural and
8986 Food Chemistry, 56, 6046-6053.

8987 Vesper HW, Caudill SP, Osterloh JD, Meyers T, Scott D and Myers GL, 2010. Exposure of the U.S.
8988 population to acrylamide in the National Health and Nutrition Examination Survey 2003-2004.
8989 Environmental Health Perspectives, 118, 278-283.

8990 Vesper HW, Sternberg MR, Frame T and Pfeiffer CM, 2013. Among 10 Sociodemographic and
8991 Lifestyle Variables, Smoking Is Strongly Associated with Biomarkers of Acrylamide Exposure in a
8992 Representative Sample of the US Population. Journal of Nutrition, 143, 995S-1000S.

8993 Vikström AC, Eriksson S, Paulsson B, Karlsson P, Athanassiadis I and Törnqvist M, 2008. Internal
8994 doses of acrylamide and glycidamide in mice fed diets with low acrylamide contents. Molecular
8995 Nutrition and Food Research, 52, 974-980.

8996 Vikström AC, Wilson KM, Paulsson B, Athanassiadis I, Grönberg H, Adami HO, Adolfsson J, Mucci
8997 LA, Balter K and Törnqvist M, 2010. Alcohol influence on acrylamide to glycidamide metabolism
8998 assessed with hemoglobin-adducts and questionnaire data. Food and Chemical Toxicology, 48,
8999 820-824.

9000 Vikström AC, Abramsson-Zetterberg L, Naruszewicz M, Athanassiadis I, Granath FN and Törnqvist
9001 MA, 2011. In vivo doses of acrylamide and glycidamide in humans after intake of acrylamide-rich
9002 food. Toxicological Sciences, 119, 41-49.

9003 Vikström AC, Warholm M, Paulsson B, Axmon A, Wirfält E and Törnqvist M, 2012. Hemoglobin
9004 adducts as a measure of variations in exposure to acrylamide in food and comparison to
9005 questionnaire data. Food and Chemical Toxicology, 50, 2531-2539.

9006 Vinci RM, Mestdagh F and De Meulenaer B, 2012. Acrylamide formation in fried potato products -
9007 Present and future, a critical review on mitigation strategies. Food Chemistry, 133, 1138-1154.

9008 von Stedingk H, Vikström AC, Rydberg P, Pedersen M, Nielsen JK, Segerbäck D, Knudsen LE and
9009 Törnqvist M, 2011. Analysis of hemoglobin adducts from acrylamide, glycidamide, and ethylene
9010 oxide in paired mother/cord blood samples from Denmark. Chemical Research in Toxicology, 24,
9011 1957-1965.

9012 Von Tungeln LS, Churchwell MI, Doerge DR, Shaddock JG, McGarrity LJ, Heflich RH, da Costa
9013 GG, Marques MM and Beland FA, 2009. DNA adduct formation and induction of micronuclei and
9014 mutations in B6C3F(1)/Tk mice treated neonatally with acrylamide or glycidamide. International
9015 Journal of Cancer, 124, 2006-2015.

9016 Von Tungeln LS, Doerge DR, da Costa GG, Matilde Marques M, Witt WM, Koturbash I, Pogribny IP
9017 and Beland FA, 2012. Tumorigenicity of acrylamide and its metabolite glycidamide in the neonatal
9018 mouse bioassay. International Journal of Cancer, 131, 2008-2015.

9019 Voogd CE, van der Stel JJ and Jacobs JJ, 1981. The mutagenic action of aliphatic epoxides. Mutation
9020 Research, 89, 269-282.

9021 Walker K, Hattis D, Russ A, Sonawane B and Ginsberg G, 2007. Approaches to acrylamide
9022 physiologically based toxicokinetic modeling for exploring child-adult dosimetry differences.
9023 Journal of Toxicology and Environmental Health A, 70, 2033-2055.

9024 Wang RS, McDaniel LP, Manjanatha MG, Shelton SD, Doerge DR and Mei N, 2010a. Mutagenicity
9025 of acrylamide and glycidamide in the testes of big blue mice. Toxicological Sciences, 117, 72-80.

9026 Wang H, Huang P, Lie T, Li J, Hutz RJ, Li K and Shi F, 2010b. Reproductive toxicity of acrylamide-
9027 treated male rats. Reproductive Toxicology, 29, 225-230.

9028 Watzek N, Bohm N, Feld J, Scherbl D, Berger F, Merz KH, Lampen A, Reemtsma T, Tannenbaum
9029 SR, Skipper PL, Baum M, Richling E and Eisenbrand G, 2012a. N7-glycidamide-guanine DNA
9030 adduct formation by orally ingested acrylamide in rats: a dose-response study encompassing human
9031 diet-related exposure levels. Chemical Research in Toxicology, 25, 381-390.

9032 Watzek N, Scherbl D, Feld J, Berger F, Doroshyenko O, Fuhr U, Tomalik-Scharte D, Baum M,
9033 Eisenbrand G and Richling E, 2012b. Profiling of mercapturic acids of acrolein and acrylamide in
9034 human urine after consumption of potato crisps. Molecular Nutrition and Food Research, 56, 1825-
9035 1837.

9036 Watzek N, Scherbl D, Schug M, Hengstler JG, Baum M, Habermeyer M, Richling E and Eisenbrand
9037 G, 2013. Toxicokinetics of acrylamide in primary rat hepatocytes: coupling to glutathione is faster
9038 than conversion to glycidamide. Archives of Toxicology, 87, 1545-1556.

9039 Wenzl T, de la Calle MB and Anklam E, 2003. Analytical methods for the determination of
9040 acrylamide in food products: a review. Food Additives and Contaminants, 20, 885-902.

9041 Wenzl T and Anklam E, 2005. Evaluation of results of an interlaboratory comparison test on
9042 determination of acrylamide in crispbread samples. Journal of the AOAC International, 88, 1413-
9043 1418.

9044 Wenzl T and Anklam E, 2007. European Union database of acrylamide levels in food: Update and
9045 critical review of data collection. Food Additives and Contaminants, 24, 5-12.

9046 Wenzl T, Szilagyi S, Rosen J and Karasek L, 2008. Validation of an analytical method to determine
9047 the content of acrylamide in roasted coffee. Report on the collaborative trial "Determination of
9048 Acrylamide in Coffee by Isotope Dilution High Performance Liquid Chromatography Tandem
9049 Mass Spectrometry." Available at: http://irmm.jrc.ec.europa.eu/activities/acrylamide/
9050 Documents/eur_23403_en.pdf

9051 WHO (World Health Organisation), 1985. WHO Task group. Acrylamide. Environmental Health
9052 Criteria 49. Worl Heatlh Organisation, Geneva, 1985.

9053 WHO (World Health Organisation), 1999. Acrylamide. International Programme on Chemical Safety.
9054 Poisons Information Monograph (PIM) 652. Acrylamide. Available at
9055 http://www.inchem.org/documents/pims/chemical/pim652.htm

9056 WHO/IPCS (World Health Organisation/International Programme on Chemical Safety), 2008.
9057 Uncertainty and data quality in exposure assessment. Harmonisation project document No. 6. ISBN
9058 978 92 4 156376 5.U.

9059 Wilson KM, Bälter K, Adami HO, Grönberg H, Vikström AC, Paulsson B, Törnqvist M and Mucci
9060 LA, 2009a. Acrylamide exposure measured by food frequency questionnaire and hemoglobin
9061 adduct levels and prostate cancer risk in the Cancer of the Prostate in Sweden Study. International
9062 Journal of Cancer, 124, 2384-2390.

9063 Wilson KM, Mucci LA, Cho E, Hunter DJ, Chen WY and Willett WC, 2009b. Dietary acrylamide
9064 intake and risk of premenopausal breast cancer. American Journal of Epidemiology, 169, 954-961.

9065 Wilson KM, Vesper HW, Tocco P, Sampson L, Rosen J, Hellenas KE, Tornqvist M and Willett WC,
9066 2009c. Validation of a food frequency questionnaire measurement of dietary acrylamide intake
9067 using hemoglobin adducts of acrylamide and glycidamide. Cancer Causes and Control, 20, 269-
9068 278.

9069 Wilson KM, Mucci LA, Rosner BA, Willett WC, 2010. A prospective study on dietary acrylamide
9070 intake and the risk for breast, endometrial, and ovarian cancers. Cancer Epidemiol Biomarkers
9071 Prev. 19, 2503-2515.

9072 Wilson KM, Giovannucci E, Stampfer MJ and Mucci LA, 2012. Dietary acrylamide and risk of
9073 prostate cancer. International Journal on Cancer, 131, 479-87.

9074 Wirfält E, Paulsson B, Törnqvist M, Axmon A and Hagmar L, 2008. Associations between estimated
9075 acrylamide intakes, and hemoglobin AA adducts in a sample from the Malmo Diet and Cancer
9076 cohort. European Journal of Clinical Nutrition, 62, 314-323.

9077 Wise LD, Gordon LR, Soper KA, et al, 1995. Developmental neurotoxicity evaluation of acrylamide
9078 in Sprague-Dawley rats. Neurotoxicology and Teratology, 17, 189-198.

9079 Witt KL, Livanos E, Kissling GE, Torous DK, Caspary W, Tice RR and Recio L, 2008. Comparison
9080 of flow cytometry- and microscopy-based methods for measuring micronucleated reticulocyte
9081 frequencies in rodents treated with nongenotoxic and genotoxic chemicals. Mutation Research-
9082 Genetic Toxicology and Environmental Mutagenesis, 649, 101-113.

9083 Working PK, Bentley KS, Hurtt ME, Mohr KL, 1987. Comparison of the dominant lethal effects of
9084 acrylonitrile and acrylamide in the male F344 rat. Mutagenesis, 2, 215-220.

9085 Xiao Y and Tates AD, 1994. Increased frequencies of micronuclei in early spermatids of rats
9086 following exposure of young primary spermatocytes to acrylamide. Mutation Research, 309, 245-
9087 253.

9088 Xie J, Terry KL, Poole EM, Wilson KM, Rosner BA, Willett WC, Vesper HW and Tworoger SS,
9089 2013. Acrylamide hemoglobin adduct levels and ovarian cancer risk: a nested case-control study.
9090 Cancer Epidemiology Biomarkers and Prevention, 22, 653-660.

9091 Xiwen H, Jing L, Tao C and Ke Y, 1992. Studies on biochemical mechanism of neurotoxicity induced
9092 by acrylamide in rats. Biomedical and Environmental Sciences, 5, 276-281.

9093 Yang HJ, Lee SH, Jin Y, Choi JH, Han DU, Chae C, Lee MH and Han CH, 2005. Toxicological
9094 effects of acrylamide on rat testicular gene expression profile. Reproductive Toxicology, 19, 527-
9095 534.

9096 Yener Y, 2013. Effects of long term low dose acrylamide exposure on rat bone marrow polychromatic
9097 erythrocytes. Biotechnic & Histochemistry: official publication of the Biological Stain
9098 Commission, 88, 356-360.

9099 Yener Y and Dikmenli M, 2009. Increased micronucleus frequency in rat bone marrow after
9100 acrylamide treatment. Food and Chemical Toxicology, 47, 2120-2123.

9101 Yener Y and Dikmenli M, 2011. The effects of acrylamide on the frequency of megakaryocytic
9102 emperipolesis and the mitotic activity of rat bone marrow cells. Journal of the Science and Food
9103 Agriculture, 91, 1810-1813.

9104 Yener Y, Kalipci E, Oztas H, Aydin AD and Yildiz H, 2013a. Possible neoplastic effects of
9105 acrylamide on rat exocrine pancreas. Biotechnic and Histochemistry, 88, 47-53.

9106 Yener Y, Sur E, Telatar T and Oznurlu Y, 2013b. The effect of acrylamide on alpha-naphthyl acetate
9107 esterase enzyme in blood circulating lymphocytes and gut associated lymphoid tissues in rats.
9108 Experimental Toxicologic Pathology, 65, 143-146.

9109 Yerlikaya FH, Toker A and Yener Y, 2013. Effects of acrylamide treatment on oxidant and
9110 antioxidant levels in rats. Kafkas Universitesi Veteriner Fakultesi Dergisi, 19, 607-612.

9111 Young JF, Luecke RH and Doerge DR, 2007. Physiologically based
9112 pharmacokinetic/pharmacodynamic model for acrylamide and its metabolites in mice, rats, and
9113 humans. Chemical Research in Toxicology, 20, 388-399.

9114 Yoshimura S, Imai K, Saitoh Y, Yamaguchi H and Ohtaki S, 1992. The same chemicals induce
9115 different neurotoxicity when administered in high-doses for short-term of low-doses for long-term
9116 to rats and dogs. Molecular and Chemical Neuropathology, 16, 59-84.

9117 Yuan Y, Zhang H, Miao Y and Zhuang H, 2014. Study on the methods for reducing the acrylamide
9118 content in potato slices after microwaving and frying processes. RSC Advances, 4, 1004-1009.

9119 Zając J, Bojar I, Helbin J, Kolarzyk E, Potocki A, Strzemecka J and Owoc A, 2013. Dietary
9120 acrylamide exposure in chosen population of South Poland. Annals of Agricultural and
9121 Environmental Medicine (AAEM), 20, 351-355.

9122 Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K and Speck W, 1987. Salmonella
9123 mutagenicity tests: III. Results from the testing of 255 chemicals. Environmental Mutagenesis, 9,
9124 1-109.

9125 Zeiger E, Recio L, Fennell TR, Haseman JK, Snyder RW and Friedman M, 2009. Investigation of the
9126 low-dose response in the in vivo induction of micronuclei and adducts by acrylamide.
9127 Toxicological Sciences, 107, 247-257.

9128 Zenick H, Hope E and Smith MK, 1986. Reproductive toxicity associated with acrylamide treatment
9129 in male and female rats. Journal of Toxicology and Environmental Health, 17, 457-472.

9130 Zhang Y, Zhang G and Zhang Y, 2005. Occurrence and analytical methods of acrylamide in heat-
9131 treated foods. Review and recent developments. Journal of Chromatography A, 1075, 1-21.

9132 Zhang JX, Yue WB, Ren YS and Zhang CX, 2010. Enhanced fat consumption potentiates acrylamide-
9133 induced oxidative stress in epididymis and epididymal sperm and effect spermatogenesis in mice.
9134 Toxicology Mechanisms and Methods, 20, 75-81.

9135 Zhang L, Gavin T, Barber DS and LoPachin RM, 2011. Role of the Nrf2-ARE pathway in acrylamide
9136 neurotoxicity. Toxicology Letters, 205, 1-7.

9137 Zhang L, Zhang H, Miao Y, Wu S, Ye H and Yuan Y, 2012. Protective effect of allicin against
9138 acrylamide-induced hepatocyte damage in vitro and in vivo. Food and Chemical Toxicology, 50,
9139 3306-3312.

9140 Zhang LL, Wang ET, Chen F, Yana HY and Yuan Y, 2013. Potential protective effects of oral
9141 administration of allicin on acrylamide-induced toxicity in male mice. Food & Function, 4, 1229-
9142 1236.

9143 Zhou PP, Zhao YF, Liu HL, Ma YJ, Li XW, Yang X and Wu YN, 2013. Dietary exposure of the
9144 Chinese population to acrylamide. Biomedical and Environmental Sciences, 26, 421-429.

9145 Zödl B, Schmid D, Wassler G, Gundacker C, Leibetseder V, Thalhammer T and Ekmekcioglu C,
9146 2007. Intestinal transport and metabolism of acrylamide. Toxicology, 232, 99-108.

9147 Zyzak DV, Sanders RA, Stojanovic M, Tallmadge DH, Eberhart BL, Ewald DK, Gruber DC, Morsch
9148 TR, Strothers MA, Rizzi GP and Villagran MD, 2003. Acrylamide formation mechanism in heated
9149 foods. Journal of Agricultural and Food Chemistry, 51, 4782-4787.

9150 **APPENDICES**

9151 **Appendix A. Identification and selection of evidence relevant for the draft risk assessment of**
9152 **acrylamide (AA) in food**

9153 For the draft risk assessment of AA in food, the CONTAM Panel applied the general principles of the
9154 risk assessment procedure as follows:

9155 - The potential health effects of AA were identified and characterised on the basis of the
9156 available scientific studies published in the open literature (hazard identification and
9157 characterisation, see Section 7).

9158 - An exposure assessment for AA in food was performed in order to compute the current level
9159 of intake of AA in the population and to cover specific consumption habits (see Sections 4, 5
9160 and 6).

9161 - The risk was characterised by comparing the reference point(s) identified with the exposure
9162 estimates to conclude on the likelihood of adverse effects (see Section 8).

9163 The principles stated in the following EFSA publications were applied in the various steps of the
9164 process:

9165 • EFSA (European Food Safety Authority), 2005b. Opinion of the Scientific Committee on a
9166 request from EFSA related to a harmonised approach for risk assessment of substances which
9167 are both genotoxic and carcinogenic. The EFSA Journal 2005, 282, 1-31.

9168 • EFSA (European Food Safety Authority), 2006. Guidance of the Scientific Committee on a
9169 request from EFSA related to uncertainties in Dietary Exposure Assessment. The EFSA
9170 Journal 2006, 438, 1-54.

9171 • EFSA (European Food Safety Authority), 2009b. Guidance of the Scientific Committee on use
9172 of the benchmark dose approach in risk assessment. The EFSA Journal 2009, 150, 1-72.

9173 • EFSA (European Food Safety Authority), 2009c. Guidance of the Scientific Committee on
9174 transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General
9175 principles. The EFSA Journal 2009, 1051, 1-22.

9176 • EFSA (European Food Safety Authority), 2011d. Use of BMDS and PROAST software
9177 packages by EFSA Scientific Panels and Units for applying the Benchmark Dose (BMD)
9178 approach risk assessment. Technical Report. EFSA Supporting Publications 2011, EN-113,
9179 190 pp.

9180 • EFSA (European Food Safety Authority), 2011e. Overview of the procedures currently used at
9181 EFSA for the assessment of dietary exposure to different chemical substances. EFSA Journal
9182 2011;9(12):2490, 33 pp. doi:10.2903/j.efsa.2011.2490

9183 • EFSA SC (EFSA Scientific Committee), 2012a. Guidance on selected default values to be
9184 used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual
9185 measured data. EFSA Journal 2012;10(3):2579, 32 pp. doi:10.2903/j.efsa.2012.2579

9186 • EFSA SC (EFSA Scientific Committee), 2012b. Scientific Opinion on Risk Assessment
9187 Terminology. EFSA Journal 2012;10(5):2664, 43 pp. doi:10.2903/j.efsa.2012.2664

9188 ## A.1. Hazard identification and characterisation

9189 ### A.1.1. Identification of scientific evidence

9190 The CONTAM Panel published in 2005 a statement based on the principal conclusions and
9191 recommendations of the summary report of the JECFA risk assessment performed in 2005 (full report
9192 published in 2006), that considered the scientific evidence available until that date. The CONTAM
9193 Panel agreed with the principal conclusions and recommendations of the JECFA summary report and
9194 concluded that an additional evaluation by the Panel was not necessary at that time (see Section 1.1).

9195 For the current risk assessment, the Panel considered the information available until the publication of
9196 the CONTAM Panel statement as well as new information that has become available since then as
9197 follows:

9198 - The background information was taken from earlier scientific evaluations on AA in food
9199 performed by national agencies, national and international independent expert advisory
9200 committees (see Section 1.1).

9201 The information provided in these assessments was complemented by a scientific literature search
9202 (Web of Science) covering the period between 1 January 1950 and 31 December 2004 using as search
9203 terms 'acrylamide' and 'cancer*' or 'carcin*' or 'develop*' or 'epidem*' or 'genotox*' or 'neur*' or '
9204 neurotox*' or ' reproductive tox*'. This search strategy identified a total of 7 479 records.

9205 - Search in scientific databases aimed at identifying studies published in the open scientific
9206 literature and in scientific peer-reviewed journals

9207 The collection of scientific studies available in the public domain published since 2005 was done
9208 through searching scientific literature databases (Web of Science and PubMed). The approach for
9209 searching was sensitive to retrieve as many studies as possible relevant for the hazard identification
9210 and characterisation of AA. The search terms contained only the term 'acrylamide' and 'glycidamide'
9211 (its main metabolite) without additional search terms. The search was performed to cover the period
9212 between 1 January 2005 and 31 December 2013. The search strategy identified a total of 14 796 (Web
9213 of Science) and 3 619 (PubMed) records relevant to AA in food, respectively. In order to update the
9214 database, a search was conducted regularly until 5 May 2014.

9215 - A consultation procedure with the EFSA Stakeholder Consultative Platform (SHP)

9216 This consultation aimed at obtaining information on research projects or risk assessments the SHP
9217 organisations were performing or had completed which were not in the public domain and could be of
9218 interest for EFSA's risk assessment on AA in food. The consultation was launched on 23 April 2013
9219 (closure date, 24 May 2013)[38]. This call resulted in the documents and information listed under the
9220 section 'Documentation provided to EFSA'.

9221 Within the comments submitted by FoodDrinkEurope (FDE), a (non-exhaustive) list of publications
9222 considered by FDE as relevant were provided. This list consisted of 20 studies published after the
9223 2010 JECFA evaluation (FAO/WHO, 2011), i.e. between 2010 and 2013.

9224 ### A.1.2. Study selection process and its results

9225 The literature database retrieved as above, included scientific peer-reviewed papers published in
9226 scientific journals and relevant non-peer reviewed papers (such as conference proceedings, risk
9227 assessment reports by national/international bodies and book chapters).

[38] http://registerofquestions.efsa.europa.eu/roqFrontend/questionsListLoader?mandate=M-2013-0002

The following criteria were considered for the inclusion of studies in the selection process:

- Experimental toxicity studies on AA or GA *in vitro*,
- Experimental toxicity studies on AA or GA in laboratory animals (oral route),
- Epidemiological studies in humans addressing associations between AA exposure and adverse health outcome(s) in the general population (via the diet) and in occupationally exposed populations (via inhalation),

The following criteria were considered for the non-inclusion of studies in the selection process:

- Studies in the field of ecotoxicity, i.e. not in the field of laboratory animals and human health,
- Studies on polyacrylamide gels or AA used as vehicle/polymers and other similar applications.

In addition, the following criteria were used for the inclusion/non-inclusion of scientific papers and reports in the selection process:

- Only studies in English were considered,
- Abstracts of conference proceedings were not considered,
- Reviews were considered as source of background information and as an additional source of scientific evidence. These were not considered as the basis for the hazard characterization.
- Book chapters were considered as sources of background information. These were not considered as the basis for the hazard characterization.

A.1.3. Result of the study selection process

The result of the study selection process was as follows:

- 1 019 studies published in the open literature were identified by the literature searches for the period indicated related to the hazard identification and characterisation of acrylamide in food.
- The 20 studies listed by FDE and submitted through the consultation with the EFSA Stakeholder Consultative Platform were all identified in the literature searches as described above.
- The studies mentioned in the proposal from organisations belonging to four EU Member States (Denmark, France, Germany and Sweden) to consider new scientific findings on the possible carcinogenicity of acrylamide sent to EFSA September 2012 were also all identified in the literature searches as described above.
- The studies were grouped according to topics of interest, e.g. toxicokinetics, general toxicity, genotoxicity, reproductive and developmental effects, and study type, i.e. human, experimental animal or *in vitro* study.
- The selection of the studies for inclusion or non-inclusion in the hazard identification and characterisation of AA was based on consideration of the extent to which the study was relevant to the assessment and general study quality considerations (e.g. sufficient details on the methodology, performance or outcome of the studies, route of administration, clear dose-response relationship, statistical description of the results (EFSA, 2009c), irrespective of whether they yielded positive, negative, or null results.
- The further application of the selection process described above resulted in the studies cited in the draft opinion.

9269 **A. 2. Exposure assessment**

9270 **A.2.1. Identification of evidence**

9271 The following sources of evidence were used:

9272 - The official monitoring data collected by the Member States/European Countries submitted in
9273 the framework of the EFSA continuous call for data[39].

9274 - An *ad-hoc* call for data entitled 'Call for acrylamide occurrence data in food and beverages
9275 intended for human consumption collected outside official controls' was launched on 25 April
9276 2013 with a closure date of 30 June 2013. This call aimed at the submission of occurrence data
9277 on AA in food from food business operators and other stakeholders collected from 2010
9278 onwards[40].

9279 - The EFSA Comprehensive European Food Consumption Database as a source of information
9280 on food consumption across the EU[41].

9281 **A.2.2. Data selection process and its results**

9282 The consideration of the selection criteria for occurrence and consumption data for the AA dietary
9283 exposure assessment and the results thereafter are described in Sections 4, 5 and 6.

9284 **A.3. Other sections of the draft scientific opinion**

9285 The sections on Introduction (Section 1 and sub-section therein), Legislation (Section 2), Sampling
9286 and methods of analysis (Section 3 and sub-section therein), Previously reported literature data on AA
9287 in food (Section 4.3) and Previously reported human exposure assessment (Section 6.3 and sub-
9288 sections therein) provide generic background information on AA to support the overall conclusions.
9289 The identification of evidence for these chapters was limited to the most relevant information.

9290 The following sources of evidence were used:

9291 - Previous scientific evaluations by national agencies, national and international independent
9292 expert advisory committees,

9293 - A consultation procedure with the EFSA Stakeholder Consultative Platform. The documents
9294 provided by BEUC, the European Consumers Association, have been considered under
9295 Section 4.3.2.

9296 - Search in scientific databases (Web of Science) aimed at identifying studies and reviews that
9297 have appeared in the open scientific literature and published in scientific peer-reviewed
9298 journals. These were identified in the search performed as explained in A.1.1.

9299 - Respective legislation on AA with special emphasis on food.

9300 The selection of the studies for inclusion in the aforementioned sections to give a comprehensive but
9301 not exhaustive background information on AA, was therefore based on consideration of the extent to
9302 which the study was informative and relevant to the assessment and general study quality
9303 considerations.

9304

9305

[39] http://www.efsa.europa.eu/en/data/call/datex101217.htm
[40] http://www.efsa.europa.eu/en/dataclosed/call/130425.htm
[41] http://www.efsa.europa.eu/en/datex/datexfoodcdb.htm

Appendix B. Distribution of acrylamide (AA) levels according to additional information from the data suppliers

Table B1: Distribution of acrylamide (AA) levels in µg/kg

	Type	N[(a)]	LC[(b)]	Mean MB [LB-UB][(c)]	P95 MB [LB-UB][(c)]
	Potato fried products				
1	Fresh	977	14.4	275 [270 - 280]	775
2	Pre-fabricate	48	20.8	197 [190 - 203]	-
	French fries				
1	Baked in the oven	161	13.7	257 [254 - 260]	1035
2	Deep fried	411	18.5	243 [238 - 249]	632
	Potato crisps made from fresh potatoes and potato dough				
1	Batch process	1 412	0.0	327	767
2	Continuous process	32 289	0.0	387	923
	Potato crisps made from fresh potatoes only				
1	Batch process	1 412	0.0	327	767
2	Continuous process	29 557	0.0	391	941
	Bread				
1	Soft bread	504	50.0	43 [36 - 49]	158
2	Toasted bread[(d)]	39	33.3	43 [37 - 48]	-
3	Crisp bread and rusk	389	22.4	163 [158 - 167]	444
	Soft bread				
1	Rye	85	37.6	57 [53 - 61]	240
2	Wheat	302	45.0	38 [33 - 44]	120
	Crisps and rusks				
1	Rye	180	1.1	245 [244 - 245]	529
2	Wheat	116	34.5	126 [119 - 133]	400
	Roasted coffee				
1	Dark	15	0	187	-
2	Medium	44	0	266	-
3	Light	45	0	374	-
	Roasted coffee				
1	Regular coffee	561	6.4	245 [244 - 247]	510
2	Decaffeinated	34	14.7	319 [317 - 321]	-
	Instant coffee				
1	Regular Coffee	788	0.8	718	1133
2	Decaffeinated	74	5.4	630 [629 - 631]	941

(a): N: number of samples.
(b): LC: percentage of censored results.
(c) Mean MB [LB-UB], P95 MB [LB-UB]: mean and 95th percentile contamination level presented as the middle bound estimate (lower bound estimate; upper bound estimate). When the middle, lower and upper bound estimates are equal, only one estimate is given. In case of too few observations (less than 60 for the 95th percentile), the estimation may be biased and is not provided.
(d): Samples taken from the market.

Appendix C. French fries and potato fried, potato crisps and coffee consumption levels estimates from the Comprehensive Database

Table C1: Overview on 'French fries and potato fried' consumption (g/day) by age group. Minimum, median and maximum of the mean and 95th percentile (P95) values across European countries and dietary surveys are shown.

	Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
All population							
Average (g/day)							
Minimum	0	6.0	9.5	11	11	7.3	6.0
Median	< 0.05	13	19	26	24	16	13
Maximum	0.7	22	57	70	58	34	30
P95 (g/day) [(a)]							
Minimum	0	32	42	58	56	43	49
Median	0	47	72	122	105	71	67
Maximum	5.4	86	185	200	155	120	107
Consumers only							
Percentage of consumers							
Minimum	0	14	21	17	15	9.9	12
Median	0.2	44	44	44	34	25	24
Maximum	8.2	61	85	83	80	58	56
Average (g/day)							
Minimum	8.0	17	20	30	27	27	24
Median	17	32	40	68	69	60	52
Maximum	26	52	88	136	104	83	92
P95 (g/day)[(a)]							
Minimum	-	38	46	75	68	58	118
Median	-	52	93	148	155	115	127
Maximum	-	102	200	265	225	143	133

Note: In order to avoid the impression of too high precision, the numbers for all consumption estimates are rounded to 2 figures. A '0' indicates the absence of consumption.
(a): The 95th percentile (P95) estimates obtained on dietary surveys/age groups with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they were not included in this table.

9323 **Table C2:** Overview on 'Potato crisps' consumption (g/day) by age group. Minimum, median and maximum of the mean and 95th percentile (P95) values
9324 across European countries and dietary surveys are shown.

	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
All population						
Average (g/day)						
Minimum	0	0	1.2	0.6	0	0
Median	1.0	3.0	4.7	2.5	0.3	0.3
Maximum	4.4	6.4	11	7.0	2.1	1.4
P95 (g/day)[(a)]						
Minimum	0	0	7.1	0	0	0
Median	2.0	17	26	17	0	0
Maximum	13	38	80	45	13	7.5
Consumers only						
Percentage of consumers						
Min	0	0	8.3	1.5	0	0
Median	6.8	17	19	9.6	3.1	1.3
Maximum	28	52	59	39	23	19
Average (g/day)						
Minimum	1.3	4.5	5.6	5.7	3.8	3.8
Median	8.3	14	20	22	13	8.6
Maximum	75	37	65	53	50	33
P95 (g/day)[(a)]						
Minimum	-	14	15	18	-	-
Median	-	32	48	50	-	-
Maximum	-	50	113	125	-	-

9325 Note: No consumption event of potato crisps was reported for the infants age groups in the Comprehensive database, so no consumption statistics were derived for this population group. In order
9326 to avoid the impression of too high precision, the numbers for all consumption estimates are rounded to 2 figures. A '0' indicates the absence of consumption.
9327 (a): The 95th percentile (P95) estimates obtained on dietary surveys/age groups with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they were not included
9328 in this table.
9329

Table C3: Overview on 'Coffee' consumption (g dry equivalent/day) by age group. Minimum, median and maximum of the mean and 95th percentile (P95) values across European countries and dietary surveys are shown.

	Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
All population							
Average (g/day)							
Minimum	0	0	0	0.1	2.5	2.4	1.9
Median	0	< 0.05	0.1	0.8	7.6	12	8.5
Maximum	< 0.05	0.2	1.0	5.5	38	47	39
P95 (g/day)[(a)]							
Minimum	0	0	0	0	6.7	13	11
Median	0	0	0	3.6	23	31	22
Maximum	0	0	3.1	13	103	100	67
Consumers only							
Percentage of consumers							
Minimum	0	0	0	2.0	50	50	34
Median	0	0.3	2.7	14	77	90	88
Maximum	0.2	2.8	14	58	89	96	96
Average (g/day)							
Minimum	< 0.05	< 0.05	0.8	1.7	3.9	4.9	4.6
Median	< 0.05	1.0	2.4	4.3	9.8	14	11
Maximum	< 0.05	6.7	7.4	10	49	49	41
P95 (g/day)[(a)]							
Minimum	-	-	3.5	6.7	8.0	17	17
Median	-	-	3.5	10	26	32	28
Maximum	-	-	3.5	30	106	100	67

Note: In order to avoid the impression of too high precision, the numbers for all consumption estimates are rounded to 2 figures. A '0' indicates the absence of consumption.

(a): In some surveys, the coffee consumption level has been reported as coffee bean or instant powder and water, whereas in some other surveys, coffee has been reported directly as a beverage. For sake of consistency, all the coffee consumption levels have been converted in dry equivalent basis, using the following dilution factors: 0.125 for coffee espresso, 0.053 for coffee Americano, 0.044 for cappuccino, 0.063 for coffee macchiato, 0.035 for iced coffee and coffee with milk, 0.017 for instant coffee beverage, and 0.06 for unspecified coffee beverage.

(b): The 95th percentile (P95) estimates obtained on dietary surveys/age groups with less than 60 observations may not be statistically robust (EFSA, 2011b) and therefore they were not included in this table.

Appendix D. Acrylamide (AA) occurrence levels used in the exposure assessment

Baseline exposure scenario

Table D1: Acrylamide (AA) occurrence levels used in the baseline exposure scenario

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
1	**Grains and grain-based products**				
2	**Grains for human consumption**	**10.4 - Grains for human consumption**	**73**	**46**	**46**
2	**Grain milling products**	**10.4 - Grains milling products**	**17**	**117**	**117**
2	**Bread and rolls**				
3	Wheat bread and rolls	4.1			
ahg 4	*Wheat bread and rolls, not toasted*	*4.1*	*302*	*39*	*39*
ahg 4	*Wheat bread and rolls, toasted*	*4.1*	*302*	*39*	*39*
4	*Unspecified wheat bread and rolls*	*4.1*	*302*	*39*	*39*
3	Rye bread and rolls				
ahg 4	*Rye bread and rolls, not toasted*	*4.2 – Rye*	*85*	*57*	*57*
ahg 4	*Rye bread and rolls, toasted*	*4.2 – Rye*	*85*	*57*	*57*
4	*Unspecified wheat bread and rolls*	*4.2 – Rye*	*85*	*57*	*57*
3	Mixed wheat and rye bread and rolls				
ahg 4	*Mixed bread and rolls, not toasted*	*4.1 – 4.2 - Rye*	*387*	*43*	*43*
ahg 4	*Mixed bread and rolls, toasted*	*4.1 – 4.2 - Rye*	*387*	*43*	*43*
4	*Unspecified mixed bread and rolls*	*4.1 – 4.2 - Rye*	*387*	*43*	*43*
3	Multigrain bread and rolls				
ahg 4	*Multigrain bread and rolls, not toasted*	*4.1 - 4.2 – 4.3*	*543*	*43*	*43*
ahg 4	*Multigrain bread and rolls, toasted*	*4.1 - 4.2 – 4.3*	*543*	*43*	*43*
4	*Unspecified multigrain bread and rolls*	*4.1 - 4.2 – 4.3*	*543*	*43*	*43*
3	Unleavened bread, crisp bread and rusk				
ahg 4	*Crisp bread, rye*	*6.2 - Crisp and rusk - rye*	*178*	*233*	*233*
ahg 4	*Crisp bread, wheat*	*6.2 - Crisp and rusk - wheat*	*101*	*107*	*107*
ahg 4	*Rusk, unspecified*	*6.2 - Crisp and rusk*	*389*	*163*	*163*
ahg 4	*Other unleavened bread (pita, matzo)*	*4.1 - 4.2 - 4.3*	*543*	*43*	*43*
4	*Unspecified unleavened bread, crisp bread and rusk*	*6.2*	*528*	*171*	*171*
3	Other bread				
4	*Extruded bread*	*6.2 - Other*	*22*	*287*	*287*
ahg 4	*Potato and potato-rye bread*	*10.5 - Bread*	*3*	*570*	*570*
ahg 4	*Rice and soya bread*	*4.2 - Other - 6.2 Other*	*34*	*193*	*193*
ahg 4	*Other other bread (corn, muesli, etc…)*	*4.2 - Other*	*12*	*8*	*33*
4	*Unspecified other bread*	*4.2 - Other - 6.2 Other*	*34*	*193*	*193*
3	Bread products				
ahg 4	*Breadcrumbs and croutons*	*6.2 - Other*	*22*	*287*	*287*
4	*Bread stuffing*	*Not taken into account*	*-*	*0.0*	*0.0*
4	*Other bread products*	*4.1 - 4.2 - 4.3 - 6.2*	*1 071*	*106*	*106*

Table continued overleaf.

9344 **Table D1:** Acrylamide (AA) occurrence levels used in the baseline exposure scenario (continued)

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
3	Unspecified bread and rolls	4.1 - 4.2 - 4.3	543	43	43
2	**Pasta (Raw)**	**10.4 - Pasta**	**9**	**< 1**	**25**
2	**Breakfast cereals**				
3	Cereal flakes				
4	*Barley, rice, millet, spelt flakes*	*5.1 - 5.3*	*730*	*180*	*180*
ahg 4	*Corn flakes*	*5.1*	*210*	*102*	*102*
4	*Millet flakes*	*5.1*	*210*	*102*	*102*
ahg 4	*Oat bran / wholemeal flakes*	*5.3*	*520*	*211*	*211*
ahg 4	*Oat flakes*	*5.1*	*210*	*102*	*102*
4	*Rye flakes*	*5.2*	*151*	*170*	*170*
ahg 4	*Wheat flakes*	*5.2 - 5.3*	*671*	*202*	*202*
4	*Wheat germs flakes*	*5.2*	*151*	*170*	*170*
4	*Mixed cereal flakes*	*5.1 - 5.3*	*730*	*180*	*180*
4	*Rice and wheat flakes with chocolate*	*5.1 - 5.2 - 5.3*	*881*	*178*	*178*
4	*Unspecified cereal flakes*	*5.1 - 5.2 - 5.3*	*881*	*178*	*178*
3	Muesli	5.1 - 5.2 - 5.3	881	178	178
3	Cereal bars	5.1 - 5.2 - 5.3	881	178	178
3	Popped cereals	5.3	520	211	211
3	Mixed breakfast cereals	5.1 - 5.2 - 5.3	881	178	178
3	Grits	10.1	9	29	29
3	Porridge	10.1	9	29	29
3	Unspecified breakfast cereals	5.1 - 5.2 - 5.3 - 10.1	890	176	176
2	**Fine bakery wares**				
3	Pastries and cakes				
ahg 4	*Gingerbread and lebkuchen*	*6.4*	*693*	*407*	*407*
4	*Waffles*	*6.3*	*682*	*201*	*201*
4	*Macaroons*	*6.3*	*682*	*201*	*201*
4	*Other and unspecified pastries and cakes*	*10.2*	*198*	*66*	*66*
3	Biscuits (cookies)				
4	*Speculaas*	*6.4*	*693*	*407*	*407*
ahg 4	*Biscuits, sticks, crackers, salty*	*6.1*	*162*	*231*	*231*
4	*Other biscuits (cookies)*	*6.3*	*682*	*201*	*201*
4	*Unspecified biscuits (cookies)*	*6.1 - 6.3 - 6.4*	*1 537*	*297*	*297*
3	Unspecified fine bakery wares	6.1 - 6.3 - 6.4 - 10.2	1 735	271	271
2	**Unspecified grains and grain-based products**	**Not taken into account**	**-**	**-**	**-**
1	**Vegetables and vegetable products**				
2	**Cocoa beans and cocoa products**				
3	Cocoa powder	10.6 – powder	13	179	179
3	Cocoa beverage-preparation, powder	10.6 – powder	13	179	179

9345 Table continued overleaf.
9346

9347 **Table D1:** Acrylamide (AA) occurrence levels used in the baseline exposure scenario (continued)

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
2	**Coffee beans and coffee products (Solid)**				
ahg 3	Coffee beans	7.1	595	249	249
3	Instant coffee, powder	7.2 – regular	788	718	718
3	Instant coffee, powder, decaffeinated	7.2 - decaffeinated	74	630	630
2	**Coffee substitutes (Solid)**				
ahg 3	Malt and barley coffee	7.3	20	510	510
3	Chicory coffee	7.4	37	2 942	2 942
3	Other and unspecified coffee substitutes (Solid)	7.3 - 7.4 - 7.5	88	1 499	1 499
2	**Other and unspecified vegetables and vegetable products**	**Not taken into account**	**-**	**-**	**-**
1	**Starchy roots and tubers**				
2	**Potatoes and potatoes products**				
ahg	French fries	1.1 - 1.2 - 1.3 – 1.4	1 598	290	290
ahg	Potato pancake (fritter, rosti, etc)	1.5	96	606	606
ahg	Non fried potato products (potato boiled, baked, mashed, etc…)	10.5 - other	37	66	75
2	**Other and unspecified starchy roots and tubers**	**Not taken into account**	**-**	**-**	**-**
1	**Legumes, nuts and oilseeds**				
2	**Legumes, beans, dried**				
3	Peanut (Arachis hypogea)	11.1	40	93	93
3	Other and unspecified legumes, beans, dried	Not taken into account	-	0	0
2	**Tree nuts**	**11.1**	**40**	**93**	**93**
2	**Oilseeds**	**11.1**	**40**	**93**	**93**
2	**Unspecified legumes, nuts and oilseeds**	**Not taken into account**	**-**	**-**	**-**
1	**Fruit and fruit products**				
2	**Citrus fruits**	**Not taken into account**	**-**	**-**	**-**
2	**Pome fruits**	**Not taken into account**	**-**	**-**	**-**
2	**Stone fruits**	**Not taken into account**	**-**	**-**	**-**
2	**Berries and small fruits**	**Not taken into account**	**-**	**-**	**-**
2	**Oilfruits**	**Not taken into account**	**-**	**-**	**-**
2	**Miscellaneous fruits**				
3	Table olives (Olea europaea)	11.2	3	454	454
3	Other and unspecified miscellaneous fruits	Not taken into account	-	-	-
2	**Dried fruits**				
3	Dried prunes (Prunus domestica)	11.3	18	89	89
3	Dried dates (Phoenix dactylifera)	11.3	18	89	89
3	Other and unspecified dried fruits	Not taken into account	-	-	-
2	**Jam, marmalade and other fruit spreads**				
3	Jam				
4	*Jam, Plums (*Prunus domestica*)*	*11.3*	*18*	*89*	*89*
4	*Other and unspecified jam*	*Not taken into account*	-	-	-

9348 Table continued overleaf.
9349

9350 **Table D1:** Acrylamide (AA) occurrence levels used in the baseline exposure scenario (continued)

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
3	Other and unspecified jam, marmalade and other fruit spreads	Not taken into account	-	-	-
2	**Other fruit products (excluding beverages)**				
3	Fruit, canned				
4	*Canned fruit, Plum (*Prunus domestica*)*	*11.3*	*18*	*89*	*89*
4	*Other and unspecified fruit, canned*	*Not taken into account*	-	-	-
3	Fruit compote				
4	*Fruit compote, Plum (*Prunus domestica*)*	*11.3*	18	89	89
4	*Other and unspecified fruit compote*	*Not taken into account*	-	-	-
3	Other and unspecified fruit products (excluding beverages)	Not taken into account	-	-	-
2	**Unspecified fruit and fruits products**	**Not taken into account**	**-**	**-**	**-**
1	**Meat and meat products**	**Not taken into account**	**-**	**-**	**-**
1	**Fish and other seafood**				
3	Fish fingers	10.4 - Composite dishes	25	129	129
3	Other and unspecified fish and other seafood	Not taken into account	-	-	-
1	**Milk and dairy products**	**Not taken into account**	**-**	**-**	**-**
1	**Eggs and egg products**	**Not taken into account**	**-**	**-**	**-**
1	**Sugar and confectionary**				
2	**Chocolate (Cocoa) products**	**10.6 - chocolate**	**31**	**73**	**73**
2	**Other and unspecified sugar and confectionary**	**Not taken into account**	**-**	**-**	**-**
1	**Animal and vegetable fats and oils**	**Not taken into account**	**-**	**-**	**-**
1	**Fruit and vegetable juices**				
2	**Fruit juice**				
3	Juice, Prune	11.3	-	-	-
3	Other and unspecified fruit juice	Not taken into account	-	-	-
2	**Other and unspecified fruit and vegetable juices**	**Not taken into account**	**-**	**-**	**-**
1	**Non-alcoholic beverages**				
2	**Coffee (Beverage)**				
3	Coffee drink, espresso	7.1 - dilution factor of 0.125	595	31	31
3	Coffee drink, café américano	7.1 - dilution factor of 0.053	595	13	13
3	Coffee drink, cappuccino	7.1 - dilution factor of 0.044	595	11	11
3	Coffee drink, café macchiato	7.1 - dilution factor of 0.063	595	16	16
3	Iced coffee	7.1 - dilution factor of 0.035	595	9	9
3	Coffee with milk (café latte, café au lait)	7.1 - dilution factor of 0.035	595	9	9
3	Instant coffee, liquid	7.2 - dilution factor of 0.017	862	12	12
3	Unspecified coffee (Beverage)	7.1 - 7.2 + dilution factor of 0.06 (median)	1 457	31	31

9351 Table continued overleaf.
9352

9353 **Table D1:** Acrylamide (AA) occurrence levels used in the baseline exposure scenario (continued)

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
2	**Coffee substitutes beverage**	**7.3 - 7.4 - 7.5 - dilution factor of 0.125**	**88**	**187**	**187**
2	**Cocoa beverage**	**10.6 – powder - dilution factor of 0.028**	**13**	**5**	**5**
2	**Other and unspecified non-alcoholic beverages**	**Not taken into account**	**-**	**-**	**-**
1	**Alcoholic beverages**	**Not taken into account**	**-**	**-**	**-**
1	**Drinking water**	**Not taken into account**	**-**	**-**	**-**
1	**Herbs, spices and condiments**	**Not taken into account**	**-**	**-**	**-**
2	**Spices**				
3	Paprika powder	11.5	30	379	379
3	Other and unspecified spices	Not taken into account	-	-	-
2	**Other and unspecified herbs, spices and condiments**	**Not taken into account**	**-**	**-**	**-**
1	**Food for infants and small children**				
Ahg 2	**Infant and follow-on formulae, powder [(a)]**	**8.1 - infant formula**	**33**	**3**	**26**
Ahg 3	Cereals which have to be reconstituted [(a)]	9.2 - flours	159	125	125
3	Biscuits, rusks and cookies	9.1	235	111	111
3	Ready-to-eat meal, cereal-based	9.2 - ready to eat meal	73	13	13
3	Unspecified cereal-based food	9.1 - 9.2 - 9.3	736	73	73
Ahg 2	**Ready-to-eat meal and desserts, not cereal-based**	**8.1 - ready to eat meal**	**291**	**14**	**27**
Agh	Fruit puree, without prunes	8.1 - puree - 8.3 - puree	34	30	44
Agh	Fruit puree, unspecified	8.1 - 8.2 - 8.3 - puree	47	55	55
2	**Fruit juice and herbal tea**	**Not taken into account**	**-**	**-**	**-**
1	**Products for special nutritional use**				
2	**Dietetic food for diabetics (labelled as such)**				
3	Fine bakery products for diabetics	10.4 – fine bakery wares for diabetics	1	139	139
3	Other and unspecified dietetic food for diabetics	Not taken into account	-	-	-
2	**Other and unspecified products for nutritional use**	**Not taken into account**	**-**	**-**	**-**
1	**Composite food**				
2	**Cereal-based dishes**				
3	Sandwich and sandwich-like meal	4.1 - 4.2 - 4.3 - Conversion factor of 0.56*	543	24	24
3	Pizza and pizza-like pies	4.1 - 4.2 - 4.3 - Conversion factor	543	24	24
3	Pasta, cooked	10.4 - Pasta	9	< 1	25
3	Other and unspecified cereal-based dishes	4.1 - 4.2 - 4.3 - conversion factor of 0.56 - 10.4 - Pasta	552	23	23
2	**Potato-based dishes**	**10.5 - other - conversion factor of 0.47***	**37**	**31**	**35**
2	**Meat-based meals**				
Ahg 3	Meat burger and meat balls	10.4 - Composite dishes	25	129	129
Ahg 3	Goulash and meat stew	Not taken into account	-	-	-
3	Unspecified meat-based meals	10.4 - Composite dishes	25	129	129

9354 Table continued overleaf.
9355

9356 **Table D1:** Acrylamide (AA) occurrence levels used in the baseline exposure scenario (continued)

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
2	**Vegetable-based meals**				
3	Vegetables, gratinated	10.4 - Composite dishes	25	129	129
3	Other and unspecified vegetable-based meals	Not taken into account	-	-	-
2	**Prepared salads**				
3	Prepared pasta salad	10.4 - Pasta	9	< 1	25
3	Prepared potato-based salad	10.5 - other - conversion factor of 0.47*	37	31	35
3	Other and unspecified prepared salads	Not taken into account	-	-	-
2	**Other and unspecified composite food**	**Not taken into account**	**-**	**-**	**-**
1	**Snacks, desserts, and other foods**				
2	**Snack food**				
3	Potato crisps	2.1 - 2.2 - 2.3	34 478	389	389
Ahg	Other potatoe snacks	2.4	23	283	283
Ahg 3	Corn snacks	10.3 - corn	114	168	168
Ahg 3	Fish-based snacks and seafood chips	Not taken into account	-	-	-
Ahg 3	Other snacks	10.3 - other	21	184	184
2	**Unspecified snack food**	**2.1 - 2.2 - 2.3 - 6.1 - 10.3**	**34 775**	**387**	**387**
2	**Desserts and other foods**	**Not taken into account**	**-**	**-**	**-**

9357 N: number of samples; LB: lower bound: UB: upper bound.
9358 (a): A conversion factor of 0.14, 0.1, 0.2 were respectively applied to 'Infant/follow-up formulae, liquid', 'Simple cereals
9359 reconstituted with milk' and 'Cereals with an added high protein food reconstituted with water' in order to express them
9360 in dry equivalent (Kersting et al., 1998).
9361 * The conversion factor applied to the sandwich and sandwich-like meal reflects the average content of bread. The
9362 conversion factor applied to the potato-based dishes and salads reflect the average content of potato. These values are
9363 derived from the draft European food conversion model.
9364

9365 Sensitivity analysis

9366 In Tables D2 to D8, only the estimates which are different from those used in the main scenario are
9367 detailed.

9368 *Influence of home-cooking*

9369 **Table D2:** Acrylamide (AA) occurrence levels used in the scenario A1 for French fries and fried
9370 potatoes

Foodex Level	Food group	Occurrence data	N	Mean LB	Mean UB
Ahg	French fries	1.3 provided by food associations (see Table 5)	316	201	201

9371 LB: lower bound; N: number of samples; UB: upper bound.
9372

Table D3: Acrylamide (AA) occurrence levels used in the scenario A2 for French fries and fried potatoes

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
Ahg	French fries	1.1 (see Table 6)	877	308	308

LB: lower bound; N: number of samples; UB: upper bound.

Table D4: Acrylamide (AA) occurrence levels used in the scenario A3 for French fries and fried potatoes

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
Ahg	French fries	95th percentile of 1.3 (see Table 6)	557	656	656

LB: lower bound; N: number of samples; UB: upper bound.

Table D5: Acrylamide (AA) occurrence levels used in the scenario B1 for toasted bread

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
ahg 4	Wheat bread and rolls, toasted	Claus et al. (2005)	-	100	100
ahg 4	Rye bread and rolls, toasted	Claus et al. (2005)	-	100	100
ahg 4	Mixed bread and rolls, toasted	Claus et al. (2005)	-	100	100
ahg 4	Multigrain bread and rolls, toasted	Claus et al. (2005)	-	100	100

LB: lower bound; N: number of samples; UB: upper bound.

Influence of brand loyalty

Table D6: Acrylamide occurrence levels used in the scenario C1 for potato crisps

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
3	Potato crisps	2.1 – continuous process (see Table B1)	29 557	391	391

LB: lower bound; N: number of samples; UB: upper bound.

Table D7: Acrylamide (AA) occurrence levels used in the scenario C2 for potato crisps

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
3	Potato crisps	2.2 (see Table 6)	2 795	338	338

LB: lower bound; N: number of samples; UB: upper bound.

9390 **Table D8:** Acrylamide (AA) occurrence levels used in the scenario D1 for coffee

Foodex level	Food group	Occurrence data	N	Mean LB	Mean UB
ahg 3	Coffee beans	7.1 – light (see Table B1)	45	374	374
3	Coffee drink, espresso	7.1 - light - dilution factor of 0.125	45	47	47
3	Coffee drink, café américano	7.1 – light - dilution factor of 0.053	45	19	19
3	Coffee drink, cappuccino	7.1 – light - dilution factor of 0.044	45	16	16
3	Coffee drink, macchiato	7.1 – light - dilution factor of 0.063	45	23	23
3	Iced coffee	7.1 – light - dilution factor of 0.035	45	13	13
3	Coffee with milk (café latte, café au lait)	7.1 – light - dilution factor of 0.035	45	13	13
3	Unspecified coffee (Beverage)	7.1 – light - 7.2 + dilution factor of 0.06 (median)	911	42	42

9391 LB: lower bound; N: number of samples; UB: upper bound.

9392 **Appendix E. Chronic exposure to acrylamide (AA) across population groups, results for the**
9393 **baseline exposure scenario**

9394 **Table E1:** Chronic exposure levels to AA resulting from the baseline exposure scenario, expressed in
9395 µg/kg b.w. per day for each survey and age group

Country	Survey acronym	N[(a)]	Mean LB-UB[(b)]	P95 LB-UB[(c)]
	Infants			
Bulgaria	NUTRICHILD	859	0.9 – 1.0	2.3 - 2.5
Germany	VELS	159	1.4 - 1.7	2.3 - 2.8
Finland	DIPP_2001_2009	499	0.7 - 0.9	1.4 - 1.7
Italy	INRAN_SCAI_2005_06	16	0.5 - 0.7	-*
	Toddlers			
Belgium	Regional_Flanders	36	1.9	-*
Bulgaria	NUTRICHILD	428	1.5	3.4
Germany	VELS	348	1.3 - 1.4	2.6 - 2.7
Spain	enKid	17	1.4	-*
Finland	DIPP_2001_2009	500	1.4 - 1.6	2.5 - 2.6
United Kingdom	NDNS-RollingProgrammeYears1-3	185	1.6 - 1.7	3.1 - 3.2
Italy	INRAN_SCAI_2005_06	36	1.1 - 1.2	-*
Netherlands	VCP_kids	322	1.3	2.3 - 2.4
	Other children			
Belgium	Regional_Flanders	625	1.6	2.8 - 2.9
Bulgaria	NUTRICHILD	433	1.4	3.2
Czech Republic	SISP04	389	1.2	2.5
Germany	EsKiMo	835	1.0 - 1.1	1.9 - 2.0
Germany	VELS	293	1.2 - 1.3	2.2
Denmark	Danish_Dietary_Survey	490	0.9	1.4 - 1.5
Spain	enKid	156	1.3 - 1.4	3.0
Spain	NUT_INK05	399	1.1	2.0
Finland	DIPP_2001_2009	750	1.0	1.7
France	INCA2	482	1.1	1.8 - 1.9
United Kingdom	NDNS-RollingProgrammeYears1-3	651	1.5	2.6 - 2.7
Greece	Regional_Crete	838	1.4	2.9
Italy	INRAN_SCAI_2005_06	193	1.0 - 1.1	2.2 - 2.4
Latvia	EFSA_TEST	187	1.2	2.3 - 2.4
Netherlands	VCP_kids	957	1.1 - 1.2	2.0
Netherlands	VCPBasis_AVL2007_2009	447	1.2	2.3
Sweden	NFA	1 473	1.2	2.0 - 2.1
	Adolescents			
Belgium	Diet_National_2004	576	0.6	1.3
Cyprus	Childhealth	303	0.7	1.4
Czech Republic	SISP04	298	0.9	2.0
Germany	National_Nutrition_Survey_II	1 011	0.4	0.9
Germany	EsKiMo	393	0.8	1.4 - 1.5
Denmark	Danish_Dietary_Survey	479	0.5 - 0.6	1.1
Spain	AESAN_FIAB	86	0.7	1.3 - 1.4
Spain	enKid	209	0.8	1.9
Spain	NUT_INK05	651	0.7	1.3
Finland	NWSSP07_08	306	0.5	0.9
France	INCA2	973	0.6	1.2
United Kingdom	NDNS-RollingProgrammeYears1-3	666	0.8 - 0.9	1.7
Italy	INRAN_SCAI_2005_06	247	0.7	1.4
Latvia	EFSA_TEST	453	0.9	2.0
Latvia	FC_PREGNANTWOMEN_2011	12	0.7	-*
Netherlands	VCPBasis_AVL2007_2009	1 142	0.9	1.7 - 1.8
Sweden	NFA	1 018	0.8	1.5 - 1.6

9396 Table continued overleaf.
9397

9398 **Table E1:** Chronic exposure levels to AA resulting from the baseline exposure scenario, expressed in
9399 µg/kg b.w. per day for each survey and age group (continued)

Country	Survey acronym	N[(a)]	Mean LB-UB[(b)]	P95 LB-UB[(c)]
	Adults			
Belgium	Diet_National_2004	1 292	0.5	1.0 - 1.1
Czech Republic	SISP04	1 666	0.5	1.1
Germany	National_Nutrition_Survey_II	10 419	0.4	0.9
Denmark	Danish_Dietary_Survey	2 822	0.5	0.8 - 0.9
Spain	AESAN	410	0.4	1.0
Spain	AESAN_FIAB	981	0.5	1.1
Finland	FINDIET2012	1 295	0.5	0.9
France	INCA2	2 276	0.4	0.7 - 0.8
United Kingdom	NDNS-RollingProgrammeYears1-3	1 266	0.5	1.0
Hungary	National_Repr_Surv	1 074	0.5	0.9
Ireland	NANS_2012	1 274	0.6	1.1
Italy	INRAN_SCAI_2005_06	2 313	0.4	0.8 - 0.9
Latvia	EFSA_TEST	1 271	0.6	1.3 - 1.4
Latvia	FC_PREGNANTWOMEN_2011	990	0.6	1.1
Netherlands	VCPBasis_AVL2007_2009	2 057	0.6	1.2
Sweden	Riksmaten 2010	1 430	0.5	0.9
	Elderly			
Belgium	Diet_National_2004	511	0.5	0.9 - 1.0
Germany	National_Nutrition_Survey_II	2 006	0.4	0.9
Denmark	Danish_Dietary_Survey	309	0.5	0.8
Finland	FINDIET2012	413	0.4 - 0.5	0.8 - 0.9
France	INCA2	264	0.4	0.7
United Kingdom	NDNS-RollingProgrammeYears1-3	166	0.5	0.8
Hungary	National_Repr_Surv	206	0.4	0.8
Ireland	NANS_2012	149	0.5	0.9
Italy	INRAN_SCAI_2005_06	290	0.3 - 0.4	0.6 - 0.7
Netherlands	VCPBasis_AVL2007_2009	173	0.5	1.0
Sweden	Riksmaten 2010	295	0.5	0.9
	Very elderly			
Belgium	Diet_National_2004	704	0.5 - 0.6	1.0
Germany	National_Nutrition_Survey_II	490	0.5	1.0
Denmark	Danish_Dietary_Survey	20	0.5	-*
France	INCA2	84	0.3 - 0.4	0.6
United Kingdom	NDNS-RollingProgrammeYears1-3	139	0.5	0.9
Hungary	National_Repr_Surv	80	0.4 - 0.5	0.9
Ireland	NANS_2012	77	0.4 - 0.5	1.0
Italy	INRAN_SCAI_2005_06	228	0.3 - 0.4	0.6 - 0.7
Sweden	Riksmaten 2010	72	0.5	0.9 - 1.0

9400 Note: In order to avoid the impression of too high precision, the numbers for all exposure estimates are rounded to 2 figures.
9401 * The 95th percentile (P95) for dietary surveys/age groups with less than 60 subjects were not reliable and therefore not
9402 presented.
9403 (a): N: number of subjects.
9404 (b): Mean LB-UB: mean lower bound – upper bound.
9405 (c): P95 LB – UB: 95th percentile lower bound – upper bound. When lower bound and upper bound are equal, only one
9406 estimate is given.
9407

9408 **Appendix F. Contribution of food groups to the acrylamide (AA) total exposure**

9409 **Table F1:** Minimum and maximum relative contribution of food groups, expressed in percentage, to the acrylamide (AA) total lower bound (LB) exposure

Food category	Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
Potato fried products	**0 - 1.6**	**12 - 32**	**11 - 49**	**10 - 51**	**9.6 - 49**	**6.3 - 29**	**5.3 - 27**
Potato crisps and snacks	**0 - 0.1**	**0.1 - 7.9**	**0.4 - 7.5**	**1.7 - 11**	**0.6 - 6.6**	**0 - 2.5**	**0 - 1.7**
Soft bread	**0 - 7.2**	**0.3 - 20**	**0.2 - 27**	**0.1 - 23**	**9.2 - 22**	**9.9 - 21**	**8.9 - 22**
Breakfast cereals	**0 - 17**	**0.2 - 21**	**0.6 - 13**	**1.5 - 12**	**1 - 11**	**0.3 - 8.9**	**0.4 - 11**
Biscuits, crackers, crisp bread	**0 - 20**	**2.3 - 24**	**2.1 - 27**	**1.6 - 24**	**1.3 - 17**	**1.5 - 20**	**1.7 - 21**
Crackers	0 - 3.6	0 – 8.0	0 - 7.7	0 - 7.4	0 - 1.8	0 - 2.4	0 - 1.4
Crisp bread	0 - 3.4	0.5 - 5.1	0.4 - 6.6	0 – 7.0	0 - 7.2	0.8 - 8.2	0.6 - 9.2
Biscuits and wafers	0 - 17	1.4 - 18	< 0.05 - 21	< 0.05 - 18	0.3 - 14	0.3 - 11	0.5 - 12
Gingerbread	0 - 0.7	0 – 6.0	0 - 7.1	0 - 8.2	0 - 6.0	0 - 8.5	0 - 3.0
Coffee and coffee substitutes	**0**	**0 - 0.2**	**0 - 6.3**	**0.1 - 3.1**	**3.2 - 27**	**3.2 - 31**	**2.3 - 33**
Roasted coffee	0	0 - 0.2	0 - 0.9	< 0.05 - 3.1	0.4 - 27	0.4 - 31	0.3 - 26
Instant coffee	0	0	0 - 0.4	0 - 1.4	0 - 11	0 - 15	0 - 13
Substitute coffee	0	0 - 0.1	0 - 5.8	0 - 0.4	0 - 3.4	0 - 11	0 - 20
Baby foods, other than processed cereal-based	**2.3 - 60**	**0 - 8.6**	**0 - 0.5**	**0 - 0.1**	**0**	**0**	**0**
Infant formulae	*0.6 - 2.3*	*0 - 0.7*	*0*	*0*	*0*	*0*	*0*
Fruit purée	*0 - 34*	*0 – 6.0*	*0 - 0.4*	*0 - 0.1*	*0*	*0*	*0*
Ready-to-eat meal and dessert	*0 - 25*	*0 - 2.5*	*0 - 0.1*	*0*	*0*	*0*	*0*
Processed cereal-based baby foods	**0 - 31**	**0 - 14**	**0 - 3.5**	**0 - 0.2**	**0 - 0.1**	**0**	**0**
Biscuits and rusks	0 - 11	0 - 8.9	0 - 3.5	0 - 0.2	0 - 0.1	0	0
Other cereal-based foods	0 - 29	0 - 14	0 - 1.6	0 - 0.1	0	0	0
Other products based on potatoes, cereals and cocoa	**16 - 77**	**27 - 51**	**23 - 67**	**20 - 71**	**18 - 37**	**24 - 41**	**30 - 45**
Porridge	0 - 2.5	0 - 1.1	0 - 1.4	0 - 0.4	0 - 5.8	0 - 11	0 - 7.5
Cake and pastry	0 - 1.4	0 - 7.7	0 - 15	0 - 15	1.2 - 12	1.8 - 12	2.2 - 13
Savoury snacks other than potato-based	0 - 1.4	0 - 4.2	< 0.05 - 3.3	0 - 1.8	< 0.05 - 0.9	0 - 0.5	0 - 0.1
Other products based on cereals	0.9 - 29	4.3 - 25	5.5 - 40	3.9 - 46	2.1 - 19	1.9 - 18	1.4 - 18
Other products based on potatoes	7.7 - 50	6.3 - 27	2.3 - 23	1.6 - 20	2.2 - 17	13 - 25	14 - 31
Other products based on cocoa	< 0.05 - 1.3	0.1 - 7.1	1.6 - 7.6	2.3 - 6.7	0.9 - 3.3	0.5 - 1.7	0.6 - 2.3
Other products	**0 - 3.2**	**0.2 - 2.5**	**0.2 - 2.9**	**0.3 - 4.2**	**1.0 - 6.0**	**0.6 - 3.1**	**0.8 - 3.1**
Roasted nuts and seeds	0 - 0.3	< 0.05 - 0.5	0.1 - 1.1	0.1 - 2	0.5 - 1.8	0.2 - 1.5	0.1 - 0.8
Black olives in brine	0	< 0.05 - 2.0	0 - 1.8	0 - 3.2	< 0.05 - 4.7	0 - 1.6	0 - 1.5
Prunes and dates	0 - 3.2	0 - 0.5	0 - 0.5	0 - 0.5	< 0.05 - 0.5	< 0.05 - 1.1	0.2 - 2.4
Paprika powder	0	0 - 0.1	0 - 0.2	0 - 0.4	0 - 1.0	0 - 1.1	0 - 1.0

9410 **Table F2:** Minimum and maximum relative contribution of food groups, expressed in percentage, to the AA total upper bound (UB) exposure

Food category	Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
Potato fried products	**0 - 1.3**	**12 - 32**	**10 - 48**	**9.7 - 50**	**9.3 - 48**	**6.1 - 28**	**5.1 - 26**
Potato crisps and snacks	**0 - 0.1**	**< 0.05 - 7.8**	**0.4 - 7.3**	**1.6 - 10**	**0.6 - 6.5**	**0 - 2.4**	**0 - 1.6**
Soft bread	**0 - 5.9**	**0.3 - 19**	**0.2 - 27**	**0.1 - 22**	**8.8 - 21**	**9.5 - 21**	**8.6 - 20**
Breakfast cereals	**0 - 14**	**0.2 - 19**	**0.5 - 13**	**1.5 - 11**	**0.9 - 11**	**0.3 - 8.7**	**0.3 - 11**
Biscuits, crackers, crisp bread	**0 - 17**	**2.1 - 23**	**2 - 26**	**1.6 - 23**	**1.2 - 16**	**1.5 - 18**	**1.6 - 20**
Crackers	0 - 2.9	0 - 7.6	0 - 7.4	0 - 7.1	0 - 1.7	0 - 2.4	0 - 1.3
Crisp bread	0 - 2.8	0.5 - 4.9	0.4 - 6.2	0 - 6.7	0 - 7.0	0.7 - 7.6	0.6 - 8.5
Biscuits and wafers	0 - 14	1.4 - 18	< 0.05 - 21	< 0.05 - 17	0.3 - 13	0.3 - 11	0.5 - 11
Gingerbread	0 - 0.6	0 - 5.8	0 – 7.0	0 - 8.0	0 - 5.9	0 - 8.4	0 - 2.9
Coffee and coffee substitutes	**0**	**0 - 0.2**	**0 - 6.2**	**0.1 - 3.0**	**3.2 - 26**	**3.2 - 30**	**2.2 - 32**
Roasted coffee	0	0 - 0.2	0 - 0.9	< 0.05 - 3.0	0.4 - 26	0.4 - 30	0.3 - 25
Instant coffee	0	0	0 - 0.4	0 - 1.3	0 - 11	0 - 14	0 - 13
Substitute coffee	0	0 - 0.1	0 - 5.7	0 - 0.4	0 - 3.3	0 - 11	0 - 19
Baby foods, other than processed cereal-based	**19 - 68**	**0 - 10**	**0 - 0.6**	**0 - 0.1**	**0**	**0**	**0**
Infant formulae	*5.1 - 19*	*0 - 6.4*	*0 - 0.1*	*0*	*0*	*0*	*0*
Fruit purée	*0 - 26*	*0 - 5.4*	*0 - 0.4*	*0 - 0.1*	*0*	*0*	*0*
Ready-to-eat meal and dessert	*0 - 33*	*0 - 4.3*	*0 - 0.3*	*0*	*0*	*0*	*0*
Processed cereal-based baby foods	**0 - 26**	**0 - 14**	**0 - 3.4**	**0 - 0.2**	**0**	**0**	**0**
Biscuits and rusks	0 - 7.6	0 - 8	0 - 3.4	0 - 0.2	0	0	0
Other cereal-based foods	0 - 24	0 - 14	0 - 1.6	0 - 0.1	0	0	0
Other products based on potatoes, cereals and cocoa	**14 - 65**	**29 - 50**	**25 - 68**	**21 - 72**	**20 - 39**	**26 - 44**	**32 - 47**
Porridge	0 - 2.0	0 - 1.1	0 - 1.4	0 - 0.4	0 - 5.6	0 - 11	0 - 7.2
Cake and pastry	0 - 1.2	0 - 7.5	0 - 14	0 - 14	1.2 - 12	1.7 - 12	2.1 - 12
Savoury snacks other than potato-based	0 - 1.1	0 - 4.1	< 0.05 - 3.3	0 - 1.8	< 0.05 - 0.9	0 - 0.5	0 - 0.1
Other products based on cereals	1.2 - 23	6.2 - 23	6.2 - 40	4.8 - 46	2.5 - 22	2.2 - 20	1.5 - 19
Other products based on potatoes	6.1 - 42	6.9 - 27	2.5 - 25	1.8 - 22	2.4 - 19	13 - 28	14 - 34
Other products based on cocoa	< 0.05 - 0.9	0.1 - 6.9	1.6 - 7.4	2.3 - 6.4	0.9 - 3.2	0.5 - 1.6	0.6 - 2.2
Other products	**0 - 2.5**	**0.2 - 2.5**	**0.2 - 2.8**	**0.3 - 4.2**	**1 - 5.9**	**0.6 - 3.0**	**0.8 - 3.0**
Roasted nuts and seeds	0 - 0.3	< 0.05 - 0.5	0.1 - 1.1	0.1 - 1.9	0.4 - 1.7	0.2 - 1.5	0.1 - 0.8
Black olives in brine	0	< 0.05 - 1.9	0 - 1.7	0 - 3.2	< 0.05 - 4.6	0 - 1.4	0 - 1.4
Prunes and dates	0 - 2.5	0 - 0.4	0 - 0.5	0 - 0.5	< 0.05 - 0.4	< 0.05 - 1.1	0.2 - 2.3
Paprika powder	0	0 - 0.1	0 - 0.2	0 - 0.4	0 - 0.9	0 - 0.1	0 - 1.0

9411

9412 **Appendix G. Chronic exposure to acrylamide (AA) across population groups – results from the other scenarios**

9413 **Table G1:** Chronic exposure levels to acrylamide (AA) resulting from the scenarios A1, A2 and A3 compared to the baseline scenario, expressed in µg/kg
9414 b.w. per day for each survey and age group

Country	Survey acronym	N[(a)]	Mean LB-UB[(b)]				P95 LB-UB[(c)]			
			BASE	A1	A2	A3	BASE	A1	A2	A3
Infants										
Bulgaria	NUTRICHILD	859	0.9 - 1.0	0.9 - 1.0	0.9 - 1.0	0.9 - 1.0	2.3 - 2.5	2.3 - 2.5	2.3 - 2.5	2.4 - 2.6
Germany	VELS	159	1.4 - 1.7	1.4 - 1.6	1.4 - 1.7	1.4 - 1.7	2.3 - 2.8	2.3 - 2.8	2.4 - 2.8	2.5 - 2.8
Finland	DIPP_2001_2009	499	0.7 - 0.9	0.7 - 0.9	0.7 - 0.9	0.7 - 0.9	1.4 - 1.7	1.4 - 1.7	1.4 - 1.7	1.4 - 1.7
Italy	INRAN_SCAI_2005_06	16	0.5 - 0.7	0.5 - 0.7	0.5 - 0.7	0.5 - 0.7	-*	-*	-*	-*
Toddlers										
Belgium	Regional_Flanders	36	1.9	1.7 - 1.8	1.9	2.4 - 2.5	-*	-*	-*	-*
Bulgaria	NUTRICHILD	428	1.5	1.4	1.5 - 1.6	1.9 - 2.0	3.4	2.8 - 2.9	3.4 - 3.5	5.5 - 5.6
Germany	VELS	348	1.3 - 1.4	1.3 - 1.4	1.4	1.5 - 1.6	2.6 - 2.7	2.4 - 2.5	2.6 - 2.7	3.1 - 3.2
Spain	enKid	17	1.4	1.3	1.4 - 1.5	2.0	-*	-*	-*	-*
Finland	DIPP_2001_2009	500	1.4 - 1.6	1.3 - 1.5	1.4 - 1.6	1.8 - 2.0	2.5 - 2.6	2.2 - 2.4	2.5 - 2.7	3.8 – 4.0
United Kingdom	NDNS-RollingProgrammeYears1-3	185	1.6 - 1.7	1.5 - 1.6	1.6 - 1.7	2.0	3.1 - 3.2	2.9 - 3.0	3.1 - 3.2	4.1 - 4.2
Italy	INRAN_SCAI_2005_06	36	1.1 - 1.2	1.0 - 1.2	1.1 - 1.2	1.3 - 1.4	-*	-*	-*	-*
Netherlands	VCP_kids	322	1.3	1.2 - 1.3	1.3	1.5	2.3 - 2.4	2.2	2.4	3.3
Other children										
Belgium	Regional_Flanders	625	1.6	1.5	1.6	2.0	2.8 - 2.9	2.5 - 2.6	2.9	3.9
Bulgaria	NUTRICHILD	433	1.4	1.3	1.4 - 1.5	1.9 - 2.0	3.2	2.6	3.3	5.3 - 5.4
Czech Republic	SISP04	389	1.2	1.1 - 1.2	1.2	1.4	2.5	2.3	2.5 - 2.6	3.2 - 3.3
Germany	EsKiMo	835	1.0 - 1.1	1.0	1.0 - 1.1	1.2	1.9 - 2.0	1.8	2.0	2.5 - 2.6
Germany	VELS	293	1.2 - 1.3	1.2	1.2 - 1.3	1.4 - 1.5	2.2	2.0 - 2.1	2.2 - 2.3	2.9
Denmark	Danish_Dietary_Survey	490	0.9	0.8	0.9	1.1	1.4 - 1.5	1.3 - 1.4	1.5	2.1
Spain	enKid	156	1.3 - 1.4	1.1 - 1.2	1.4	2.2	3.0	2.5	3.1	5.3 - 5.4
Spain	NUT_INK05	399	1.1	1.0	1.1	1.3	2.0	1.8	2.0 - 2.1	2.8 - 2.9
Finland	DIPP_2001_2009	750	1.0	0.9 - 1.0	1.0	1.2	1.7	1.5 - 1.6	1.7	2.5
France	INCA2	482	1.1	1.0	1.1	1.3 - 1.4	1.8 - 1.9	1.7	1.8 - 1.9	2.5 - 2.6
United Kingdom	NDNS-RollingProgrammeYears1-3	651	1.5	1.3 - 1.4	1.5	2.0	2.6 - 2.7	2.4	2.7 - 2.8	4.0
Greece	Regional_Crete	838	1.4	1.2	1.4 - 1.5	2.2	2.9	2.3 - 2.4	3.0	5.1 - 5.3
Italy	INRAN_SCAI_2005_06	193	1.0 - 1.1	1.0	1.0 - 1.1	1.3 - 1.4	2.2 - 2.4	2.0 - 2.1	2.3 - 2.4	3.2 - 3.3

9415 Table continued overleaf.
9416

9417 **Table G1:** Chronic exposure levels to acrylamide (AA) resulting from the scenarios A1, A2 and A3 compared to the baseline scenario, expressed in µg/kg
9418 b.w. per day for each survey and age group (continued)

Country	Survey acronym	N[(a)]	Mean LB-UB[(b)]				P95 LB-UB[(c)]			
			BASE	A1	A2	A3	BASE	A1	A2	A3
Latvia	EFSA_TEST	187	1.2	1.1	1.2	1.5	2.3 - 2.4	2.2	2.4 - 2.5	3.4
Netherlands	VCP_kids	957	1.1 - 1.2	1.1	1.1 - 1.2	1.3 - 1.4	2.0	1.9	2.1	2.9
Netherlands	VCPBasis_AVL2007_2009	447	1.2	1.1	1.2 - 1.3	1.6	2.3	2.0	2.3	3.7
Sweden	NFA	1 473	1.2	1.1 - 1.2	1.2	1.4	2.0 - 2.1	1.9 - 2.0	2.0 - 2.1	2.8 - 2.9
			Adolescents							
Belgium	Diet_National_2004	576	0.6	0.6	0.6	0.8	1.3	1.2	1.3	1.8
Cyprus	Childhealth	303	0.7	0.6	0.7 - 0.8	1.1 - 1.2	1.4	1.1 - 1.2	1.5	2.5 - 2.6
Czech Republic	SISP04	298	0.9	0.9	0.9	1.1	2.0	1.8 - 1.9	2.0 - 2.1	2.9
Germany	National_Nutrition_Survey_II	1 011	0.4	0.4	0.4	0.5	0.9	0.9	0.9 - 1.0	1.2 - 1.3
Germany	EsKiMo	393	0.8	0.8	0.8	0.9	1.4 - 1.5	1.4	1.5	1.9
Denmark	Danish_Dietary_Survey	479	0.5 - 0.6	0.5	0.6	0.7	1.1	1.0	1.1	1.5 - 1.6
Spain	AESAN_FIAB	86	0.7	0.6	0.7	1.1	1.3 - 1.4	1.1	1.4	2.1
Spain	enKid	209	0.8	0.7	0.8 - 0.9	1.3 - 1.4	1.9	1.5	2.0	3.3
Spain	NUT_INK05	651	0.7	0.6	0.7	0.9	1.3	1.2	1.3 - 1.4	1.9
Finland	NWSSP07_08	306	0.5	0.5	0.5	0.6	0.9	0.8	0.9 - 1.0	1.3
France	INCA2	973	0.6	0.6	0.6	0.8	1.2	1.1	1.2	1.6 - 1.7
United Kingdom	NDNS-RollingProgrammeYears1-3	666	0.8 - 0.9	0.7 - 0.8	0.9	1.2	1.7	1.4 - 1.5	1.7	2.6 - 2.7
Italy	INRAN_SCAI_2005_06	247	0.7	0.6	0.7	0.9	1.4	1.3	1.5	2.2
Latvia	EFSA_TEST	453	0.9	0.8	0.9	1.2	2.0	1.7	2.0	3.1
Latvia	FC_PREGNANTWOMEN_2011	12	0.7	0.6 - 0.7	0.7	0.8	-*	-*	-*	-*
Netherlands	VCPBasis_AVL2007_2009	1 142	0.9	0.8	0.9	1.1	1.7 - 1.8	1.5	1.8	2.7
Sweden	NFA	1 018	0.8	0.8	0.8 - 0.9	1.0	1.5 - 1.6	1.4	1.6	2.2
			Adults							
Belgium	Diet_National_2004	1 292	0.5	0.5	0.5	0.6	1.0 - 1.1	0.9 - 1.0	1.1	1.5
Czech Republic	SISP04	1 666	0.5	0.5	0.5	0.6	1.1	1.1	1.1 - 1.2	1.5 - 1.6
Germany	National_Nutrition_Survey_II	10 419	0.4	0.4	0.4	0.5	0.9	0.8 - 0.9	0.9	1.2
Denmark	Danish_Dietary_Survey	2 822	0.5	0.5	0.5	0.6	0.8 - 0.9	0.8	0.9	1.1
Spain	AESAN	410	0.4	0.4	0.5	0.7	1.0	0.8 - 0.9	1.0	1.8
Spain	AESAN_FIAB	981	0.5	0.4	0.5	0.8	1.1	0.9	1.1	1.9

9419 Table continued overleaf.
9420

9421 **Table G1:** Chronic exposure levels to acrylamide (AA) resulting from the scenarios A1, A2 and A3 compared to the baseline scenario, expressed in µg/kg
9422 b.w. per day for each survey and age group (continued)

Country	Survey acronym	N[(a)]	Mean LB-UB[(b)]				P95 LB-UB[(c)]			
			BASE	A1	A2	A3	BASE	A1	A2	A3
Finland	FINDIET2012	1 295	0.5	0.5	0.5	0.5	0.9	0.9	0.9	1.2
France	INCA2	2 276	0.4	0.4	0.4	0.5	0.7 - 0.8	0.7	0.8	1.0
United Kingdom	NDNS-RollingProgrammeYears1-3	1 266	0.5	0.5	0.5	0.7	1.0	0.9	1.0	1.6
Hungary	National_Repr_Surv	1 074	0.5	0.4	0.5	0.6	0.9	0.8	0.9 - 1.0	1.5
Ireland	NANS_2012	1 274	0.6	0.5	0.6	0.8	1.1	1.0	1.1	1.7
Italy	INRAN_SCAI_2005_06	2 313	0.4	0.4	0.4	0.5	0.8 - 0.9	0.7 - 0.8	0.8 - 0.9	1.3
Latvia	EFSA_TEST	1 271	0.6	0.5	0.6	0.7 - 0.8	1.3 - 1.4	1.2	1.4	2.0
Latvia	FC_PREGNANTWOMEN_2011	990	0.6	0.6	0.6	0.7 - 0.8	1.1	1	1.1	1.7
Netherlands	VCPBasis_AVL2007_2009	2 057	0.6	0.5	0.6	0.7	1.2	1.0 - 1.1	1.2	1.8
Sweden	Riksmaten 2010	1 430	0.5	0.5	0.5	0.5 - 0.6	0.9	0.9	0.9	1.1
			Elderly							
Belgium	Diet_National_2004	511	0.5	0.5	0.5	0.6	0.9 – 1.0	0.9	1.0	1.4 - 1.5
Germany	National_Nutrition_Survey_II	2 006	0.4	0.4	0.4	0.5	0.9	0.8 - 0.9	0.9	1.1
Denmark	Danish_Dietary_Survey	309	0.5	0.5	0.5	0.5 - 0.6	0.8	0.8	0.8 - 0.9	1.0
Finland	FINDIET2012	413	0.4 - 0.5	0.4	0.4 - 0.5	0.5	0.8 - 0.9	0.8	0.8 - 0.9	1.1
France	INCA2	264	0.4	0.3 - 0.4	0.4	0.4 - 0.5	0.7	0.7	0.7	0.9
United Kingdom	NDNS-RollingProgrammeYears1-3	166	0.5	0.4	0.5	0.6	0.8	0.8	0.8	1.3
Hungary	National_Repr_Surv	206	0.4	0.4	0.4	0.5	0.8	0.7	0.8	1.1 - 1.2
Ireland	NANS_2012	149	0.5	0.4 - 0.5	0.5	0.6	0.9	0.9	0.9	1.3
Italy	INRAN_SCAI_2005_06	290	0.3 - 0.4	0.3	0.3 - 0.4	0.4	0.6 - 0.7	0.6	0.7	0.9 - 1.0
Netherlands	VCPBasis_AVL2007_2009	173	0.5	0.5	0.5	0.6	1.0	1.0	1.0	1.5
Sweden	Riksmaten 2010	295	0.5	0.5	0.5	0.6	0.9	0.9	0.9	1.1
			Very elderly							
Belgium	Diet_National_2004	704	0.5 - 0.6	0.5	0.5 - 0.6	0.6	1.0	1.0	1.0	1.3
Germany	National_Nutrition_Survey_II	490	0.5	0.4 - 0.5	0.5	0.5	1.0	1.0	1.1	1.3
Denmark	Danish_Dietary_Survey	20	0.5	0.5	0.5	0.5	-*	-*	-*	-*
France	INCA2	84	0.3 - 0.4	0.3	0.3 - 0.4	0.4	0.6	0.6	0.6	0.9 - 1.0
United Kingdom	NDNS-RollingProgrammeYears1-3	139	0.5	0.4	0.5	0.6	0.9	0.8	0.9	1.4
Hungary	National_Repr_Surv	80	0.4 - 0.5	0.4	0.4 - 0.5	0.6	0.9	0.8	0.9	1.5

9423 Table continued overleaf.
9424

9425 **Table G1:** Chronic exposure levels to acrylamide (AA) resulting from the scenarios A1, A2 and A3 compared to the baseline scenario, expressed in µg/kg
9426 b.w. per day for each survey and age group (continued)

Country	Survey acronym	N[(a)]	Mean LB-UB[(b)]				P95 LB-UB[(c)]			
			BASE	A1	A2	A3	BASE	A1	A2	A3
Ireland	NANS_2012	77	0.4 - 0.5	0.4	0.5	0.5	1.0	0.9	1.0	1.3
Italy	INRAN_SCAI_2005_06	228	0.3 - 0.4	0.3	0.3 - 0.4	0.4	0.6 - 0.7	0.6	0.6 - 0.7	0.9 - 1.0
Sweden	Riksmaten 2010	72	0.5	0.5	0.5	0.6	0.9 – 1.0	0.9	0.9 - 1.0	1.1 - 1.2

9427 Note: In order to avoid the impression of too high precision, the numbers for all exposure estimates are rounded to 2 figures.
9428 * The 95th percentile (P95) for dietary surveys/age groups with less than 60 subjects were not reliable and therefore not presented.
9429 (a): N: number of subjects.
9430 (b): Mean LB-UB: mean lower bound – upper bound.
9431 (c): P95 LB – UB: 95th percentile lower bound – upper bound. When lower bound and upper bound estimates are equal, only one estimate is provided.

9432

Table G2: Chronic exposure levels to acrylamide (AA) resulting from the scenarios B1, C1, C2 and D1 compared to the baseline (BASE) scenario, expressed in µg/kg b.w. per day for each survey and age group

Country	Survey acronym	N[(a)]	Mean LB-UB[(b)]					P95 LB-UB[(c)]				
			BASE	B1	C1	C2	D1	BASE	B1	C1	C2	D1
Infants												
Bulgaria	NUTRICHILD	859	0.9 - 1.0	0.9 - 1.0	0.9 - 1.0	0.9 - 1.0	0.9 - 1.0	2.3 - 2.5	2.3 - 2.5	2.3 - 2.5	2.3 - 2.5	2.3 - 2.5
Germany	VELS	159	1.4 - 1.7	1.4 - 1.7	1.4 - 1.7	1.4 - 1.7	1.4 - 1.7	2.3 - 2.8	2.3 - 2.8	2.3 - 2.8	2.3 - 2.8	2.3 - 2.8
Finland	DIPP_2001_2009	499	0.7 - 0.9	0.7 - 0.9	0.7 - 0.9	0.7 - 0.9	0.7 - 0.9	1.4 - 1.7	1.4 - 1.7	1.4 - 1.7	1.4 - 1.7	1.4 - 1.7
Italy	INRAN_SCAI_2005_06	16	0.5 - 0.7	0.5 - 0.7	0.5 - 0.7	0.5 - 0.7	0.5 - 0.7	-*	-*	-*	-*	-*
Toddlers												
Belgium	Regional_Flanders	36	1.9	1.9	1.9	1.9	1.9	-*	-*	-*	-*	-*
Bulgaria	NUTRICHILD	428	1.5	1.5	1.5	1.5	1.5	3.4	3.4	3.4	3.4	3.4
Germany	VELS	348	1.3 - 1.4	1.3 - 1.4	1.3 - 1.4	1.3 - 1.4	1.3 - 1.4	2.6 - 2.7	2.6 - 2.7	2.6 - 2.7	2.6 - 2.7	2.6 - 2.7
Spain	enKid	17	1.4	1.4	1.4	1.4	1.4	-*	-*	-*	-*	-*
Finland	DIPP_2001_2009	500	1.4 - 1.6	1.4 - 1.6	1.4 - 1.6	1.4 - 1.6	1.4 - 1.6	2.5 - 2.6	2.5 - 2.6	2.5 - 2.6	2.5 - 2.6	2.5 - 2.6
United Kingdom	NDNS-RollingProgrammeYears1-3	185	1.6 - 1.7	1.6 - 1.7	1.6 - 1.7	1.6	1.6 - 1.7	3.1 - 3.2	3.1 - 3.2	3.1 - 3.2	3.1 - 3.2	3.1 - 3.2
Italy	INRAN_SCAI_2005_06	36	1.1 - 1.2	1.1 - 1.2	1.1 - 1.2	1.1 - 1.2	1.1 - 1.2	-*	-*	-*	-*	-*
Netherlands	VCP_kids	322	1.3	1.3	1.3	1.3	1.3	2.3 - 2.4	2.3 - 2.4	2.3 - 2.4	2.3 - 2.4	2.3 - 2.4
Other children												
Belgium	Regional_Flanders	625	1.6	1.6	1.6	1.6	1.6	2.8 - 2.9	2.8 - 2.9	2.8 - 2.9	2.8	2.8 - 2.9
Bulgaria	NUTRICHILD	433	1.4	1.4	1.4	1.4	1.4	3.2	3.2	3.2	3.2	3.2
Czech Republic	SISP04	389	1.2	1.2	1.2	1.2	1.2	2.5	2.5 - 2.6	2.5	2.5	2.5
Germany	EsKiMo	835	1.0 - 1.1	1.0 - 1.1	1.0 - 1.1	1.0 - 1.1	1.0 - 1.1	1.9 - 2.0	1.9 - 2.0	2.0	1.9 - 2.0	1.9 - 2.0
Germany	VELS	293	1.2 - 1.3	1.2 - 1.3	1.2 - 1.3	1.2 - 1.3	1.2 - 1.3	2.2	2.2	2.2	2.2	2.2
Denmark	Danish_Dietary_Survey	490	0.9	0.9	0.9	0.9	0.9	1.4 - 1.5	1.5	1.4 - 1.5	1.4 - 1.5	1.4 - 1.5
Spain	enKid	156	1.3 - 1.4	1.3 - 1.4	1.3 - 1.4	1.3	1.3 - 1.4	3.0	3.0	3.0 - 3.1	2.9 - 3.0	3.0
Spain	NUT_INK05	399	1.1	1.1	1.1	1.0 - 1.1	1.1	2.0	2.0	2.0	2.0	2.0
Finland	DIPP_2001_2009	750	1.0	1.0	1.0	1.0	1.0	1.7	1.7	1.7	1.7	1.7
France	INCA2	482	1.1	1.1	1.1	1.1	1.1	1.8 - 1.9	1.8 - 1.9	1.8 - 1.9	1.8 - 1.9	1.8 - 1.9
United Kingdom	NDNS-RollingProgrammeYears1-3	651	1.5	1.5	1.5	1.4 - 1.5	1.5	2.6 - 2.7	2.6 - 2.7	2.6 - 2.7	2.6	2.6 - 2.7
Greece	Regional_Crete	838	1.4	1.4	1.4	1.4	1.4	2.9	2.9	2.9	2.9	2.9
Italy	INRAN_SCAI_2005_06	193	1.0 - 1.1	1.0 - 1.1	1.0 - 1.1	1.0 - 1.1	1.0 - 1.1	2.2 - 2.4	2.3 - 2.4	2.2 - 2.4	2.2 - 2.4	2.2 - 2.4
Latvia	EFSA_TEST	187	1.2	1.2	1.2	1.2	1.2	2.3 - 2.4	2.3 - 2.4	2.3 - 2.4	2.3 - 2.4	2.3 - 2.4
Netherlands	VCP_kids	957	1.1 - 1.2	1.1 - 1.2	1.1 - 1.2	1.1	1.1 - 1.2	2.0	2.0	2.0	2.0	2.0

Table continued overleaf.

9437 **Table G2:** Chronic exposure levels to acrylamide (AA) resulting from the scenarios B1, C1, C2 and D1 compared to the baseline (BASE) scenario,
9438 expressed in µg/kg b.w. per day for each survey and age group (continued)

Country	Survey acronym	N[(a)]	Mean LB-UB[(b)]					P95 LB-UB[(c)]				
			BASE	B1	C1	C2	D1	BASE	B1	C1	C2	D1
Netherlands	VCPBasis_AVL2007_2009	447	1.2	1.2	1.2	1.2	1.2	2.3	2.3	2.3	2.2 - 2.3	2.3
Sweden	NFA	1 473	1.2	1.2	1.2	1.1 - 1.2	1.2	2.0 - 2.1	2.0 - 2.1	2.0 - 2.1	2.0 - 2.1	2.0 - 2.1
					Adolescents							
Belgium	Diet_National_2004	576	0.6	0.6	0.6	0.6	0.6	1.3	1.3	1.3	1.3	1.3
Cyprus	Childhealth	303	0.7	0.7	0.7	0.7	0.7	1.4	1.4	1.4	1.4	1.4
Czech Republic	SISP04	298	0.9	0.9	0.9	0.9	0.9	2.0	2.0	2.0	1.9	2.0
Germany	National_Nutrition_Survey_II	1 011	0.4	0.4	0.4	0.4	0.4	0.9	0.9 - 1.0	0.9	0.9	0.9 - 1.0
Germany	EsKiMo	393	0.8	0.8	0.8	0.8	0.8	1.4 - 1.5	1.4 - 1.5	1.4 - 1.5	1.4 - 1.5	1.4 - 1.5
Denmark	Danish_Dietary_Survey	479	0.5 - 0.6	0.6	0.5 - 0.6	0.5 - 0.6	0.5 - 0.6	1.1	1.1 - 1.2	1.1	1.1	1.1
Spain	AESAN_FIAB	86	0.7	0.7	0.7	0.7	0.7	1.3 - 1.4	1.3 - 1.4	1.3 - 1.4	1.3	1.3 - 1.4
Spain	enKid	209	0.8	0.8	0.8	0.8	0.8	1.9	1.9	1.9	1.9	1.9
Spain	NUT_INK05	651	0.7	0.7	0.7	0.7	0.7	1.3	1.3	1.3	1.3	1.3
Finland	NWSSP07_08	306	0.5	0.5	0.5	0.5	0.5	0.9	0.9	0.9	0.9	0.9
France	INCA2	973	0.6	0.6	0.6	0.6	0.6	1.2	1.2	1.2	1.2	1.2
United Kingdom	NDNS-RollingProgrammeYears1-3	666	0.8 - 0.9	0.8 - 0.9	0.8 - 0.9	0.8 - 0.9	0.8 - 0.9	1.7	1.7	1.7	1.6 - 1.7	1.7
Italy	INRAN_SCAI_2005_06	247	0.7	0.7	0.7	0.7	0.7	1.4	1.4	1.4	1.4	1.4
Latvia	EFSA_TEST	453	0.9	0.9	0.9	0.9	0.9	2.0	2.0	2.0	1.9	2.0
Latvia	FC_PREGNANTWOMEN_2011	12	0.7	0.7	0.7	0.7	0.7	-*	-*	-*	-*	-*
Netherlands	VCPBasis_AVL2007_2009	1 142	0.9	0.9	0.9	0.8 - 0.9	0.9	1.7 - 1.8	1.7 - 1.8	1.7 - 1.8	1.7	1.7 - 1.8
Sweden	NFA	1 018	0.8	0.8	0.8	0.8	0.8	1.5 - 1.6	1.5 - 1.6	1.5 - 1.6	1.5 - 1.6	1.5 - 1.6
					Adults							
Belgium	Diet_National_2004	1 292	0.5	0.5	0.5	0.5	0.5	1.0 - 1.1	1.0 - 1.1	1.0 - 1.1	1.0 - 1.1	1.0 - 1.1
Czech Republic	SISP04	1 666	0.5	0.5	0.5	0.5	0.5	1.1	1.1	1.1	1.1	1.1 - 1.2
Germany	National_Nutrition_Survey_II	10 419	0.4	0.4	0.4	0.4	0.4 - 0.5	0.9	0.9	0.9	0.9	0.9
Denmark	Danish_Dietary_Survey	2 822	0.5	0.5	0.5	0.5	0.5	0.8 - 0.9	0.9	0.8 - 0.9	0.8 - 0.9	1.0
Spain	AESAN	410	0.4	0.4 - 0.5	0.4	0.4	0.4 - 0.5	1.0	1.0	1.0	1.0	1.0
Spain	AESAN_FIAB	981	0.5	0.5	0.5	0.5	0.5	1.1	1.1	1.1	1.1	1.1

9439 Table continued overleaf.
9440

Table G2: Chronic exposure levels to acrylamide (AA) resulting from the scenarios B1, C1, C2 and D1 compared to the baseline (BASE) scenario, expressed in µg/kg b.w. per day for each survey and age group (continued)

Country	Survey acronym	N[(a)]	Mean LB-UB[(b)]					P95 LB-UB[(c)]				
			BASE	B1	C1	C2	D1	BASE	B1	C1	C2	D1
Finland	FINDIET2012	1 295	0.5	0.5	0.5	0.5	0.5	0.9	0.9	0.9	0.9	1
France	INCA2	2 276	0.4	0.4	0.4	0.4	0.4	0.7 - 0.8	0.8	0.7 - 0.8	0.7 - 0.8	0.8
United Kingdom	NDNS-RollingProgrammeYears1-3	1 266	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.0
Hungary	National_Repr_Surv	1 074	0.5	0.5	0.5	0.5	0.5	0.9	0.9	0.9	0.9	0.9
Ireland	NANS_2012	1 274	0.6	0.6	0.6	0.6	0.6	1.1	1.1	1.1	1.1	1.1
Italy	INRAN_SCAI_2005_06	2 313	0.4	0.4	0.4	0.4	0.4 - 0.5	0.8 - 0.9	0.8 - 0.9	0.8 - 0.9	0.8 - 0.9	0.9
Latvia	EFSA_TEST	1 271	0.6	0.6	0.6	0.6	0.6	1.3 - 1.4	1.3 - 1.4	1.3 - 1.4	1.3 - 1.4	1.3 - 1.4
Latvia	FC_PREGNANTWOMEN_2011	990	0.6	0.6	0.6	0.6	0.6	1.1	1.1	1.1	1.1	1.1
Netherlands	VCPBasis_AVL2007_2009	2 057	0.6	0.6	0.6	0.6	0.6	1.2	1.2	1.2	1.2	1.2 - 1.3
Sweden	Riksmaten 2010	1 430	0.5	0.5	0.5	0.5	0.5 - 0.6	0.9	0.9	0.9	0.9	1.0 - 1.1
Elderly												
Belgium	Diet_National_2004	511	0.5	0.5	0.5	0.5	0.5	0.9 - 1.0	0.9 - 1.0	0.9 - 1.0	0.9 - 1.0	0.9 - 1.0
Germany	National_Nutrition_Survey_II	2 006	0.4	0.4	0.4	0.4	0.4 - 0.5	0.9	0.9	0.9	0.9	0.9 - 1.0
Denmark	Danish_Dietary_Survey	309	0.5	0.5	0.5	0.5	0.6	0.8	0.8 - 0.9	0.8	0.8	0.9 - 1.0
Finland	FINDIET2012	413	0.4 - 0.5	0.4 - 0.5	0.4 - 0.5	0.4 - 0.5	0.5	0.8 - 0.9	0.8 - 0.9	0.8 - 0.9	0.8 - 0.9	0.9
France	INCA2	264	0.4	0.4	0.4	0.4	0.4	0.7	0.7	0.7	0.7	0.7 - 0.8
United Kingdom	NDNS-RollingProgrammeYears1-3	166	0.5	0.5	0.5	0.4 - 0.5	0.5	0.8	0.8	0.8	0.8	0.8
Hungary	National_Repr_Surv	206	0.4	0.4	0.4	0.4	0.4	0.8	0.8	0.8	0.8	0.8
Ireland	NANS_2012	149	0.5	0.5	0.5	0.5	0.5	0.9	0.9	0.9	0.9	0.9
Italy	INRAN_SCAI_2005_06	290	0.3 - 0.4	0.3 - 0.4	0.3 - 0.4	0.3 - 0.4	0.3 - 0.4	0.6 - 0.7	0.6 - 0.7	0.6 - 0.7	0.6 - 0.7	0.7
Netherlands	VCPBasis_AVL2007_2009	173	0.5	0.5	0.5	0.5	0.5 - 0.6	1.0	1.0	1.0	1.0	1.0
Sweden	Riksmaten 2010	295	0.5	0.5	0.5	0.5	0.6	0.9	0.9	0.9	0.9	1.1
Very elderly												
Belgium	Diet_National_2004	704	0.5 - 0.6	0.5 - 0.6	0.5 - 0.6	0.5 - 0.6	0.5 - 0.6	1.0	1.0	1.0	1.0	1.0
Germany	National_Nutrition_Survey_II	490	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.1
Denmark	Danish_Dietary_Survey	20	0.5	0.5	0.5	0.5	0.5 - 0.6	-*	-*	-*	-*	-*

Table continued overleaf.

Table G2: Chronic exposure levels to acrylamide (AA) resulting from the scenarios B1, C1, C2 and D1 compared to the baseline (BASE) scenario, expressed in µg/kg b.w. per day for each survey and age group (continued)

Country	Survey acronym	N[(a)]	Mean LB-UB[(b)]					P95 LB-UB[(c)]				
			BASE	B1	C1	C2	D1	BASE	B1	C1	C2	D1
France	INCA2	84	0.3 - 0.4	0.3 - 0.4	0.3 - 0.4	0.3 - 0.4	0.4	0.6	0.6	0.6	0.6	0.6
United Kingdom	NDNS-RollingProgrammeYears1-3	139	0.5	0.5	0.5	0.5	0.5	0.9	0.9	0.9	0.9	0.9
Hungary	National_Repr_Surv	80	0.4 - 0.5	0.4 - 0.5	0.4 - 0.5	0.4 - 0.5	0.4 - 0.5	0.9	0.9	0.9	0.9	0.9
Ireland	NANS_2012	77	0.4 - 0.5	0.4 - 0.5	0.4 - 0.5	0.4 - 0.5	0.4 - 0.5	1.0	1.0	1.0	1.0	1.0
Italy	INRAN_SCAI_2005_06	228	0.3 - 0.4	0.3 - 0.4	0.3 - 0.4	0.3 - 0.4	0.4	0.6 - 0.7	0.6 - 0.7	0.6 - 0.7	0.6 - 0.7	0.7
Sweden	Riksmaten 2010	72	0.5	0.5	0.5	0.5	0.6	0.9 - 1.0	0.9 - 1.0	0.9 - 1.0	0.9 - 1.0	1.0 - 1.1

Note: In order to avoid the impression of too high precision, the numbers for all exposure estimates are rounded to 2 figures.

* The 95th percentile (P95) for dietary surveys/age groups with less than 60 subjects were not reliable and therefore not presented.

(a): N: number of subjects.

(b): Mean LB-UB: mean lower bound – upper bound.

(c): P95 LB – UB: 95th percentile lower bound – upper bound. When lower bound and upper bound estimates are equal, only one estimate is provided.

9452 **Appendix H. Previously reported human exposure assessments**

9453 **Table H1**: Previous dietary exposure estimates for acrylamide (AA) for the adult population as published in the literature since 2010

Country	Sampling year	Type of survey	Mean (P95) exposure (µg/kg b.w. per day)	Comments	Reference
31 countries	2004-2009	Different depending on the country	1 (4)	The estimates include children	FAO/WHO (2011)
Poland	2005-2007	24-h dietary recall	0.33 (0.69)	19-96 years old	Mojska et al. (2010)
Poland	2006	Semi-quantitative FFQ	0.85 (1.70)	Chosen population (South of Poland)	Zając et al. (2013)
Belgium	2002-2007	Repeated non-consecutive 24-h recall and self-administered FFQ	0.35 (1.58[(a)])	-	Claeys et al. (2010)
Finland	Published data (2002, 2004)	48-h recall and 3-d food record	0.44[(b)] (1.16[(a)]) 0.41[(b)] (0.87[(a)])	Women Men	Hirvonen et al. (2011)
France	2007-2009	7-d food record diary	0.43 (1.02)	TDS	Sirot et al. (2012)
France	2007-2009	FFQ in the year before pregnancy and during the last 3 months of pregnancy	0.404 (0.969) 0.285 (0.712)	Before pregnancy Third trimester of pregnancy	Chan-Hon-Tong et al. (2013)
10 European countries	EU monitoring database (JRC-IRMM) (2002-2006) and US-FDA database (2002-2004)	24-h dietary recall	12 - 41 µg per day (women)[(c)(d)] 15-48 µg per day (men)[(c)(d)]	EPIC study 27 centers of 10 European countries	Freisling et al. (2013)
United States	2002-2004	24-h dietary recall and FFQ	0.33 (0.75) (women) 0.39 (0.91) (men)	> 20 years old	Tran et al. (2010)
China	2007	3-day household dietary survey and 24-h recall	0.29 (0.49)	TDS Medium bound	Zhou et al. (2013)
Hong Kong	n.s.	FBQ, FFQ and 24-h dietary recalls	0.13 (0.69[(a)])	-	FEHD (2012)
New Zealand	2011	24-h dietary recall	1.36 1.01 0.84	11-14 years old 19-24 years old > 25 years old	MAF (2012)

9454 b.w.: body weight; FBQ: food behaviour questionnaire; FFQ: food frequency questionnaire; h : hour(s) ; n.s.: not specified; P95: 95th percentile; TD: typical diet; TDS: total diet study.
9455 (a): P97.5 (97.5th percentile).
9456 (b): Median.
9457 (c): Minimally adjusted by gender.
9458 (d): Corresponding to 0.17-0.58 µg/kg b.w. per day for women and to 0.21-0.68 µg/kg b.w. per day for men when using a default body weight of 70 kg (EFSA SC, 2012b).

9459 **Table H2:** Previous dietary exposure estimates for acrylamide (AA) for infants, children and adolescents as reported in the literature

Country	Sampling year	Dietary survey method	Mean (P95) exposure (µg/kg b.w. per day)	Comments	Reference
31 countries	2004-2009	Different depending on the country	1 (4)	The estimates include children	FAO/WHO (2011)
United States	2002-2004	24-h dietary recall and FFQ	0.86 (2.39)	3-12 years old	Tran et al. (2010)
United States	2002-2004	24-h dietary recall	0.44 (0.64) 0.50 (0.73)	Teenagers - Western diet Teenagers - Guideline based diet	Katz et al. (2012)
Finland	Published data (2002, 2004)	3-d food record	0.4[(b)] 1.01[(b)] (1.95[(a)]) 0.87[(b)] (1.53[(a)])	1 year old 3 years old 6 years old	Hirvonen et al. (2011)
Poland	2005-2007	24-h dietary recall	0.75 (2.88) 0.62 (2.45)	1-6 years old 7-18 years old	Mojska et al. (2010)
Spain	Published data (2005-2007). Other foods: n.s.	Design diets based on eating pattersn and following the recommended Intakes for the Spanish Population	0.53	11-14 years old (males)	Delgado-Andrade et al. (2012)
Poland	2006	Semi-quantitative FFQ	1.51 (2.86)	6-12 years old Chosen population (South of Poland)	Zając et al. (2013)
France	2007-2009	7-d food record diary	0.89 (1.86) 0.69 (1.80)	3-6 years old 3-17 years old	Sirot et al. (2012)
Poland	2007-2011	Theoretical infant daily intake of individual foodstuff	0.4 - 0.6 2.1 - 4.3 7.5 - 12.4	Minimum AA levels Non breast-fed infants (6-12 months old) Average AA levels Non breast-fed infants (6-12 months old) Highest AA levels Non breast-fed infants (6-12 months old)	Mojska et al. (2012)
Turkey	2012	24-h dietary recall	1.43 (3.76)	1-3 years old	Cengiz and Gündüz (2013)
Canada	n.s.	2-d food record FFQ	0.58 (2.19) 0.20 (0.44)	10-17 year old non-smoking adolescents	Normandin et al. (2013)

9460 b.w.: body weight; FFQ: food frequency questionnaire; n.s.: not specified; P95: 95th percentile.
9461 (a): P97.5.
9462 (b): Median.

9463 **Appendix I. Studies in the literature reporting potential protective effects against acrylamide (AA) and glycidamide (GA) toxicity**

9464 **Table I1:** Overview of publications reporting on the potential protective effects of drugs, natural compounds and other chemicals towards biochemical and
9465 adverse effects of acrylamide (AA) and glycidamide (GA). This overview covers studies published from 2010 and does not claim for completeness. The
9466 conclusions are those expressed by the authors of the studies in the publications.

Reference	Title of the study	Conclusions from the authors as indicated in the abstract of the studies
Mehri et al. (2014)	Chrysin reduced AA-induced neurotoxicity in both *in vitro* and *in vivo* assessments	AA decreased cell viability and pre-treatment with chrysin (0.5-5 μM) significantly decreased AA-induced cytotoxicity in the time- and dose-dependent manner. In Wistar rats, exposure to AA significantly induced severe gait abnormalities, but treatment with chrysin (50 mg/kg) reduced AA-induced neurotoxicity in animals. The authors concluded that '*In the current study, chrysin exhibited neuroprotective effect on PC12 cells as an in vitro model and also on Wistar rats'*.
Siahkoohi et al. (2014)	The effects of vitamin E on the liver integrity of mice fed with AA diet	Following AA consumption, the serum levels of liver enzymes significantly increased and light microscopy showed lymphocytes infiltration, inflammation of portal space and central vein, apoptosis, chromatolysis and fibrous expansion in some portal areas in AA-treated mice. There was a statistically considerable difference between biochemical parameters, index apoptosis and histological features when the AA plus vitamin E-treated group was compared with acrylamide-treated group. The authors concluded that '*Acrylamide induced disturbance in hepatocytes activity and increased the serum levels of liver and structural changes in the liver. Administration of vitamin E significantly reduced the increased level of serum aminotransferase and the pathological changes, also effectively suppressed the acrylamide–induced liver injury*'
Zhang et al. (2013)	Potential protective effects of oral administration of allicin on AA-induced toxicity in male mice	Orally administered allicin could significantly decrease TBARS and MPO levels, and remarkably increased the SOD activity, GST and GSH levels in the kidney, liver, and brain of the AA-treated mice. Oral administration of allicin significantly decreased AST, ALT, LDH, BUN, TNF-α, IL-1β, IL-6, ROS and 8-OHdG, and increased IL-10 in the serum of AA-treated mice. The authors concluded that '*oral administration of allicin had a significant* in vivo *protective effect against the AA induced toxicity'*.
Song et al. (2013)	Protection of cyanidin-3-glucoside (Cy-3-glu) against oxidative stress induced by AA in human MDA-MB-231 cells	Compared to MDA-MB-231 cells treated with AA only, pre-treatment of Cy-3-glu significantly inhibited AA-induced cytotoxicity, reduced ROS generation, recovered GSH depletion and decreased the activities of GPx and GST. The expression of GPx1, GSTP1 and γ-GCS were enhanced, and CYP2E1 expression was inhibited by the pre-treatment of Cy-3-glu. The authors concluded that '*Cy-3-glu presents the protective role against oxidative stress induced by AA in MDA-MB-231 cells*'.

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9469 **Table I1:** Overview of publications reporting on the potential protective effects of drugs, natural compounds and other chemicals towards biochemical and
9470 adverse effects of acrylamide (AA) and glycidamide (GA). This overview covers studies published from 2010 and does not claim for completeness. The
9471 conclusions are those expressed by the authors of the studies in the publications (continued).

Reference	Title of the study	Conclusions from the authors as indicated in the abstract of the studies
Shinomol et al. (2013)	Prophylaxis with *Bacopa monnieri* attenuates AA induced neurotoxicity and oxidative damage via elevated antioxidant function	Pretreatment with *Bacopa monnieri* protected the N27 cells against AA-induced cell death and associated oxidative damage. Co-treatment and pre-treatment of *Drosophila melanogaster* with *Bacopa monnieri* extract protected against AA-induced locomotor dysfunction and GSH depletion. The authors concluded that 'Bacopa monnieri *displays prophylactic effects against AA induced oxidative damage and neurotoxicity with potential therapeutic application in human pathology associated with neuropathy*'.
Prasad and Muralidhara (2013)	Neuroprotective efficacy of eugenol and isoeugenol in AA-Induced neuropathy in rats: behavioral and biochemical evidence.	Treatment of rats with AA and with spice active principles caused marked improvement in gait score and responses in a battery of behavioral tests. Both spice active principles markedly attenuated AA-induced markers of oxidative stress viz., ROS, MDA and NO in SN as well as brain regions (cortex Ct, cerebellum Cb). Treatment with eugenol restored the reduced glutathione levels in SN and brain regions. Interestingly, both spice active principles effectively diminished AA-induced elevation in cytosolic calcium levels and acetylcholinesterase activity in SN and Ct. The diminished activity of ATPase among AA rats was enhanced in SN and restored in brain regions. Eugenol treatment significantly offset AA-induced depletion in dopamine levels in brain regions. The authors concluded '*on the propensity of these spice active principles to attenuate AA-induced neuropathy'*.
Hasseeb et al. (2013)	Impacts of grape seed oil supplementation against the AA induced lesions in male genital organs of rats.	Compared to the group of AA-intoxication, similar scales of lesions were seen in the group administrated by AA with low levels of grape seed oil, while the lesions in the other group of administration by AA with the high levels of grape seed oil were of less scales. The authors concluded on '*the occurrence of a less and level-dependent impact ameliorating effect of grape seed oil supplementation against AA-induced lesions in male genital organs of the adult rats'*.
Chen et al. (2013b)	Myricitrin inhibits AA-mediated cytotoxicity in human Caco-2 cells by preventing oxidative stress	Myricitrin can effectively scavenge multiple free radicals (including DPPH free radical, hydroxyl radical, and ABTS free radical) in a concentration-dependent manner. The presence of myricitrin (2.5–10 μg/mL) was found to significantly inhibit AA-induced cytotoxicity in human gastro-intestinal Caco-2 cells. Myricitrin was able to suppress AA toxicity by inhibiting ROS generation. The authors concluded that '*these results demonstrate that myricitrin had a profound antioxidant effect and can protect against AA-mediated cytotoxicity*'.

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Table I1: Overview of publications reporting on the potential protective effects of drugs, natural compounds and other chemicals towards biochemical and adverse effects of acrylamide (AA) and glycidamide (GA). This overview covers studies published from 2010 and does not claim for completeness. The conclusions are those expressed by the authors of the studies in the publications (continued).

Reference	Title of the study	Conclusions from the authors as indicated in the abstract of the studies
Alturfan et al. (2012a)	Resveratrol ameliorates oxidative DNA damage and protects against AA-induced oxidative stress in rats	In the resveratrol-treated AA group, oxidant responses reversed significantly. Serum enzyme activities, cytokine levels and leukocyte late apoptosis which increased following AA administration, decreased with resveratrol treatment. The authors concluded that '*supplementing with resveratrol can be useful in individuals at risk of AA toxicity*'.
Alturfan et al. (2012b)	Protective effect of N-acetyl-L-cysteine against AA-induced oxidative stress in rats.	In the AA group, GSH levels decreased significantly, while the MDA levels, MPO activity, and collagen content increased in the tissues suggesting oxidative organ damage. In the NAC + AA group, oxidant responses reversed significantly. Serum enzyme activities, cytokine levels, and leukocyte apoptosis, which increased following AA administration, decreased with NAC treatment. The authors concluded that '*supplementing with NAC can be useful when there is a risk of AA toxicity, as NAC inhibited neutrophil infiltration, balanced the oxidant-antioxidant status, and regulated the generation of infl ammatory mediators to protect tissues*'.
Dobrowolski et al. (2012)	Potato fiber protects the small intestinal wall against the toxic influence of AA	The two potato fiber preparations that were used abolished the negative influences of AA on the small intestinal wall and had no influence on the hemoglobin adduct levels of AA. The authors concluded that '*the negative impact of AA on the histologic structure, regeneration, and innervation of the small intestinal wall and the absorptive function of the small intestinal mucosa can be abolished by dietary potato fiber preparations*'.
El-Halim and Mohamed (2012)	Garlic powder attenuates AA-induced oxidative damage in multiple organs in rat	The administration of AA resulted in significant elevation in kidney, spleen, testes and brain malondialdehyde level (MDA) and significant reduction in the level of reduced glutathione (GSH) and the activity of copper-zinc superoxide dismutase (Cu/Zn SOD) in the same organs. Also serum urea and creatinine levels and LDH and alkaline phosphatase activities were significantly elevated whereas serum total proteins and albumin were significantly reduced in AA-treated rats as compared with negative control. The authors concluded that '*treatment with garlic prior to AA produced protective effects and attenuated these biochemical changes*'
El-Kholy et al. (2012)	A trail of using green tea for competing toxicity of AA on liver function	The authors concluded that '*in rats, supplementation with antioxidant (green tea) reduced the effect of AA on the hepatological markers in serum AST and ALT and ALP, and that green tea enhanced antioxidative abilities in liver and protected liver cells membrane against AA action'*.

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9479 **Table I1:** Overview of publications reporting on the potential protective effects of drugs, natural compounds and other chemicals towards biochemical and
9480 adverse effects of acrylamide (AA) and glycidamide (GA). This overview covers studies published from 2010 and does not claim for completeness. The
9481 conclusions are those expressed by the authors of the studies in the publications (continued).

Reference	Title of the study	Conclusions from the authors as indicated in the abstract of the studies
Lakshmi et al. (2012)	Ameliorating effect of fish oil on AA induced oxidative stress and neuronal apoptosis in cerebral cortex	AA administered rats showed increased levels of lipid peroxidative product, protein carbonyl content, hydroxyl radical and hydroperoxide which were significantly modulated by the supplementation of fish oil. The activities of enzymic antioxidants and levels of reduced glutathione were markedly lowered in AA-induced rats. Fish oil treatment augmented these antioxidant levels in cortex. Free radicals generated during AA administration reduced the activities of membrane adenosine triphosphatases and acetylcholine esterase. Fish oil enhanced the activities of these enzymes near normal level. Histological observation represented the protective role of fish oil in AA-induced neuronal damage. Fish oil reduced the AA-induced apoptosis through the modulation in expressions of B-cell lymphoma 2 (Bcl2)-associated X protein and Bcl2-associated death promoter. Further, fish oil increases the expression of heat shock protein 27 (Hsp27). The authors concluded that '*there is evidence for the neuroprotective effect of fish oil on AA-induced neurotoxicity by reducing oxidative stress and apoptosis with modulation in the expression of Hsp27*'.
Mehri et al. (2012)	Neuroprotective effect of crocin on AA-induced cytotoxicity in PC12 cells	Crocin significantly attenuated AA cytotoxicity in a dose-dependent manner. Crocin inhibited the downregulation of Bcl-2 and the upregulation of Bax and decreased apoptosis in treated cells, and inhibited ROS generation in cells exposed to AA. The authors concluded that '*pre-treatment with crocin protected cells from AA-induced apoptosis partly by inhibition of intracellular ROS production*'.
Sadek (2012)	Antioxidant and immunostimulant effect of *carica papaya* linn. aqueous extract in AA intoxicated rats	Administration of *Carica papaya* fruit aqueous extract significantly ameliorated the increased levels of MDA and decline of GSH, SOD and CAT activity in the stomach, liver and kidney tissues caused by AA toxicity. *Carica papaya* fruit aqueous extract significantly increased immune functions (IgG and IgM) while AA significantly decrease it specially IgG. The authors concluded that '*AA-induced oxidative stress in rats can be ameliorated by administration of Carica papaya fruit aqueous extract*'.
Rahangadale et al. (2012)	Evaluation of protective effect of Vitamin E on AA induced testicular toxicity in Wistar rats	At recovery period, there was significant increase in the total sperm count of vitamin-E-treated group of animals as compared to untreated toxicated rats. But, values were significantly lower than control animals. Somewhat better architecture of the seminiferous tubules was also observed. Late spermatids were seen in few seminiferous tubules and other revealed starting of spermatogenesis. The authors concluded that '*Vitamin E is not able to protect testes from AA toxicity during active feeding, but after cessation of AA feeding treatment with vitamin E revealed faster recovery as compare to not treated group'*.

9482 Table continued overleaf.
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Table I1: Overview of publications reporting on the potential protective effects of drugs, natural compounds and other chemicals towards biochemical and adverse effects of acrylamide (AA) and glycidamide (GA). This overview covers studies published from 2010 and does not claim for completeness. The conclusions are those expressed by the authors of the studies in the publications (continued).

Reference	Title of the study	Conclusions from the authors as indicated in the abstract of the studies
Zhang et al. (2012)	Protective effect of allicin against AA-induced hepatocyte damage *in vitro* and *in vivo*	Allicin significantly decreased the levels of MDA and 8-OHdG both *in vitro* and *in vivo* study. Allicin markedly increased the activity of total SOD and level of GSH. The authors concluded that '*the protective effects of allicin against AA-induced hepatocyte damage may be due to its ability to scavenge free radicals and its effective recovery of the antioxidative defense system, and its ability to block the epoxidation process of AA to GA by inhibiting P450 enzyme'*.
Rodríguez-Ramiro et al. (2011a)	Olive oil hydroxytyrosol reduces toxicity evoked by AA in human Caco-2 cells by preventing oxidative stress	AA cytotoxicity was counteracted by hydroxytyrosol by powerfully reducing ROS generation, recovering the excited enzyme antioxidant defences and decreasing phospho-Jun kinase concentration and caspase-3 activity induced by AA. The authors concluded that '*the olive oil natural dietary antioxidant hydroxytyrosol was able to contain AA toxicity by improving the redox status of Caco-2 cells and by partly restraining the apoptotic pathway activated by AA'*.
Rodríguez-Ramiro et al. (2011b)	Procyanidin B2 and a cocoa polyphenolic extract inhibit AA-induced apoptosis in human Caco-2 cells by preventing oxidative stress and activation of JNK pathway	AA cytotoxicity was counteracted by cocoa polyphenolic extract or Procyanidin B2 by inhibiting GSH consumption and ROS generation, increasing the levels of gamma-glutamyl cysteine synthase and glutathione-S-transferase and blocking the apoptotic pathways activated by AA. The authors concluded that '*natural dietary antioxidant such as Procyanidin B2 and cocoa polyphenolic extract were able to suppress AA toxicity by improving the redox status of Caco-2 cells and by blocking the apoptotic pathway activated by AA'*.
Khan et al. (2011)	Protective potential of methanol extract of *Digera muricata* on AA induced hepatotoxicity in rats	Treatment of methanol extract of *Digera muricata* dose dependently ameliorated the toxicity of AA and the studied parameters were reversed towards the control level. Hepatic lesions induced with AA were reduced with treatment with methanol extract of *Digera muricata*. Phytochemical screening indicates the presence of flavonoids, alkaloids, terpenoids, saponins, tannins, phlobatanin, coumarins, anthraquinones and cardiac glycosides. The authors concluded that '*the results obtained suggested that the hepatoprotective effects of the methanol extract of* Digera muricata *against AA-induced oxidative injuries could be attributed to the phenolics and flavonoids'*.
Alzahrani (2011)	Protective effect of L-carnitine against AA-induced DNA damage in somatic and germ cells of mice	Treatment with AA induced a statistically significant increase in the % of chromosomal aberrations and micronuclei in bone- marrow cells. This was reduced significantly in all groups treated with AA and the protective agent L-carnitine. The morphological sperm abnormalities observed in the AAA treated animals were reduced in the group treated with the same dose of AA and L-carnitine. The authors concluded that '*the results confirmed the protective role of L-carnitine against the mutagenicity of AA*'.

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9489 **Table I1:** Overview of publications reporting on the potential protective effects of drugs, natural compounds and other chemicals towards biochemical and
9490 adverse effects of acrylamide (AA) and glycidamide (GA). This overview covers studies published from 2010 and does not claim for completeness. The
9491 conclusions are those expressed by the authors of the studies in the publications (continued).

Reference	Title of the study	Conclusions from the authors as indicated in the abstract of the studies
Mohareb et al. (2011)	Development of new indole-derived neuroprotective agents	Treatment with the indole derivatives 9b, 12c, 14a, and 17 (i.p., 50 mg kg/b.w.) prior to AA produced neuroprotective activity with various intensities depending on the structure of each compound. Compound 17 in which the tetrazole ring was attached to the L-tryptophan moiety ranked as the strongest neuroprotective agent. The authors concluded that '*all the tested compounds have been shown to possess antioxidant properties offering promising efficacy against oxidative stress induced by AA administration*'.
Ghareeb et al. (2010)	Ameliorated effects of garlic (*Allium sativum*) on biomarkers of subchronic AA hepatotoxicity and brain toxicity in rats	Co-administration of garlic powder with AA significantly attenuated oxidative stress, MAO activity, and inflammation in brain and hepatic tissues but did not ameliorate AChE activity. The authors concluded that '*the results obtained emphasized the role of garlic as a potential adjuvant therapy to prevent AA neurotoxicity and hepatotoxicity*'.
Ahmed et al. (2010)	Potent neuroprotective role of novel melatonin derivatives for management of central neuropathy induced by AA in rats	Treatment with melatonin derivatives prior to AA produced significant decrease in brain MDA level and LDH activity with concomitant significant increase in brain monoamines and antioxidant enzymes activity. The authors concluded that '*the new synthesized melatonin derivatives exhibited promising protective activity against AA-induced neurotoxicity*'.

9492 γ-GCS: gamma-glutamyl cysteine synthase; 8-OHdG: 8-hydroxy-desoxyguanosine; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; GPx:
9493 glutathione peroxidase; GSH: glutathione; GST: glutathione S-transferase; IL-1β: interleukin-1β; IL-6: interleukin-6; IL-10: interleukin-10; LDH: lactate dehydrogenase; MDA:
9494 malondialdehyde; MPO: myeloperoxidase; NO: nitric oxide; ROS: reactive oxygen species; SOD: superoxide dismutase; SN: sciatic nerve;TBARS: thiobarbituric reactive substances; TNF-α:
9495 tumour necrosis factor α.
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9497 **Appendix J. Epidemiological studies**

9498 **Table J1**: Features of epidemiological studies on dietary acrylamide (AA) and cancer risk

Reference	Study design	Country	Exposure measurement	Details of exposure measurement	Used AA data	Validity and reproducibility
Mucci et al. (2003a,b)	Population-based case-control study	Sweden	FFQ with 188 items	Foods were ranked according to AA content (1,2,4,8), multiplied with frequency and summed up	Swedish data	not reported
Mucci et al. (2004)	Population-based case-control study	Sweden	FFQ with 63 items		Swedish and US data	not reported
Mucci et al. (2005)	Prospective cohort study	Sweden (Women's Lifestyle and Health Cohort)	FFQ with 80 items		Swedish data	not reported
Mucci et al. (2006); Larsson et al. (2009a,b,c)	Prospective cohort study	Swedish Mammography Cohort (SMC)	FFQ with 67 items (at baseline) and with 96 items (in 1997)		Swedish and US data	Validity 1st FFQ: correlation 0.6 for coffee, 0.5 for whole grain bread and 0.6 and breakfast cereals/muesli
Pelucchi et al. (2006, 2007, 2011b)	Hospital-based case-control study	Italy and Switzerland	FFQ with 78 items		WHO, French and Swiss data	Reproducibility: correlation 0.52-0.75 for AA-containing foods

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9501 **Table J1**: Features of epidemiological studies on dietary acrylamide (AA) and cancer risk (continued)

Reference	Study design	Country	Exposure measurement	Details of exposure measurement	Used AA data	Validity and reproducibility
Hogervorst et al. (2007, 2008a,b, 2009a,b); Pedersen et al. (2010); Schouten et al. (2009); Bongers et al. (2012); Hogervorst et al. (2014)	Prospective cohort study	Netherlands Cohort Study (NLCS) on Diet and Cancer	FFQ with 150 items		Dutch data	Reproducibility: correlation 0.66-0.71 for carbohydrates and fiber. Validity: correlation 0.65-0.80 for carbohydrates, fiber, energy intake, potates, bread, cakes and cookies.
Olesen et al.(2008)	Nested case-control study	Danish Diet, Cancer and Health Study	AA-Hb and GA-Hb adducts		n.a.	n.a.
Larsson et al. (2009d,e)	Prospective cohort study	Cohort of Swedish Men	FFQ with 96 items		Swedish data	not reported
Wilson et al. (2009a)	Population-based case-control study	Cancer of the Prostate in Sweden Study (CAPS)	FFQ with 261 items and AA-Hb adducts in subgroups		Swedish data	Correlation 0.25 with AA-HB adducts (0.15 in cases and 0.35 in controls)
Wilson et al. (2009b)	Prospective cohort study	USA, Nurses' Health Study (NHS) II	FFQ with >130 items, at baseline and repeated every 4 years		US data and US foods measured in Sweden	Validity correlation 0.6-0.8 for potato crisps, French fries, coffee and breakfast cereals
Wilson et al. (2010)	Prospective cohort study	USA, NHS	FFQ with 61 to 116 items at baseline and repeated every 2-4 years	Frequency and individual portion size	US data and US foods measured in Sweden	Correlation 0.34 with AA- and GA-HB adducts (see Wilson et al., 2009c)

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9504 **Table J1**: Features of epidemiological studies on dietary acrylamide (AA) and cancer risk (continued)

Reference	Study design	Country	Exposure measurement	Details of exposure measurement	Used AA data	Validity and reproducibility
Hirvonen et al. (2010)	Prospective cohort study	Finland, Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study	Self-administered modified diet-history method with 276 items	Frequency and individual portion size	Mainly Finnish data, some Swedish data	Reproducibility correlation 0.73 Validity correlation 0.43
Burley et al. (2010)	Prospective cohort study	United Kingdom, UK Women's Cohort Study	FFQ with 217 items	Individual frequency and standard portion size	EU data	Reproducibility: correlation 0.61 for dietary AA
Lin et al. (2011)	Case-control study	Sweden	FFQ with 63 items	Individual frequency and sex-specific portion size	Swedish data	Not reported
Olsen et al. (2012)	Prospective cohort study	Denmark, Danish Diet, Cancer and Health Study	AA-Hb and GA-Hb adducts	Prediagnostic measurements	n.a.	n.a.
Wilson et al. (2012)	Prospective cohort study	USA, Health Professionals Follow-up Study	FFQ with >130 items, at baseline and repeated every 4 years	Frequency and individual portion size	US data and US foods measured in Sweden	Correlation 0.34 with AA-en GA-Hb adducts (see Wilson et al., 2009c)

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9507 **Table J1**: Features of epidemiological studies on dietary acrylamide (AA) and cancer risk (continued)

Reference	Study design	Country	Exposure measurement	Details of exposure measurement	Used AA data	Validity and reproducibility
Xie et al. (2013)	Prospective cohort study	USA, NHS and NHS II	AA-Hb and GA-Hb adducts		n.a.	Correlation 0.34 with AA- and GA-Hb adducts (see Wilson et al., 2009c)
Obón-Santacana et al. (2013); Lujan-Barroso et al. (2014)	Prospective cohort study	European Prospective Investigation into Cancer (EPIC)	Country-specific FFQs	Frequency and individual portion size	Database using data from 7 European countries, completed with data from the USA	Validity: correlation 0.17. Correlation 0.08 between FFQ and Hb-AA adducts (see Ferrari et al., 2013)

9508 FFQ: food frequency questionnaire; GA: glycidamide; Hb: hemoglobin; n.a.: not available; US: United States.
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9510 **Table J2:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of reproductive organs

Reference	Cancer site	Study design and size	Range of intake	Overall Results: OR/RR (95 % CI)	Subgroup analyses	Confounders allowed for in the analyses
BREAST CANCER						
Mucci et al. (2005)	Breast, mainly pre-menopausal	Cohort: 667 cases; 43 404 cohort members	12-44 µg/day (mean Q1-Q5)	Q2: 0.9 (0.7-1.1) Q3: 1.0 (0.8-1.3) Q4: 1.0 (0.8-1.3) Q5: 1.2 (0.9-1.6)	n.a.	Age, education, smoking,alcohol, fiber, saturated fat, family history of breast cancer, energy intake, parity, OAC use, age first child birth, menopausal status
Pelucchi et al. (2006)	Breast, pre- and post-menopausal	Case-control study: 2 900 cases 3 122 controls	11-34 µg/day (p20-p80)	Q2: 1.01 (0.85-1.20) Q3: 1.01 (0.85-1.20) Q4: 1.09 (0.92-1.31) Q5: 1.06 (0.88-1.28) *p*-trend, 0.37	n.a.	Age, study center, education, BMI, energy intake, family history of breast or ovarian cancer, parity
Hogervorst et al. (2007)	Breast, post-menopausal	Cohort: 1 835 cases; 62 573 cohort members	10-37 µg/day (median Q1-Q5)	Q2: 0.80 (0.64-1.02) Q3: 0.92 (0.72-1.17) Q4: 0.86 (0.67-1.10) Q5: 0.93 (0.73-1.19) *p*-trend, 0.79	Comparable results for never-smokers	Age, age menarche and menopause,age first child birth, OAC use, post-menopausal hormone use, BMI, height, cigarette smoking, SES,education, energy intake, saturated fat, carbohydrates, family history ofbreast cancer, benign breast disease
Olesen et al. (2008)	Breast, post-menopausal	Nested case-control study in a cohort; 374 cases; 374 controls	AA-Hb adducts 20-209 pmol/g globin GA-Hb adducts 9-99 pmol/g globin (p5-p95)	IRR: 1.9 (0.9-4.0) and IRR: 1.3 (0.6-2.8) per 10-fold increase AA-Hb adducts and GA-Hb adducts, respectively.	ER+ tumours: IRR: 2.7 (1.1-6.6) per 10-fold increase AA-Hb adducts. Stronger associations for smokers	Age, BMI, age at first child birth, parity, postmenopausal hormone use, education, alcohol, tobacco use, past smoking, smoking years and mutually adjusted for AA-Hb and GA-Hb adducts

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9513 **Table J2:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of reproductive organs (continued)

Reference	Cancer site	Study design and size	Range of intake	Overall Results: OR/RR (95 % CI)	Subgroup analyses	Confounders allowed for in the analyses
Larsson et al. (2009a)	Breast, pre- and post-menopausal	Cohort: 2 592 cases; 61 433 cohort members	20-29 µg/day (p25-p75)	Q2: 1.02 (0.92-1.14) Q3: 0.95 (0.85-1.06) Q4: 0.91 (0.80-1.02) p-trend, 0.06	Comparable results for ER- and PR- subgroups and according to smoking status	Age, education, BMI, height, parity, age first child birth, age menarche and menopause, OAC use, PMH treatment, family history of breast cancer, benign breast disease, and intakes of alcohol, coffee, cereal fiber, and total energy
Wilson et al. (2009b)	Breast, pre-menopausal	Cohort: 1 179 cases; 90 628 participants	11-38 µg/day (mean Q1-Q5)	Q2: 0.95 (0.79-1.14) Q3: 0.94 (0.78-1.13) Q4: 1.03 (0.87-1.24) Q5: 0.92 (0.76-1.11) *p*-trend, 0.61	Comparable results according to hormone receptor and smoking status	Age, calendar year, BMI, height, OAC use, parity and age at first birth, age at menarche, family history of breast cancer, history of benign breast disease, smoking, physical activity, animal fat, glycemic load, alcohol and total energy intake
Pedersen et al. (2010)	Breast, post-menopausal	Cohort: 2 225 cases; 62 573 cohort members	10-37 µg/day (median Q1-Q5)	Q2: 0.91 (0.73-1.23) Q3: 0.96 (0.76-1.19) Q4: 0.89 (0.72-1.12) Q5: 0.92 (0.73-1.15) *p*-trend, 0.48	Positive associations for never-smokers and for ER+, PR+ and ER+PR+	Age, age menarche and menopause, age first child birth, parity, BMI, family history of breast cancer, benign breast disease, OAC use, PMH use, energy intake, cigarette smoking

9514 Table continued overleaf.
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9516 **Table J2:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of reproductive organs (continued)

Reference	Cancer site	Study design and size	Range of intake	Overall Results: OR/RR (95 % CI)	Subgroup analyses	Confounders allowed for in the analyses
Wilson et al. (2010)	Breast, post-menopausal	Cohort: 6 301 cases; 88 672 participants	9-26 µg/day (mean Q1-Q5)	Q2: 0.93 (0.86-1.01) Q3: 0.98 (0.91-1.06) Q4: 0.98 (0.90-1.06) Q5: 0.95 (0.87-1.03) *p*-trend, 0.50	Comparable results in strata of smoking, menopausal status, and BMI	Age, calendar year, smoking, BMI, height, menopausal status/ age at menopause/ PMH use, parity and age at first birth, family history of breast cancer, benign breast disease physical activity, glycemic index, folate, animal fat, alcohol, energy intake
Burley et al. (2010)	Breast, pre- and post-menopausal	Cohort: 1 084 cases; 33 731 participants	6-32 µg/day (mean Q1-Q5)	Q2: 1.06 (0.83-1.35) Q3: 1.05 (0.82-1.34) Q4: 1.12 (0.87-1.45) Q5: 1.16 (0.88-1.52) *p*-trend, 0.1	Premenopausal cases: Q2: 1.06 (0.71-1.59) Q3: 1.15 (0.77-1.71) Q4: 1.15 (0.76-1.73) Q5: 1.47 (0.96-2.27) *p*-trend, 0.008 No association for Post-menopausal cases	Age, smoking status, weight, height, physical activity, parity, OAC use, PMH use,age at menarche, alcohol intake, energy intake, level of education.
Olsen et al. (2012)	Breast, post-menopausal	Cohort: 420 cases; 80 breast cancer deaths (survival)	AA and GA-Hb adducts: non-smokers: 30-137 pmol/ g globin Smokers: 60-389 pmol/g globin (p5-p95)	*Non-smoking women* AA-Hb adducts: 1.21 (0.98-1.50) per 25 pmol/g globin. GA-Hb adducts: 1.63 (1.06-2.51) per 25 pmol/g globin.	Significant associations ER+ cases No or slightly weaker associations in smoking women	Time from blood draw to diagnosis, baseline levels of alcohol intake, PMH use

9517 Table continued overleaf.
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9519 **Table J2:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of reproductive organs (continued)

Reference	Cancer site	Study design and size	Range of intake	Overall Results: OR/RR (95 % CI)	Subgroup analyses	Confounders allowed for in the analyses
ENDOMETRIAL CANCER						
Hogervorst et al. (2007)	Endometrial	Cohort: 221 cases; 62 573 cohort members	10-37 µg/day (median Q1-Q5)	Q2: 0.95 (0.59-1.54) Q3: 0.94 (0.56-1.56) Q4: 1.21 (0.74-1.98) Q5: 1.29 (0.81-2.07) *p*-trend, 0.18	Never-smokers: Q2: 1.16 (0.63-2.15) Q3: 1.35 (0.73-2.51) Q4: 1.30 (0.69-2.46) Q5: 1.99 (1.12-3.52) *p*-trend, 0.03	Age, age at menarche, age at menopause, age at first childbirth, parity, duration OAC use, duration PMH use, BMI, height, cigarette smoking, physical acitivity, energy, transunsaturated fat acid, and carbohydrate intake, alcohol intake
Larsson et al. (2009b)	Endometrial	Cohort: 687 cases; 61 226 participants	17-33 µg/day (median Q1-4)	Q2: 1.10 (0.89-1.36) Q3: 1.08 (0.88-1.34) Q4: 0.96 (0.59-1.78) *p*-trend, 0.72	Never-smokers: Q2: 1.31 (0.85-2.04) Q3: 1.30 (0.83-2.02) Q4: 1.20 (0.76-1.90) *p*-trend, 0.52 no association in smoking women	Age, education, BMI, parity, age at first birth, age at menarche, age at menopause, OAC use, PMH use, history of diabetes, smoking status, physical activity, carbohydrate intake, total energy intake
Wilson et al. (2010)	Endometrial	Cohort: 484 cases; 88 672 participants	9-26 µg/day (mean Q1-Q5)	Q2: 1.12 (0.83-1.50) Q3: 1.31 (0.97-1.77) Q4: 1.35 (0.99-1.84) Q5: 1.41 (1.01-1.97) *p*-trend, 0.03	Never-smokers: Q2: 0.97 (0.64-1.46) Q3: 1.35 (0.90-2.02) Q4: 1.47 (0.97-2.24) Q5: 1.43 (0.90-2.28) *p*-trend, 0.04 Comparable results in strata of menopausal status. Significant association in women with normal BMI (<25 kg/m^2)	Age, calendar year, smoking, BMI, height, menopausal status/ age at menopause/ PMH use, OAC use, age at menarche, high blood pressure, diabetes, physical activity, caffeine and energy intake

9520 Table continued overleaf.
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9522 **Table J2:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of reproductive organs (continued)

Reference	Cancer site	Study design and size	Range of intake	Overall Results: OR/RR (95 % CI)	Subgroup analyses	Confounders allowed for in the analyses
OVARIAN CANCER						
Pelucchi et al. (2006)	Ovarian	Case-control study: 1 031 cases 2 411 controls	10-32 µg (p20-p80)	Q2: 1.03 (0.79-1.34) Q3: 1.09 (0.83-1.44) Q4: 1.01 (0.76-1.34) Q5: 0.97 (0.73-1.31) *p*-trend, 0.80	n.a.	Age, study center, education, BMI, energy intake, family history of breast and/or ovarian cancer, parity
Hogervorst et al. (2007)	Ovarian	Cohort: 195 cases; 62 573 cohort members	10-37 µg/day (median Q1-Q5)	Q2: 1.22 (0.73-2.01) Q3: 1.12 (0.65-1.92) Q4: 1.28 (0.77-2.13) Q5: 1.78 (1.10-2.88) *p*-trend, 0.02	Never-smokers: Q2: 1.60 (0.85-3.02) Q3: 1.64 (0.84-3.19) Q4: 1.86 (1.00-3.48) Q5: 2.22 (1.20-4.08) *p*-trend, 0.01	Age, age at menarche, age at menopause, parity, duration OAC use, duration PMH use, BMI, height, cigarette smoking, saturated fat intake, trans-unsaturated fatty acid intake
Larsson et al. (2009c)	Ovarian	Cohort: 368 cases; 61 057 cohort members	17-33 µg/day (median Q1-Q4)	Q2: 0.91 (0.68-1.21) Q3: 0.97 (0.73-1.29) Q4: 0.86 (0.63-1.16) *p*-trend, 0.39	No association for serous ovarian cancer cases	age, education, BMI, parity, age at first childbirth, age at menarche, age at menopause, OAC use, PMH use, total energy intake, dietary fat, carbohydrate, fiber
Wilson et al. (2010)	Ovarian	Cohort: 416 cases; 88 672 participants	9-26 µg/day (mean Q1-Q5)	Q2: 0.93 (0.68-1.29) Q3: 1.29 (0.94-1.76) Q4: 1.17 (0.84-1.64) Q5: 1.25 (0.88-1.77) *p*-trend, 0.12	Never-smokers: Q2: 1.17 (0.72-1.88) Q3: 1.04 (0.63-1.74) Q4: 1.11 (0.63-1.94) Q5: 1.19 (0.66-2.15) *p*-trend, 0.63 Comparable results in strata of menopausal status. Significant association in women with normal BMI (<25 kg/m^2)	Age, calendar year, smoking, BMI, parity, OAC use, menopausal status/PMH use, tubal ligation, physical activity, caffeine intake, energy intake

9523 Table continued overleaf.
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9525 **Table J2:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of reproductive organs (continued)

Reference	Cancer site	Study design and size	Range of intake	Overall Results: OR/RR (95 % CI)	Subgroup analyses	Confounders allowed for in the analyses
Xie et al. (2013)	Ovarian	Cohort, 263 cases and 526 matched controls	AA+GA adducts 74-226 pmol/ g Hb (p10-p90)	T-2: 0.83 (0.56-1.24) T-3: 0.79 (0.50-1.24) *p*-trend, 0.08	Comparable results in non-smokers and for histological subtypes	Matching factors and height, family history of ovarian cancer, tubal ligation, OAC use, BMI, parity, alcohol, smoking, physical activity, caffeine intake

9526 AA: Acrylamide; BMI: Body Mass Index; ER: Estrogen receptor; GA: Glycidamide; Hb: Hemoglobin; IRR: Incidence Rate Ratio; n.a.: not applicable; OAC: Oral contraceptives; OR: Odds
9527 Ratio; p: percentile; PMH: post- menopausal hormones; pmol: picomol; PR: Progesteron receptor; Q1-Q4: quartile 1-4; Q1-Q5: Quintile 1-5; RR: relative risk; SES: Social Economic Status. T:
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9530 **Table J3:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of the gastro-intestinal tract

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
OESOPHAGEAL CANCER						
Pelucchi et al. (2006)	Oesophagus Men and women	Case-control study: 395 cases 1 066 controls	13-40 µg/day (p20-p80)	Q2: 1.16 (0.75-1.81) Q3: 1.20 (0.75-1.93) Q4: 0.74 (0.44-1.24) Q5: 1.10 (0.65-1.86) *p*-trend, 0.67	n.a.	Age, sex, study center, education, BMI, energy intake, alcohol consumption, smoking habits
Hogervorst et al. (2008a)	Oesophagus Men and women	Cohort: 216 cases 120 852 cohort members	M: 10-42 µg/day W: 9-40 µg/day (median Q1-Q5)	Q2: 0.73 (0.47-1.15) Q3: 0.86 (0.56-1.33) Q4: 0.83 (0.54-1.28) Q5: 0.83 (0.54-1.30) *p*-trend, 0.68	Comparable results in never- and former smokers, and in oesophageal adenocarcinomas or squamous cell carcinomas. Increased risks in overweight and obese persons	Age, sex, BMI, consumption of tea, vegetables, fruits, dairy and alcohol, cigarette smoking, family history of oesophageal cancer
Lin et al. (2011)	Oesophagus and Gastrooesophag-eal junction Men and women	Case-control study: 618 cases 820 controls	27-44 µg/day (p25-p75)	Q2: 1.35 (0.96-1.99) Q3: 1.12 (0.91-1.58) Q4: 1.23 (1.02-1.75) *p*-trend, 0.46	Comparable results for adenocarcinoma, squamous cell carcin oma and gastro-eso phageal junction. Stronger associations in overweight persons, and non-smokers only	Age, sex, smoker, alcohol intake, fruit intake, BMI, education, reflux, H.Pylori infection

9531 Table continued overleaf.
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9533 **Table J3:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of the gastro-intestinal tract (continued)

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
Luján-Barroso et al. (2014)	Oesophagus Men and women	Cohort: 341 cases >500,000 Cohort members	14-37 µg/day (p20-p80)	Q2: 1.75 (1.12-2.74) Q3: 1.66 (1.05-2.61) Q4: 1.41 (0.86-2.71)	Comparable results for adenocarcinoma and squamous-cell carcinoma, and never-smokers. Attenuated HRs using energy-adjusted AA intake	Sex, total energy intake, fruit, smoking intensity, processed meat, and stratified for age and country
STOMACH CANCER						
Hogervorst et al. (2008a)	Stomach Men and women	Cohort: 563 cases 120 852 cohort members	M: 10-42 µg/day W: 9-40 µg/day (median Q1-Q5)	Q2: 1.09 (0.81-1.47) Q3: 1.09 (0.81-1.48) Q4: 1.18 (0.87-1.60) Q5: 1.06 (0.78-1.45) *p*-trend, 0.77	No increased risks in never- and former smokers, and in gastric cardia or other stomach cancers	Age, sex, BMI, energy intake, consumption of tea,vegetables, fruits and fish, socioeconomic status, cigarette smoking, family history of stomach cancer
Hirvonen et al. (2010)	Stomach Men	Cohort: 224 cases; 27 111 cohort members	22-56 µg/day (median Q1-Q5)	Q2: 1.42 (0.94-2.13) Q3: 0.78 (0.49-1.26) Q4: 1.34 (0.88-2.05) Q5: 0.96 (0.60-1.53) *p*-trend, 0.78	n.a.	Age, supplementation group, cigarette smoking, physical activity, alcohol intake, BMI, energy-adjusted fiber intake
COLORECTAL CANCER						
Mucci et al. (2003a)	Colorectal cancer Men and women	Case-control study: 591 cases; 538 controls	28 (0.6) µg/day Mean (SE)*	Q2: 0.9 (0.6-1.3) Q3: 0.6 (0.4-0.9) Q4: 0.6 (0.4-1.0) *p*-trend, 0.01	Comparable results for nonsmokers and current smokers	Age, gender, smoking, BMI, alcohol intake, fruit and vegetable intake, saturated fat density, red meat density and total energy

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9535 **Table J3:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of the gastro-intestinal tract (continued)

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
Mucci et al. (2006)	Colorectal cancer Women	Cohort: 741 cases; 61 467 cohort members	13-38 µg/day (mean Q1-Q5)	Q2: 1.1 (0.9-1.4) Q3: 1.2 (0.9-1.5) Q4: 1.1 (0.8-1.4) Q5: 0.9 (0.7-1.3) *p*-trend, 0.85	Comparable results for colon and rectal cancer	Age at screening, BMI, education, alcohol intake, energy intake, saturated fat intake, fiber intake.
Pelucchi et al. (2006)	Colorectal cancer Men and women	Case-control study: 2 280 cases 4 765 controls	12-40 µg/day (p20-p80)	Q2: 0.89 (0.75-1.05) Q3: 1.06 (0.89-1.26) Q4: 1.05 (0.88-1.26) Q5: 0.97 (0.80-1.18) *p*-trend, 0.56	Comparable results for colon and rectal cancer	Age, sex, study center, education, BMI, energy intake, alcohol consumption, smoking habits, physical activity
Hogervorst et al. (2008a)	Colorectal cancer Men and women	Cohort: 2 190 cases 120 852 cohort members	M: 10-42 µg/day W: 9-40 µg/day (median Q1-Q5)	Q2: 0.96 (0.81-1.15) Q3: 1.06 (0.89-1.27) Q4: 0.96 (0.80-1.14) Q5: 1.00 (0.84-1.20) *p*-trend, 0.94	No increased risks in never-smokers, and in colon or rectal cancer	Age, sex, BMI, height, energy, fiber and vitamin B-6 intake, consumption of vegetables, fruits, dairy, meat and alcohol, physical activity, smoking and family history of colorectal cancer
Larsson et al. (2009d)	Colorectal cancer Men	Cohort: 676 cases; 45 306 cohort members	25-49 µg/day(median Q1-Q4)	Q2: 1.02 (0.83-1.25) Q3: 1.03 (0.83-1.28) Q4: 0.95 (0.74-1.20) *p*-trend, 0.69	Comparable results for different subsites of colorectal cancer, and in never-, past and current smokers	Age, education, family history of colorectal cancer, BMI, exercise, history of diabetes, cigarette smoking, aspirin use, total energy intake, alcohol, calcium and dietary fiber intake
Hirvonen et al. (2010)	Colorectal cancer Men	Cohort: 316 cases; 27 111 cohort members	22-56 µg/day (median Q1-Q5)	Q2: 0.93 (0.66-1.32) Q3: 0.89 (0.62-1.26) Q4: 0.95 (0.67-1.36) Q5: 0.93 (0.65-1.34) *p*-trend, 0.75	n.a.	Age, supplementation group, cigarette smoking, physical activity,alcohol intake, BMI

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9538 **Table J3:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of the gastro-intestinal tract (continued)

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
Hogervorst et al. (2014)	Colorectal cancer Men and women	Cohort: 733 cases 120 852 cohort members	M: 10-42 µg/day W: 9-40 µg/day (median Q1-Q5)	Subset of cases from Hogervorst (2008a)	AA intake is positively associated with an activating mutation in the KRAS gene in CRC among men, and has a statistically significant inverse association with the risk of tumors with a truncating mutation in the APC gene among women.	Age, sex, smoking, BMI, family history of colorectal cancer, total energy intake
PANCREATIC CANCER						
Hogervorst et al. (2008a)	Pancreatic cancer Men and women	Cohort: 349 cases 120 852 cohort members	M: 10-42 and W: 9-40 µg/day (median Q1-Q5)	Q2: 1.02 (0.72-1.44) Q3: 0.96 (0.66-1.38) Q4: 0.87 (0.60-1.27) Q5: 0.98 (0.68-1.40) *p*-trend, 0.75	No increased risks in never- and former smokers, and in microscopically confirmed cancers	Age, sex, BMI, height, energy intake, consumption of vegetables, fruits and alcohol, cigarette smoking, diabetes, family history of pancreatic cancer
Hirvonen et al. (2010)	Pancreatic cancer Men	Cohort: 192 cases; 27 111 cohort members	22-56 µg/day (median Q1-Q5)	Q2: 0.98 (0.61-1.56) Q3: 1.08 (0.69-1.71) Q4: 1.06 (0.66-1.69) Q5: 1.00 (0.62-1.62) *p*-trend, 0.89	n.a.	Age, supplementation group, cigarette smoking, physical activity, alcohol intake, BMI, comsumption of vegetables

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9541 **Table J3:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of the gastro-intestinal tract (continued)

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
Pelucchi et al. (2011)	Pancreatic cancer Men and women	Case-control study 326 cases; 652 controls	32 (20) µg/day Mean (SD)	Q2: 1.48 (0.88-2.50) Q3: 1.57 (0.91-2.69) Q4: 1.70 (0.98-2.96) Q5: 1.49 (0.83-2.70) *p*-trend, 0.21	n.a.	study center, sex, age, year of interview, education, tobacco smoking, history of diabetes, energy intake
Obón-Santacana et al. (2013)	Pancreatic cancer Men and woemen	Cohort: 865 cases >500 000 cohort members	14-37 µg/day (p20-p80)	Q2 :0.90 (0.71-1.15) Q3 : 0.78 (0.60-1.01) Q4 : 0.68 (0.52-0.90) Q5 : 0.77 (0.58-1.04)	Comparable results across trata of smoking. Lower risks in women and in obese persons (BMI ≥30 kg/m^2)	Stratified by age at recruitment and center, adjusted for sex, total energy intake, smoking intensity, diabetes and alcohol intake

9542 BMI: Body Mass Index; n.a.: not applicable; OR: Odds Ratio; p: percentile; Q1-Q4: quartile 1-4; Q1-Q5: Quintile 1-5; RR: relative risk; SD: Standard Deviation; SE: Standard Error.
9543 * among controls.
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9545 **Table J4:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of the urinary tract

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
PROSTATE CANCER						
Pelucchi et al. (2006)	Prostate cancer Men	Case-control study: 1 294 cases 1 451 controls	12-36 µg/day (p20-p80)	Q2: 1.00 (0.77-1.30) Q3: 1.22 (0.94-1.58) Q4: 1.01 (0.77-1.33) Q5: 0.92 (0.69-1.23) *p*-trend, 0.65	n.a.	Age, study center, education, BMI, energy intake, alcohol consumption, smoking habits, family history of prostate cancer, physical activity
Hogervorst et al. (2008b)	Prostate cancer Men	Cohort: 2 246 cases 58 279 male cohort members	10-42 µg/day (median Q1-Q5)	Q2: 1.07 (0.88-1.31) Q3: 1.01 (0.82-1.24) Q4: 1.02 (0.83-1.26) Q5: 1.06 (0.87-1.30) *p*-trend, 0.69	Comparable results in never- and former smokers, possibly an inverse association with advanced cancer in never-smokers	Age, socioeconomic status, prostate cancer in the family, alcohol intake, smoking status
Larsson et al. (2009c)	Prostate cancer Men	Cohort: 2 696 cases; 45 306 cohort members	28-43 µg/day (p20-p80)	Q2: 0.86 (0.71-1.04) Q3: 1.02 (0.84-1.23) Q4: 0.90 (0.73-1.10) Q5: 0.88 (0.70-1.09) *p*-trend, 0.34	Comparable results in never-smokers, in localized and in advanced cancer	Age, education, smoking status, BMI, height, physical activity, history of diabetes, family history of prostate cancer, intakes of total energy, alcohol, dietary calcium and red meat.
Wilson et al. (2009a)	Prostate cancer Men	Case-control study: 170 cases; 161 controls	32-56 pmol/g globin AA adducts (median Q1-Q4)	Q2: 0.74 (0.37-1.49) Q3: 0.98 (0.50-1.93) Q4: 0.93 (0.47-1.85)	Comparable results in advanced and localized cancers; in high- and low-grade cancers.	age, region, laboratory batch, BMI, former smoking age, region, education, former and current smoking, BMI, zinc intake, energy intake
		1 499 cases 1 118 controls	33-56 µg/day (p20-p80)	Q2: 1.14 (0.89-1.47) Q3: 0.99 (0.76-1.28) Q4: 1.06 (0.82-1.37) Q5: 0.97 (0.75-1.27)	n.a.	

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9548 **Table J4:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of the urinary tract (continued)

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
Hirvonen et al. (2010)	Prostate cancer Men	Cohort: 799 cases; 27 111 cohort members	22-56 µg/day (median Q1-Q5)	Q2: 0.95 (0.76-1.19) Q3: 1.03 (0.83-1.29) Q4: 1.06 (0.84-1.33) Q5: 1.05 (0.83-1.32) *p*-trend, 0.43	n.a.	age, supplementation group, cigarette smoking, physical activity, alcohol intake, BMI
Wilson et al. (2012)	Prostate cancer Men	Cohort: 5 025 cases; 47 896 cohort members	12-35 µg/day (median Q1-Q5)	Q2: 1.10 (1.01-1.20) Q3: 1.08 (0.99-1.18) Q4: 1.06 (0.97-1.16) Q5: 1.02 (0.92-1.13) *p*-trend, 0.90	Comparable results in advanced and localized cancers; in high- and low-grade cancers	age, calendar time, race, height, BMI at age 21, current BMI, vigorous physical activity, smoking, diabetes, family history of prostate cancer, multivitamin use, intakes of red meat, tomato sauce, calcium, alpha linolneic acid, suppl. Vitamin E, alcohol intake, energy intake, PSA testing
BLADDER CANCER						
Mucci et al. (2003a)	Bladder cancer Men and women	Case-control study: 263 cases; 538 controls	28 (0.6) µg/day Mean (SE)*	Q2: 1.1 (0.7-1.8) Q3: 0.7 (0.4-1.3) Q4: 0.8 (0.5-1.5) *p*-trend, 0.26	Comparable results for nonsmokers and no association in smokers	Age, gender, smoking, BMI, alcohol intake, fruit and vegetable intake, saturated fat density, red meat density and total energy
Hogervorst et al. (2008b)	Bladder cancer Men and women	Cohort: 1 210 cases 120 852 cohort members	M: 10-42 and W: 9-40 µg/day (median Q1-Q5)	Q2: 0.96 (0.77-1.20) Q3: 0.89 (0.71-1.12) Q4: 1.01 (0.81-1.26) Q5: 0.91 (0.73-1.15) *p*-trend, 0.60	Indications for increased risk in heavy smokers. Indications for for decreased risks in women	Age, sex, vegetables, fruits, tea, bladder cancer in the family, smoking status
Hirvonen et al. (2010)	Urothelial cancer Men	Cohort: 365 cases; 27 111 cohort members	22-56 µg/day (median Q1-Q5)	Q2: 0.91 (0.65-1.27) Q3: 1.06 (0.77-1.47) Q4: 0.78 (0.55-1.11) Q5: 0.99 (0.71-1.39) *p*-trend, 0.71	n.a.	Age, supplementation group, cigarette smoking, physical activity, alcohol intake, BMI, consumption of vegetables

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9551 **Table J4:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of the urinary tract (continued)

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
RENAL CELL CANCER						
Mucci et al. (2003a)	Kidney cancer Men and women	Case-control study: 133 cases; 538 controls	28 (0.6) µg/day Mean (SE)	Q2: 1.0 (0.6-1.9) Q3: 1.1 (0.6-2.0) Q4: 0.8 (0.4-1.7) *p*-trend, 0.64	Slightly increased risks for smokers, although not statistically significant	Age, gender, smoking, BMI, alcohol intake, fruit and vegetable intake, saturated fat density, red meat density and total energy
Mucci et al. (2004)	Renal cell cancer Men and women	Case-control study: 379 cases; 353 controls	20-32 µg/day (p25-p75)	Q2: 1.1 (0.7-1.8) Q3: 1.0 (0.7-1.6) Q4: 1.1 (0.7-1.8) *p*-trend, 0.8	no difference between smokers and non-smokers	Age, sex, smoking, education, BMI and total energy
Pelucchi et al. (2007)	Renal cell cancer Men and women	Case-control study: 767 cases and 1 534 controls	20-44 µg/day (p25-p75)	Q2: 1.21 (0.94-1.57) Q3: 1.14 (0.86-1.51) Q4: 1.20 (0.88-1.63) *p*-trend, 0.35	n.a.	Study centre, sex, age, year of interview, education, smoking habit, alcohol consumption, BMI, occupational physical activity, family history of kidney cancer, energy intake
Hogervorst et al. (2008b)	Renal cell cancer Men and women	Cohort: 339 cases 120 852 cohort members	M: 10-42 and W: 9-40 µg/day (median Q1-Q5)	Q2: 1.25 (0.86-1.83) Q3: 1.48 (1.02-2.15) Q4: 1.23 (0.83-1.81) Q5: 1.59 (1.09-2.30) *p*-trend, 0.04	Stronger association in long-term smokers	Age, sex, hypertension, BMI, energy intake, fruit and vegetable consumption, smoking habits
Hirvonen et al. (2010)	Renal cell cancer Men	Cohort: 184 cases; 27 111 cohort members	22-56 µg/day (median Q1-Q5)	Q2: 0.94 (0.55-1.62) Q3: 1.65 (1.02-2.67) Q4: 1.47 (0.89-2.41) Q5: 1.28 (0.76-2.15) *p*-trend, 0.12	n.a.	Age, supplementation group, cigarette smoking, physical activity, alcohol intake, BMI, consumption of vegetables

9552 BMI: Body Mass Index; n.a.: not applicable; OR: Odds Ratio; p: percentile; Q1-Q4: Quartile 1-4; Q1-Q5: Quintile 1-5; RR: relative risk; SD: Standard Deviation; SE: Standard Error.
9553 * among controls.
9554

9555 **Table J5:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of the respiratory tract

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
ORAL CAVITY AND PHARYNGEAL CANCER						
Pelucchi et al. (2006)	Oral cavity and pharynx Men and women	Case-control study: 749 cases 1 772 controls	13-40 µg (p20-p80)	Q2: 1.10 (0.78-1.57) Q3: 1.27 (0.89-1.81) Q4: 1.04 (0.72-1.51) Q5: 1.12 (0.76-1.66) *p*-trend, 0.70	n.a.	Age, sex, study center, education, BMI, energy intake, alcohol consumption, smoking habits.
Schouten et al. (2009)	Oral cavity Men and women	Cohort: 101 cases 120 852 cohort members	M: 10-42 and W: 9-40 µg/day (median Q1-Q5)	Q2: 0.70 (0.37-1.33) Q3: 0.77 (0.39-1.52) Q4: 0.77 (0.39-1.53) Q5: 0.72 (0.36-1.42) *p*-trend, 0.49	Positive association in non-smoking women (21 cases): HR 1.28 (1.01-1.62) per 10 µg/day	Age, sex, cigarette smoking, energy intake, alcohol intake niacin intake
Schouten et al. (2009)	Oro- and hypopharynx Men and women	Cohort: 83 cases 120 852 cohort members	M: 10-42 and W: 9-40 µg/day (median Q1-Q5)	T-2: 0.44 (0.23-0.85) T-3: 0.61 (0.33-1.12) *p*-trend, 0.17	No significant differences between men and women	Age, sex, cigarette smoking, energy intake, alcohol intake niacin intake
LARYNGEAL CANCER						
Pelucchi et al. (2006)	Larynx	Case-control study: 527 cases 1 297 controls	13-38 µg (p20-p80)	Q2: 1.04 (0.70-1.57) Q3: 0.85 (0.56-1.29) Q4: 0.89 (0.59-1.36) Q5: 1.23 (0.80-1.90) *p*-trend, 0.54	n.a.	Age, sex, study center, education, BMI, energy intake,alcohol consumption, smoking habits.

9556 Table continued overleaf.
9557

9558 **Table J5:** Results of epidemiological studies on dietary acrylamide (AA) intake and cancers of the respiratory tract (continued)

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
Schouten et al. (2009)	Larynx Men and women	Cohort: 180 cases 120 852 cohort members	M: 10-42 and W: 9-40 µg/day (median Q1-Q5)	Q2: 0.66 (0.38-1.16) Q3: 1.06 (0.62-1.80) Q4: 1.02 (0.60-1.74) Q5: 0.93 (0.54-1.58) *p*-trend, 0.85	No significant differences between men and women	Age, sex, cigarette smoking, energy intake, alcohol intake niacin intake
LUNG CANCER						
Hogervorst et al. (2009a)	Lung Men	Cohort: 1 600 cases 58 279 male cohort members	M: 10-42 and (median Q1-Q5)	Q2: 1.05 (0.81-1.38) Q3: 0.94 (0.71-1.26) Q4: 1.00 (0.75-1.34) Q5: 1.03 (0.77-1.39) *p*-trend, 0.85	No significant differences between histological subtypes	Age, BMI, energy intake, alcohol, vegetables and fruit intake, processed meat (in males only), family history of lung cancer, non-occupational physical activity,
	Lung Women	Cohort: 295 cases 62 573 female cohort members	W: 9-40 µg/day (median Q1-Q5)	Q2: 0.66 (0.42-1.04) Q3: 0.60 (0.38-0.96) Q4: 0.58 (0.36-0.95) Q5: 0.45 (0.27-0.76) *p*-trend, 0.01	Strongest inverse association observed for adenocarcinomas	Education, niacin, and cigarette smoking
Hirvonen et al. (2010)	Lung, men	Cohort: 1 703 cases; 27 111 cohort members	22-56 µg/day (median Q1-Q5)	Q2: 1.01 (0.86-1.18) Q3: 1.11 (0.95-1.29) Q4: 0.93 (0.79-1.10) Q5: 1.18 (1.01-1.38) *p*-trend, 0.11	n.a.	Age, supplementation group, cigarette smoking, physical activity, alcohol intake, BMI

9559 BMI: Body Mass Index; HR: hazard ratio; n.a.: not applicable; OR: Odds Ratio; p: percentile; Q1-Q4: Quartile 1-4; Q1-Q5: Quintile 1-5; RR: Relative risk; SD: Standard Deviation; SE:
9560 Standard Error; T-3: Tertile 1-3.
9561

9562 **Table J6:** Results of epidemiological studies on dietary acrylamide (AA) intake and other cancers

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
BRAIN CANCER						
Hogervorst et al. (2009b)	Brain cancer Men and women	Cohort: 216 cases 120 852 cohort members	M: 10-42 and W: 9-40 µg/day (median Q1-Q5)	Q2: 0.92 (0.59-1.44) Q3: 1.20 (0.78-1.83) Q4: 1.07 (0.68-1.68) Q5: 0.87 (0.54-1.41) *p*-trend, 0.61	No association in never-smokers and subgroups like microscopically verified cancers, and astrocytic gliomas	Age, sex, educational level, BMI, height, energy intake, cigarette smoking.
THYROID CANCER						
Schouten et al. (2009)	Larynx Men and women	Cohort: 66 cases 120 852 cohort members	M: 10-42 and W: 9-40 µg/day (median Q1-Q5)	T-2: 1.14 (0.58-2.26) T-3: 1.33 (0.70-2.53) *p*-trend, 0.42	No significant associations in women or in non-smokers	Age, sex, cigarette smoking, energy intake, vegetable intake niacin intake
LYMPHATIC MALIGNANCIES						
Hirvonen et al. (2010)	Lymphomas men	Cohort: 175 cases; 27 111 cohort members	22-56 µg/day (median Q1-Q5)	Q2: 0.93 (0.56-1.53) Q3: 1.17 (0.73-1.88) Q4: 0.98 (0.59-1.61) Q5: 1.10 (0.67-1.80) *p*-trend, 0.67	n.a.	Age, supplementation group, cigarette smoking, physical activity, alcohol intake, BMI

9563 Table continued overleaf.
9564

9565 **Table J6:** Results of epidemiological studies on dietary acrylamide (AA) intake and other cancers (continued)

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
Bongers et al. (2012)	Multiple myeloma, men	Cohort: 170 cases 58 279 male cohort members	M: 10-42 and (median Q1-Q5)	Q2: 0.65 (0.36-1.16) Q3: 1.14 (0.67-1.94) Q4: 1.14 (0.67-1.94) Q5: 1.54 (0.92-2.58) *p*-trend, 0.02	Statistical significant positive association in never-smoking men, HR: 1.98 per 10 µg/day	Age, height, education level, fiber, total fatty acids, trans unsaturated fat acids, mono unsaturated fat acids, poly unsaturated fat, carbohydrates and niacin
	Multiple myeloma, women	Cohort: 153 cases 62 573 female cohort members	W: 9-40 µg/day (median Q1-Q5)	Q2: 1.46 (0.85-2.49) Q3: 1.19 (0.67-2.12) Q4: 0.73 (0.39-1.37) Q5: 0.93 (0.50-1.73) *p*-trend, 0.22	No clear association in never-smoking women	age, height, education level, fiber, total fatty acids, trans unsaturated fat acids, mono unsaturated fat acids, poly unsaturated fat, carbohydrates and niacin
Bongers et al. (2012)	Diffuse large-cell lymphoma, men	Cohort: 159 cases 58 279 male cohort members	M: 10-42 and (median Q1-Q5)	Q2: 0.93 (0.54-1.59) Q3: 1.23 (0.74-2.04) Q4: 1.26 (0.74-2.17) Q5: 1.06 (0.61-1.38) *p*-trend, 0.73	n.a.	age, height, education level, fiber, total fatty acids, trans unsaturated fat acids, mono unsaturated fat acids, poly unsaturated fat, carbohydrates and niacin
	Diffuse large-cell lymphoma, women	Cohort: 100 cases 62 573 female cohort members	W: 9-40 µg/day (median Q1-Q5)	Q2: 1.05 (0.51-2.15) Q3: 1.71 (0.87-3.36) Q4: 1.72 (0.84-3.50) Q5: 1.38 (0.63-3.02) *p*-trend, 0.43	No clear association in never-smoking women	age, height, education level, fiber, total fatty acids, trans unsaturated fat acids, mono unsaturated fat acids, poly unsaturated fat, carbohydrates and niacin

9566 Table continued overleaf.
9567

9568 **Table J6:** Results of epidemiological studies on dietary acrylamide (AA) intake and other cancers (continued)

Reference	Cancer site and sex	Study design and size	Range of intake	Overall Results: OR/RR (95 %CI)	Subgroup analyses	Confounders allowed for in the analyses
Bongers et al. (2012)	Chronic lymphocytic leukaemia, men	Cohort: 134 cases 58 279 male cohort members	M: 10-42 and (median Q1-Q5)	0.88 (0.74-1.09) per 10 µg/day	no assocation in never-smoking men	age, height, education level, fiber, total fatty acids, trans unsaturated fat acids, mono unsaturated fat acids, poly unsaturated fat, carbohydrates and niacin
	Chronic lymphocytic leukaemia, women	Cohort: 66 cases 62 573 female cohort members	W: 9-40 µg/day (median Q1-Q5)	0.83 (0.64-1.09) per 10 µg/day	No association in never-smoking women	age, height, education level, fiber, total fatty acids, trans unsaturated fat acids, mono unsaturated fat acids, poly unsaturated fat, carbohydrates and niacin
Bongers et al. (2012)	Follicular lymphoma, Men and women	Cohort: 42 male and 47 female cases, 58 279 male and 62 573 female cohort members	M: 10-42 and W: 9-40 µg/day (median Q1-Q5)	M: 1.28 (1.03-1.61) F: 1.12 (0.80-1.57) per 10 µg/day	No statistical significant association in never-smoking women	age, height, education level, fiber, total fatty acids, trans unsaturated fat acids, mono unsaturated fat acids, poly unsaturated fat, carbohydrates and niacin
Bongers et al. (2012)	Waldenström Macroglobulin-emia and immu-nocytoma, Men and women	Cohort: 54 male and 35 female cases, 58 279 male and 62 573 female cohort members	M: 10-42 and W: 9-40 µg/day (median Q1-Q5)	M: 1.21 (0.93-1.50) F: 1.21 (0.88-1.66) per 10 µg/day	No statistical significant association in never-smoking women	age, height, education level, fiber, total fatty acids, trans unsaturated fat acids, mono unsaturated fat acids, poly unsaturated fat, carbohydrates and niacin

9569 BMI: Body Mass Index; F: Female; HR: hazard ratio; M: Male; n.a.: not applicable; OR: Odds Ratio; p: percentile; Q1-Q4: Quartile 1-4; Q1-Q5: Quintile 1-5; RR: Relative risk; SD: Standard
9570 Deviation; SE: Standard Error; T-: Tertile.
9571

9572 **Appendix K. Benchmark Dose Modelling (BMD)**

9573 **Previous studies reporting dose-response modelling for AA**

9574 Several studies report on dose response modelling for data on AA. The CONTAM Panel noted that the
9575 experimental design including dose and time regime for the exposure may vary between the different
9576 studies requiring correction of doses used for the modelling to reflect daily lifetime exposure.

9577 Bolger et al. (2010) applied the MOE approach to AA and derived BMD and BMDL values for a wide
9578 range of reported tumour types taking data from the 2-year carcinogenicity studies of Friedman et al.
9579 (1995) and Johnson et al. (1986) as compiled by Rice (2005). The CONTAM Panel noted that the
9580 NTP data were not available at that time. The $BMDL_{10}$ values, taken as reference point for calculation
9581 of the MOE values by Bolger et al. (2010) were derived using the tumour types with the lowest
9582 BMDLs. These $BMDL_{10}$ values amounted to 1.00 mg/kg b.w. per day for male rat peritesticular
9583 mesotheliomas and 0.16 mg/kg b.w. per day for female rat mammary gland tumours.

9584 BMD modelling of the data from the NTP (2012) study was previously performed by Beland et al.
9585 (2013). BMD modelling of neoplastic incidences in male and female $B6C3F_1$ mice and F344/N rats
9586 administered AA in the drinking water for two years (NTP, 2012) resulted in the lowest $BMDL_{10}$
9587 values for the Harderian gland adenomas in male and female mice and for the mammary gland
9588 adenomas in female rats. In male mice the log-logistic and log-probit models resulted in $BMDL_{10}$
9589 values for Harderian gland adenomas of 0.173 and 0.159 mg/kg b.w. per day, respectively and for
9590 female mice these values amounted to 0.282 and 0.230 mg/kg b.w. per day, respectively. In female
9591 rats $BMDL_{10}$ values for mammary gland fibroadenomas varied from 0.296-0.649 mg/kg b.w. per day
9592 depending on the model used. The value of 0.008 mg/kg b.w. per day obtained using the log-probit
9593 model was not taken into further account by Beland et al. (2013) due to the disparity between the
9594 $BMDL_{10}$ obtained from the log-probit model and the $BMDL_{10}$ values obtained from other models
9595 (Beland et al., 2013).

9596 **Dose-response modelling for AA as performed by the CONTAM Panel**

9597 The CONTAM Panel performed BMD analyses on:

9598 (i) Data on neurotoxicity, consisting of incidences of peripheral nerve (sciatic) axonal
9599 degeneration in F344 male rats exposed to AA in drinking water for 2 years (NTP, 2012) (see
9600 Table 20), and

9601 (ii) The tumour incidences of AA from the 2-year-studies in mice and rats (see Tables 23 and 24).

9602 Details on these BMD analyses and the data used for further dose-response modeling and the
9603 subsequent risk assessment are shown in Tables K1 to K12.

9604 The BMD/L values were calculated by means of the software BMDS v.2.4 (US-EPA). All models for
9605 dichotomous (quantal) data available there were selected for the BMD analysis using the default
9606 benchmark response (BMR) of 10 % extra risk as advised by the EFSA guidance on the use of
9607 benchmark dose (EFSA, 2009b). In the first instance all models were run without restrictions.

9608 Since the BMDL is the lower 95 % one-sided confidence bound of the BMD and the BMDU is the
9609 upper 95 % confidence bound of the BMD, the interval BMDL/BMDU represents the 90 %
9610 confidence interval of the BMD. This interval provides a measure for the accuracy of the BMD
9611 estimate, which for an acceptable BMDL value should not be larger than one order of magnitude
9612 (EFSA, 2009b). It was also noted (EFSA, 2011d) that when the BMD/BMDL ratios for the different
9613 models are very large or when the BMDL values from different models are very different the data are
9614 not informative enough to derive an RP. EFSA also stated (EFSA 2009b) that as a general rule, dose-
9615 response data should not result in a range of BMDL values from different accepted models that
9616 substantially exceeds one order of magnitude. EFSA also indicated (EFSA, 2009b) that when this

value is exceeded, several options are available and should be considered on a case-by-case basis, e.g. increasing the BMR, re-evaluating the set of models, or model averaging. Acceptance of a model (and its BMDL) was defined through the log-likelihood value and its goodness-of-fit test with a p-value > 0.05. The lowest BMDL of all accepted models fitted to the data of one endpoint and one dose-response data set, was determined as the BMDL of that data set.

For the BMD analysis of the data on incidences of Harderian gland adenomas in female B6C3F$_1$ mice exposed to AA for 2 years (NTP, 2012) (Table K7), the data on incidences of Harderian gland adenomas and adenocarcinomas in male B6C3F$_1$ mice exposed to AA for 2 years (NTP, 2012) (Table K8) and the data on incidences of mammary gland fibroadenomas in female rats exposed to AA for 2 years (NTP, 2012) (Table K9), the results revealed several models for which the BMD/BMDL ratio exceeded one order of magnitude, or for which there was disparity between the BMDL$_{10}$ obtained for a specific model and the BMDL$_{10}$ values obtained from other models. This indicated that the set of models applied did not result in an adequate reference point. Therefore, the set of models was re-evaluated choosing restricted models for further BMD analysis of the data.

The results obtained using restricted models applying default values for the incidences of Harderian gland adenomas in female B6C3F$_1$ mice exposed to AA for 2 years (NTP, 2012) (Table K10), of Harderian gland adenomas and adenocarcinomas in male B6C3F$_1$ mice exposed to AA for 2 years (NTP, 2012) (Table K11) and of mammary gland fibroadenomas in female rats exposed to AA for 2 years (NTP, 2012) (Table K12) did no longer exceed one order of magnitude, and disparity between the BMDL$_{10}$ values obtained by different models was no longer observed.

Thus, from the results obtained, the CONTAM Panel selected the value of 0.43 mg/kg b.w. per day derived as the lowest BMDL$_{10}$ from the data data on incidences of peripheral nerve (sciatic) axonal degeneration in male F344 rats exposed to AA in drinking water for 2 years (NTP, 2012) obtained using default settings and unrestricted models (Table K1 and Figure K1) as RP for non-neoplastic effects.

For neoplastic effects, the CONTAM Panel selected as RP the value of 0.17 mg/kg b.w. per day derived as the lowest BMDL$_{10}$ from data on incidences of Harderian gland adenomas and adenocarcinomas in male B6C3F$_1$ mice exposed to AA for 2 years (NTP, 2012) (Table K11 and Figure K2).

Table K1: Results from the BMD analysis of the data on incidences of peripheral nerve (sciatic) axonal degeneration in male F344 rats exposed to AA in drinking water for 2 years (NTP, 2012). The benchmark dose (BMD$_{10}$), the 95 % benchmark dose lower confidence limit (BMDL$_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit. Model with lowest BMDL$_{10}$ is given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD$_{10}$ (mg/kg b.w. per day)	BMDL$_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	114.99	-	-	-	-
Null (reduced) model	n.a.	1	126.71	-	-	-	-
Probit	n.a.	2	115.14	0.96	yes	0.91	0.74
LogProbit	none	3	115.17	0.83	yes	1.19	0.48
Logistic	n.a.	2	115.09	0.97	yes	0.96	0.79
LogLogistic	none	3	115.12	0.87	yes	1.15	0.46
Quantal-Linear	**n.a.**	**2**	**115.84**	**0.63**	**yes**	**0.61**	**0.43**
Multistage Cancer	n.a.	3	115.09	0.90	yes	1.55	0.48
Multistage	none	3	115.09	0.90	yes	1.08	0.47
Weibull	none	3	115.11	0.88	yes	1.12	0.44
Gamma	none	3	115.13	0.87	yes	1.14	0.43

b.w.: body weight; n.a.: not applicable.

Table K2: Results from the BMD analysis of the data on incidences of mesothelioma of the testes tunica albuginea in male F344 rats exposed to AA for 2 years (Johnson et al., 1986). The benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	89.14	-	-	-	-
Null (reduced) model	n.a.	1	99.70	-	-	-	-
Probit	n.a.	2	96.85	0.00	no	-	-
LogProbit	none	3	93.51	0.01	no	-	-
Logistic	n.a.	2	96.94	0.00	no	-	-
LogLogistic	none	3	93.81	0.01	no	-	-
Quantal-Linear	n.a.	2	96.14	0.00	no	-	-
Multistage Cancer	n.a.	2	96.14	0.00	no	-	-
Multistage	none	3	92.32	0.04	no	-	-
Weibull	none	3	93.88	0.01	no	-	-
Gamma	none	3	93.95	0.01	no	-	-

b.w.: body weight; n.a.: not applicable.

Table K3: Results from the BMD analysis of the data on incidences of mesothelioma of the testes tunica in male F344 rats exposed to AA for 2 years (Friedman et al., 1995). The benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit. Model with lowest $BMDL_{10}$ is given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	116.39	-	-	-	-
Null (reduced) model	n.a.	1	123.00	-	-	-	-
Probit	n.a.	2	116.58	0.82	yes	1.59	1.19
LogProbit	none	3	116.39	0.97	yes	1.31	0.63
Logistic	n.a.	2	116.63	0.79	yes	1.63	1.25
LogLogistic	none	3	116.40	0.87	yes	1.34	0.66
Quantal-Linear	n.a.	2	116.41	0.98	yes	1.37	0.84
Multistage Cancer	n.a.	2	116.41	0.98	yes	1.37	0.84
Multistage	**none**	**3**	**116.40**	**0.88**	**yes**	**1.32**	**0.51**
Weibull	none	3	116.41	0.86	yes	1.35	0.67
Gamma	none	3	116.41	0.86	yes	1.35	0.68

b.w.: body weight; n.a.: not applicable.

Table K4: Results from the BMD analysis of the data on incidences of mesothelioma of the epididymis or testes tunica vaginalis in male F344 rats exposed to AA for 2 years (NTP, 2012). The benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit. Model with lowest $BMDL_{10}$ is given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	62.06	-	-	-	-
Null (reduced) model	n.a.	1	66.42	-	-	-	-
Probit	n.a.	2	63.09	0.56	yes	2.21	1.60
LogProbit	none	3	62.98	0.40	yes	2.19	1.21
Logistic	n.a.	2	63.09	0.56	yes	2.25	1.68
LogLogistic	none	3	63.02	0.38	yes	2.21	1.21
Quantal-Linear	n.a.	2	63.26	0.50	yes	2.15	1.21
Multistage Cancer	n.a.	3	63.05	0.37	yes	2.25	1.26
Multistage	**none**	**3**	**63.05**	**0.37**	**yes**	**2.25**	**1.13**
Weibull	none	3	63.03	0.38	yes	2.23	1.22
Gamma	none	3	63.02	0.38	yes	2.22	1.23

b.w.: body weight; n.a.: not applicable.

Table K5: Results from the BMD analysis of the data on incidences of various types of sarcomas in female $B6C3F_1$ mice exposed to AA for 2 years (NTP, 2012). The benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit. Model with lowest $BMDL_{10}$ is given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	52.44	-	-	-	-
Null (reduced) model	n.a.	1	65.57	-	-	-	-
Probit	n.a.	2	60.51	0.00	no	-	-
LogProbit	none	2	56.40	0.05	no	-	-
Logistic	n.a.	2	60.90	0.00	no	-	-
LogLogistic	none	2	56.72	0.04	no	-	-
Quantal-Linear	n.a.	1	56.96	0.06	yes	4.09	2.86
Multistage Cancer	n.a.	1	56.96	0.06	yes	4.09	2.86
Multistage	**none**	**2**	**55.91**	**0.07**	**yes**	**2.80**	**1.56**
Weibull	none	2	56.83	0.03	no	-	-
Gamma	none	2	56.83	0.03	no	-	-

b.w.: body weight; n.a.: not applicable.

Table K6: Results from the BMD analysis of the data on incidences of lung tumours in male B6C3F$_1$ mice exposed to AA for 2 years (NTP, 2012). The benchmark dose (BMD$_{10}$), the 95 % benchmark dose lower confidence limit (BMDL$_{10}$) values for a BMR of 10% extra risk with characteristics of the model fit. Model with lowest BMDL$_{10}$ is given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD$_{10}$ (mg/kg b.w. per day)	BMDL$_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	62.06	-	-	-	-
Null (reduced) model	n.a.	1	66.42	-	-	-	-
Probit	n.a.	2	63.09	0.56	yes	2.21	1.60
LogProbit	none	3	62.98	0.40	yes	2.19	1.21
Logistic	n.a.	2	63.09	0.56	yes	2.25	1.68
LogLogistic	none	3	63.02	0.38	yes	2.21	1.21
Quantal-Linear	n.a.	2	63.26	0.50	yes	2.15	1.21
Multistage Cancer	n.a.	3	63.05	0.37	yes	2.25	1.26
Multistage	**none**	**3**	**63.05**	**0.37**	**yes**	**2.25**	**1.13**
Weibull	none	3	63.03	0.38	yes	2.23	1.22
Gamma	none	3	63.02	0.38	yes	2.22	1.23

b.w.: body weight; n.a.: not applicable.

Table K7: Results from the BMD analysis of the data on incidences of Harderian gland adenomas in female B6C3F$_1$ mice exposed to AA for 2 years (NTP, 2012). The benchmark dose (BMD$_{10}$), the 95 % benchmark dose lower confidence limit (BMDL$_{10}$) values for a BMR of 10% extra risk with characteristics of the model fit. Model with lowest BMDL$_{10}$ is given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD$_{10}$ (mg/kg b.w. per day)	BMDL$_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	108.36	-	-	-	-
Null (reduced) model	n.a.	1	152.85	-	-	-	-
Probit	n.a.	2	124.13	<0.01	no	-	-
LogProbit	none	2	109.78	0.42	yes	0.52	0.23
Logistic	n.a.	2	124.25	<0.01	no	-	-
LogLogistic	none	2	109.74	0.43	yes	0.42	0.20
Quantal-Linear	n.a.	1	112.07	0.12	yes	0.57	0.47
Multistage Cancer	n.a.	1	112.07	0.12	yes	0.57	0.47
Multistage	none	2	109.30	0.60	yes	0.39	0.30
Weibull	none	2	110.57	0.22	yes	0.29	0.088
Gamma	**none**	**2**	**110.78**	**0.18**	**yes**	**0.26**	**0.054**

b.w.: body weight; n.a.: not applicable.

Table K8: Results from a BMD analysis of the data on incidences of Harderian gland adenomas and adenocarcinomas in male B6C3F$_1$ mice exposed to AA for 2 years (NTP, 2012). The benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values for a BMR of 10% extra risk with characteristics of the model fit. Model with lowest $BMDL_{10}$ is given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	113.44	-	-	-	-
Null (reduced) model	n.a.	1	161.48	-	-	-	-
Probit	n.a.	2	127.93	<0.01	no	-	-
LogProbit	none	3	114.84	0.25	yes	0.39	0.16
Logistic	n.a.	2	127.00	<0.01	no	-	-
LogLogistic	**none**	**3**	**114.64**	**0.30**	**yes**	**0.37**	**0.15**
Quantal-Linear	n.a.	2	117.24	0.055	yes	0.38	0.31
Multistage Cancer	n.a.	2	117.24	0.055	yes	0.38	0.31
Multistage	none	3	114.39	0.39	yes	0.26	0.20
Weibull	none	3	115.72	0.10	yes	0.17	0.05
Gamma	none	3	115.98	0.08	yes	0.14	0.02

b.w.: body weight; n.a.: not applicable

Table K9: Results from the BMD analysis of the data on incidences of mammary gland fibroadenomas in female F344 rats exposed to AA for 2 years (NTP, 2012). The benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values for a BMR of 10% extra risk with characteristics of the model fit. Model with lowest $BMDL_{10}$ is given in bold.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	157.83	-	-	-	-
Null (reduced) model	n.a.	1	163.80	-	-	-	-
Probit	n.a.	2	158.89	0.55	yes	0.91	0.65
LogProbit	none	3	158.72	0.41	yes	0.43	0.008
Logistic	n.a.	2	158.90	0.55	yes	0.91	0.65
LogLogistic	none	3	158.72	0.41	yes	0.41	0.006
Quantal-Linear	n.a.	2	158.80	0.59	yes	0.71	0.44
Multistage Cancer	n.a.	2	158.80	0.59	yes	0.71	0.44
Multistage	**none**	**3**	**158.76**	**0.39**	**yes**	**0.58**	**0.24**
Weibull	none	3	158.69	0.42	yes	0.40	0.004
Gamma	none	3	158.69	0.42	yes	0.38	0.002

b.w.: body weight; n.a.: not applicable.

Table K10: Results from the BMD analysis of the data on incidences of Harderian gland adenomas in female B6C3F$_1$ mice exposed to AA for 2 years (NTP, 2012). The benchmark dose (BMD$_{10}$), the 95 % benchmark dose lower confidence limit (BMDL$_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit. Model with lowest BMDL$_{10}$ is given in bold. Where possible models were restricted using default restrictions.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD$_{10}$ (mg/kg b.w. per day)	BMDL$_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	108.36	-	-	-	-
Null (reduced) model	n.a.	1	152.85	-	-	-	-
LogProbit	default	1	112.51	0.08	yes	0.91	0.77
LogLogistic	**default**	**2**	**109.74**	**0.43**	**yes**	**0.47**	**0.28**
Multistage	default	1	112.07	0.12	yes	0.57	0.47
Weibull	default	1	112.07	0.12	yes	0.57	0.47
Gamma	default	1	112.07	0.12	yes	0.57	0.47

b.w.: body weight; n.a.: not applicable.

Table K11: Results from the BMD analysis of the data on incidences of Harderian gland adenomas and adenocarcinomas in male B6C3F$_1$ mice exposed to AA for 2 years (NTP, 2012). The benchmark dose (BMD$_{10}$), the 95 % benchmark dose lower confidence limit (BMDL$_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit. Model with lowest BMDL$_{10}$ is given in bold. Where possible models were restricted using default restrictions.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD$_{10}$ (mg/kg b.w. per day)	BMDL$_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	113.44	-	-	-	-
Null (reduced) model	n.a.	1	161.48	-	-	-	-
LogProbit	default	2	116.15	0.14	yes	0.62	0.51
LogLogistic	**default**	**3**	**114.64**	**0.30**	**yes**	**0.37**	**0.17**
Multistage	default	2	117.24	0.055	yes	0.38	0.31
Weibull	default	2	117.24	0.055	yes	0.38	0.31
Gamma	default	2	117.24	0.055	yes	0.38	0.31

b.w.: body weight; n.a.: not applicable.

Table K12: Results from a BMD analysis of the data on incidences of mammary gland fibroadenomas in female F334 rats exposed to AA for 2 years (NTP, 2012). The benchmark dose (BMD_{10}), the 95 % benchmark dose lower confidence limit ($BMDL_{10}$) values for a BMR of 10 % extra risk with characteristics of the model fit. Model with lowest $BMDL_{10}$ is given in bold. Where possible models were restricted using default restrictions.

Models	Restriction	N of parameters	Minus Log-likelihood	P-value	Accepted	BMD_{10} (mg/kg b.w. per day)	$BMDL_{10}$ (mg/kg b.w. per day)
Full model	n.a.	5	157.83	-	-	-	-
Null (reduced) model	n.a.	1	163.80	-	-	-	-
LogProbit	default	2	159.28	0.41	yes	1.31	0.85
LogLogistic	**default**	**2**	**158.74**	**0.61**	**yes**	**0.55**	**0.30**
Multistage	default	2	158.80	0.59	yes	0.71	0.44
Weibull	default	2	158.80	0.59	yes	0.71	0.44
Gamma	default	2	158.80	0.59	yes	0.71	0.44

b.w.: body weight; n.a.: not applicable.

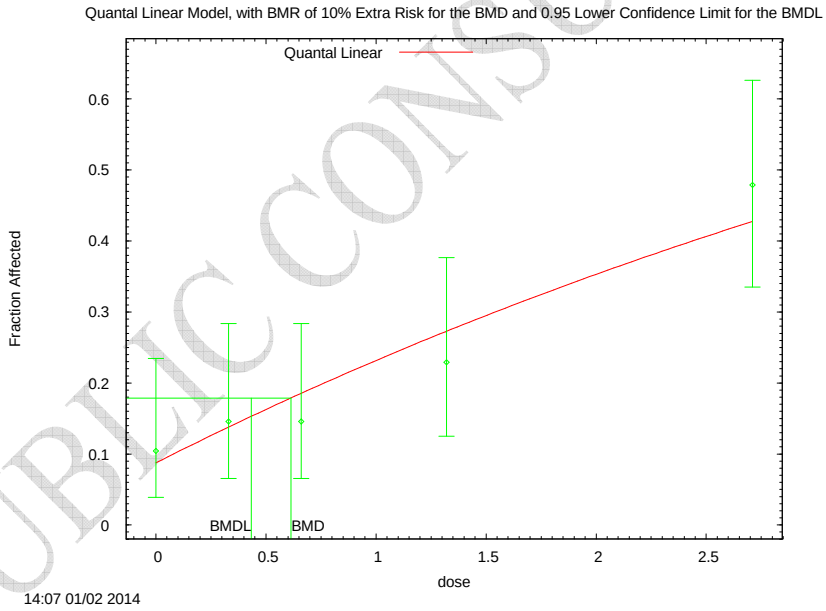

Figure K1: Graphical display of the result from the BMD analysis using the quantal linear model of the data on incidences of peripheral nerve (sciatic) axonal degeneration in male F344 rats exposed to AA in drinking water for 2 years (NTP, 2012).

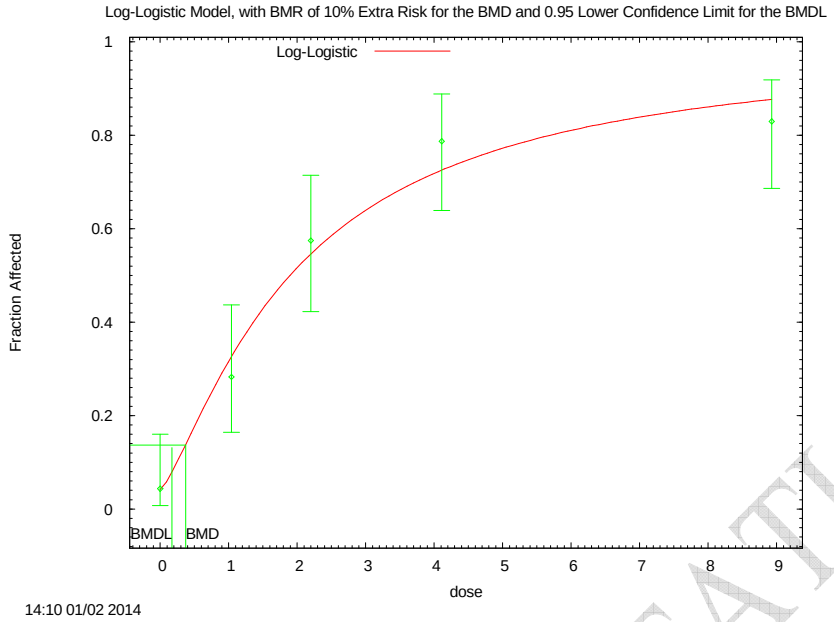

Figure K2: Graphical display of the result from the BMD analysis using the LogLogistic model of the data on incidences of Harderian gland adenomas and adenocarcinomas in male B6C3F$_1$ mice exposed to AA for 2 years (NTP, 2012).

ABBREVIATIONS

α-A-NDELA	α-acetoxy-N-nitroso-diethanolamine
γ-GCS	Gamma-glutamyl cysteine synthase
ΣAA	Sum of AA+AAMA+AAMA-sulfoxide
ΣGA	Sum of GA+GAMA
1-CE-dAdo	1-(2-carboxyethyl)-dAdo
3-HPMA	N-acetyl-S-(3-hydroxypropyl)cysteine
3-MCPD	3-monochloropropanediol
7-FAE-Gua	7-(2-formamidoethyl)-Gua
8-OHdG	8-hydroxy-deoxyguanosine
AA	Acrylamide
AACF	Atypical acinar cell foci
AA-GSH	Glutathione adduct of AA
AAMA	N-acetyl-S-(2-carbamoylethyl)-L-cysteine
AAMA-SO	Sulfoxide of AAMA
AC	Acrolein
ACEG	Aberrant cells excluding gaps
ACF	Aberrant crypt foci
$AccD_{AA}$	Accumulated *in vivo* dose of AA throughout the duration of employment
ACGIH	American Conference of Governmental Industrial Hygienists
AChE	Acetylcholinesterase
ADU	Alkaline DNA unwinding
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AN	Acrylonitrile
ANSES	Agency for Food, Environmental and Occupation Health and Safety, former Afssa
AOM	Azoxymethane
APC	Adenomatous polyposis coli
APCI	Atmospheric pressure chemical ionisation
ASCGE	Alkylating single cell electrophoresis
AST	Aspartate aminotransferase
AUC	Area under the curve
AUC/D	Dose-adjusted AUC
ATBC	Alpha-Tocopherol, Beta-Carotene Cancer Prevention
ATSDR	Agency for Toxic Substances and Disease Registry
BAM	German Federal Institute for Materials Research and Testing
BCF	Bioconcentration factor
BER	Base excision repair
BEUC	European Consumers Association
BfR	German Federal Institute for Risk Assessment
BMD	Benchmark dose
BMDL	95 % benchmark dose lower confidence limit
BMI	Body Mass Index
BMR	Benckmark response
BN	Binucleated
BSO	Buthionine sulfoximine
BPDE	Anti-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide
BUN	Blood urea nitrogen
BVL	German Federal Office for Consumer Protection and Food Safety
b.w.	Body weight
C+	Positive control
C-	Negative control

9797	CA	Chromosomal aberration
9798	CAPS	Cancer of the Prostate in Sweden
9799	Cbl(I)	Cob(l)alamin
9800	CE	Capillary electrophoresis
9801	CEA	Carcino-embryonic antigen
9802	cDNA	Complementary DNA
9803	CEN	European Committee for Standardization
9804	CERHR	Center for the Evaluation of Risks to Human Reproduction
9805	CI	Confidence interval
9806	CNS	Central nervous system
9807	CONTAM Panel	EFSA Scientific Panel on Contaminants in the Food Chain
9808	CRM	Certified reference material
9809	CYP	Cytochrome P450
9810	Cys	Cysteine residues
9811	CZE	Capillary zone electrophoresis
9812	DDC	Diethyldithiocarbamate
9813	DMSO	Dimethylsulfoxide
9814	dpt	Days post treatment
9815	DRAG	Detection of repairable adducts by growth inhibition
9816	DSB	Double stand break
9817	DTU	Danish National Food Institute
9818	EEA	European Economic Area
9819	EC	European countries
9820	DAD	Diode array detector
9821	DDC	Diethyldithiocarbamate
9822	DHPA	2,3-dihydroxypropionamide or glyceramide
9823	DTU	Danish National Food Institute
9824	EFSA	European Food Safety Authority
9825	EH	Epoxide hydrolase
9826	EI	Electron ionisation mode
9827	EM	Electron microscope
9828	EPIC	European Prospective Investigation into Cancer
9829	ER	Estrogen receptor
9830	ESI	Electrospray ionisation
9831	EU	European Union
9832	F	Female
9833	FA	Food Associations
9834	FAO	Food and Agriculture Organization of the United Nations
9835	FASI	Field amplified sample injection
9836	FBQ	Food behaviour questionnaire
9837	FDE	FoodDrinkEurope
9838	FFQ	Food frequency questionaire
9839	FITC	Fluorescein isothiocyanate
9840	fpg	formamidopyrimidine-DNA glycosylase
9841	FSANZ	Food Standards Australia New Zealand
9842	FSH	Follicle-stimulating hormone
9843	FT-IR	Fourier Transform Infrared Analysis
9844	GA	Glycidamide
9845	GA-Cbl	GA-alkylcobalamin
9846	GA-GSH	Glutathione adduct of glycidamide
9847	GAMA	N-acetyl-S-(2-carbamoyl-2-hydroxyethyl)-L-cysteine
9848	GC	Gas chromatography
9849	G:C	guanine:cytosine
9850	GD	Gestation day
9851	GI	Gastro-intestinal

GPx	Glutathione peroxidase
GSH	Glutathione
GSH-EE	GSH-monoethyl ester
GST	Glutathione-S-transferases
HACCP	Hazard Analysis and Critical Control Points
Hb	Hemoglobin
HED	Human equivalent dose
HOMA-IR	Homeostasis model assessment of insulin resistance
HPG	Hypothalamic-pituitary-testes
HPFS	The Health Professionals' Follow-up study
HR	Hazard ratio
HSAB	Hard-soft-acid-base
HT	Heritable translocation
HTE	Hemithyroidectomy
HPLC	High performance liquid chromatography
IARC	Insitute Research on Cancer
IL	Interleukin
ILUAE	Ionic Liquid Based Ultrasonic Assisted Extraction
IRA	Incremental repeat acquisition
IRMM	Institute for Reference Materials and Measurements
IRR	Incidence Rate Ratio
Iso-GAMA	N-acetyl-S-(1-carbamoyl-2-hydroxyethyl)-L-cysteine
ISQ	1,5-isoquinolinediol
i.p.	Intraperitoneal
i.v.	Intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
K_m	Michaelis-Menten constant
LB	Lower bound
LC	Percentage of censored results
LDH	Lactate dehydrogenase
LH	Luteinizing hormone
LMW	Low molecular weight
LOAEL	Lowest-observed-adverse-effect level
LOD	Limit of detection
LOH	Loss of heterozygosity
LOQ	Limit of quantification
M	Male
MA	Mercapturic acid
MB	Middle bound
MCB	Monochlorobimane
MDA	Malondialdehyde
MEKC	Micellar electrokinetic chromatography
MF	Mutant frequency
MN	Micronucleus
MN-NCE	Micronucleated normochromatic erythrocytes
MN-RET	Micronucleated reticulocytes
MNBN	Micronucleated binucleated cells
MoA	Mode of action
MOE	Margin of exposure
MPO	Myeloperoxidase
MRM	Multiple reaction mode
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MSPD	Matrix solid phase dispersion

N	Number of samples/number of population groups used to derive the corresponding Statistics
N1-GA-dA	N1-(2-carboxy-2-hydroxyethyl)-2'deoxyadenosine)
N3-GA-Ade	GA (N3-(2-carbamoyl-2-hydroxyethyl) adenine
N7-GA-Gua	GA, N-7-(2-carbamoyl-2-hydroxyethyl)guanine
n.a.	Not applicable
NACE	Non aqueous capillary electrophoresis
NCEs	Normochromatic erythrocytes
NCTR	National Centre for Toxicological Research
ND	Not determined
NDI	Nuclear division index
NEM	N-ethylmaleimide
NER	Nucleotide excision repair
NFA	National Food Administration
NG	Normally growing
NHANES	National Health and Nutrition Examination Survey
NHS	Nurses' Health Study
NLCS	Netherlands Cohort Study
NMA	*N*-methylolacrylamide
NMR	Nuclear magnetic resonance
NO	Nitric oxide
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
NQ	Not quantified
n.s.	Not specified
NTP	National Toxicology Programe
OAC	Oral contraceptives
Ogg1	8-oxoguanine DNA glycosylase
OR	Odds ratio
P	Percentile
PBS	Phosphate-buffered saline
PCE	Polychromatic erythrocytes
PCI	Positive chemical ionisation
PBPK	Physiologically Based PharmacoKinetic
PD	Pharmacodynamic
PMH	Post-menopausal hormones
pmol	Picomol
PMTDI	Provisional maximum tolerable daily intake
PND	Postnatal day
PR	Progesteron receptor
PRL	Prolactin
PSA	Prostate-specific antigen
Py-GC-MS	Pyrolysis gas chromatography/mass spectrometry
Q1-Q4	Quartile 1-4
Q1-Q5	Quintile 1-5
RA	Radioactivity
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
REL	Recommended exposure limit
RET	Reticulocytes
RfC	Reference concentration
RfD	Reference dose
ROS	Reactive oxygen species
RR	Relative risk
RT-PCR	Reverse transcriptase polymerase chain reaction
S	Scenario

9962	*s.c.*	Subcutaneous
9963	SCE	Sister Chromatid Exchange
9964	SCF	Scientific Committee on Food
9965	SES	Social economic status
9966	SG	Slowly growing
9967	sGC	Soluble guanylate cyclase
9968	SGZ	Subgranular zone
9969	SHP	EFSA Stakeholder Consultative Platform
9970	SIM	Selected Ion Monitoring
9971	SMC	Swedish Mammography Cohort
9972	SME	Small and medium size enterprise
9973	SMR	Standardised Mortality Ratio
9974	SN	Sciatic nerve
9975	SOD	Superoxide dismutase
9976	SPE	Solid phase extraction
9977	SPFF	Sweetpotato French Fries
9978	SPME	Solid phase micro extraction
9979	SSB	Single strand breaks
9980	SVHC	Substances of Very High Concern
9981	T3	Thriiodothyronine
9982	T4	Thyroxine
9983	T-	Tertile-
9984	T:A	thymine:adenine
9985	TBARS	Thiobarbituric acid-reactive substance
9986	TD	Typical diet
9987	TDI	Tolerable daily intake
9988	TDS	Total Diet Study
9989	TG	Thyroid gland
9990	TNF-α	Tumour necrosis factor α
9991	TPA	12-*O*-Tetradecanoylphorbol-13-acetate
9992	TRH	Thyrotropin-releasing hormone
9993	TSH	Thyroid stimulating hormone
9994	TVM	Tunica Vaginalis Mesothelioma
9995	UB	Upper bound
9996	UDS	Unscheduled DNA synthesis
9997	UF	Uncertainty factor
9998	UPLC	Ultra-performance liquid chromatography
9999	US-EPA	United States Environmental Protection Agency
10000	US-FDA	United States Food and Drug Administration
10001	Val	Valine
10002	V_{max}	Maximum formation rate
10003	VU	Vibration units
10004	WHO	World Health Organization